Laying hen welfare

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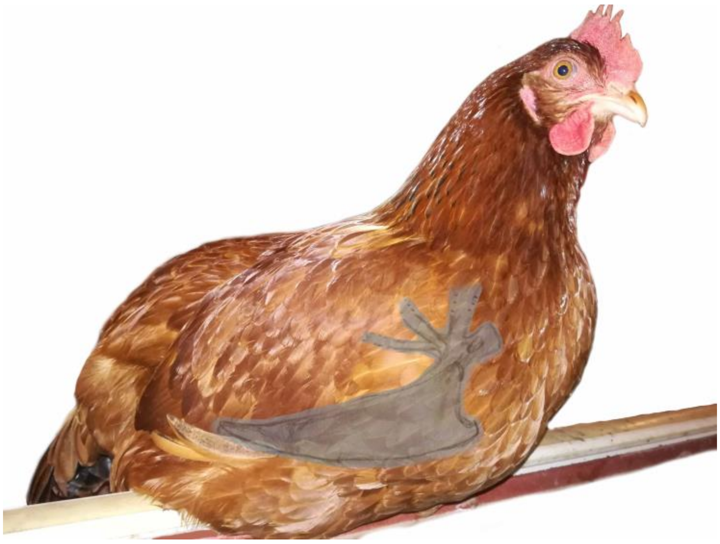
The prevention of feather pecking and keel bone damage

Dissertation zur Erlangung des akademischen Grades
eines Doktors der Agrarwissenschaften (Dr. agr.)



Submitted to the Faculty of Organic Agricultural Sciences
University of Kassel, Germany

by Lisa Jung. M.Sc. agr.
Farm Animal Behaviour and Husbandry Section

Gutachter: Prof. Dr. Ute Knierim, Prof Dr. Lars Schrader
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Summary

Animal welfare is an important topic in animal husbandry. Two main welfare problems in laying hen housing, in both organic and conventional systems, are feather pecking (FP) and keel bone damage (KBD).

The main aims of this PhD project were to detect: (1) whether recommendations for the prevention of FP are based on scientific evidence, (2) whether there is further evidence for influencing factors on FP, (3) whether there is a significant correlation between the quantitative fulfilment of recommendations and the occurrence of FP and, in the second part of this work (4) which management and housing factors are associated with KBD in organic laying hens.

Despite extensive research and increasing sources of advice for farmers, FP remains an important animal welfare problem in laying hens. An overview over results from experimental and epidemiological studies which investigated influencing effects on FP is given in Chapter 2. Here, studies which were carried out in non-cage systems, covering the rearing or laying phase are included. The investigated factors were categorised into good, contentious or no evidence regarding the prevention of FP. Moreover, it was checked to what extent recommendations for farmers are based on scientific evidence. In total, 62 potential preventive factors were extracted out of 88 experimental and 21 epidemiological studies. 17 factors during rearing, and 32 factors during the laying phase significantly affected the risk to develop FP or plumage damage. Factors were counted as significant if other studies found no or, at most, one opposite result. Seven factors during rearing and 16 factors during laying were confirmed by more than one study with no or, at most, one opposite result. Highly influencing factors were for example the provision of dry litter on the floor, sufficiently high perches during rearing and laying and a high use of the free-range area during the laying phase. In the 15 practice recommendations which were reviewed, almost all of these factors have been taken up. However, no recommendation comprised all factors, while most recommendations missed more than the half of them, therefore leaving ample room for improvement. Altogether, 15 contentious as well as eight non-significant or 12 not yet investigated factors were recommended.

The analysis in Chapter 3 partly bases on results, gained in Chapter 2. Data from three cross-sectional studies were pooled, to conduct a case control study, resulting in 224 flocks. After a group-matching 165 flocks were then allocated to cases (FP flocks) or controls (no FP). It was expected, that the prevention of FP does not only depend on certain factors (analysed in step one), but also on the extent of compliance with recommendations (analysed in step two). Out of 32 potentially preventive factors that had been recorded in all data sets, 18 factors passed a univariable pre-selection and were further analysed with a logistic regression analysis. The resulting model for 137 flocks (reduced number due to missing values) was able to explain 41% of the variance, while correctly classifying 77% of cases and comprising four variables with an effect size of f = 0.8. While a higher stocking density increased the likelihood of a 'FP-problem', the presence of wooden perches and a littered veranda lowered it. Unexpectedly, a higher ratio of drinking place/hen also predicted a FP problem. The results concerning wooden perches and ratio of drinking place/hen might be due to indirect effects and should be investigated further. In non-FP flocks, on average 46.5% of recommendations (out of a list of 13 factors) were fulfilled, while FP flocks only complied with 42.5%, resulting in a significant difference (P= .036, U= 2,537.500, N= 165, Mann-Whitney U Test, d_{Cohen}= 0.327). In conclusion, the number of fulfilled recommendations as well as the combination of specific measures such as provision of a covered veranda with dry litter or reduced stocking density are important in order to prevent FP.

Chapter 4 focusses on the topic KBD, by analysing possible risk factors for KBD in organic hens. Cross-sectional data of 107 flocks were assessed in eight European countries. Due to partly missing data, the final multiple regression model was based on data of 50 flocks. Keel bone damage included fractures and/or deviations and was recorded, alongside other animal based measures, by palpation and visual inspection of at least 50 randomly collected hens per flock between 52 and 73 weeks of age. Management and housing data were obtained by interviews, inspection and feed analysis. Flock prevalence for KBD ranged from 3% to 88%. Based on literature and practical experience, 26 factors potentially associated to KBD were included in an univariable selection by Spearman correlation analysis or Mann-Whitney U Test (with P<0.1 level). The resulting nine factors were further analysed with stepwise forward linear regression modelling. Aviary vs. floor systems, absence of natural daylight in the hen house, high proportion of underweight birds as well as a high laying performance were found to be significantly associated with an increased number of KBD cases. The final model was able to explain 32% of the variation in KBD between farms. The moderate explanatory value of the model underlines the multifactorial nature of KBD. Based on these results, increased attention should be paid to adequate housing design and lighting which enables easy orientation and safe manoeuvring for the birds in the system. An important objective regarding feeding management is a sufficient live weight, fulfilling the breeder weight standards. In order to achieve a better understanding of the relationships between laying performance, feeding management and KBD, further investigations are needed.

The results of this thesis show that the prevalence of FP and KBD are very high. But for both, FP and KBD, influencing management factors could be identified, even though the underlying mechanisms are not fully understood until now and further research is needed in both cases.

Zusammenfassung

Tierwohl ist ein wichtiges Thema in der landwirtschaftlichen Nutztierhaltung. Zwei gravierende Tierwohl-Probleme in der Legehennenhaltung, auch in alternativen Systemen, sind Federpicken (FP) und Brustbeinschäden.

Im Rahmen dieser Arbeit galt es zu ermitteln, ob: (1) Empfehlungen zur Prävention von FP auf Ergebnissen wissenschaftlicher Untersuchungen basieren, (2) welche möglichen Faktoren das Auftreten von FP beeinflussen können, (3) ob es einen signifikanten Zusammenhang zwischen dem Umfang eingehaltener Empfehlungen und dem Auftreten von FP gibt und im zweiten Teil der Arbeit (4) welche Management- und Haltungsfaktoren Einfluss auf das Auftreten von Brustbeinschäden bei ökologisch gehaltenen Legehennenherden haben.

Ungeachtet umfangreicher Untersuchungen und einer steigenden Anzahl von Ratgebern, bleibt FP bis heute ein großes Tierwohlproblem in der Legehennenhaltung. Kapitel 2 zeigt eine Übersicht der Ergebnisse aus experimentellen und epidemiologischen Studien, die Einflussfaktoren auf FP untersucht haben. Einbezogen wurden hierbei Untersuchungen aus „Nicht-Käfig-Haltungssystemen“, die die Aufzucht oder Legephase beinhaltet haben. Dabei wurden die Faktoren in durch Untersuchungsergebnisse bestätigte, umstrittene und nicht signifikante oder nicht untersuchte Einflussfaktoren unterschieden. Die auf dieser Grundlage identifizierten Einflussfaktoren wurden 15 deutsch- oder englischsprachigen publizierten Praxisempfehlungen gegenübergestellt. Insgesamt wurden 62 potenzielle Faktoren in 88 experimentellen und 21 epidemiologischen Untersuchungen analysiert. Daraus resultierten 17 Faktoren während der Aufzucht und 32 Faktoren während der Legephase als signifikant für die Entstehung von FP oder Gefiederschäden. Faktoren, die sich in Untersuchungen als signifikant erwiesen hatten, und in weiteren Untersuchungen höchstens ein gegensätzliches oder nicht signifikantes Ergebnis zeigten, wurden als „bestätigt“ definiert. Sieben Faktoren während der Aufzucht und 16 Faktoren während der Legephase wurden durch mehr als eine Untersuchung mit keinem oder höchstens einem gegensätzlichen Ergebnis bestätigt. Die Bereitstellung von trockener Einstreu im Stall und ausreichend hohe Sitzstangen während der Aufzucht und der Legephase und die Nutzung des Auslaufs durch möglichst viele Hennen in der

Legephase sind Beispiele für diese bestätigten Faktoren. Die einbezogenen 15 Empfehlungen haben insgesamt fast alle der bestätigten Faktoren aufgenommen, wenn auch keine einzelne Empfehlung alle Einflussfaktoren berücksichtigt. In den meisten Empfehlungen wird mehr als die Hälfte möglicher Einflüsse nicht genannt. Zum einen lässt das Raum für die Nachbesserung der Empfehlungen, zum anderen wurden Maßnahmen empfohlen, die in 15 Fällen unterschiedliche Ergebnisse in den Untersuchungen zeigten, in acht keinen signifikanten Einfluss hatten und in 12 Fällen noch gar nicht wissenschaftlich untersucht worden sind. Hierzu wären weitere Studien notwendig.

In Kapitel 3 wurden Daten aus drei Querschnittsstudien gepoolt, um qualitative und quantitative Unterschiede bezüglich Risikofaktoren und Einhaltung von Empfehlungen zwischen Herden mit Federpickproblem und solchen ohne zu ermitteln. Daten von 224 Herden wurden für die Durchführung einer Fall-Kontroll-Studie genutzt, einem Gruppenmatching unterzogen und die 165 verbliebenen Herden entweder Fallherden (mit FP Problem) oder Kontrollherden (kein FP) zugeordnet. Aus 32 potenziell präventiven Faktoren (basierend auf Erkenntnissen aus Kapitel 2), die in allen drei Untersuchungen erhoben wurden, werden 18 Faktoren die univariabel vorselektiert und weiter mit einer logistischen Regression analysiert. Das Endmodell für 137 Herden (reduzierte Anzahl aufgrund fehlender Werte) erklärt 41% der Varianz e, klassifiziert 77% der Fälle richtig und umfasst vier Variablen bei einer Effektstärke von f= 0,8. Während die Wahrscheinlichkeit eines FP Problems durch eine höhere Besatzdichte stieg, senkten Sitzstangen aus Holz und ein eingestreuter Wintergarten die Wahrscheinlichkeit einer FP-Herde zu bekommen. Unerwarteterweise wurde FP auch durch ein höheres Trinkplatz/Henne Verhältnis vorhergesagt. Die Ergebnisse bezüglich der Sitzstangen aus Holz und dem Trinkplatzverhältnis basieren möglicherweise auf indirekten Effekten und sollten weiter untersucht werden. Es wurde zudem angenommen, dass die Entstehung von FP neben bestimmten Faktoren auch durch die Anzahl der eingehaltenen Empfehlungen beeinflusst wird. Im Durchschnitt wurden in Kontrollherden (ohne FP) 46,5% der Empfehlungen eingehalten (von einer Liste aus 13 Faktoren), während Fallherden (mit FP) nur 42,5% Empfehlungen erfüllten, der Mann-Whitney U Test zeigte einen signifikanten Unterschied (P= .036, U= 2,537.500, N= 165, d_{Cohen}= 0.327). Schlussfolgernd kann sowohl eine hohe

Anzahl eingehaltener Empfehlungen als auch die Kombination bestimmter Managementfaktoren helfen, das Auftreten von FP zu vermeiden.

Kapitel 4 fokussiert das Thema Brustbeinschäden, wobei mögliche Risikofaktoren für Brustbeinschäden von 107 ökologisch gehaltenen Legehennen aus acht europäischen Ländern analysiert wurden. Aufgrund fehlender Werte basiert das Endmodell der Multiplen Regression auf Daten aus 50 Herden. Brustbeinschäden umfassten Brüche und/oder Deformationen und wurden, neben weiteren tierbasierten Indikatoren, durch Palpation und Adspektion bei mindestens 50 zufällig gefangenen Hennen/Herde in der 52. bis 73. Lebenswoche erhoben. Management und Stalldaten wurden durch Interviews, Begehung und Futteranalysen erfasst. Die Herdenprävalenzen bei Brustbeinschäden lagen zwischen 3% und 88%. Basierend auf Literaturangaben und eigener Erfahrung wurden 26 potenzielle mit Brustbeinschäden assoziierte Faktoren univariabel mittels der Spearman Korrelation oder Mann-Whitney U-Test (mit $P<0.1$) vorselektiert. Die neun resultierenden Faktoren wurden mit einer linearen Regression (schrittweise Vorwärtsselektion) weiter analysiert. Volieren vs. Bodenhaltung, die Abwesenheit von natürlichem Tageslicht im Stall, ein hoher Anteil an untergewichtigen Hennen und eine hohe Legeleistung resultierten als Faktoren, die signifikant mit einer erhöhten Anzahl Brustbeinschäden verbunden waren. Das Endmodell erklärt 32% der Variation der Brustbeinschäden zwischen den Betrieben. Dieser moderate Wert unterstreicht die multifaktorielle Natur der Brustbeinschäden. Basierend auf diesen Ergebnissen sollte erhöhte Aufmerksamkeit auf eine adäquate Stalleinrichtung und Stallbeleuchtung gelegt werden, die den Hennen eine einfache Orientierung und sicheres Manövrieren im System ermöglichen. Ein wichtiges Ziel hinsichtlich des Fütterungsmanagements sind ausreichendhohe Gewichte der Hennen, die die Gewichtsvorgaben der Züchter erfüllen. Um Zusammenhänge von Legeleistung, Fütterung und Brustbeinschäden besser zu verstehen, sind weitere Untersuchungen notwendig.

Die Ergebnisse dieser Dissertation zeigen, dass die Prävalenzen von Federpicken und Brustbeinschäden sehr hoch sind. Für beide Tierwohlprobleme konnten Einflussfaktoren identifiziert wurden, auch wenn die zu Grunde liegenden Mechanismen bisher noch nicht völlig verstanden sind und in beiden Fällen weitere Forschung nötig ist.

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List of abbreviations

BIC	Bayesian information criterion
C°	centigrade
Ca	calcium
CI	confidence interval
CL	confidence level
cm	centimeter
cm^2	square centimeter
df	degree of freedom
e.g	exempli gratia (for example)
Exp(B)	exponentiation of the beta coefficient
F	value of the difference between means of a group of variables
familiariz.	familiarization
for l	factor in rearing significant for laying
FP	feather pecking
FPL	foot pad lesions
GFP	gentle feather pecking
HFP	high feather pecking
HH	Healthy Hens project
HPA-axis	hypothalamic-pituitary-adrenal axis
HZ	hertz
IB	infectious bronchitis
IOR	inter observer reliability
κ	minimum-variance unbiased estimator
KBD	keel bone damage
lay	laying
LFP	low feather pecking
lux	unit of illuminance
m^2	square metre
Max	maximum
mg	milligram
Min.	minimum
min	minute
MIS	minimum standards
MRT	magnetic resonance tomography

N	sample size
n.s.	not significant
NFP	non feather pecking
NH3	ammonia
NIRS	near Infra-Red Spectrometry
No.	number
ns^l	not significant in laying
ns^r	not significant in rearing
OR	odds ratio
PABAK	prevalence-adjusted bias-adjusted Kappa
PD	plumage damage
ppm	parts per million
P-value	probability value
R^2	coefficient of determination
rear	rearing
r_s	Spearman rank correlation coefficient
$\|r_s\|$	absolute Spearman rank correlation coefficient
SD	standard deviation
SE	standard error
SFP	severe feather pecking
sign.	significant
ß	beta type II error rate (1-power)
suff.	sufficient
t	difference of the means of a variable between two populations
U	total count of smaller observations and the half of equal observations from sample 2 considered to sample 1
UK	United Kingdom
uninterr.	uninterrupted
UV	ultraviolet
VB	Verbund Projekt
VIF	variance inflation factor
vs.	versus
$\tilde{x}$	sample median
χ^2	Chi-squared test
Z	number of standard deviations from the sample mean
↑	increasing factor

1 General Introduction

This thesis tackles two important animal welfare topics regarding laying hens. Even though consumers believe that the welfare of laying hens is enhanced in non-cage systems (Pettersson et al., 2016), two welfare problems are found also in alternative egg production systems: feather pecking (FP) and keel bone damage (KBD). The level of animal welfare differs substantially between farms, depending on their specific housing and management conditions.

Animal welfare is defined by the ability to successfully cope with the environment, as well as the extent of experiencing positive emotions in addition to the absence of pain, suffering and physical harm. Consequently, any option to actively and successfully interact with the environment may increase animal welfare (Knierim, 2001).

The knowledge about natural habitats, behaviours and physiology of the chicken‘s wild ancestors, the junglefowl, is vital for the understanding of behavioural and physiological needs and disorders of laying hens. The domestication of the chicken began in Southeast Asia, where it descended from the red junglefowl (Gallus gallus). Under natural conditions, the red junglefowl lives in small mixed flocks at the edge of the forest. Within their home range, red junglefowl use regular roosting sites, including branches high up in the trees (Appleby et al., 2004). In springtime, the stronger cocks claim a territory together with three to five hens, whereas younger cocks live in groups of two or three animals. After mating, hens lay four to six eggs per clutch (Anwar et al., 2016). At twelve weeks of age, the offspring has to leave their parents and look for a social group of their own. Naturally, chickens spend around 60% of their active time with scratching and pecking to search for seeds, fruit and insects (Savory et al., 1978; Dawkins, 1989). The time spent foraging increases during daytime and hens execute around 14,000 to 15,000 pecks per day (Webster, 2002). Most behavioural patterns remained the same throughout the domestication process and according to Špinka (2006), behavioural problems are less likely if natural behaviours can be performed without restrictions. In this way, behavioural patterns associated with positive affective experience can bring long term benefits for the animal and therefore enhance welfare. The domestication of chickens started approximately around 8,000-10,000 years ago (Potts, 2012). However, molecular studies suggest evidence of earlier domestication dating as far back as 58,000 years ago (Sawai et al., 2010). Chickens were used for religious practices (e.g. in Rome 250 BC, where they were used to tell the future by scratching), for cock fighting (e.g. in Egypt 1350, where afterwards artificial incubation techniques were developed, by using camel dung and straw to warm eggs) and for food (e.g. in Greece 500 BC). With the decline of the Roman Empire, also the importance of chickens weakened (Elson, 2011). Even though chickens regained significance at the end of the middle age for a limited amount of time, eggs remained in a minor role well into the 20th century. In the 1930s, industrial egg production started with caged hens in the USA and the UK. Laying performance steadily increased from 70 eggs/year in backyard housing, to 150 eggs/year in cage systems up to 250 eggs/year in cages after the development of hybrid lines like H&N Nick Chick. Simultaneously, chicken mortality decreased from 40% in back yard production to less than 10% in cage systems (Windhorst, 2014). In 1966, about 90% of laying hens were housed in cages in the UK while 1972 80% of layers were housed in cages in Germany (Windhorst, 1979). The consequences of cage housing were profound for the individual chickens. Under such barren environments, hens were not able to perform basic behaviours such as nesting, foraging or perching. With the expansion of cage batteries in the 1970s, discussions about the welfare of laying hens were on the rise. In 1992, cage systems were first banned in Switzerland and the development of lower confinement housing systems, like floor and aviary housing started. By 2012, conventional cage-housing was banned in all EU-member countries (Council directive 1999/74/EC, 1999). They were replaced by alternative systems like group cages, floor and aviary systems. Floor systems are defined as one level systems, which are partly or completely covered with litter or with perforated floors. Aviaries are multi-tier systems with a littered ground floor. Both can be designed as indoor systems only, or they can be combined with a covered veranda and/or an outdoor run. The percentage of non-cage systems in 2017 ranged from 46.9% in Europe (Wing, 2018) to 90.5% in Germany (destatis, 2018). Organic egg production systems can also be floor or multi-tier systems, but they are obliged to include a free-range. In 2017, organic egg production comprised a market share of 5.1% in Europe and 10.5% in Germany (Wing, 2018). FP occurs in all housing systems, but outbreaks have a higher prevalence in non-cage systems compared to battery cages (Appleby and Hughes, 1991; Gunnarsson et al., 1999), probably because larger numbers of potential victims are present. Thus, FP represents a serious problem, also under conditions with enhanced animal welfare, that needs to be solved. Bone density was found to be higher in alternative systems but due to less collisions, KBD levels are also lower in cage systems. Until now, there is a lack of knowledge concerning causes and solutions for both FP and KBD and further investigations are urgently needed.

1.1 Feather pecking

Feather Pecking can be subdivided into gentle and severe FP. While in gentle FP, chickens peck without the removal of feathers, severe FP leads to plumage damage (PD) and feather loss (Bilĉík and Keeling, 2000; Savory, 1995). Feather loss leaves the skin exposed to physical injury and can be followed by tissue pecking, a form of cannibalistic behaviour (Rodenburg et al., 2013). Also heat loss is a problematic issue once skin is exposed after feather loss. FP is observed in production birds like laying hens, turkeys (Erasmus, 2018), ducks (Rodenburg et al., 2005), Japanese quail (Pizzolante et al., 2007) pheasants (Deeming et al., 2011) and ostriches (Muvhali, 2018). Abnormal behaviour like stereotyped gentle FP or severe FP has not been reported to appear in the behavioural repertoire of hens living under natural conditions (Brunberg at al., 2016). Fraser and Broom (1990) defined a behaviour as abnormal if it differs in pattern, frequency, or context from that which is shown by most members of the species in conditions that allow a full range of behaviour and if it serves no function and is caused by mental or physical disorder. Further examples for FP-related abnormal behaviours are self-pecking in parrots or tail biting in pigs. To minimize the damage caused by FP, it was a common practice to trim the beaks. However, the beak tip contains the bill tip organ which is made of highly sensitive mechanoreceptors. Therefore, beak trimming is considered to be painful. Partially debeaked chickens show a significant increase in guarding behaviour, i.e., tucking their bill under their wing, as well as diminished pecking and preening behaviour after the procedure. These pain-related avoidances may continue for approximately months (Duncan et al., 1989; Gentle et al., 1990, 1991). For these welfare reasons, beak trimming is heavily debated and is or will be prohibited in several European countries. However, two studies found that flocks with intact beaks showed higher PD and were at higher risk for cannibalism outbreaks (Riber and Hinrichsen, 2017; Sepeur et al., 2017). With these findings, the question arises, whether commercial flocks can be kept with intact beaks without further compromising their welfare through an increase in pain and suffering of the animals accompanied by an associated decrease of economic return.

The prevalence of FP in non-cage systems is alarmingly high. In a cross-sectional study of Green et al. (2000) which was carried out in none-cage systems, 46.6% of farmers reported that FP occurred regularly in their flocks with 10% to 75% (median 30%) affected birds during lay. Newer studies found FP prevalence of 60% (de Haas et al., 2014) up to 86% (Lambton et al., 2010) affected flocks at the end of the laying period.

1.1.1 Causation

As mentioned above, in nature, hens spend a large amount of their daytime with pecking and scratching for food. FP is hypothesized to be a redirected behaviour of foraging or dustbathing (Blockhuis, 1986; Vestergaard and Lisborg, 1993). In an experiment of Dixon et al. (2008), significant variations in pecking patterns between ground pecking during foraging and dustbathing could be shown, revealing that the pattern of severe feather pecks were similar to foraging pecks. This result confirmed the assumption that FP is a redirected foraging behaviour. Blockhuis (1986) observed, that hens without litter access redirect pecking to other attractive objects like feathers. It can be concluded that factors such as the possibility to forage and the provision of occupational material are important preventive measures against FP. In these regards, also high stocking density and an increased group size in commercial laying hen housing has to be considered as it changes the social structure of the animals dramatically. The presence of unfamiliar hens and reduced resources under constricted conditions can lead to resource-fights and social stress (Williams et al., 1977). El-Lethey et al. (2000) and Hedlund et al. (2019) hypothesized, that stress also increases the risk of FP. Besides, in large flocks with limited resources, hens are not able to synchronize their behaviour and resting hens may easily become victims of feather peckers. For this reason, the provision of perches is hypothesized to have a preventive effect, because they offer the possibility to rest in safe distance to active animals. Farmers from Finland mentioned optimal feeding and optimal lighting as the most important prevention measures against FP, whereas additional enrichment was only of low value to them (Kaukonen and Valros, 2019). A further hypothetical influence on FP examines poor health/gut microbiota (Birkl et al., 2018). Several measures are supposed to be preventive for FP, but to date there is no systematic overview of recommended prevention measures and their scientific base. Chapter 2 gives detailed information about possible influencing factors and scientific evidence for recommended prevention measures.

1.1.2 The assessment of feather pecking

Differences between assessment methods should be kept in mind when comparing and judging study outcomes. While in some studies direct observations, partly differentiating between different forms of FP are used, others assess PD using different scoring systems. Parts of the applied plumage scoring systems are adapted based on Gunnarsson (1995), who divided the body into 11 regions (see Figure 1) and Tauson et al. (2005), who established a more practical scoring system that comprises 6 body parts (see Figure 2).

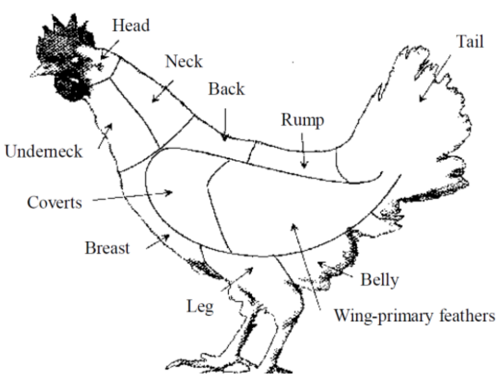

Figure 1: Body parts after Gunnarson (1995)

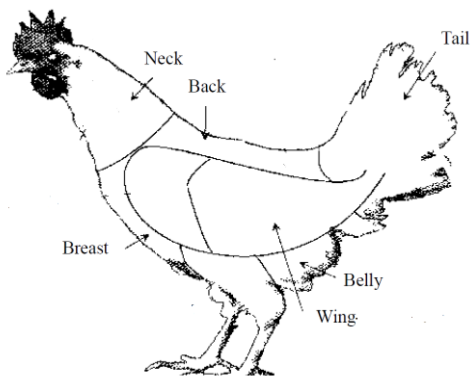

Figure 2: Body parts after Tauson et al. (2005)

The following Figures are examples for excellent plumage conditions as well as damage occurring on different body parts. Figure 3 shows a belly without PD in comparison with highly damaged plumage shown in Figure 4. Figure 5 shows a hen with a very good plumage condition, whereas Figure 6 shows PD at the back and tail. Differentiating between damage levels can be difficult and also depends on light conditions, experience, sensual ability of the assessor and on clear definitions in the scoring scheme. Interobserver reliability tests (IOR) are a necessary precondition to ensure reliability of scoring schemes (Haradhan, 2017).

Figure 3: Belly without feather damage

Figure 4: Belly with feather damage

Figure 5: Neck, breast and back without feather damage

Figure 6: Back and tail with feather damage

1.1.3 Aims and approach concerning feather pecking

The high prevalence of FP indicates that improved recommendations for the prevention of FP are urgently needed, especially for farmers housing birds with intact beaks. An important information would be if recommendations for the prevention of FP are available and in how far the advices are complete and right.

The first part of this thesis focusses on the following research questions: (1) Are practice recommendations in line with results of epidemiological and experimental studies? (2) What are possible risk factors for feather pecking in commercial farms? (3) Do FP and non-FP flocks differ in the quantitative fulfilment of recommendations?

In order to answer these questions, first a literature research is conducted. Results of experimental and epidemiological investigations concerning FP are compared and recommendations are checked for scientific evidence and completeness. The results are presented and discussed in Chapter 2.

Based on the findings in Chapter 2, data of three cross sectional studies are used to design a case control study with FP and non-FP flocks in Chapter 3. A logistic regression is used to confirm or to detect possible risk factors. The difference of the compliance with recommendations between FP and non-FP flocks is then analysed using a Mann-Whitney U Test.

Finally, in Chapter 5, results are discussed taking into account confirmed and contentious factors as well as open research questions. The main aim of improving practice recommendations for the prevention of FP remains paramount throughout the Chapter.

1.2 Keel bone damage

The keel, also called sternum or breast bone, is a single large bone on the ventral surface of the body. It runs axially along the midline and extends outward, perpendicular to the plane of the ribs (see Figure 7). The keel provides a large surface where the muscles used for wing motion, the pectoralis minor and pectoralis major are anchored.

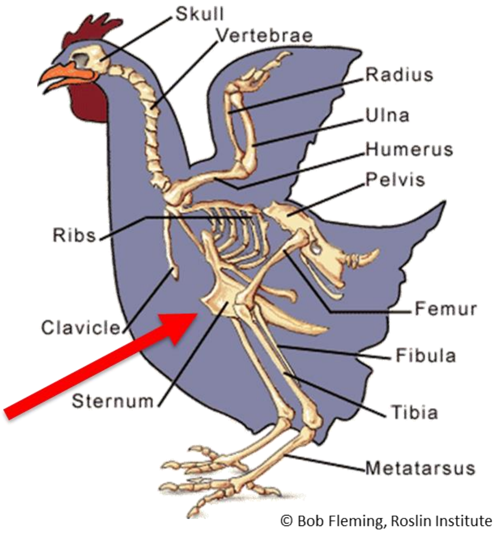

Figure 7: Position of the sternum in the hen skeleton

Keel bone fractures and/or deviations are found in all systems with nearly 100% affected flocks and 3%-97% affected hens/flock (Richards et al., 2012; Heerkens et al., 2016; Rufener et al., 2018). KBD therefore is a serious welfare problem as it may be a source of chronic pain (Nasr et al., 2012a and 2013a) and negatively affect mobility (Nasr et al., 2012b; Richards et al., 2012; Casey-Trott and Widowski, 2016).

1.2.1 Causation

Bone is a living tissue, continuously adapting to external mechanical stimuli as well as internal metabolic calcium demands (Kerschnitzki et al., 2014). The keel bone is exposed to the pressure of perches or wing flapping. In intact bones, wing flapping improves bone structure with long lasting effects (bone loading). The keel is a pneumatic, structural bone. During sexual maturation, the oestrogen level rises and stimulates the deposition of calcium and osteoblasts to change from forming structural bone to producing a woven bone (medullary bone), unique to birds and dinosaurs (Whitehead, 2004). Medullary bones (e.g. long bones of the wings, legs, vertebrae and keel) are a source of calcium for eggshell formation. Laying hens transfer around 10% of their total body calcium volume for egg production on a daily basis (Bar, 2009). The flow of calcium from the intestine to the plasma stops 4 to 5 h after the last feeding. Because eggshell formation occurs while the intestine lacks dietary calcium, bone resorption is activated during the second half of the dark period (Bar, 2009). The progressive change of structural bone into medullary bone is characteristic of osteoporosis. It is caused by a mineral loss from the bone as well as architectural losses of normal bone structure and results in the weakening of the skeleton and increased risk of fractures.

As soon as the hen goes out of lay, medullary bone gradually disappears and structural bone formation recommences (Whitehead, 2004). Thus, laying performance is hypothesized to have an impact on keel bone formation. Interestingly, neither Gebhardt-Henrich and Fröhlich (2012) nor Heerkens et al. (2013) or Candelotto et al. (2017) could find a correlation of egg production and prevalence of KBD. Eusemann et al. (2018) found significantly more hens with fractures in a high performing line (BLA) compared to a low performing line (L68). However, no difference was found between high and low performing white lines. Gebhardt-Henrich and Fröhlich (2012) reported more fractures in hens which started laying at an earlier age. A negative correlation between early egg number and good palpation score was found by Andersson (2017), too.

Other potential influences linked to bone metabolism are for example feed ingredients, hormones and the combination thereof. Therefore, feed ingredients (such as calcium, phosphorus, vitamin D3, vitamin K, vitamin A, vitamin C, and minerals like nitrate, sodium, magnesium, zinc, copper and iron) play an important role in maintaining bone health and improving the breaking strength of bones in laying hens, especially in combination with hormones (such as oestrogen, calcitonin, androgen, somatotropin, glucocorticoids and thyroid hormones). In different studies, various influences of nutrition were investigated. Tarlton et al. (2013) for example, found a significant reduction in keel bone breakage rate (40-60%) in omega-3 supplemented hens. Hens which were fed a diet high in energy and low in protein, showed an upregulation in bone turnover and aggravated skeletal damage in the investigation of Jiang et al. (2013). Eusebio-Balcazar et al. (2018) found, that the keel of white pullets which were fed coarse limestone was positively influenced at 54 weeks of age. On one hand, bone weakness is influenced by hormones, calcium demand and nutrition, on the other hand there are several external hazards which can also result in bone fractures. Studies showed higher KBD prevalence in aviaries than in floor systems (Riber and Hinrichsen, 2016), predominantly due to unsuccessful landings on perches or tiers followed by collisions and falls (Stratmann et al., 2015a). KBD can be partly decreased by adding ramps (Stratmann et al., 2015a, Heerkens et al., 2016b) and using soft perches in stables (Stratmann et al., 2015b). Also, genetics can have an influence on KBD (Andersson, 2017). Stratmann et al. (2016a) found genetic influences in this regard and concluded that specific bone strength and other genetically associated body morphologies lead to different predisposition for KBD. Next to foot pad lesions (Gebhardt-Henrich and Fröhlich, 2015) and poor feather cover (Riber and Hinrichsen, 2017), also the rearing system (Casey-Trott et al., 2017) and perch design (Tauson et al., 1994; Pickel et al., 2011) are further possible influencing factors.

1.2.2 Assessment of keel bone damage

The assessment of KBD is divided into deviations and fractures. Following the definition of Casey-Trott et al. (2015), a deviated bone is characterized by an atypically shaped structure that contains section(s) that vary from a perfectly 2-dimensional straight plane (Figure 8). Fractures however, are characterized by dislocations, fragments, sharp bends or shearing of the keel bone. Fractures may extend from the ventral to the dorsal surface in the sagittal plane, though they can also occur cranial to caudal, or as a combination of both (Casey-Trott et al., 2015).

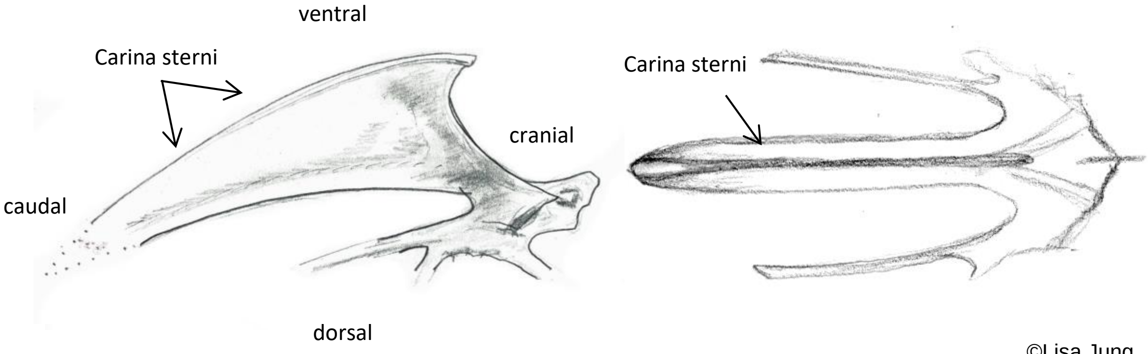

Figure 8: Schematic of a straight keel bone from lateral and ventral view

Following the initial trauma, the bone heals either through direct intramembranous or indirect fracture healing. The most common pathway is through indirect healing, since direct bone healing requires highly stable conditions. Direct healing allows the bone structure to immediately regenerate without the necessity of any remodelling steps. Indirect bone healing involves an acute inflammatory response and primarily generates cartilaginous callus. This primary callus later undergoes revascularization and calcification and is finally remodelled to fully restore a normal bone.

In literature, different kinds of scoring systems for the assessment of KBD can be found. Most studies used 3-point scoring systems (Fleming et al., 2004; Heerkens et al., 2016a; Hinrichsen et al., 2016; Grafl et al., 2017), sometimes verifying results by dissection. Some studies differentiated between fractures and deviations, while others did not make this distinction or exclusively examined fractures only. Scholz et al. (2008) found histological indications for fractures in 100% of severe and 80% of moderate deviations. Deviations can be felt as curves in lateral or dorsal direction. Palpable callus material indicates healing fractures, whereas new fractures are only palpable if they are accompanied by dislocations or sharp bends. Personal experience showed, that the caudal tip is damaged in most cases at least at the end of lay (Figure 9).

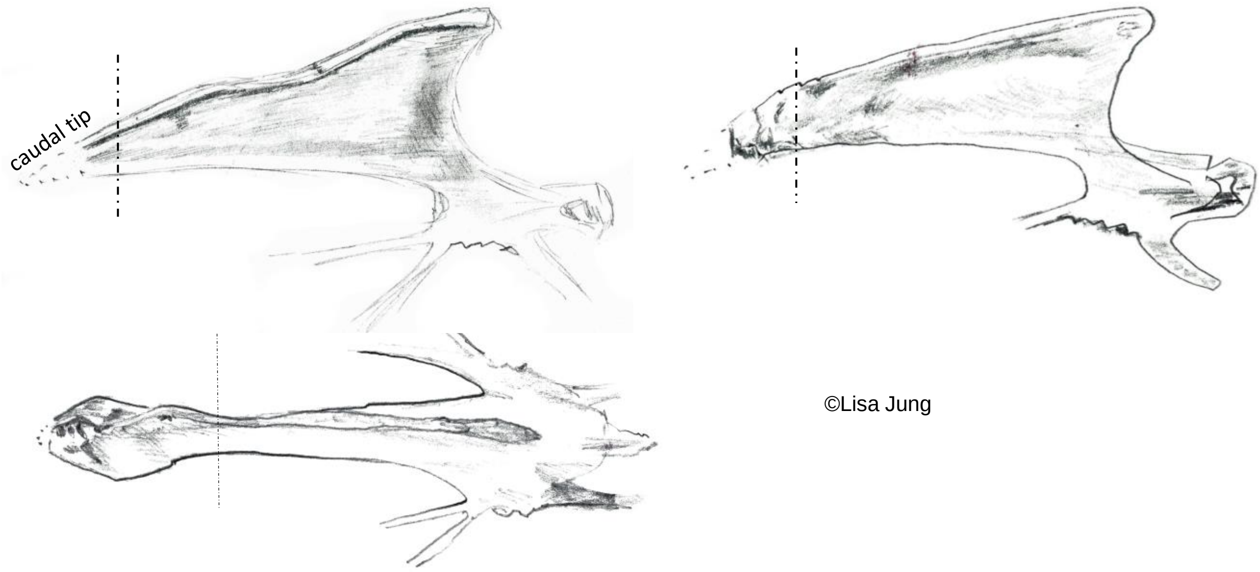

Figure 9: Fractured and deviated keel bones with broken and compressed tips

1.2.3 Aims and approach concerning keel bone damage

Previous research showed a high prevalence of KBD in laying hens. The overall aim of this part of the thesis was the identification of possible risk factors for KBD in organic egg production systems. By analysing the data of 107 flocks in eight countries, common European housing and management practices are considered. Using linear regression modelling on the data of 50 flocks (complete data sets, without missing values), possible risk factors for the occurrence of KBD in organic egg production systems were identified.

2 Are practice recommendations for the prevention of feather pecking in laying hens in non-cage systems in line with the results of experimental and epidemiological studies?

2.1 Introduction

Feather pecking (FP) is still a significant problem in laying hens (e.g. Heerkens et al., 2015; Nicol et al., 2013; Rodenburg et al., 2013). In literature, distinction is being made between six different types of allopecking behaviour: (1) aggressive pecking which is to be differentiated from FP (Savory, 1995), (2) gentle FP without removal of feathers (Bilĉík and Keeling, 2000; Savory, 1995), (3) severe FP leading to feather loss (Bilĉík and Keeling, 2000; Savory, 1995), injurious (4) tissue pecking in denuded areas, (5) vent pecking (Savory, 1995) and (6) pecking at toes, which can also be regarded as a type of cannibalistic allopecking behaviour (Krause et al., 2011). Furthermore, regarding gentle FP, Rodenburg et al. (2004) suggest distinguishing (1) 'normal' gentle feather pecking from (2) stereotyped gentle feather pecking, and (3) gentle pecking at particles on the plumage (which is no FP behaviour). It is still unclear whether only some or all forms of gentle FP may develop into severe FP (Newberry et al., 2007). Severe feather pecking may result in economic losses because of increased food consumption in defeathered birds (Leeson and Morrison, 1978; Tullett et al., 1980), increased mortality (El-Lethey et al., 2000) as well as in reduced animal welfare since FP is painful (Gentle and Hunter, 1990). Consequently, it can lead to cannibalism (Green et al., 2000) and the victims' death (Fossum et al., 2009; Heerkens et al., 2015). Only severe FP behaviour or the resulting PD will be considered in this paper. Non-cage systems are increasingly used in the EU, making up about 45% of the hen places in 2014 (Wing, 2015); and having increased from 26.7% in 2005 to 89.3% in 2014 in Germany (Statistisches Bundesamt, 2015). While the risk of problems due to FP is increased in these systems (Rodenburg et al., 2004), the major symptomatic measure to control damage due to FP, namely beak trimming, is heavily debated for animal welfare reasons (Defra animal welfare team, 2015). In several European countries beak trimming is either already forbidden by law (Sweden, Norway and Finland) or by label guidelines

(Austria), or shall be phased out in the near future, with dates between 2017 (UK, Germany) and 2018 (Netherlands). Alternatively, individual egg producers voluntarily refrain from beak trimming, like in Denmark since 2014 (Defra animal welfare team, 2015). Therefore, the demand for practice recommendations on how to prevent FP is increasing.

There is an abundance of experimental studies on possible risk factors for this undesirable behaviour (e.g. reviewed by Kjaer and Bessei, 2013; Nicol et al., 2013; Rodenburg et al., 2013). However, as FP is a multifactorial problem and the various influencing factors may interact differently on each individual farm, the successful transfer of the results of experimental studies into farm practice is difficult (Lambton et al., 2013). This is one reason why epidemiological studies have been increasingly undertaken. For this review we examined (1) epidemiological and (2) experimental studies as well as (3) practice recommendations which are easily accessible to laying hen farmers in terms of consistency within and between the three categories. On this basis we aimed to identify influencing factors regarding FP for which there is either good, contentious or no evidence.

2.2 Material and Methods

For the search of epidemiological and experimental studies in the electronic databases Web of Science, scienceDirect, CAB Abstracts, pub.med. and organic eprints the keywords ‘laying hens’ in combination with ‘feather pecking’ or ‘plumage damage’ were applied. Only studies concerning the species Gallus gallus domesticus in non-cage systems and the topics ‘damaging feather pecking’ or ‘plumage damage’ were included. In addition, reference lists of retrieved papers were searched for further studies.

Recommendations were sought using the internet search engine ‘Google’ with the keywords ‘laying hens’ and ‘feather pecking’ which were used in combination with ‘recommendations’, ‘management guidelines’ or ‘references’. Also, the German keywords ‘Federpicken’ and ‘Legehennenhaltung’ were applied in combination with ‘Empfehlungen’, ‘Prävention’, ‘Managementempfehlungen’ or ‘Haltungsempfehlungen’. Selection criteria for the recommendations were that they must be freely available, that they covered rearing, placement or the laying period and that they are related to non-cage systems. Recommendations which were directly derived from an individual epidemiological study were excluded. Influencing factors (for the sake of clarity concerning the direction of influence, we call them preventive factors in this paper) which were found in the reviewed studies were grouped into categories and listed in tables, together with further relevant information, e.g. whether FP or PD had been studied, size of the study, age of hens at scoring or beak status.

2.3 Results

We identified 21 epidemiological, 88 experimental studies and 15 recommendations fulfilling the criteria described above. Altogether 82 potential preventive factors regarding FP were extracted from the reviewed recommendations and studies. The housing and management systems investigated included organic or conventional systems with barn, aviary or free-range housing, and beak trimmed as well as non-beak-trimmed birds. Sometimes no information about housing systems or beak status was given. The dependent variables were FP (yes/no), the amount of FP observed (total number of feather pecks), partly with differentiation of forms of FP, plumage damage scores, the percentage of birds with PD or the time when FP started.

2.3.1 Epidemiological studies

From the identified epidemiological studies, 17 are published peer reviewed articles, two are conference papers, one is a PhD-thesis and one pilot study is available as pdf in the Internet. Table 1 gives information on important aspects of the study designs. Most studies (20) focused on the laying phase while eight also considered rearing. Huber-Eicher and Audigé (1999) focused only on rearing. Lambton et al. (2010a) as well as Pötzsch et al. (2001) additionally collected data concerning rearing, without showing them. Two studies explicitly included information about the placement of the hens (Bestman, 2000; Bestman and Wagenaar, 2003). The number of potential preventive factors taken into account per study varied from one to 28, leading to altogether 51 factors, from which 46 were found to significantly affect FP or PD in at least one study (Table 2)

Table 1: Characterization of the identified epidemiological studies

No.	Reference	Age (weeks) of birds at visit	Beak trimmed (no. of flocks)	Dependent variable	Number of hens scored	Number of flocks	System[1] (number of flocks)
1	Bestman 2000	50	No information	PD	40	36 lay	Organic
2	Bestman and Wagenaar 2003	≥50	No information	PD	40 (20 in small flocks)	63 lay	Organic
3	Bestman and Wagenaar 2014	50-60	No information	PD	50	49 lay (information about rearing of 35 flocks)	Organic aviary (22 lay) and floor (27 lay); cage (6 rear), loose house (27 rear) with free-range (26), unknown (2)
4	Bestman et al. 2009	7,12,16,30	No information	PD	100	28 rear, 51 lay	Organic
5	Bright et al. 2011	No information[2]	Yes (161), No (1)	PD	50	162 lay	Free-range
6	de Haas et al. 2014a	1,5,10, 15, 40	Yes	SFP and PD	20 rear, 50 lay	35 rear, 35 lay	Conventional floor (7 lay), level (3 rear), aviary (32 rear, 28 lay),
7	Drake et al. 2010	<17,18-22,23-30,50	Yes	PD	200	12 rear, 84 lay	Conventional barn (10 lay) and free-range (55 lay); organic (19 lay)
8	Gilani et al. 2013	1,8,16,35	Yes (12), No (22)	GFP and SFP and PD	20	34 rear, 34 lay	Conventional barn (17 rear, 1 lay) and free-range (1 rear, 16 lay); organic (16 rear, 17 lay)
9	Gilani et al. 2014	8,16,35	Yes (11), No (22)	GFP and SFP	0	33 rear, 33 lay	Conventional barn (17 rear) and free-range (1 rear, 17 lay); organic (15 rear, 16 lay)
10	Green et al. 2000	No information	No information	Any FP or PD[1]	No information	198 lay	Conventional barn (26) and free-range (172)
11	Gunnarsson et al. 1999	35	No	PD	100	59 rear/lay	Floor and aviary
12	Häne et al. 2000	40-80	Yes and No (no information)	PD	No information	96 lay	Floor, aviary and free-range
13	Heerkens et al. 2015	58-64	Yes (46), No (1)	PD	50	47 lay	Conventional aviary (47) with free-range (9)
14	Huber-Eicher and Audigé 1999	No information	No information	Any FP or PD	No information	64 rear	Non-cage system

Table 1 continued: Characterization of the identified epidemiological studies

No.	Reference	Age (weeks) of birds at visit	Beak trimmed (no. of flocks)	Dependent variable	Number of hens scored	Number of flocks	System[1] (number of flocks)
15	Huber-Eicher and Sebö 2001a	5, 14, 20, 32, 50	Yes (13), No (12)	Any FP and PD	10%	25 rear, 19 lay	Conventional floor (15 rear, 7 lay) and aviary (10 rear, 12 lay)
16	Lambton et al. 2010b	20-30, 35-45	Yes (79), No (21)	GFP and SFP and PD	100	119 lay	Conventional barn (3 lay) and free-range (50 lay); organic (66 lay)
17	Lambton et al. 2010a	25, 40	No information	SFP and PD	100	75 lay	Free-range
18	Lugmair 2009	16, 21-82	No	PD	20	42 rear, 115 lay	Conventional floor (32 rear, 33 lay), aviary (9 rear) and free-range (56 lay); organic (1 rear, 26 lay)
19	Nicol et al. 2003	23-74	Yes	PD	15	112 lay	Free-range
20	Pötzsch et al. 2001	No information	No information	Any FP, PD[2]	No information	198 lay	Conventional barn (26) and free-range (172)
21	Velik et al. 2005	No information	No information	PD	20	21 (no infomation)	Conventional (9), organic (12)

PD= plumage damage, GFP= gentle feather pecking, SFP= severe feather pecking, FP= feather pecking, lay= laying, rear= rearing,

[1] information as provided in the publications, [2] study based on information from questionnaires and assessments as reported by the farmer s

On average a factor was investigated by three studies. The factors most frequently considered were ‘small flock size’ (9x), ‘high use of range’ (9x), ‘suitable hybrid’ (7x), ‘access to perches’ (7x), ‘low stocking density’ (6x) and ‘low light intensity’ (6x).

The preventive potential of quite a number of factors was unanimously confirmed in different studies at least concerning one phase of the hens’ life, during the rearing or laying phase. These were ‘use of pullets without FP in rear’ (5x), ‘high percentage of sheltered areas’ in the free-range during laying (4x) and ‘measures encouraging hens to go outside’ (3x), ‘low stocking density’ during rearing (3x), but not always during laying (1x significant (sign.) and 3x non-significant (n.s.)), ‘prevention of diseases’ during laying (3x), feeding ‘mash instead of pellets’ (3x laying, 1x rearing), ‘low sound level’ during laying (2x), but not unequivocally during rearing (2x sign., 1x n.s.) and ‘provision of dry litter on the floor’ during rearing (2x), but not unequivocally during laying (3x sign., 1x n.s.). For ‘spreading grain on floor’ during laying a significant effect was confirmed, but two of three times as risk increasing, while during rearing no effect was found (2x). Predominantly a ‘high use of range’ was found to be significantly beneficial in the laying period (6x sign, 1x n.s), but not during rearing (2x n.s.).

‘Early placement before 20 weeks of age’, ‘different barn areas/levels in the laying house’, ‘nests without lighting’ and an ‘appropriate feed company’ were all identified as preventive factor in two studies, while one found no significant effect. For ‘less feed phases’ during laying results are balanced (2x sign., 1x n.s., 1x increased risk). Several other factors had only been investigated in one study during rearing or laying, but were found to significantly affect FP or PD (‘rearing own pullets’, ‘sufficient uniformity in weight’, ‘presence of cockerels’, ‘adjusted management’, ‘sufficient litter height’, ‘sufficiently high perches (> 35 cm) ’, ‘wood as material for perches’, ‘uninterrupted light period’, ‘no flickering light’ (during rearing), ‘spelt as nest material’, ‘provision of a platform in front of the nests’, ‘sufficient drink places/hen’, ‘more sugar, less starch in ration’, ‘less feed phases’ (during rearing). In one case, ‘even distribution of light’, the significant effect was contrary to expectations.

For the remaining factors displayed in bold in Table 2, however, different studies yielded balanced (1x sign, 1x n.s.) or predominantly non-significant results or sometimes contrary effects. They comprised ‘suitable hybrid’, ‘good expert knowledge’, ‘regular checks of hens’, ‘small flock size’, ‘good air quality’, ‘suitable air temperature’, ‘provision of hay and straw’, ‘access to perches’, ‘daylight’, ‘low light intensity’, ‘dawn phase’, ‘individual nest boxes’, ‘chain feeders’, ‘nipple drinkers’, ‘provision of feeders/drinkers in litter area’, ‘sufficient methionine in the laying period’ and ‘daily access to range’. For the five factors ‘start of lay not before 20 weeks of age’ (1x), ‘matching of rearing and laying environment’ (2x), ‘early access to litter’ in the rearing unit (1x), ‘additional vitamins’ (2x) and ‘spreading seashells on floor’ (2x) studies yielded only non-significant results.

Table 2: Potential preventive factors investigated in epidemiological studies (numbers of studies according to Table 1); factors in bold have been found to significantly affect feather pecking (FP) or plumage damage in at least one study (l = laying, r = rear)

	Factors / Studies	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Management	**Suitable hybrid**	ns^{l}	ns^{l}			l	ns^{l}					ns^{l}							l	ns^{l}		
	Use of pullets without FP in rearing				l		l		l							l	l					
	Rearing own pullets							l														
	Good expert knowledge	l	ns^{l}						r		l			ns^{l}								
	Regular checks of hens														ns^{r}				r/l	ns^{l}		
	Low stocking density	l	ns^{l}		r							ns^{l}			r				ns^{l}/r			
	Sufficient uniformity in weight																		r			
	Low sound level						ns^{r}	r/l	r										l			
	[1]Small flock size	l	l		ns^{r}	ns^{l}	ns$^{r/l}$		ns^{r}			ns^{l}		ns^{l}					l(↑)[1]			
	Prevention of diseases like IB or egg peritonitis										l			l					l			
	Start of lay not before 20 weeks of age										ns											
	Presence of cockerels		l																			
	Early placement before 20 weeks of age	l	l																	ns^{l}		
	[2]Adjusted management						l															
	Matching of rearing and laying environment							ns^{l}				ns^{l}										
	Good air quality							r/l	ns$^{r/l}$		ns^{l}				ns^{r}				l			
	Suitable air temperature (>20 C°)							ns$^{r/l}$	ns^{r}		l						ns^{l}					
	Different barn areas (different levels)						l		r		ns^{l}								l			
Litter	Early access to litter											ns^{r}										
	Provision of dry litter on the floor				r		ns^{l}		r		l		l						l			
	Provision of straw and hay																ns^{l}		l			
	Sufficient litter height																		l			
Perch	**Access to perches**				ns^{r}				ns$^{r/l}$		ns^{r}	ns^{r}			r		ns^{l}		ns^{r}/l			
	Sufficiently high perches (>35 cm)														r				l			
	Wooden perches																		l			

Table 2 continued: Potential preventive factors investigated in epidemiological studies (numbers of studies according to Table 1); factors in bold found to have been significantly affect feather pecking (FP) or plumage damage in at least one study (l = laying, r = rearing, ns = non-significant, ↑ = increases risk)

	Factors / Studies	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Light	**Uninterrupted light period**								r													
	Daylight				r			r	ns^{r}													
	No flickering light							r↑										l		ns^{l}		
	Low light intensity						ns^{l}	l			ns^{l}				ns^{r}			l		ns^{l}		
	Dawn phase								ns^{r}										l	ns^{l}		
	Even distribution of light																		l↑			
Nest	**Individual nest boxes**	l									ns^{l}											
	Nests without lighting										l									ns^{l}	l	
	Spelt as nest material																		l			
	Provision of a platform in front of the nests													l								
Feed and water	**Mash instead of pellets**																l	l	r/l			
	Chain feeders (instead of pan feeders or mixed feed systems)	l↑						r/l↑	l										ns^{l}			
	Nipple drinkers (instead of bell drinkers)						l↑				l								l↑	ns^{l}	l	
	Sufficient drink places/hen																		l			
	Provision of feeders/ drinkers in litter area	l																		ns^{l}		
	More sugar, less starch in ration																		l			
	Additional vitamins										ns^{l}									ns^{l}		
	Sufficient methionine																		l			ns^{l}
	Spreading grain on floor	l			ns^{r}												l↑		l↑	ns^{l}		
	Spreading seashells on floor										ns^{l}									ns^{l}		
	Less feed phases								r		l			l↑						ns^{l}	l	
	Appropriate feed company																l		l	ns^{l}		
Free-range	**High use of range**	l		l	ns^{r}		ns^{r}			ns$^{r/l}$	l			l			l			l		
	Daily access to range	l	ns^{l}																			
	Measures encouraging hens to go outside	l	l																l			
	High percentage of sheltered areas	l	l			l													l			

[1]No linear relationship was found, 1,001-2,999 hens showed more FP than less than 1,000 hens or more than 3,000 hens [2]Adjusted management: radio, pecking blocks, round drinkers and/or roosters

2.3.2 Experimental studies

The majority of the included 88 experimental studies are peer-reviewed articles; six are conference papers, two are PhD-theses and two are research reports.

The experimental studies were carried out during rearing in 48 cases and during the laying phase in 52 cases. Nine times the effects of management strategies during rearing on the laying period were investigated. The observed birds were between 1 day and 69 weeks old.

Of the 29 factors in total, most frequently investigated were ‘suitable hybrid’ in 22, ‘provision of dry litter on the floor’ in 16 and ‘provision of enrichment material’ in nine experiments; ten factors were only taken into account once (Table 3).

The factors unanimously confirmed as reducing FP or PD by all respective experiments were ‘provision of dry litter on the floor’ in rearing (13x) and laying (4x), ‘provision of enrichment material’ during rearing (4x), but not unequivocally during laying (4x sign., 1x n.s.), ‘access to range’ during laying (3x), but not during rearing (1x n.s.), access to ‘sufficiently high perches (> 60 cm)’, provision of ‘nests without lighting’, ‘nipple instead of bell drinkers’ and ‘roughage feeding’ (all 4x during laying) as well as ‘low stocking density’ in rearing (2x), but not in laying (1 sign., 1x increased risk, 3x n.s.). The preventive effect of the ‘use of dark brooders in rearing’ was confirmed three times, but in one further study only on FP, not on PD.

‘Mash instead of pellets’ (during laying) was identified as preventive factor in two studies, while one found no effect.

A number of studies found lasting effects of rearing conditions on the laying period. These were ‘provision of dry litter on the floor’ (4x, 1x n.s.), ‘use of dark brooders in rearing’ (2x) and ‘spreading grain on the floor’ (1x).

Six factors were only investigated once, but significantly affected FP or PD (‘familiarization of hens with people’, ‘feeding ad libitum’ and ‘spreading grain on the floor’ during rearing; ‘provision of refuge sites’ and ‘no flickering light’ during laying). For ‘no exclusion from litter after placement’ a significant, but risk increasing effect was found. For the remaining factors displayed in bold in Table 3 results of different studies were rather variable. These were ‘suitable hybrid’ for rearing (9x sign., 5x n.s.) and for laying (8x sign., 4x n.s.), ‘small flock size’ during rearing (1x sign., 1x n.s.) and during laying (3x sign. but once risk increasing, 3x n.s.), ‘low light intensity’ in rearing (1x sign., 2x n.s.) and in laying (1x sign., 1x n.s.), as well as the ‘high amounts of certain essential amino acids’ during laying (2x sign., 2x n.s.) or rearing (2x n.s.), which included from 25 weeks of age onwards a generally higher protein content (Dixon and Nicol 2008; Elwinger et al., 2008), a higher methionine and cystine content (Elwinger et al., 2002, 2008) or only a higher methionine content (Elwinger et al., 2008; Kjaer and Sørensen, 2002; van Krimpen et al., 2015) or an increase of dietary L-tryptophan (van Hierden et al., 2004) as well as the use of animal protein and synthetic amino acids (Keppler et al., 2001). Further ambiguous preventive factors during laying were ‘access to perches’ (1x sign., 1x n.s.) and ‘low energy and non-starch polysaccharide content in feed’ (2x sign. but once increasing risk, 1x n.s.).

No significant effects were found for: ‘use of broody/mother hens’ (2x), ‘provision of dust-bath’ (1x), ‘less feed phases’ (2x), all during rearing, and the ‘use of pullets without FP in rearing’ (1x), the ‘presence of cockerels’ (1x) during laying, as well as ‘high amounts of certain minerals’ during rearing (1x) and laying (1x) and ‘animal protein’ during rearing (1x) and laying (2x). The investigated minerals were Aluminium, Barium, Chromium, Copper, Lead, Molybdenum, Nickel, Silver, Tin, Titanium and Zirconium (Willimon and Morgan, 1953).

Table 3: Potential preventive factors investigated in experimental studies; factors in bold have been found to significantly affect feather pecking (FP) or plumage damage (PD) in at least one study in the expected direction (for l = effects of rearing conditions on laying, ↑ = increases risk)

		Rearing		Laying	
	Factors	Significant	Not significant	Significant	Not significant
Management	**Suitable hybrid** (mostly high versus low feather pecking lines)	Bright 2007, de Haas et al. 2014b, Harlander-Matauschek et al. 2010, Keeling et al. 2004, Kjaer 2011, Kjaer and Sørensen 1997, Kjaer and Sørensen 2002, Klein et al. 2000, Rodenburg and Koene 2003	Albentosa et al. 2003, Hocking et al. 2004, Keppler et al. 2001, Rodenburg et al. 2003, van Hierden et al. 2002	Benda 2008, Elwinger et al. 2008, Harlander-Matauschek et al. 2010, Keppler et al. 2001, Kjaer 2000, Kjaer and Sørensen 2002, Rodenburg and Koene 2003, Wahlström et al. 2001	Albentosa et al. 2003, Mahboub 2004, Jensen et al. 2005, Rodenburg et al. 2003
	Use of pullets without FP in rearing				Newberry et al. 2007
	Low stocking density	Hansen and Braastad 1994, Keppler 2008		Hansen and Braastad 1994, ↑Zimmerman et al. 2006	Carmichael et al. 1999, Nicol et al.1999, Nicol et al. 2006
	Small flock size	Keppler 2008	Liste et al. 2015	Bilĉík and Keeling 1999, Bilĉík and Keeling 2000, ↑Zimmerman et al. 2006	de Haas et al. 2013, Nicol, et al. 1999,Nicol et al. 2006
	No exclusion from litter after placement			↑Alm et al. 2015	
	Use of broody/mother hens		Roden and Wechsler 1998, Shimmura. et al. 2010		
	Presence of cockerels				Odén et al. 1999
	Familiarization of hens with people	de Haas et al. 2014a		de Haas et al. 2014a	
Housing	**Provision of enrichment material such as pecking blocks, strings, vegetables, baskets, hay bales**	Huber-Eicher and Wechsler 1998, Klein et al. 2000, Mc Adie et al. 2005, Zeltner et al. 2000	Hartcher et al. 2015 (for l)	Norgaard-Nielsen et al. 1993, Steenfeldt et al. 2007, Wechsler and Huber-Eicher 1997, Wechsler and Huber-Eicher 1998	Daigle et al. 2014
	Provision of dust-bath		Huber-Eicher and Wechsler 1997		

Table 3 continued: Potential preventive factors investigated in experimental studies; factors in bold have been found to significantly affect feather pecking (FP) or plumage damage (PD) in at least one study in the expected direction (for l = effects of rearing conditions on laying, ↑ = increases risk)

		Rearing		Laying	
	Factors	Significant	Not significant	Significant	Not significant
Housing	**Use of dark brooders in rearing**	Brinch Jensen et al. 2006 (also for l), Gilani et al. 2012 (also for l), Johnsen and Kristensen 2001 for FP	Johnsen and Kristensen 2001 for PD		
	Provision of refuge sites			Freire et al. 2003	
Litter	**Provision of dry litter on the floor**	Aerni et al. 2000, Blokhuis 1989 (also for l), Blokhuis and van der Haar 1989 (also for l), de Haas et al. 2014b, de Jong et al. 2013b, El-Lethey et al. 2000, 2001, Huber-Eicher and Sebö 2001b, Huber-Eicher and Wechsler 1997, Johnsen et al. 1998 (for l), Mathlouthi at al. 2011, Nicol et al. 2001 (also for l), Zeltner et al. 2000	de Jong et al. 2013a,b (for l)	Aerni et al. 2000, Blokhuis 1989, Blokhuis 1986, Blokhuis and van der Haar 1989	
Perch	**Access to perches**			Wechsler and Huber-Eicher 1998	Donaldson and O`Connell 2012
	Sufficiently high perches (>60 cm)			Wechsler and Huber-Eicher 1997, 1998	
Light	**No flickering light**			Mohammed et al. 2010	
	Low light intensity	Kjaer and Vestergaard 1999	Kjaer and Sørensen 2002, Keppler 2008	Mohammed et al. 2010	Kjaer and Vestergaard 1999
Nest	**Nests without lighting**			Nicol et al. 2006, Zimmerman et al. 2006	
Feed and water	**Mash instead of pellets**			Aerni et al. 2000, El-Lethey et al. 2000	Wahlström et al. 2001
	Nipple drinkers (instead of bell drinkers)			Nicol et al. 2006, Zimmerman et al. 2006	
	Feeding ad libitum	Mathlouthi et al. 2011			
	Low energy and non-starch polysaccharide content in feed			van der Lee et al. 2001, ↑van Krimpen et al. 2009	van Krimpen et al. 2008

Table 3 continued: Potential preventive factors investigated in experimental studies; factors in bold have been found to significantly affect feather pecking (FP) or plumage damage (PD) in at least one study in the expected direction (for l = effects of rearing conditions on laying, ↑ = increases risk)

	Rearing		Laying	
Factors	Significant	Not significant	Significant	Not significant
High amounts of certain minerals		Willimon and Morgan 1953		Willimon and Morgan 1953
High amounts of certain essential amino acids or protein		van Hierden et al. 2004, Dixon and Nicol 2008	Elwinger et al. 2002, Elwinger et al. 2008	Kjaer and Sørensen 2002, van Krimpen et al. 2015
Roughage feeding			Kalmendal and Wall 2012, Steenfeldt et al. 2007	
Spreading grain on floor	Blokhuis and van der Haar 1992 (for l)			
Less feed phases		Dixon and Nicol 2008, Dixon et al. 2006		
Access to range		Kjaer and Sørensen 2002	Mahboub 2004, Petek 2015, Shimmura et al. 2008	

2.3.4 Recommendations

The 15 identified recommendations relate specifically to the prevention of FP or PD as a whole (11) or in parts (4) (Table 4). They are either internet resources or available in printed form; eight are in English, seven in German. They were published by administrations (5), associations (4), universities (3), breeding companies (2) and a food label (1). Only information explicitly referring to the prevention of FP was extracted, although we realized that a general improvement of management could be regarded as a preventive factor, too. And some recommendations provide extensive general management guidance.

Table 4: Identified recommendations with number of recommended factors either confirmed by epidemiological or experimental studies with at maximum one opposite or non-significant result or being contentious or not confirmed or not yet investigated

No.	Reference	System[1]	Number of recommended factors			
			Confirmed Rearing	Confirmed Laying	Contentious/not confirmed/not investigated	Total
1	AssureWel project no year	No information	3	13	7/3/6	32
2	Bassett 2009	No information	1	12	4/2/2	21
3	Big Dutchman International et al. 2004	Non-cage	0	4	6/0/2	12
4	Defra 2005	No information	5	9	5/3/2	24
5	FAWAC 2011	Barn/alternative	0	7	4/1/0	12
6	Klosterhalfen 2010	No information	8	10	5/1/3	27
7	LAVES 2013	No information	15	22	10/0/14	61
8	Lohmann Tierzucht 2011	Non-cage	2	5	6/0/0	13
9	Lugmair et al. 2005	Non-cage	8	18	7/1/5	39
10	Macey 2009	Organic	6	13	8/0/6	33
11	Michael 2013	No information	3	4	4/0/1	12
12	Pickett 2008	No information	7	15	4/2/2	30
13	Staack et al. 2010	Organic	7	11	5/2/5	30
14	Thiele and Pottgüter 2008	Barn, free-range	0	2	2/0/0	4
15	University of Bristol 2013	Non-cage	8	13	7/2/7	37

[1]information as provided in the recommendations

About half of the recommendations do not refer to a specific housing or management system and all except two include the rearing period (Table 4). Five sources provide information about different pecking forms (Bassett, 2009; Lugmair et al., 2005; Staack et al., 2010; University of Bristol, 2013).

On average, 36 potential preventive factors were counted per recommendation, summing up to a total of about 100 different, partly very detailed measures. We classified them into 62 more general factors, based on the ones defined in Tables 2 and 3 plus 12 factors which were not investigated yet. The following information about the contents of the recommendations is subdivided into three categories: recommended factors supported by study results which means that there is no more than one opposing result (Tables 5 and 6), recommended factors based on contentious evidence (Table 7), and recommended factors not supported by any study result, either because they have never been investigated or their effects could not be confirmed.

Almost all preventive factors confirmed in the studies have been taken up in the recommendations. Only two factors, each confirmed by only one study, ‘more sugar, less starch in ration’ and ‘provision of a platform in front of the nests’ as well as two further factors with balanced results (1x sign, 1x n.s.), ‘individual nest boxes’ and ‘provision of feeders/drinkers in litter area’, were not mentioned. However, no single recommendation includes all factors. Most frequently cited preventive factors (in 12 recommendations) are ‘prevention of diseases like IB or egg peritonitis’, ‘provision of dry litter on the floor’, ‘high use of range’ and aspects concerning feed ingredients, phases and form. On the other hand, recommendations comprise 15 contentious preventive factors (Table 7), and eight factors not confirmed by study results: ’start of lay not before week 20’, ‘matching of rearing and laying environment’, ‘provision of dust-bath’, ‘early access to litter’, ‘higher amounts of certain minerals’, ‘additional vitamins’, ‘spreading seashells’, ‘access to free-range in rearing’. Further 12 factors have not yet been investigated: ‘minimizing stress at placement’, ‘sufficient perch length per pullet’, ‘uninterrupted period of darkness’, ‘no direct sunlight in laying house’, ‘no reduction of length of daylight during laying’, ‘sufficient nest space per hen’, ‘sufficient sodium’, ‘provision of grit’, ‘trough should be completely empty once a day’, ‘nipple instead of bell drinkers during rearing’, ‘access to covered veranda’, ‘provision of good shelter in free-range during rearing’.

Table 5: Proposed preventive factors for rearing concerning feather pecking from different recommendations which have been confirmed in epidemiological or experimental studies with at maximum one opposing result. Factors in bold have been confirmed in at least two studies, figures are presented as far as available

Preventive factors for rearing		Recommendations (numbered according to Table 4)														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Management	**Good expert knowledge**	×			×			×		×		×	×			×
	Regular check of hens	×	×		×		×	×		×		×	×	×		×
	Low stocking density (birds/m² ground surface)						18[1]	35[2]			10[2]			13		
	Sufficient uniformity in weight	×			×		×	×	×	×	×		×			×
	Low sound level				×											
	Adjusted management[3]						×	×		×	×		×	×		×
	Provision of enrichment material such as pick blocks, strings, vegetables, baskets, hay bales						×	×		×	×		×	×		×
	Familiarization of hens with people				×		×	×					×			×
	Use of dark brooders in rearing										×					
	Different barn areas (levels)							×		×			×			
Litter	**Provision of dry litter on the floor**						×	×	×	×	×	×	×	×		×
Perch	**Sufficiently high perches**							×		×						
Light	**Uninterrupted light period (hours)**							8								
	Daylight							×						×		
Feed and water	**Mash instead of pellets**							×						×		×
	Feeding ad libitum						×	×								

[1]for chicks older than 10 weeks, [2]for chicks older than 5 weeks, [3]radio, pecking blocks, round drinkers and/or roosters

Table 6: Proposed preventive factors for laying concerning feather pecking from different recommendations which have been confirmed in epidemiological or experimental studies with at maximum one opposing result. Factors in bold have been confirmed in at least two studies, figures are presented as far as available

Preventive factors for laying		Recommendations (numbered according to Table 4)														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Management	**Use of pullets without FP in rearing**				×			×		×	×		×			
	Rearing own pullets	×	×													
	Regular check of hens		×	×	×		×	×	×	×			×	×		×
	Low sound level		×													
	Prevention of diseases	×	×	×	×		×	×	×	×	×		×		×	×
	Presence of cockerels										×		×			
	Early placement before 20 Weeks							18		18				17		
	Adjusted management[1]	×	×				×	×		×	×		×	×		×
	Familiarization of hens with people				×		×	×					×			×
Housing	**Provision of enrichment material such as pick blocks, strings, vegetables, baskets, hay bales**	×	×			×	×	×		×	×	×	×	×		×
	Different levels	×						×		×			×			
Litter	**Provision of dry litter**	×	×		×	×	×	×		×	×	×	×	×		×
	Provision of straw hay				×			×		×			×	×		
	Sufficient litter height (cm)					10		1-2								
Perch	**Sufficiently high perches (cm)**	50				×				35			70			40
	Perch with grip/wood as perch material							×		×						
Light	Dawn phase							×								
	No flickering light			×				×	×	×				×	×	
Nest	**Nests without lighting**	×						×		×	×					×
	Spelt as nest material					×		×								
Feed and water	**Mash instead of pellets**	×	×					×	×	×	×		×	×		×
	Sufficient drink places/hen			1/10				×	×	1/10	0.9/1			×		
	Roughage feeding	×	×				×	×		×	×	×	×	×		×
Free-range	**High use of range**	×	×		×	×	×	×		×	×	×	×	×		×
	Encouraging hens to go outside	×	×		×	×	×	×		×	×		×			×
	High percentage of sheltered areas	×	×		×		×	×			×		×			×

[1]radio, pecking blocks, round drinkers and/or roosters

Table 7: Proposed preventive factors concerning feather pecking from different recommendations with contentious results from epidemiological or experimental studies (l = laying, r = rearing), Figures are presented as far as available

Contentious preventive factors		Recommendations (numbered according to Table 4)														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Management	Suitable hybrid	r/l		l	l	l	LT/LB[1]				l	White	l	r/l	l	
	Good expert knowledge (laying)	l	l		l	l		l								
	Small flock size in thousand							6 r/l			l		5 l	3 r		
Housing	Low stocking density (laying)			l		l	l		l	l	l					
	Good air quality	l		l			l	r/l	l	l		l		r/l		r/l
	Suitable temperature (in C°, laying)		l	18			l	16	18	16						
Perch	Access to perches (rearing)	r			r		r	r		r	r	r	r	r		r
Light	No flickering light (>2000 Hz)			l				r/l	l	l					l	
	Low light intensity (lux)				l			20	15		20					
Feed and water	Chain feeder															r
	Nipple drinker															r
	High amount of essential amino acids or protein	l	l	l				l	l	l	l			l		r
	Spreading grain on floor	l						r/l		l	l			r/l		r
	Less feed phases	l	3		l			l			l		l			l
Free-range	Daily access											l				

[1] LT= Lohmann Tradition, LB= Lohman Brown

2.4 Discussion

Results of our review clearly underline the notion that FP and PD are multifactorial caused (e.g. Bestman, 2000; Hartcher et al., 2013; Nicol et al., 2013). Of the 51 factors investigated in epidemiological studies, 46 were found to be affecting FP or PD significantly in at least one study. Of the 29 factors addressed in experimental studies, 21 were influencing FP significantly. Altogether, these results led to a list of 62 different factors, whereof 17 factors regarding the rearing unit and 32 factors with respect to laying were confirmed by experimental or epidemiological studies with no or at most one opposite result. Seven factors regarding the rearing unit and 16 factors with respect to the laying unit were confirmed by at least two studies with no or at most one opposite result.

This overview has certain methodological limitations owing to the manageability of the broad body of literature. First, we refrained from a systematic quality control of the studies included. The aim was, to provide an overview over the scientific work done, and on tendencies regarding the evidence provided. We do not claim to finally proof validity or invalidity of any of the potentially preventive factors, as we secondly have not assessed power and effect sizes. This would have been a tremendous undertaking, as rather different indicators and measures of FP with different scales have been used and often relevant information is missing in the papers. Thus, we do not conclude in case of non-significant study results that no influence exists, but rather that further investigation is necessary, as non-significant results can just be due to insufficient power, confounding factors or the specific combination of different factors in the individual study. Moreover, the different methods assessing FP or PD might have caused different results. In addition to the different dependent variables used (e.g. pecking behaviour vs. PD), the methods of assessment varied, e.g. plumage scoring was done in different body areas (2–11 areas) with three to six point scoring scales, from the distance or after taking hens up, from samples of 20 hens per group or farm (Lugmair, 2009; Velik et al., 2005) to 200 hens, and often without reported reliability testing.

Nevertheless, we allocated the potentially influencing factors to three categories: firstly, those supported by study results with no or at maximum one opposite result, secondly, those with contentious results and lastly factors not supported by any study result, either because they have never been investigated or could not be confirmed. This should provide some structure and orientation, but it is obvious that other possibilities of categorisation exist (e.g. requiring a minimum of studies or not accepting opposite or non-significant results). We also had to categorise partly comprehensive recommendations into distinct factors. More detailed information, e.g. concerning the design of the outdoor run (bushes, shelters, pop holes), was lost by applying this categorisation.

As said, contentious results may be due to a multitude of interactions between the different factors (Gunnarsson et al., 1999). For instance, investigated group sizes may have affected outcomes concerning further factors (such as the availability of different resources), and are likely confounded with factors such as housing design, feeding technique or human-bird interactions in practice. In some experimental studies (e.g. Liste et al., 2015; Nicol et al., 1999), for instance stocking density and group size were confounded. Further examples are feeding trough and drinker form, where interactions can be expected with bird to feeding or drinking place ratios, ad libitum or restricted feeding, height of feeders and drinkers, their location and the general system design or management. For instance, water troughs in littered areas may lead to wet litter by spilling of water, (Green et al., 2000) which could in turn result in fewer opportunities for foraging and dust-bathing (Kim-Madslien and Nicol 1999). On the other hand, feeders and drinkers in the litter area may allow birds waiting for access to redirect pecks at litter instead of other birds. Alternatively, they may be related to smaller farm systems in general, with a number of further factors being concurrently different. The latter was the assumption of Bestman (2000) who found certain effects of type and location of feeders and drinkers.

Also concerning the importance of essential amino acids, study results were contentious and thus contrary to expectations in 60% of the recommendations. Interestingly, van Krimpen et al. (2005) came to the same conclusion, also including experiments in cage systems in their review. Again, reasons may be interaction effects, for instance between diet and strain (Al Bustany and Elwinger, 1986; Ambrosen and Petersen, 1997; Hughes and Duncan, 1972), diet and brooding temperature (Hughes and Duncan, 1972) or between methionine and energy content (Lugmair, 2009). However, also ceiling effects may play an important role. No further plumage improvement was found when reaching a lysine level of 850–950 mg/hen/day (Al Bustany and Elwinger, 1987a, b) or a protein level of 15.2% (Ambrosen and Petersen, 1997); van Krimpen et al. (2015) postulated a methionine content of at least 356 mg/hen/day to prevent PD. Therefore, the range of the investigated factors will often affect results but was in general frequently not reported in epidemiological studies.

Other indications for non-linear relationships relate for example to flock size. Lugmair (2009) found a higher PD risk in flocks with 1,001–2,999 hens, compared to flocks of 3,000 hens or more. No differences were found in flocks with 1,000 hens or less and 3,000 hens or more. These results are in accordance with findings of Zimmerman et al. (2006) who observed higher FP rates in flocks of 2,400 compared to 4,200 hens but did not investigate smaller flock sizes. In general, experimental studies used smaller group sizes, ranging from two to three hens (Dixon and Nicol, 2008) to a maximum of around 4,000 hens (Donaldson and O'Connell, 2012) and 30,000 chicks (de Haas et al., 2014b). The most common group sizes used in experiments were around 10–150 hens. In epidemiological research, group sizes varied from 80 to 5,400 hens (Bestman and Wagenaar, 2014) up to between 500 and more than 60,000 hens (Heerkens et al., 2015).

There was also a great variety concerning age of the investigated birds (1–74 weeks of age). As it can be expected that FP and PD increase with age (Lambton et al., 2010b; Nicol et al., 1999; Pötzsch et al., 2001), possible effects might therefore have been more or less conspicuous. For instance, the effect of broody hens on FP was only investigated up to an age of 28 days (Shimmura et al., 2010) or 8 weeks (Roden and Wechsler, 1998), while it cannot be excluded that effects become apparent also later in life, as found for the use of dark brooders (Brinch Jensen et al., 2006; Gilani et al., 2012). Furthermore, contradictory results of epidemiological studies concerning effects of the number of feed phases may relate to the way the feed is changed rather than to the feed change itself. A feed change involves risks, but this may also be true for feed not adjusted to different needs of the hens in their lifecycle.

While in scientific studies and recommendations genetic aspects were most frequently addressed, studies yielded contentious results with an especially high number of nondetectable effects in epidemiological studies. Beside the likely important interaction effects mentioned above, this may be due to rather different hybrids being compared in experimental and epidemiological studies or very uneven distributions of different hybrids on the farms (e.g. Bright et al., 2011), but also to genetic changes and differences within birds with the same plumage colour or even within the same hybrid line over the years. It thus appears that the scientific basis for hybrid recommendations is very weak, even though experimental evidence clearly shows the general importance of genetics for the predisposition to develop FP.

Finally, our categorisation of factors may have been responsible for some contentious results. For instance, the category 'air quality' comprised various measures which reflect different aspects of air condition, namely ammonia and carbon dioxide concentrations in ppm at bird height (Drake et al., 2010), ammonia concentrations at human height (Lugmair 2009), scores concerning dust levels or difficulty to breathe at human height (Huber-Eicher and Audigé, 1999; Gilani et al., 2013) or the presence of natural ventilation (Green et al., 2000).

On a similar line, some factors not empirically confirmed, but with a theoretically high preventive potential like 'good expert knowledge' during laying or 'minimizing stress at placement', are difficult to operationalize. Epidemiological studies assessed the years of experience as a laying hen holder (Bestman, 2000; Bestman and Wagenaar, 2003; Heerkens et al., 2015), the number of people working with the hens (Gilani et al., 2013) or if inspections are done by one person or more (Green et al., 2000). We summarized these factors under 'good expert knowledge', although it is questionable whether all of them are true indicators of the extent and depth of the specific biological and farming knowledge. In the same way, there is scientific evidence (though not without exception) of an association of FP with stress or fear (de Haas et al., 2014b; El-Lethey et al., 2000; Johnsen et al., 1998; Rodenburg et al., 2004). However, minimizing stress at placement is a much broader and rather vague recommendation that is difficult to test scientifically.

'Spreading grain on the floor' was recommended six times as a preventive measure, although in two epidemiological studies even opposing effects were observed. It is possible though, that the associations were due to this measure being used in case of a pre-existing FP problem. Moreover, frequency, amount and place of scattering grain, as well as stocking density must be observed, in order to avoid stress and smothering risks for the hens.

The recommendations for which scientific evidence is contentious or not available pose a future task for research and practice to be either validated or discarded. In our view, especially the areas of feeding and caretaking deserve deeper investigation. Also, more recent research showing connections between gut health and FP (Brunberg et al., 2016; Meyer et al., 2013) should be heeded. At the same time, in scientific studies reporting of study conditions, quality control such as reliability testing and of descriptive statistics should be improved. It is remarkable that existing recommendations include almost all preventive factors confirmed in studies. At the same time however, no recommendation refers to all of them. In fact, apart from two (Laves, 2013: 37 confirmed factors; Lugmair et al., 2005: 26 confirmed factors) the recommendations listed less than 50% of the confirmed factors. According to results from Lambton et al. (2013), farms following a higher number of recommendations have a decreased risk of FP in their flock. Therefore, there is room for improvement of recommendations available for farmers. We are, however, aware of constant development in this area. For instance, two new rather comprehensive recommendations were published (Keppler et al., 2017; Landwirtschaftskammer Niedersachsen, 2016) in Germany recently, which were not taken into account in this overview.

2.5 Conclusion

FP is influenced by a wide range of interacting factors. The comparison of 15 practice recommendations with results of 109 empirical studies revealed that on average each recommendation contained less than 50% of the 49 confirmed preventive factors. In total they also comprised 15 contentious and 12 not yet investigated factors. Therefore, on the one hand, recommendations should be amended. On the other hand, in future research unconfirmed factors from practice recommendation, e.g. in the areas of feeding or caretaking, should be further investigated.

3 Differences between feather pecking and non-feather pecking laying hen flocks regarding their compliance with recommendations for the prevention of feather pecking – A matched concurrent case-control design

3.1 Introduction

Egg production systems in Europe are undergoing dynamic changes. One driving force is an increasing concern of consumers, legislators and producers with respect to laying hen welfare (Pettersson et al., 2016). In a number of EU countries beak trimming/treating is or will be banned by legal provisions (Sweden, Norway, Finland, UK and the Netherlands), voluntary agreements (Germany) or label guidelines (Austria). However, feather pecking (FP) is still a problem in all housing systems (e.g. Kjaer and Bessei, 2013; Nicol et al., 2013). Reported percentages of affected flocks at the end of lay range from 60% (de Haas et al., 2014: flocks with more than 10% of hens with moderate or severe feather damage in one body region) to 86% (Lambton et al., 2010: flocks in which severe FP was observed).

Severe feather pecking leading to feather loss can result in economic losses as a result of increased food consumption in defeathered birds (Leeson and Morrison, 1978; Tullett et al., 1980) and increased mortality (El-Lethey et al., 2000; Fossum et al., 2009; Heerkens et al., 2015) as well as in reduced animal welfare since FP is painful (Gentle and Hunter, 1990) and hens with feather damage are more susceptible to cannibalistic pecking (Green et al., 2000). A large number of studies have tried to identify risk factors for the occurrence of this undesirable behaviour (reviews by Nicol et al., 2013; Jung and Knierim, 2018). In brief, FP is influenced by the interaction between numerous environmental and genetic factors which mainly affect foraging behaviour and the ability to cope with stress (Rodenburg et al., 2013). Jung and Knierim (2018) listed 32 risk factors during the laying period whose significance were supported by epidemiological or experimental study results with at most one contradictory result. An additional 21 factors had heterogeneous effects in the laying period or they were not confirmed yet, and nine potential preventive factors proposed in practice recommendations have not been studied yet.

At the same time, for multifactorially caused problems, the number of risk factors on a farm may be at least as important as the presence of specific single risks. Support for this idea can be found in the results of Lambton et al. (2013) that farms employing more recommended management strategies had lower FP problems. Similarly, we assume that farmers have higher chances of maintaining a 'non-FP-flock' when they comply with a greater number of recommendations how to prevent feather pecking.

To analyse which potential set of variables may be decisive for a flock to become case or control we used a logistic regression. At the same time, we expected that the prevention of feather pecking not only depends on certain factors, but also quantitatively on the extent of compliance with recommendations.

3.2 Animals, material and methods

3.2.1 Description of data used

Data from three cross-sectional studies on laying hens in non-cage systems recorded in the years 2004–2014 were available (Table 1). In all studies, flocks were visited when hens were between 30 and 78 weeks of age (Table 1). Management data were collected by interviews using questionnaires. This included general farm information (e.g. number of hen places), flock information (e.g. age at placement, hybrid), data on vaccinations and medical treatments, feeding (e.g. composition, phases), housing and range management. Housing conditions regarding hen house and, if applicable, covered veranda and free range area were recorded through inspection and measurements.

After training and testing of inter-assessor agreement (reaching acceptable to very good PABAK or kappa values of 0.41–1), plumage condition was assessed in samples of 30–120 individual hens. Two differing four-point scales were used on four to six body parts (Table 1). For the present analysis only assessments of back and vent were included.

Table 8: Characteristics of the three data sets included in the analysis

Project name	HealthyHens (HH)	Verbundprojekt (VB)	Minimum Standards (MS)
Aim of the study	Identification of major risk factors for disease and other welfare problems	Elaboration of management recommendations for furnished cages and aviary systems concerning health, behaviour, economy, hygiene and emissions	Development of minimum standards for rearing and laying in order to minimise feather pecking and cannibalism in non-cage systems
Included flocks	112 organic flocks	47 conventional flocks in aviaries (19 flocks in furnished cages not included)	100 laying flocks (45 organic, 55 conventional) (50 rearing flocks -23 organic, 27 conventional- not included)
Countries (number of flocks)	Austria (20), Belgium (8), Denmark (20), Germany (20), Italy (14), Netherlands (10), Sweden (10) and the United Kingdom (10)	Germany	Austria (50), Germany (50)
Age at assessment (week)	52-73	48-78	30-40 (16-18 not included)
Scored hens/flock	50 (in 9 cases 100 to 120)	100	30
Scoring system	4: very good plumage condition, no or very few feathers damaged 3: Completely or almost completely feathered, few feathers damaged, featherless areas <5 cm² 2: Highly damaged feathers and/or featherless areas ≥5 cm² up to ≤75 % featherless 1: Very high graded damage of feathers with no or very few feather covered areas. Featherless area ≥ 5 cm² AND almost bare (>75 % featherless)		0: very good plumage condition, ≤2 damaged feathers 1: ≥3 damaged feathers, no featherless area 2: single feathers missing 3: ≥25 cm² featherless areas
Assessed body parts	neck, back, vent, tail	neck, back, vent, wings, breast	head/neck, back, vent, tail, wings, breast
Included flocks in the in total 60 'controls' (no-FP-problem)	36 (organic)	5 (conventional)	19 (6 conventional, 13 organic)
Included flocks in the in total 105 'cases' (FP-problem)	44 (organic)	12 (conventional)	49 (27 conventional, 22 organic)

In total 259 flocks were investigated and classified as having a 'FPproblem' or 'no FP-problem'. A 'no FP-problem' flock had at least 98% of hens with a very good or nearly complete feather cover (hens with score 4 or 3 in the 'HealthyHens project' (HH) and 'Aviary project' (AP) or with score 0 or 1 in the 'Minimum standards project' (MS), see Table 1). In a case flock ('FP-problem'), 10% or more of the hens had highly damaged feathers or featherless areas ≥ 5 cm² (score 2 or 1 in the HH and AP project or score 2 or 3 in the MS project, see Table 1) in at least one body region. A total of 224 flocks could be classified as either case or control flock.

In a second step, frequency matching was carried out for the following variables: age, trimmed beaks, provision of a free range and brown/white layer (Table 2). The classification in case and control flocks and the matching procedure resulted in a reduction of flocks to 165, namely 60 no FP and 105 FP flocks. Table 1 shows the distribution of case and control flocks regarding the original studies.

Table 9: Results of frequency matching of laying hen case and control flocks for age of assessment, beak trimming, provision of free-range and white layers

	N	Age			N of	N of	N of
		minimum	maximum	mean	beak trimmed	free-range	white layer*
Controls: ‘no FP-problem’	60	30	69	47	13	53	5
Cases: ‘FP-problem’	105	30	67	47	36	83	10
% controls					22	88	10
% case					34	79	10

*3 case and 2 control flocks had mixed flocks of brown and white layers

3.2.2 Statistical analysis

A binominal logistic regression with the dependent dichotomous variable ‘FP-problem’ (yes/no) was used to identify variables that predict case and control flocks. Based on a list of potential preventive factors regarding FP (Jung and Knierim, 2018), all corresponding variables that were consistently available in all three data sets (Tables 3 and 4) were subjected to a pre-selection procedure. Beforehand, variables with more than 10% missing values, with insufficient distribution over categories (less than 10% of flocks in a category), those used for frequency matching and highly correlating variables (r>0.5) were excluded (Table 5). The pre-selection was carried out by chi² tests in case of dichotomous variables (Table 4) and by Mann-Whitney-U tests in case of continuous variables (Table 3). Variables with a P-value below 0.25 were further analysed by stepwise forward logistic regression. Due to missing values, the data set was reduced to 50 ‘no FPproblem’ and 87 ‘FP-problem’ flocks. Model diagnostics were done by Likelihood ratio test (overall model evaluation) and Hosmer & Lemeshow (goodness of fit test). The absence of strong collinearity was checked using variance inflation factor, where the values should not exceed 5 (Menard, 1995). The effect size was measured by Cohen's f.

Table 10: Potential influencing factors regarding feather pecking in laying hens; continuous variables and their definitions with minimum, maximum and mean as well as P-value in the Mann-Whitney U Test ('FP-problem' versus 'no FP-problem' flocks), carried out for the pre-selection of variables (pre-selected variables in bold)

Variables (metric)	Definition	Minimum	Maximum	Mean	Median	*P*
Number of cockerels/1000 hens	Number of cockerels*1000/total number of hens	0	60	2.10	0	.489
Number of workers/1000 hens	**Number of persons caring for hens. Number of workers*1000/total number of hens**	**0.1**	**1.8**	**1.6**	**1**	**.021**
Flock size	**Number of hens living in a separate area, if applicable separated from other flocks by visual barriers**	**350**	**24,578**	**2,841**	**2,200**	**.149**
Stocking density	**Hens/m² accessible area (excluding elevated surfaces without dunging pit/belt)**	**2.4**	**16.4**	**6.7**	**6.0**	**.010**
Age at placement	**Age in weeks at placement in laying facility**	**8.8**	**21.0**	**17.7**	**18.0**	**.206**
Litter height hen house	Mean in cm, measurement with folding rule at three different places in the hen house at areas without plaque	0	25.0	4.3	3.0	.899
Perch length	**Total perch length in cm/hen**	**0**	**53.7**	**14.2**	**14.4**	**.107**
Perch height	**Perch height in cm above closest usable area in the hen house**	**0**	**220.0**	**90.3**	**80.0**	**.240**
Drinkplace/hen	**Number of drinking places per placed hen (based on EU-legislation: 10 hens per nipple, 2.5 cm on one side of continuous trough, 1 cm at circular trough)**	**0.10**	**3.7**	**1.2**	**1.1**	**.085**
Feedplace/hen	**Number of feeding places per placed hen (based on EU-legislation: 10 cm on one side of linear feeder, 4 cm at circular feeder)**	**0.2**	**2.7**	**1.0**	**1.0**	**.150**

Table 11: Potential influencing factors regarding feather pecking in laying hens; dichotomous variables and their definitions with percentages (and number) of flocks as well as P-value in the chi² test ('FP-problem' versus 'no FP-problem' flocks), carried out for the pre-selection of variables (pre-selected variables in bold)

Factors (dichotomous)	Definition		Percentage of flocks (number)	*P*
Aviary system	**1 = more than one-tier, 0 = floor system**	**yes** **no**	**34.5 (57)** **65.5 (108)**	**.231**
Monitoring of feed consumption	Daily monitoring of feed consume per flock, group or subgroup	yes no	43.0 (71) 56.4 (93)	.660
Same housing type in rearing and laying	Floor to floor or multitier to multitier	yes no	36.4 (60) 56.4 (93)	.593
Uniformity in weight	≥80 % of hens are in the range of the mean ±10 %	yes no	31.5 (52) 68.5 (113)	.918
Good air quality (hen height)	**1 = no or acceptable smell of dung/ammonia or if measured NH_3 ≤10 ppm**	**yes** **no**	**67,9 (112)** **31.5 (52)**	**.117**
Provision of occupational material	Hens receive additional grain, vegetables, strings, pick blocks or bottles on the floor, in the covered veranda or in the outdoor run	yes no	49.7 (82) 48.5 (80)	.516
Litter topping up	**Fresh litter is provided within the laying phase**	**yes** **no**	**66.1 (109)** **33.9 (56)**	**.077**
Provision of straw or hay	**Straw or hay are used as litter material**	**yes** **no**	**54.5 (90)** **45.5 (75)**	**.193**
Perch material metal	**Provision of metal perches**	**yes** **no**	**53.3 (88)** **46.7 (77)**	**.233**
Perch material wood	**Provision of wooden perches**	**yes** **no**	**59.4 (98)** **40.6 (67)**	**.003**
Perch material plastic	Provision of plastic perches	yes no	15.2 (25) 84.8 (140)	.653
High frequency light	**Incandescent or high frequency light source in the hen house**	**yes** **no**	**43.0 (71)** **52.7 (87)**	**.199**
Natural daylight	**Unblocked windows or openings in the hen house allow entering of natural light**	**yes** **no**	**75.8 (125)** **24.2 (40)**	**.055**
Dawn phase	Gradual change of light for at least 5 minutes after dark period	yes no	48.5 (80) 50.3 (83)	.778
Littered nest	**Nest is littered with hults and not lined with Astroturf**	**yes** **no**	**32.7 (54)** **66.1 (109)**	**.248**
Feeding of mash	Feed is ground: coarse, medium or fine, instead of pellets	yes no	77.6 (128) 19.4 (32)	.619
Little feed change	Not more than two feeding phases during laying	yes no	31.5 (52) 53.3 (88)	.504
Feed chain	Feed chain system present	yes no	77.6 (128) 21.8 (36)	.686
Round trough or pan feeder	Round through or pan feeder present	yes no	28.5 (47) 70.3 (116)	.307
Covered veranda with dry litter	**Covered veranda with dry litter versus no veranda or veranda without dry litter**	**yes** **no**	**55.2 (91)** **44.2 (73)**	**.001**
Covered veranda with perches	Covered veranda with perches versus no veranda or veranda without perches	yes no	10.9 (18) 88.5 (146)	.789
High use of the free-range area	More than 30 % of hens are using the free-range area	yes no	27.3 (45) 72.1 (119)	.809

Table 12: Excluded variables, their definitions and reasons for exclusion from the logistic regression

Variable	Definition	Reason for exclusion
Regular check of hens	Control hours/1000 hens	21.8% of records missing
Start of lay not before week of age 20	Start of lay means 50% of hens are laying eggs at three sequent days.	36.4% of records missing
Prevention of diseases	Number of vaccinations	> 30% of records missing
Sufficient hen weight	Breeder weight standard fulfilled (mean of weight per flock)	11.5% of records missing
Adjusted management	Presence of radio, pecking blocks, round drinkers and/or roosters	No information assessed
Provision of dry litter on the floor	Dry and friable without conglomerates or plaques	Correlation with ‘Litter topping up’
Access to perches	Provision of perches in the hen house	Only 6.6% of flocks without access to perches
Bell drinker	Provision of bell drinker in the hen house	Only 8.4% of flocks had bell drinker
Nipple drinker	Provision of nipple drinker in the hen house	Only 6.6% of flocks had no nipple drinker
Roughage feeding	Feeding of vegetables or silage	Included in provision of occupational material
Provision of covered veranda	Provision of covered veranda	Correlation with ‘Covered veranda with dry litter’
Access to range	Access to outdoor run	Matched

In order to analyse whether high compliance with recommendations decreased the risk of a ‘FP-problem’, we used the list in Jung and Knierim (2018) of 26 proposed preventive factors from different recommendations which have been confirmed in epidemiological or experimental studies with at maximum one opposing result. The current data yielded information regarding 13 factors (Table 6). For each flock, each variable was dichotomised into ‘recommendation fulfilled (yes/no)’, and the percentage of fulfilled recommendations calculated. Where single values were missing (0.6 to 3.6% of values for nine factors), percentage compliance was calculated in relation to the actual number of factors. FP and no FP flocks were then compared regarding the % fulfillment of recommendations by Mann-Whitney-U-Test, as data showed no normal distribution. Effect size was measured by Cohen’s d. All analyses were done in SPSS Version 24.0.

Table 13: Variables and limits used for summarizing the percentage of fulfilled recommendations per laying hen flock for the Mann-Whitney U Test

Variable	Recommendation
Number of cockerels /1000 hens	≥ 10 cockerels/1000 hens
Early placemen at laying unit	≤ 17 weeks of age
Different levels	multitier recommended versus floor system
Occupational material	Additional grain, vegetables, strings, pick blocks or bottles are provided on the floor, in the covered veranda or in the outdoor run
Provision of dry litter	dry and friable without conglomerates or plaques
Sufficient litter height in hen house	≥ 5 cm
Straw or hay	provided as litter material
Sufficient perch height	≥70 cm above closest usable area present
Perches with grip	Wood as perch material
Dawn phase	Dawn phase of at minimum 5 min. in light program
Spelt as nest material	Nests are littered and not lined with Astroturf
Feeding of mash	Feed consistency: mash coarse, mash medium or mash fine
High use of range	> 30 % of hens are using the free-range area

3.3 Results

Plumage damage (score 2 or 1 in the HH and AP project or score 2 or 3 in the MS project) was found on average in 53% of individual birds from case flocks and in 0.5% of hens from control flocks. The study flocks are described with respect to the different independent variables in Tables 3 and 4.

In total, 18 of the original 32 variables passed the pre-selection procedure (Tables 3 and 4) and were presented to forward logistic regression analysis. The resulting significant model ($\chi2$ (4, n=137) =49.05, $P < 0.001$; Table 7) explained 41% (Nagelkerke R2) of the variance in plumage damage between flocks and correctly classified 77% of cases (Table 8). It comprised the four variables 'drinking places/hen', 'stocking density', 'perch material wood' and' covered veranda with litter' with the effect size f=0.8. A higher drinking place/hen ratio and a higher stocking density increased the likelihood of a 'FP-problem' (odds ratio Exp(B)>1) whereas the presence of wooden perches and a littered veranda lowered it (Table 7). The highest variance inflation factor was 1.245. Goodness of fit was insignificant (x^2=5.47, df=8, P= 0.706), suggesting that the model fit the data well.

Table 14: Final logistic regression model regarding predictors of feather pecking case flocks (N= 137, 50 no FP flocks, 87 FP flocks)

Predictor	ß	SE (ß)	Wald's x^2	df	*P*	Exp(B)	95% CI for OR	
							Lower	Upper
Stocking density	0.37	0.13	7.56	1	<0.05	1.45	1.11	1.88
Wooden perches	-1.77	0.50	12.61	1	<0.001	0.17	0.06	0.45
Drinking places/hen	3.00	0.74	16.64	1	<0.001	20.17	4.76	85.43
Littered veranda	-1.89	0.51	13.54	1	<0.001	0.15	0.06	0.41
Constant	-2.97	1.14	4.24	1	<0.05	0.05		

Table 15: Sensitivity and specificity of the model prediction regarding the classification into FP flocks or no-FP flocks

Observed	Predicted		% Correct
	No-FP flock	FP flock	
No-FP flock	27	23	54%
FP flock	9	78	90%
Overall % correct			77%

Sensitivity=78/(9+78)= 90%; Specificity=27/(27+23)= 54%

Recommendation fulfillment regarding the 13 compiled factors was significantly lower in FP flocks (mean: 42.5%, range: 7.7% to 76.9%) than in no FP flocks (mean: 46.5%, range: 15.4% to 69.2%; P=.036, U=2537.500, n=165, dCohen = 0.327; Fig. 1).

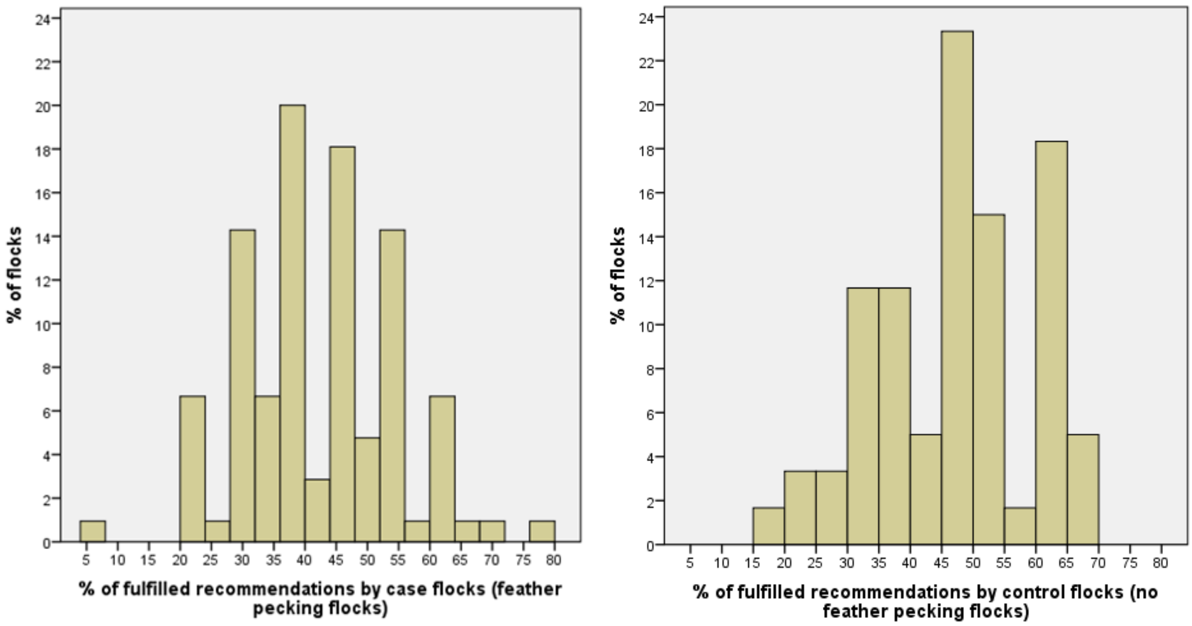

Figure 10: Percent of fulfilled recommendations by case and control flocks

3.5 Discussion

This study used data from three cross-sectional studies to examine risk factors for the development of FP. The three included studies focused on the main challenges for laying hen farms regarding disease and animal welfare, one aspect being the problem of FP. The 165 included flocks were convenience samples that, however, reflected a wide and well distributed diversity of major conventional and organic non-cage egg production conditions in some European countries, while excluding frequently relocated mobile housing systems and farms with less than 500 hen places.

The relatively high number of 'FP-problem' flocks in the pooled sample (with on average 53% of birds with plumage damage in these flocks) agrees with results from other studies in Europe that found mean prevalences of 49% affected flocks (de Haas et al., 2014), 64% affected hens (Bestman and Wagenaar, 2014) and in the case-control study of Nicol et al. (2013) of 50% affected hens in case flocks.

Pooling data from three different projects provided a higher analytical power and also greater external validity but introduced challenges regarding the appropriate handling of different assessment methods. First of all, the scoring systems for plumage damage differed. To ensure comparability we only included the body areas back and vent, which were assessed in all three projects. Besides, feather loss at the neck, wings and tail can have other causes than FP, e.g. moult or abrasion due to housing fixtures (Bilĉík and Keeling, 1999). Our definition of a no FP flock does not ensure that all flocks with a FP problem have been excluded. Hence, it is possible that in some flocks FP was just starting, especially because in one project assessments had been carried out at peak of lay. It is well established that plumage damage usually increases until the end of the laying phase (Nicol et al., 1999; Lambton et al., 2010; Pötzsch et al., 2001). Nicol et al. (2003) defined a flock as control where no feather damage was found and where no feather pecking behaviour was observed during the visit. However, in our sample only 13 flocks had no plumage damage at all. In addition, instantaneous assessment of one flock may not always reflect the overall likelihood of FP-problems on the individual farm. From batch to batch there might be large deviations and FP-outbreaks are often hard to predict (Defra, 2005). In particular, differing conditions in rearing play an important role for the later FP-risk (Gilani et al., 2013; Jung and Knierim, 2018). Unfortunately, this aspect could not be included in this analysis, because only in one project detailed information about pullet rearing was available.

Furthermore, the wide range of study conditions could have biased results. For instance, plumage damage increases with increasing age (Huber-Eicher and Sebö, 2001), because the impact of FP is additive. Also, beak trimming influences feather damage, but has no causal relation with FP. We therefore frequency matched case and control samples for age and trimmed beaks to avoid confounding. We also frequency matched flocks with and without free-range access, although free-range use is a preventive factor (Jung and Knierim, 2018). However, in two of the data sets, all flocks either had or had no free-range access, so that we could not test for possible effects on the risk of FP. Lastly, the proportion of white layers versus brown layers was unbalanced, so that we matched flocks concerning this parameter, too. Our model explained only 41% variation of plumage damage, but the effect size (f=0.8) indicates a high effect of the factors identified.

Unexpectedly, a higher drinking places/hen ratio was significantly associated with a higher likelihood of FP-problems. The odd ratio 20.17 indicates that the odds for FP increase 20.17 times when the value of drinking places/hen increased by one unit. It is possible that drinker bars were used for perching at unfavourable heights or the position of drinkers played a role. For instance, drinkers in littered areas may lead to wet litter by spilling of water (Green et al., 2000) which could in turn result in fewer opportunities for foraging and dust-bathing (Kim-Madslien and Nicol, 1999). However, the large confidence interval of 4.76–85.43 suggests that a higher sample size would be needed to achieve higher external validity. Because of this result, we did not include a drinking place recommendation in the recommendation list, although one investigation had found that a higher percentage of drinking places based on the recommendation of maximum 10 animals/nipple drinker or minimum 1.5 cm round drinker/ animal led to less plumage damage (Lugmair, 2009).

Different from earlier studies (Gunnarsson et al., 1999; Bestman and Wagenaar, 2003; Lugmair, 2009), but in line with Bestman (2000) and Zepp et al. (2018), stocking density in the laying phase was significantly associated with FP. The different investigated ranges of stocking densities as well as possible interactions with flock size and resources may explain the different results. In our sample, the range was considerable, from 2.4 to 16.4 hens/m2 accessible area. Furthermore, the large number of flocks with free-range access in the data set may have played a role. Lower stocking densities may lead to a higher use of the free range (Gilani et al., 2014), and a high use of the free-range area in turn was found to be beneficial for the prevention of FP in six epidemiological studies (Jung and Knierim, 2018).

In line with Lugmair (2009), we found wooden perches to be a preventive factor for FP. Lugmair (2009) assumed that wooden perches provide better grip and that hens therefore use them better than metal or plastic perches in order to avoid being pecked by conspecifics. However, a system-related effect cannot completely be excluded, as wooden perches are more prevalent in smaller houses where many further conditions may be different, too. Among them may be factors such as aviary vs. floor systems, maximum perch height or perch length, although for the single factors we found no effect on FP in this analysis. This may be due to complex systems like aviaries making it difficult to record meaningful measures such as perch height when, for example many different perch heights in different areas are provided. Furthermore, the heterogeneity of the investigated systems might preclude detection of smaller design effects.

The importance of dry litter on the floor as a prevention measure for FP has been confirmed experimentally (Blokhuis, 1986, 1989; Blokhuis and van der Haar, 1989; Aerni et al., 2000) as well as by epidemiological studies (Green et al., 2000; Häne et al., 2000; Lugmair, 2009). However, to our knowledge no other study looked at the effect of a covered veranda in combination with provision of dry litter. Our results suggest that a covered veranda is more beneficial when it contains dry litter, in comparison to a covered veranda without litter. This can lead to more foraging behaviour and may encourage the hens to use the covered veranda which additionally decreases the stocking density in the hen house. Litter topping up trended to significance in the pre-selection but was not further relevant in the regression model. Thus, our results confirm the general importance of dry litter, but provision in a covered veranda yields additional positive effects.

Some of the factors that did not contribute to the final model may have inadequately reflected the condition in question. Additionally, some factors were summarized from different assessments. For instance, the independent variable 'good air quality' was measured in NH3 ppm in the AP- and MS-project, whereas in the HH-project the assessment was subjective. We categorised concentrations below 10 ppm NH3 as 'good air quality', but that does not necessarily correspond well with the subjective evaluation which additionally takes into account factors like air moisture, temperature or dust level. Another example is light quality (natural daylight and high frequency lights) where a differentiation according to light source appears inadequate to find specific light influences on FP. Considering the marked differences between poultry and humans regarding UV-A radiation, brightness, flicker frequency and spectral colours (Kämmerling et al., 2017), more detailed measurement methods are warranted to detect more effects in future investigations.

The specificity of the regression model of 54% shows that there must be other factors with preventive influence on FP which were not included in our study. For instance, factors during rearing like the use of dark brooders (Brinch Jensen et al., 2006; Gilani et al., 2012) or the provision of dry litter on the floor (e.g. Blokhuis, 1989; Johnsen et al., 1998; Nicol et al., 2001) will largely affect FP behaviour during lay.

While our regression results reflect the particular relevance of certain factors, we could at the same time confirm that the number of effective factors plays a role. Our finding that a higher compliance with recommendations increases the chance to prevent FP is in line with the results of Lambton et al. (2013) on free-range farms in the UK. The more management strategies had been applied, the lower were the rates of severe FP as well as plumage damage in the 40th week of age. However, in our data there was no clear-cut differentiation between case and control flocks and the effect size was relatively low. This might be caused by the relatively low number of included recommended factors. Moreover, the list of Jung and Knierim (2018) included factors with, at maximum, one non-confirming study result. However, for the factors 'number of cockerels', 'dawn phase' and 'provision of straw or hay as litter material' only one study had found a significant effect while a second had not. Thus, their effect was not clearly confirmed. In the current analysis, the first two factors did not pass the pre-selection procedure. 'Provision of straw or hay' had not been included in the modelling because of correlation with 'litter topping up'. In general, it is possible that the inclusion of management and housing factors from the rearing phase (Janczak and Riber, 2015) as well as data regarding feed ingredients like essential amino acids (Kjaer and Bessei, 2013), would lead to clearer results. On the other hand, the list of potential preventive factors should be amended based on our regression results, namely regarding the factors 'provision of dry litter in a covered veranda' and 'lower stocking density'.

3.6 Conclusions

The provision of dry litter in a covered veranda and lower stocking density in the hen house were identified as preventive measures against FP. Wooden perches were found to have a preventive effect on FP too, but it is not clear if this was rather due to indirect system related effects or the actual wooden perches. An unexpected negative impact of more drinking places needs further investigation. Our results showed that the chance to maintain a non-FP flock increases, the more recommendations for the prevention of FP are fulfilled.

4 Possible risk factors for keel bone damage in organic laying hens

4.1 Introduction

Over the last decade, evidence is accumulating that keel bone damage in laying hens constitutes a serious welfare problem in commercial egg production systems. Although non-cage and free-range systems decrease the risk of inactivity osteoporosis (Rodenburg et al., 2008; Wilkins et al., 2011), it has become apparent that prevalence of KBD associated to collisions with housing structures or other environmental impact may be very high, especially in aviaries (e.g. Stratmann et al., 2015a). Reported average flock prevalences range from about 12% fractures and 14% deviations in Denmark, with no differences between organic and conventional hens (Riber and Hinrichsen, 2016) to about 83% fractures and 59% deviations in conventional Belgian flocks (Heerkens et al., 2016). Staack et al. (2009) similarly did not find prevalence differences between organic and conventional flocks in Austria and Germany (28% vs. 27% birds with deviations or fractures). In another study in Dutch organic egg productions systems, the average flock prevalence of keel bone deviations or fractures was 21% (Bestman and Wagenaar, 2014). Keel bone fractures cause chronic pain (Nasr et al., 2012a, 2013a) and can negatively affect economic performance (Nasr et al., 2012b). However, the number of scientific studies, especially epidemiological research, on potential risk factors for KBD is still limited. Apparently, this welfare problem has a multifactorial origin: the main underlying problem has been suggested to be a progressive osteoporosis, starting after the onset of sexual maturity where a switching from structural to medullary bone formation occurs. Both bone types are resorbed as a calcium source, leading to a gradual decline of the proportion of the stronger structural bones (Fleming, 2008). In addition, influencing environmental factors for KBD have been identified, such as floor vs. aviary systems (e.g. Riber and Hinrichsen, 2016), perch height (Staack et al., 2009; Wilkins et al., 2011) and material (Käppeli et al., 2011; Stratmann et al., 2015b) or stocking density (Staack et al., 2009). Moreover, important feeding factors are in particular calcium supply (Fleming, 2008) and supplementation of n-3 fatty acids (Tarlton et al., 2013). In terms of the birds' preconditions, genetics (e.g. Käppeli et al., 2011; Heerkens et al., 2016; Riber and Hinrichsen, 2016) and foot health (Gebhardt-Henrich and Fröhlich, 2015), were found to be influential. However, other suggested associated factors have to date not been confirmed, namely perch form (Donaldson et al., 2012; Bestman and Wagenaar, 2014), flock size (Wilkins et al., 2005; Nicol et al., 2006), access to free-range (Sherwin et al., 2010; Heerkens et al., 2016), vitamin D supplementation (Käppeli et al., 2011), body weight (Fleming et al., 2004) or body weight uniformity (Petrik et al., 2015). Many farmers are presumably not yet fully aware of the extent of this welfare problem, since it is not easily visible from the outside, nor related to conspicuous behavioural or performance changes. The aim of this study was to identify potential factors of housing and management associated with KBD in European organic production systems.

4.2 Material and methods

Epidemiological data from 107 organic layer flocks across eight European countries (Austria, Belgium, Denmark, Germany, Italy, the Netherlands, Sweden and the United Kingdom), collected within the CORE Organic II project ‘HealthyHens’ between February 2012 and March 2014, were available for analyses. It was the aim to cover a range of systems that are typical for the majority of organic egg production in each country. Therefore, only farms with stationary housing or mobile houses relocated only after the laying period, as well as farms with at least 500 hen places were included (see Table 16). Spatial distribution of study farms within countries was partly limited due to driving time restrictions. Moreover, inclusion of farmers depended on their willingness to participate in the study. Farms purchasing compound feed were prioritised in order to use feed declarations as an information source. Per country one to two assessors with different experience in the assessment of resource and animal based measures recorded the data. Before the start of the farm visits, all 12 assessors were trained regarding all measurements. Inter assessor agreement was tested for animal-based measures (Table 17).

Table 16: Distribution of strains, group sizes, stocking densities and keel bone damage prevalences of laying hen flocks over the 8 countries sorted by housing system floor or aviary

Country	Housing system	Strain (no. flocks Brown	White)	Group size (mean)	Stocking density[1] (mean)	Keel bone damage % (mean)
Austria	Floor	18	0	1971	4.9	36.7
	Aviary	7	0	3007	5.0	45.1
Belgium	Floor	6	0	2585	4.7	73.3
	Aviary	1	0	3000	4.6	76.0
Denmark	Floor	4	7	2922	5.9	15.5
	Aviary	0	4	3000	5.4	34.4
Germany	Floor	7	0	1611	5.7	40.9
	Aviary	12	0	2511	5.0	53.3
Italy	Floor	13	0	2141	5.7	35.1
	Aviary	2	0	2891	6.2	46.0
Netherlands	Floor	1	2	2814	4.8	53.3
	Aviary	3	1	2922	5.9	71.5
Sweden	Floor	0	1	3200	4.5	74.0
	Aviary	0	8	2770	5.7	66.3
UK	Floor	8	2	1946	6.9	43.8
	Aviary	0	0	-	-	-

[1]number of hens per m² usable area including the covered veranda (if present)

Table 17: Scoring systems for keel bone damage, plumage damage and foot pad lesions of laying hens and achieved pairwise inter-assessor agreement between twelve assessors

Measure/score			PABAK (median, rage)
Keel bone deviation			0.8 (0.4-1)
3	Straight or deviation ≤ 0.5 cm		
2	Deviation > 0.5 cm to ≤ 1 cm		
1	Deviation > 1 cm		
Keel bone fracture			0.7 (0.5-1)
2	No callus/pieces of fractured bone palpable		
1	Callus/pieces palpable		
Keel bone tip damage (last 1.5 cm caudal)			0.4 (-0.25-1)
2	No callus/pieces of fractured bone palpable, no compression or angle		
1	Callus/pieces of fractured bone palpable, compressed or angled		
Plumage damage: neck, back, belly,		tail	0.8 (0.4-1)
4	No or very few feathers damaged	Tail feathers ≤ 5 damaged	
3	Completely or almost completely feathered, few feather damaged, featherless areas < 5 cm²	6-10 tail feathers damaged	
2	Highly damaged feathers and/or featherless areas. Featherless areas ≥ 5 cm² (up to 75% featherless)	9-12 tail feathers highly damaged	
1	No or very few feather-covered areas. Featherless area ≥ 5 cm²/almost bare 75 % featherless–completely featherless	≥ 13 tail feathers highly damaged and/or almost bare	
Foot pad lesions			0.9 (0.8-1)
4	No lesion		
3	Small lesion < 0.2 cm		
2	Larger lesion > 0.2 cm		
1	Larger lesion ≥ 0.2 cm and dorsal swelling		

4.2.1 Animal-based measurements

Keel bone damage, hen weight, PD and foot pad lesions (FPL) of at least 50 hens/flock (in nine cases 100 to 120 hens/flock) were scored at the age of 52 to 73 weeks (mean 62.4). The hens were caught in different litter and slat areas, aiming to cover the whole hen house and to achieve a random sample. Assessed hens were marked on their leg in order to exclude double scoring. Keel bones were scored by visual inspection and palpation with respect to keel bone deviations and fractures (Table 17). For data analysis both categories were afterwards merged to KBD absent (score 3 for deviations and score 2 for fractures) or present (all other scores and combinations). Assessments of the keel bone tip (last 1.5 cm of the keel bone) were excluded from analyses in general, because of poor inter-assessor agreement. Hens were weighed and results compared with breeder weight standards for the specific genetic strain and age. If weighing less than 90% of the recommended weight, a hen was considered underweight. The percentage of underweight hens was calculated per flock. Four-point scales were used for the assessment of plumage condition (Bestman et al., 2017) and FPL (Table 17). Foot pad lesions were scored according to Tauson et al. (2005) and comprised scabs, ulcerations of the foot pad and visible swellings of the foot. Plumage condition per flock was expressed as mean feather score, calculated from the individual average scores of each bird, while the flock status regarding FPL was based on the percentage of hens per flock with a score <4. Use of the free-range was estimated at an age of 30 to 40 weeks (peak of lay) as well as 52 to 73 weeks (towards end of lay) by three instantaneous scan samples. Thus, each flock was assessed in spring/summer as well as autumn/winter. Numbers of birds in three different zones (0 to 20 m, 20 to 40 m and >40 distance from pop holes) were counted, starting at 0445, 0300 and 0145 h before sunset, irrespective of the time the lights went off in the barn. The variable ‘percentage of hens counted in the outdoor run’ was the mean of the six scans.

4.2.2 Further data recording

Management data were collected by an interview using a standardised questionnaire (included items see Tables 18 and 19). Housing conditions regarding hen house, covered veranda and free-range area were recorded through inspection and measurement. Information on feed composition originated either from feed declarations in case of compound feed, or for on-farm-mixed feed from laboratory analyses using NIRS or Weender analysis.

4.2.3 Statistical analysis

The dependent variable was KBD prevalence per flock in per cent. The independent variables, potential associated factors regarding KBD, were based on scientific literature and the authors' practical experience. Parameters with more than 5% missing values and categorical variables with less than 10% values per category were excluded from analyses. In order to handle missing values without losing too many cases, univariable pre-selection of variables was carried out in two steps, each time using Spearman correlation analysis for continuous variables and Mann–Whitney U Test for dichotomous variables. Variables with a *P*-value below 0.1 in the first tests (total sample, descriptive data see Tables 18 and 19) were analysed again with Spearman correlation or Mann–Whitney U Tests after excluding all flocks with a missing value for one of the variables of interest (final sample, descriptive data see Tables 18 and 19). The variables which once again had a *P*-value below 0.1 were further analysed by forward stepwise selection in the linear regression model. Model diagnostics were carried out graphically, for evaluating linearity of variables and homogeneity of variance by scatter plots of studentised residuals and unstandardized predicted values. Normal distribution of residuals was evaluated with histograms and p-p plot. The absence of strong collinearity between factors was checked using collinearity statistics and variance inflation factor, where values should not be >5 (Menard, 1995). The absence of influential data points were checked by Cooks' distance (⩽1.0; Cohen et al., 2003). The Bayesian information criterion was a criterion for model selection. All statistics were carried out using the software SPSS (version 24).

4.3 Results

4.3.1 Flocks

The average group size in aviary systems was 2,785 (minimum 1,000, max. 3,200 hens), in floor systems 2,222 (minimum 318, max. 4,500 hens). Table 16 shows the distribution of aviary and floor systems, brown and white layers, group sizes and stocking densities for the eight countries. Protein content in the feed rations at week 55 of the hens' life varied from 14.6% to 22.2% (*N*= 91), with energy contents between 10.2 and 12.8 MJ of metabolisable energy per kilogram of dry matter (MJ ME/kg) (*N*= 54) and crude fibre contents from 3.0% to 8.5% (*N*= 84). Keel bone damage prevalence values ranged from 3% to 88% affected hens per flock (mean 44.5%). Due to missing values for different variables in the data set, the final data set for the regression model comprised 50 independent flocks, with 2 to 19 flocks per country.

Table 18: Range (minimum, maximum), mean and median of potential metric risk factors regarding keel bone damage in laying hens for the total and the final sample

	Total sample (107 flocks)				Final sample[1] (50 flocks)			
Variables (continuous)	Min.	Max.	Mean	Median	Min.	Max.	Mean	Median
Age (weeks)*	52	73	62	62	59	69	62	62
Group size	318	4,500	2,422	2,998	318	3,250	2,216	2,507
Stocking density	2.38	12.23	5.46	5.34	2.38	12.23	5.37	5.21
Feather score[2]*	1.52	3.98	3.12	3.37	1.53	3.94	3.25	3.55
Target weight fulfilment[3] (%)	79	177	98	97	80	120	99	98
Underweight hens/flock[4] (%)*	3	38	13	12	3	24	12	12
Foot pad lesions (% of hens/flock)*	0	80	31	29	0	78	31	27
Perch height maximum (from floor or tiers to underside perch in cm)	0.36	220	96	80	0.36	220	97	86
Perch diameter (cm)	1.40	9.00	3.81	3.69	2.00	9.00	3.85	3.56
Hens counted in the outdoor run (%)	0	77	25	26	0	76	30	29
P[5] in feed at 55 weeks of age (%)*	0.47	0.98	0.59	0.60	0.47	0.70	0.58	0.60
Ca[6] in feed at 55 weeks of age (%)	3.20	4.65	3.75	3.70	3.20	4.51	3.73	3.70
Ca:P ratio in feed at 55 weeks of age	3.74	9.06	6.45	6.36	4.57	8.72	6.49	6.20
50% laying performance (week of age)*	19	24	21	21	19	24	21	21
Average laying performance until week of age of scoring (%)*	67	95	88	89	75	95	88	89

(factors in bold were pre-selected from the total sample, those with * from the final sample for inclusion in the multiple regression modelling). [1] only flocks without missing values for the pre-selected variables; [2] scores see Table 1; [3] hen weight/target weight for the specific week of age according to guidelines of the breeding company*100; [4] percentage of hens with ≥ 11% underweight in comparison to breeder weight standards for the specific week of age, [5] P = Phosphorus, [6] Ca = Calcium

Table 19: Distribution of flocks with regard to potential dichotomous risk factors concerning keel bone damage in laying hens for the total and the final sample

Variables (dichotomous)		Percentage of flocks (number) total sample	Percentage of flocks (number) final sample[1]
Brown strain	no	23% (25)	24% (12)
	yes	77% (82)	76% (38)
Perch material wood or plastic	no	70% (75)	66% (33)
	yes	30% (32)	34% (17)
Circular perch form	no	38% (41)	40% (20)
	yes	62% (66)	60% (30)
Rearing system aviary	no	34% (32)	34% (17)
	yes	66% (61)	58% (29)
Laying system aviary*	no	65% (69)	72% (36)
	yes	36% (38)	28% (14)
Natural daylight*	no	21% (21)	16% (8)
	yes	80% (85)	84% (42)
Dawn phase of at minimum 10 minutes	no	38% (41)	42% (21)
	yes	62% (66)	58% (29)
Daily access to the outdoor run	no	66% (71)	54% (27)
	yes	34% (36)	46% (23)
Calcium addition peak of lay	no	43% (46)	54% (27)
	yes	57% (61)	46% (23)
Calcium addition end of lay*	no	41% (38)	44% (22)
	yes	59% (54)	56% (28)
Calcium addition peak and end of lay	no	56% (56)	60% (30)
	yes	44% (44)	40% (20)

(factors in bold were pre-selected from the total sample, those with * from the final sample for inclusion in the multiple regression modelling).[1] only flocks without missing values for the pre-selected variables

4.3.2 Variable pre-selection

From the 26 variables offered for pre-selection (Tables 18 and 19), 12 were selected in the first step from the total sample. In the final sample (after exclusion of flocks with missing values), nine variables remained and entered the multivariable analysis (Table 20, marked in bold).

Table 20: Pre-selection of variables potentially related to keel bone damage in laying hens by Spearman correlation analysis and Mann-Whitney U Test in the total and final sample (P< 0.1). Variables in bold were included in the regression modelling

Variables (continuous)	r_s		*P*-value		N	
Sample	total	final	total	final	total	final
Age in weeks	-0.184	-0.374	**0.058**	**0.007**	106	50
Group size	0.064		0.515		107	
Stocking density	-0.153		0.123		103	
Feather score	-0.201	-0.146	**0.038**	0.311	107	
Target weight fulfilment (%)	0.062		0.528		107	
Underweight hens/flock (%)	0.300	0.272	**0.025**	**0.056**	107	50
Foot pad lesions (% hens/flock)	0.269	0.267	**0.005**	**0.061**	106	50
Perch height maximum in cm	-0.221	-0.224	**0.027**	0.117	100	
Perch width mean in cm	-0.046		0.643		106	
Hens counted in the outdoor run (%)	-0.096		0.329		105	
P in feed at 55 weeks of age (%)	-0.229	-0.244	**0.039**	**0.087**	82	50
Ca in feed at 55 weeks of age (%)	0.111		0.316		83	
Ca:P ratio in feed at 55 weeks of age	0.205	0.184	**0.065**	0.201	82	
50% laying performance (week of age)	-0.291	-0.265	**0.006**	**0.062**	88	50
Average laying performance until week of age of scoring (%)	0.192	0.299	**0.090**	**0.035**	79	50
Variables (dichotomous)	*z*		*P*-value		N	
Sample	total	final	total	final	total	final
Brown strain	0.372		0.710		107	
Circular perch form	1.530		0.126		107	
Perch material wood or plastic	-0.330		0.741		107	
Rearing system aviary	0.554		0.579		93	
Laying system aviary	3.040	2.110	**0.002**	**0.035**	107	50
Daylight	-1.740	-2.411	**0.082**	**0.015**	107	50
Dawn phase	-1.142		0.253		106	
Daily access to the outdoor run	-0.188		0.851		107	
Calcium addition peak of lay	-0.652		0.515		107	
Calcium addition end of lay	-1.829	-1.761	**0.067**	**0.078**	92	50
Calcium addition peak and end of lay	-1.529		.126		100	

r_s = Spearman correlation value, N= number of observations, *z*= standard score

4.3.3 Multiple linear regression model

The significant final model ($F(4, 45)$= 6.708, P <0.001, N= 50) explained about 32% of the variation in KBD prevalences between flocks and contained the four factors natural daylight in the house (yes/no), aviary vs. floor system, the proportion of underweight hens and average laying performance in percent (Table 21). This means that a higher proportion of hens that suffered KBD was seen in case of absence of natural daylight, in aviary systems, when there were more hens with underweight and with higher laying performance.

Table 21: Final linear regression model regarding keel bone damage in laying hens in %

Variable	Estimate	SE	*t*	*P*-value
Intercept	-75.97	54.864	-1.385	0.173
Daylight present (yes/no)	-18.41	7.398	-2.488	0.017
Laying system aviary (yes/no)	15.03	5.850	2.568	0.014
Underweight hens/flock (%)	1.61	0.652	2.463	0.018
Average laying performance until week of age of scoring (%)	1.26	0.585	2.152	0.037

R^2 = coefficient of determination, SE= standard error, t = hypothesis test statistic
R^2 = 0.374, R^2 adjusted = 0.318, F (4, 45) = 6.708, $P \leq 0.001$; all variance inflation factors < 1.13; N = 50.

4.4 Discussion

The ‘HealthyHens’ project focused on the main challenges for organic laying hen farms regarding animal welfare, particularly health, one aspect being KBD. Within the 107 flocks there was no flock without KBD. The lowest prevalence was 3% and the highest 88% affected hens/flock. The high percentage of affected birds as well as the large range of KBD prevalences was similar to those found in other studies (e.g. Rodenburg et al., 2008; Wilkins et al., 2011). The most common techniques to assess KBD are palpation and visual inspection (e.g. Gebhardt-Henrich and Fröhlich, 2015; Heerkens et al., 2016; Riber and Hinrichsen, 2016). The presence of callus material (palpable at the ventral and lateral surfaces of the keel), sharp bends or fragmented bone segments indicate fractures, and mere aberrances from the straight axis between the caudal and cranial points of the keel or indentations are commonly termed deviations (Casey-Trott et al., 2015). In agreement with the recommendations of Casey-Trott et al. (2015) we decided to pool different severity grades for KBD into a binary system. Moreover, we summarised fractures and deviations to KBD in general, as the distinction between them is not always clear-cut. Scholz et al. (2008) histologically found indications for fractures in all cases of severe keel bone deviations, in most cases (90%) of moderate deviations and even in about 50% of slight deviations. Thus, for the final binary scores used in this study, inter-assessor reliability was likely higher than achieved PABAK-values reflect. Poor inter-assessor agreement was, however, reached for the keel bone tip. Consequently, due to its exclusion from further analyses, the resulting associated factors only relate to damage at the main area of the keel. Including eight EU member states in the study, lea to a high number (12) of involved assessors with varying prior experience in the assessments. However, the joint training led to acceptable to very good inter-assessor agreement regarding the animal-based individual scoring. The average level of agreement among assessors was comparable to that found in other studies (e.g. Petrik et al., 2013). It was not feasible to extend the testing of inter-assessor agreement to housing measures, although joint training took place, because it would have required a large number of test farms. Consequently, an assessor bias cannot completely be excluded, although the risk concerning housing and management measures is likely smaller than for animal-based measures. Based on earlier projects and the joint training, definitions of each measure were laid down as precisely as possible. In cases of doubt, data were not used which contributed to a relatively large number of missing values. This partly aggravated the problem of an unbalanced study design with uneven numbers of farms per country owing to differing national funding. Moreover, typical organic husbandry conditions partly differed between countries. For example, aviaries were rare in the United Kingdom and therefore not present in the sample. Similarly, in our data set no brown layers were included in Sweden, nor white layers in Austria, Belgium, Germany and Italy. We refrained from using country as a covariate in the data analysis because it might have overruled such factors that were rather homogenous within country. Therefore, confounding with certain additional effects present in individual countries, such as use of certain feed ingredients or the already mentioned assessor bias, cannot completely be excluded. However, we expect only minor such effects as we minimised assessor bias by training and reliability testing. We furthermore checked that results regarding housing system were still consistent after excluding the data from the United Kingdom.

4.4.1 Absence of natural daylight

According to the regression model, farms without natural daylight in the hen house had about 18% points more hens with KBD than those with natural daylight. The EU regulation on organic farming (EU, 2008) requires the provision of daylight in the house. However, in ten of the 21 houses without daylight, windows were shaded because of feather pecking or cannibalism problems, reasons concerning the other houses are unknown. Thus, there are several possible mechanisms underlying the association found. In flocks with feather pecking or cannibalism problems, the disturbance in the flock may contribute to more escape behaviour and accidents in the house. Poor feathering may at the same time impair flying abilities of the hens. Riber and Hinrichsen (2017) reported an association between plumage and KBD, with hens with poor plumage being more likely to have KBD (31.5% vs. 22.2%). The small but significant univariable correlation between feather score and KBD in our pre-selection from the total data set points into this direction, too. However, an effect was not conspicuous in the final sample anymore, so that the factor was not selected for the final modelling. Another possible explanation for the association between natural daylight and KBD may be lower light levels in houses without daylight, with negative effects on the birds' jumping and landing behaviour (Taylor et al., 2003). Unfortunately, light intensities were not measured in the current study. It could also be that the part of UV-A radiation that penetrates windows, increases brightness perception in the birds (Kämmerling et al., 2017). A last option might be positive effects of UV-radiation on bone strength, as found by Fleming (2008) in UV-treated broilers. However, it appears less likely that this mechanism was involved in our study, because on one hand only small percentages of UV-radiation penetrates windows; and on the other, organic birds have access to outside. However, if birds are more active under daylight conditions, this might indirectly improve bone structure (Whitehead, 2004; Fleming, 2008).

4.4.2 Aviary systems

In line with earlier studies, we found aviary systems to be associated with increased KBD prevalences compared to floor systems (e.g. Rodenburg et al., 2008; Riber and Hinrichsen, 2016; for raised perches, Wilkins et al., 2011), while Bestman and Wagenaar (2014) found no relation between KBD and floor or aviary housing systems. According to our regression model, farms with aviary systems had about 15% points more hens with KBD than those with floor systems. However, within aviaries there may be considerable variation. Other studies have found that certain design aspects are negatively associated with KBD prevalence. These comprise among others type of system (portal instead of row aviaries, Heerkens et al., 2016), tier flooring material (plastic slats instead of wire mesh; Heerkens et al., 2016), adding of ramps (Stratmann et al., 2015a), provision of plastic instead of metal perches (Käppeli et al., 2011), provision of soft perches (Stratmann et al., 2015b) or lower maximum perch heights above both slats and litter (Wilkins et al., 2011). In our data set (including more floor than aviary systems) we could not detect any significant influences of perch height, form or softness. Possibly, they were confounded with other aspects of the housing system. Nevertheless, also a number of other studies did not find significant effects of round vs. rectangular perches (Donaldson et al., 2012; Bestman and Wagenaar, 2014), of wooden vs. plastic or metal perches (Bestman and Wagenaar, 2014), or three-dimensional perch positioning (Donaldson et al., 2012). In general, complex systems such as aviaries make it difficult to record meaningful measures, such as perch height or angles between different tiers, because of the multitude of these measures in different areas of the aviary and interactions with other factors such as type and shape of the different structures. Thus, the investigated systems might have been too heterogeneous to detect minor design effects.

4.4.3 Underweight and high laying performance

The regression model revealed an association between KBD and body weight as well as laying performance. Keel bone damage prevalence increased about 1.6% points with each percent point more of underweight hens in the flock and 1.3% points with every additional percent point of laying performance until the week of scoring. At the same time, we found no association with target weight fulfilment (hen weight/target weight for the specific week of age according to guidelines of the breeding company ×100). The percentage of birds with insufficient weight may better reflect the adequacy of nutrient supply. However, no conclusions about causal relationship can be drawn from the present epidemiological study: on the one hand there is the possibility that hens with painful KBD were feeding less, although this has not been confirmed experimentally (Nasr et al., 2012b and 2013b). On the other hand, fracture healing processes may require energy that leads to loss of body weight. Another explanation for the identified relation would be a protective effect of an adequate nutritious state, by better cover of the keel bone by muscles or by better nutrient supply relevant for the maintenance of bone structure. Our finding that flocks with a higher laying performance had higher KBD prevalence may point into the same direction of a higher risk of inadequate nutrition, but the low variance inflation in the regression model indicates that prevalence of underweight hens and laying performance were independently related to KBD prevalence. Although it has been suggested that high egg production increases osteoporosis, previous studies have found no correlation between laying performance and keel bone fractures (Gebardt-Henrich and Fröhlich, 2015; Heerkens et al., 2016). Whitehead (2004) found that a negative correlation between performance and KBD is more distinct at lower performance levels with longer individual periods out of lay in which bone reformation can take place. Indeed, within the laying period Gebardt-Henrich and Fröhlich (2015) found more new fractures occurring during the time when laying rates were highest. However, average laying performance in our investigated flocks was not particularly low, but roughly in line with breeder standards (e.g. Lohmann Tierzucht GmbH, without year). These areas are still rather inconclusive and should be further investigated. While in the univariable selection we found indications for higher KBD prevalences with lower phosphorus levels in the diet, no calcium addition at end of lay and conforming to findings of Gebhardt-Henrich and Fröhlich (2015), an earlier onset of laying (in this study achievement of 50% laying performance), these factors did not stay in the multivariable regression model. Probably, overruling factors such as percentage of birds with underweight or average laying performance played a role. The moderate explanatory value of the model underlines the multifactorial nature of KBD. More detailed feeding data (e.g. on kind, time and amount of calcium provision), including feeding during the rearing period, should be taken into account in future investigations.

4.4.4 Other factors

We expected that flocks with a higher age would have higher KBD prevalences due to accumulation of impacts on the keel. In contrast, the univariable pre-selection revealed an opposite association which is hard to explain. However, this factor did not contribute to the final model, probably because of the relatively small range of ages of the different flocks. Gebhardt-Henrich and Fröhlich (2015) found a positive association between bumble foot occurrence and keel bone fractures on individual bird level. In our data there was a low univariable correlation between flock prevalences of FPL and KBD in the expected direction (positive correlation), but FPL did not significantly contribute to the final regression model. In line with Wilkins et al. (2005) and Nicol et al. (2006) we could not detect any relation between group size, stocking density and the occurrence of KBD. Conforming with other studies (Sherwin et al., 2010; Käppeli et al., 2011; Heerkens et al., 2016), we also found no relation between the percentage of hens using the outdoor run and KBD. In conclusion, we found aviary systems, absence of natural daylight in the hen house, a higher proportion of underweight birds, as well as a higher laying performance to be positively associated with KBD in organic layers. It is likely that these factors similarly play a role for the presence of KBD in conventional flocks. Thus, in general particular attention should be paid to an adequate housing design and lighting that allows the birds to orient and manoeuvre safely in the system. Furthermore, the feeding management should aim at achieving a sufficient bird live-weight that fulfils breeder weight standards. Relations between laying performance, feed management and KBD should be further investigated.

5 General discussion

In order to improve laying hen welfare by preventing FP and KBD in non-cage egg production systems, a review (Chapter 2) and investigations of influencing farm practice factors on FP (Chapter 3) and KBD (Chapter 4) were undertaken in this thesis. Also, this study aimed to enhance practice recommendations with regards to FP and KBD and to identify further important research questions. Confirmed, contentious or not yet investigated factors were found in practice recommendations for the prevention of FP. This factor-variety underlined that FP is not yet fully understood, recommendations must be completed and further research is needed. Logistic regression revealed new possible risk factors and showed that high compliance with recommendations reduced the risk of FP. In Chapter 4, analysis showed high KBD prevalence, with new findings contributing to the discussion about causes of KBD.

5.1 Feather pecking

A high number of researchers are investigating FP. In spite of the large number of studies that amounted over the last 50 years, FP is still a problem in many flocks. A case control study (Chapter 3) showed, that in more than half of the laying hen flocks FP problems occured with in average 53% victims/flock. These findings are comparable with earlier investigations, if 38% (Huber-Eicher, 1999), 54% (Bestman et al., 2009) and 77% (Huber-Eicher and Sebö, 2001a) of flocks were affected with an average of 52%-75% victims/flock (Heerkens et al., 2015). One purpose of this thesis was to evaluate the results of scientific studies, in order to improve existing recommendations for the prevention of FP in non-cage systems. In the following, the results of Chapter 2 and 3 will be discussed including the influence of age, assessment methods and sample sizes and suggest further challenging topics for future research. Furthermore, epigenetic mechanisms e.g. the exposure to stress, the impact of the hypothalamic-pituitary-adrenal (HPA) axis as well as gut-health parameters will be examined.

5.1.1 Assessment of feather pecking

Animal based measurements require reasonable assessor training and periodic verification with inter-observer and intra-observer reliability tests (agreement on ratings between and within trained assessors). Commonly used test statistics in this regard are Cohen's Kappa or PABAK. Kappa was previously criticized for being highly dependent on prevalence and bias effects. Therefore, the prevalence-adjusted-bias-adjusted kappa (PABAK) value has been developed. PABAK coefficients may range between -1 and 1. Values can be interpreted as poor <0.20, fair 0.21-0.40, moderate 0.41-0.60, good 0.61-0.80 and very good 0.81-1.00 (Landis and Koch, 1997).

When carrying out behavioural observations, it is important to determine the correct form of FP, which is the focus of the observation. Gentle FP and PD were not found to be significantly correlated (McKeegan and Savory, 1999) as opposed to severe FP and PD (e.g. Bilĉík and Keeling, 1999). Four studies that are included in Chapter 2 did not distinguish between different FP forms. Because gentle and severe FP may have different origins, these results could therefore be biased. McAdie and Keeling (2002) suggested that stereotyped gentle FP may develop into severe FP, which was not confirmed. Still, gentle FP may be a sign of maladaptation and could be an early sign of reduced welfare.

Body regions which are mostly affected by FP are the breast, tail, back, wings, belly and neck (Mielenz et al, 2010; Habig and Distl, 2014). However, tail-, wing- and laryngeal neck feathers may also be damaged due to technopathies. Feather damage on the breast can also be caused by abrasion, while the areas next to the brood patch are naturally defeathered. Thus, depending on scoring scheme and assessor, different levels of FP prevalence could be assessed in the same flock. Divergent study results can be explained by differences in housing and management as well as interaction effects or overruling factors. Also, unreliable scoring schemes can lead to a biased assessment of animal-based indicators, and therefore also result in biased study outcomes. To avoid these shortcomings, IOR tests should be carried out and resulting values should be presented in the results section. Only one study which was included in Chapter 2 stated an IOR value with a PABAK of 0.55 (Heerkens et al., 2015). PABAK values between 0.4 and 1.0 show fair to very good assessor agreement in the studies which were used for the logistic regression in Chapter 3. In general, study results can be biased either by a high scoring-variance of different assessors, or sample sizes which are too low to represent the true prevalence.

The review in Chapter 2 would have been more systematic, if sample size (the number of individuals in a studied group), as well as the factors' biological significance would have also been considered in addition to the statistical significance. Sample size plays an important role in the detection of an effect and in reaching a desired estimate precision for the factors of interest. The evaluation of study design would have been a tremendous undertaking, since information was missing in most studies. As it is shown in Table 1, only five epidemiological studies scored ≥ 100 hens/flock for the assessment of PD, while sometimes the number of scored hens comprised only 15 (Nicol et al., 2003) or 20 (Velik et al., 2005; Lugmair, 2009; Gilani et al., 2013) hens/flock. Determining the required sample size for a flock of 3,000 hens with a confidence level of 95%, a confidence interval of 5 and a probability of 50% damage, a sample size of 341 animals would at least be needed to ensure that the assessed damage truly reflects the damage which is present in the whole flock. The formula n= N*X/(X+N-1) was used, where X = Zα/22 -*p*(1-p)/MOE2 and Zα/2 is the critical value of the normal distribution at α/2, MOE is the margin of error, p is the sample proportion, and N is the population size (Daniel, 1999). Hence, it is questionable whether study results based on low sample sizes allow drawing reliable conclusions on the prevalence of FP in the population, even though hens were randomly selected.

5.1.2 Influencing factors on feather pecking

There are numerous mismatches when the natural environment of chickens is compared to the housing environment of modern laying hens. For instance, the absence of male birds and mother hens, higher group sizes and stocking densities, reduced possibilities to forage and longer light periods under artificial light can negatively affect the behaviour of the chickens at every age from the breeding until the laying phase. It is known, that natural maternal care strongly influences the behavioural development of the chicks (Edgar et al., 2016). The mother hen shows the chicks what to peck at, synchronises activity and resting phases and influences how chicks react to stressors. Several studies have shown that chicks which were brooded by a hen are less fearful (Perré et al., 2002; Wauters et al., 2002; Riber et al., 2007; Shimmura et al., 2010; Edgar et al., 2011, 2013, 2015), more behaviourally synchronised (Edgar et al., 2016) and also less likely to perform FP. In two experiments, no decrease of severe FP was found for chicks accompanied by a mother hen (Roden and Wechsler, 1998 and Shimmura. et al., 2010), but in both studies there was nearly no occurrence of severe FP at all. Preventive effects were found for chicks brooded in dark brooders (Brinch Jensen et al., 2006; Gilani et al., 2012). Apart from that, experimental studies showed that FP behaviour can already be influenced in the hatchery, for instance through effects of varying corticosterone levels (Janczak et al., 2006; Davis et al., 2008) even down to the laying unit. Brinch Jensen et al. (2006) found, that the use of dark brooders can also affect FP behaviour much later in life. The same was found for the provision of dry litter during rearing, which then affected FP in lay (e.g. Blockhuis, 1989; Blockhuis and van der Haar, 1989; Johnsen et al., 1998; Nicol et al., 2001). The studies included in the review in Chapter 2 were carried out with birds of 1 to 69 weeks of age. Some factors might influence FP at specific phases of a hens' life. The flocks which were included in the analysis in Chapter 3 were 30 to 69 weeks of age. This was a wide, but nearly the same range for case and control flocks. However, information about rearing was unfortunately not complete. It is possible, that rearing factors like pullets without FP (e.g. Gilani et al., 2013; de Haas et al, 2014a), the use of dark brooders (Brinch Jensen et al., 20006; Gilani et al., 2012) or the provision of dry litter on the floor (e.g. Johnsen et al., 1998; Nicol et al., 2001) led to differences between the case and control flocks in the laying unit. It would be assumed, that results concerning the preventive effect of fulfilling recommendations to a large extent (Chapter 3) would be clearer, if we could have included how FP during laying is affected by the extent to which the recommendations are already fulfilled during rearing. The age of hens, as well as their early life history plays an important role which should be considered when comparing and interpreting study results. When trying to summarize confirmed influencing factors for the prevention of FP (Chapter 2), four main strategies can be formulated: good health, occupation of hens, prevention of stress and no birds which are attractive victims for feather peckers. When deducing recommendations for the laying unit, hen holders should use pullets with intact plumage and good health. Due to their natural behaviour, it is obvious that laying hens need possibilities to forage (Dawkins, 1989). Hen holders should firstly provide sheltered areas and encourage hens to use the outdoor run, secondly, decrease the stocking density in the hen house and lastly, enhance the possibility to forage. The provision of dry litter on the floor, enrichment material and the feeding of mash helps to keep the hens occupied (see Chapter 2, Table 6). Providing a covered veranda with litter was found to be preventive in Chapter 3, probably because it is a further possibility to forage and stocking density in the hen house can be decreased. Through sufficiently high perches, the provision of nests without lighting and resting areas, hens can be protected from being pecked by pen mates. Chapter 3 revealed that wooden perches are preventive and preferable, maybe because they are more comfortable and less slippery than metal and plastic ones. Next to sufficient occupation and shelter areas, the prevention of stress, like for instance ensuring low noise levels and adequate group sizes, are further measures to reduce the risk of FP. The correlation of group size and FP is not linear, meaning that in low group sizes with less than 1,000 hens (Lugmair, 2009), and in high group sizes with more than 3,000 hens (Zimmerman et al., 2006; Lugmair, 2009) less PD or FP was found. In this case it is possible that effects related to the housing system play a role, whereas smaller housing systems with lower stocking densities may be found in lower group sizes. In systems with more than 3,000 hens optimized management could be a reason for less FP in comparison to group sizes between 1,000 and 3,000 hens. In fact, group size is sometimes confounded by stocking density. Stocking density mostly showed non-significant results for the laying unit in Chapter 2. However, this was not the case in the analyses of Chapter 3. Different ranges as well as interactions with group size and resources may lead to the difference in results. The odd ratio of 1.45 indicates, that the odds for FP increase 1.45 times when the value of hens/m² is increased by one unit. A high stocking density may increase the risk of FP if resources like litter, feeding place, perch length and nest places are limited. It could be assumed, that a high stocking density induces stress for the animals while, at the same time, hindering escape behaviour. In experiments during rearing, low stocking density correlated with lower FP or PD rates, while access to a free-range area during rearing was not influential. A high stocking density, a covered veranda without litter, more drinking places/hen and no wooden perches were identified as risk factors in the analysis in Chapter 3. FP increased 20.17 times when the number of drinking places/hen increased by one unit. In this case, the high confidence interval of 4.76-85.43 suggests that an increased sample size would be needed to get more distinctive results. However, it is also possible that drinker bars were misused for perching in an unfavourable height, or drinkers in littered areas may have led to wet litter through water spillage (Green et al., 2000), in turn leading to fewer opportunities for foraging and dust-bathing (Kim-Madslien and Nicol, 1999). When interpreting the influence of wooden perches, a system related effect cannot be excluded, as wooden perches were mostly found in smaller systems with lower stocking densities and lower group sizes. Those influences can also be expected with regards to the form of drinker and feeding trough. Height, location and ratio of feeding and drinking places, ad libitum or restricted feeding, the systems general design and the management may lead to significant interactions. Following this idea, drinker or through form are not as decisive, but rather three of the four main criteria mentioned before: occupation of hens (e.g. mash instead of pellets), the prevention of stress (e.g. enough drinker and feeding places), and the avoidance of attractiveness for feather peckers (e.g. drinker lines should be installed at a favourable place). In non-FP flocks, significantly more recommendations were fulfilled than in FP flocks (46.5% vs. 42.5%; Chapter 3). These findings are concordant with the project of Lambton et al. (2013), who studied the benefits of management strategies and was able to show that the more management strategies were employed, the lower the rates of severe FP. Nicol et al. (2003) found, that specific management practices (restriction of bird access to the litter area and/or the outside range) have a higher influence on FP than others, but cluster analysis indicated that FP was not associated with any particular husbandry system. However, as mentioned before, preventive management strategies should consider the chickens whole life cycle and include a wide range of preventive effects. The findings of Nicol et al. (2003) support the general advice for laying hen holders to provide foraging opportunities (litter area, range use) and to minimize stress (lower stocking density in the hen house if > 20% of hens use the free-range area) in order to reduce FP behaviour. Based on the findings in Chapter 2, confirmed factors with a maximum of one contradicting result for the prevention of FP in rearing and laying are presented in Figure 11. Yellow and blue factors were found to be preventive in both life-phases, others were only investigated in one phase of the hen's life or influences were solely found in either, the rearing, or laying unit (blue for rearing or yellow for laying). The factors can be assigned to the four prevention categories: 'occupation', 'prevention of stress', 'good health' and 'avoidance of attractiveness as a victim'. Figure

11 presents a flowing system indeed, and factors may fit to multiple categories (e.g. 'good knowledge' of the farmer fits in both categories 'good health' as well as 'prevention of stress', because the farmers' knowledge is crucial for the management in both categories), which can also be correlating with each other (e.g. familiarization of hens with people and regular check of hens).

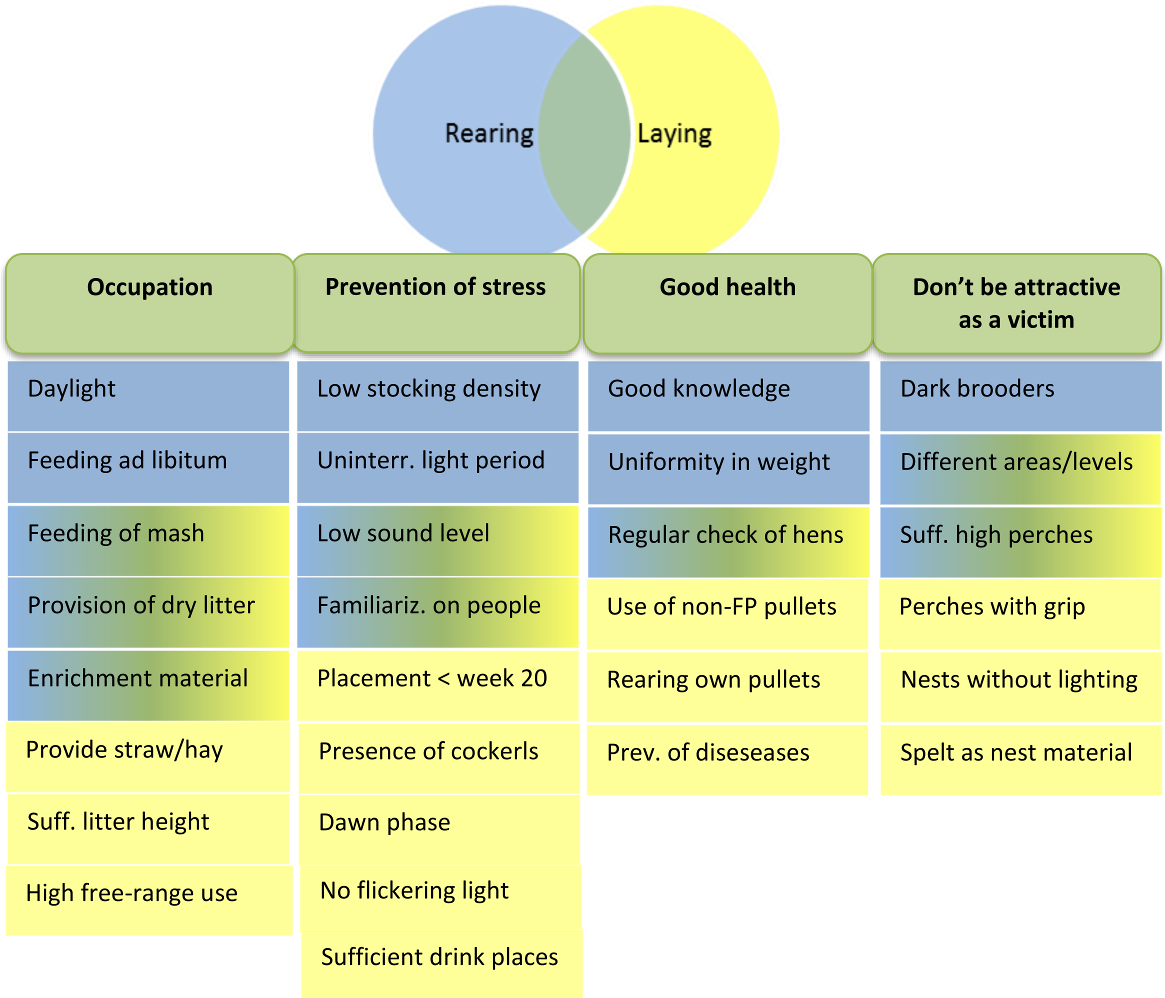

Figure 11: Recommended factors for the prevention of feather pecking for rear and lay (confirmed by at minimum one study result with no or at maximum one contradicting result)

Contentious factors were included in all 15 recommendations (see Chapter 2, Table 7). Even though a FP increasing effect was found in three epidemiological studies for the practice of spreading grain on the floor, it was still recommended. The same was true for the recommendation of using less feed phases, for which one study found FP increasing effects. As mentioned in Chapter 2, grain can be spread out by farmers who are aware of a commencing FP problem. Concerning feed phases, it can be assumed, that the timing and the change of feed affects FP rather than the number of feed phases itself. Quite to the contrary, nutrient supply should be adapted to physiological requirements. 12 factors resulted as non-significant in more than one study, but preventive effects are not to be excluded. Especially in epidemiological studies, factors like 'good expert knowledge in laying' or 'good air quality' are difficult to test scientifically. Nevertheless, for a number of factors like flock size, stocking density in laying and light properties, no accordance could be found. Consistent results were expected for nutrition, which has direct effects on metabolism. The feeding of mash and roughage were confirmed as influencing factors, as both encouraged the birds to spend more time with feeding and foraging. Effects of energy content, amino acids and minerals are known to have an indirect influence on FP. Nevertheless, a common practice is to provide NaCl when FP outbreaks occur and it is assumed that amino acids like tryptophan can influence the brain metabolism which in turn is linked with FP. Study results however, could not confirm influences of most investigated factors linked with nutrition.

5.1.3 Internal influences on feather pecking

The ability of individual hens to cope with fear and stress may play an important role for the development of FP. Recommendations state the prevention of stress at placement as an important measurement for the prevention of FP. However, this is difficult to measure empirically. There is no research that is specifically investigating stress at placement, but some studies investigate stress in general. Stress is a biological response to internal or external events (stressors) which jeopardize homeostasis. Stress responses can cause behavioural and immunological changes, affect the autonomous nervous system and influence responses in the hypothalamic-pituitary-adrenal (HPA) axis (von Eugen et al., 2019). The HPA-axis controls reactions to stress and regulates many processes, including digestion, the immune system, mood and emotions, sexuality and energy storage. Severe and continuous stress can have negative effects for individual animals. There is growing evidence, that laying birds live in a state of chronic stress for the duration of their productive life. Stress leads to increased levels of glucocorticoids including cortisol and corticosterone (Downing and Bryden, 2008), which can cause behavioural changes. Even prenatal stress is found to influence HPA regulation in the offspring. In humans, prolonged maternal stress during gestation is associated with behavioural disorders such as anxiety and depression in offspring (Weinstock, 2008). Similar evidence was found for chicks from mothers that were stressed during egg laying (Janczak et al., 2007). The chicks were more fearful and less competitive than chicks from control mothers, even though the control chicks were also reared without their mother. Eggs treated with corticosterone generated more fearful chicks (Freire et al., 2006; Janczak et al., 2006), indicating that the level of stress hormones in the egg influences the behaviour of the chicks after hatching. In most species, higher fearfulness is accompanied by an increased HPA-axis reactivity (Forkman et al., 2007). A higher risk for developing FP was found for more fearful birds in an experiment of Uitehaag et al. (2009). Also, according to Jensen et al. (2005) fearful birds, which are proactively coping with their environment are more likely to develop FP. However, Rodenburg et al. (2010) found that the fear response of chickens of lines selected for high feather pecking (HFP) did not differ from that of low feather pecking lines (LFP).

There is also evidence that FP outbreaks in commercial flocks often coincide with outbreaks of E.coli infections or chronic enteritis. Accordingly, disease-prevention was stated in 12 of the 15 recommendations. E.coli is shown to activate the HPA-axis, increase gut permeability and activate the immune response. Moreover, probiotics lead to an increase in tryptophan levels and reduction of HPA-axis activity. Probiotics have also been used in gut-treatment for depression in humans. Meyer et al. (2012) found indications that HFP and LFP lines differ in gut microbiota (metabolites). Animals of HFP lines were also more motivated to eat feathers, thereby increasing gut mobility, having the same effect as insoluble fibres (Harlander-Matauschek et al., 2006). Feather eating leads to a change in microbiota composition (Meyer et al., 2013). These findings indicate a relationship between gut microbiotica, immune system, HPA-axis reactivity, coping with stress and FP behaviour.

Interestingly, some individuals in a flock are not involved in FP outbreaks, neither as peckers nor as victims (Daigle et al., 2015). Breeders are highly interested in these neutral animals, who could be a relevant group for further research.

Deducing the recommendations, it is important that the breeders are aware of the fact that stress of the mother hen can affect FP behaviour in the offspring. Stress should therefore be avoided at every stage of the hens' lifecycle.

It was shown, that a multitude of factors and their interactions may play a role for the development of this undesirable behaviour. There is a large amount of evidence showing that FP results from a complex interaction between the internal state of an animal and its external environment.

5.2 Keel bone damage

Study outcomes concerning the occurrence of KBD reveal an alarmingly high prevalence of fractured and deviated keel bones in laying hens. In Chapter 4, KBD was found in 100% of flocks, with 3%-88% affected hens/flock. Previous studies found an average of 15% (Riber and Hinrichsen, 2016) up to 50% or even 98% affected hens/flock (Freire et al., 2003; Wilkins et al., 2004; Nicol et al., 2006; Wilkins et al., 2011; Heerkens et al., 2016a).

KBD mostly occurs during the laying period (e.g. Willkins et al., 2005). Also management strategies during rearing influence bone strength and the ability of birds to move in different housing systems (Casey-Trott et al., 2017). Therefore, rearing also has to be considered as influential on the occurrence of KBD. The height of damage increases from the beginning to the peak of the laying period (Fleming et al., 2004; Donaldson et al., 2012; Daigle and Siegford, 2014; Blatchford et al., 2016).

In the following, different assessment methods for KBD and their implication will be discussed. Results of these investigations will be brought together, and it will be identified where further research is still needed.

5.2.1 Methods for the assessment of keel bone damage

Depending on the research question, varying methods for the assessment of KBD are used. Table 22 (appendix) gives an overview about the applied evaluation systems. By far, palpation and visual inspection are the most commonly applied methods for the assessment of KBD. However, these methods often suffer from poor accuracy and repeatability (Casey-Trott et al., 2015). Nevertheless, Wilkins et al. (2004) reported palpation as a reliable method for determining the prevalence of keel bone fractures for a trained person, using a 2-point scale. Reliability was checked by comparing palpation scores with the actual damage of the dissected keels. Table 22 shows values for accuracy, sensitivity and specificity. Andersson (2017) took into consideration that different deformity levels are difficult to distinguish. Because distinction between fractures and deviations is not always clear-cut, it was merged together for analyses in Chapter 4. The easiest applied assessment method, especially for on-farm assessment, was palpation as it is owing to low cost, holds a low level of invasiveness and can because it be applied rapidly. Palpation focusses on the detection of deviation and/or callus and therefore, in addition to dislocations, considers healing or healed fractures. The variation of methods assessing KBD on dead birds is greater than the ones for live birds and a wider range of scales can be used. Identification of fractures by x-ray is more accurate than palpation, as the number of fractures, the severity as well as the healing status can be described (e.g. Richards et al., 2011). Still, this method is very costly and time-consuming. A different but not yet validated method is the use of ultra sound, that would be easier to apply and might have lower costs than x-ray.

5.2.2 Influencing factors on keel bone damage

KBD increases with age (e.g. Richards et al., 2012; Blatchford et al., 2016; Stratmann et al., 2016), whereby nearly no damage could be found during rearing (Wilkins et al., 2005; Käppeli et al., 2011). Age is directly related to sexual maturity and an increase of KBD (when traumas may sum up). Most studies found, that damage begins with start of lay. Casey-Trott et al. (2017) for instance, found hardly any damage in birds at 16 weeks of age, but already 44% damaged keels in week 30 and 75% in week 70. Analyses in Chapter 4 revealed an association between KBD and laying performance. With every additional 1% in laying performance, KBD prevalence increased by 1.3%. This result is supported by Petow et al. (2018), who demonstrated that hens with normal productivity throughout the laying cycle showed a high fracture prevalence, whereas hens with suppressed egg production did not develop fractures. Also, Rufener et al. (2018a) concluded, that older hens with high laying performance are more susceptible to fractures.

Even though KBD mostly occurs during laying, it may also be influenced by the rearing system. Flocks that were reared in aviary systems had a lower percentage of fractures compared to hens reared in cages, if they were further housed in cages or furnished cages (Casey-Trott et al., 2017). Next to their higher bone loading, birds reared in aviaries are better at using different levels and preventing collisions (Campbell et al., 2019). Further research is needed with regard to feeding during rearing, and its effects on bone structure and bone density in lay as optimal nutrition influences bone growth.

Influences of the housing system persist during the whole laying period. Birds originating from non-cage and free-range systems had higher bone strength and greater cortical density of the keel compared to birds housed in cages (Rodenburg et al., 2008; Regmi et al., 2016). However, Petrik et al. (2015) found higher damage rates in floor housed hens, compared to hens that were housed in cages. In line with earlier studies (Wilkins et al., 2011; Riber and Hinrichsen, 2016), higher damage rates for hens housed in aviaries compared to hens that were housed in floor systems were found in Chapter 4. According to the regression model, farms with multi-tier systems had more hens with KBD (15%) than those with floor systems. These findings indicate that non-cage systems may have a beneficial impact on the keel bone integrity compared to conventional cages, but the improvement may not be sufficient to prevent fractures or deformities altogether. Nevertheless, several studies found differences in KBD prevalence depending on the aviary design like e.g. aviary type (Heerkens et al., 2016a; Stratmann et al., 2016), corridor width (Harlander-Matauschek et al., 2015; Heerkens et al., 2016a), perch material (Käppeli et al., 2011, Stratmann et al., 2015b), or the provision of ramps (Stratmann et al., 2015a). In Chapter 4 however, no significant influences of perch height, form or softness were found. Confounding effects and interactions with other system related aspects and heterogeneous structures probably hindered the detection of minor design effects.

A further influence on the bone structure of chickens is nutrition. Approximately 2.4 g of Ca is required to produce a 60 g egg, but only 60-75% of Ca is available through intestinal absorption from feed for egg shell production (Fleming, 2008). For this reason, calcium absorption from bones is enhanced during egg production. On the univariable level of analysis in Chapter 4, lower KBD prevalence were found when hens received additional calcium at the end of lay. This factor however did not stay in the multivariable regression model. Calcium additives as well as calcium source, particle size or feeding time were not considered in the analysis, but these influences can still affect calcium absorption and thus bone weakness (Fleming, 2008). Several studies show an increased bone strength, when calcium was increased in the diet (Narvaez-Solarte et al., 2006; Nascimento et al., 2014). Kerschnitzki et al. (2014) found, that hens absorbed large amounts of calcium between 5 am to 11 am. Measures to increase availability of night calcium include a last feeding of large particle sized calcium one to two hours before the lights are turned off. Large particles remain in the gizzard for a longer period of time compared to the powder form (Bar et al., 2002). Numbers of active osteoclasts were found to be reduced in hens that were fed particulate limestone, reducing overall bone resorption (Fleming, 2008). However, Whitehead (2001) proposed an increased amount of medullary bone through the provision of calcium with improved digestive characteristics, without impacting the loss of structural bone. This indicates, that calcium deficiency alone is not a primary cause of osteoporosis.

Phosphorus (as phosphate ions) represents a structural bone element in combination with Ca, whereas lower phosphorus levels in the diet correlated with higher KBD in the univariable analysis in Chapter 4, it did not remain significant in the final model. Results regarding the phosphorus influence on bone strength were inconsistent. In the study of Sohail and Roland (2002), a positive effect of non-phytate P increase in the diet was found, and also Mansoori and Modirsanei (2015) reported increased breaking strength by the addition of phytase. Phytase is an enzyme that enhances the digestion and absorption of P. Still, others found no effect of non-phytate P or phytase supplementation on breaking strength (Nie et al., 2013; Englmaierova et al., 2014). Whitehead (2011) recommended a Ca:P ratio of 2:1. The Ca:P ratio correlated with KBD when the whole data set was analysed but did not stay significant when flocks with missing values were excluded. The absorption of Ca and P absorption strongly depends on vitamin D3, which was unfortunately not recorded for analyses in Chapter 4.

Vitamin D3 (cholecalciferol) is an essential, fat-soluble vitamin and aids in calcium and phosphorus uptake in the small intestine, bone mineralization, inhibition of calcium excretion in the urine and immune system modulation (Whiting and Calvo, 2013). Cholecalciferol is produced endogenously in the skin when exposed to sunlight. The absence of natural light in the hen house increased KBD about 18.4% in the final model. As all flocks had access to the free range it is not clear if day light in the hen house is connected with vitamin D3 and bone. Still, poultry housed indoors generally need vitamin D3 supplementation in the feed. Vitamin D enhances Ca absorption and reduces the Ca concentration in the gut, which in turn enhances the utilisation of phytin P through increasing mucosal phytase. The vitamin D3 need of hens is therefore increased by inadequate levels of calcium or phosphorus or by improper ratios of these minerals in the diet. A deficiency of vitamin D3 results in clinical signs similar to those of a lack of calcium (Ramp et al., 1989) or phosphorus or both, as all affect good bone formation. Most studies concerning vitamin D3 in poultry are carried out on broilers, or, if laying hens are used, usually egg shell formation is the topic of interest. The interrelations of vitamin D3 and other nutrients are very complex, especially when thinking of different vitamin D3 metabolites. Recommended vitamin D3 supplementation is 2,000-3,500 µg/kg feed (e.g. Lohmann, 2011). More research is needed with regards to the interaction and impact of vitamin D3, also concerning the importance of natural sunlight and vitamin D3 assimilation.

For every 1% point of underweight hens in the flock, KBD increased about 1.6% points. It is assumable, that underweight hens suffer from a nutrient deficiency, leading to an interference in bone formation. Conforming to other studies, no association with mean body weight (Fleming et al., 2004; Nasr et al., 2013b) or weight uniformity (Petrik et al., 2015) was found. Insufficient weight may reflect the adequacy of nutrient supply therefore in a better way.

Most laying hen holders are unfortunately not aware of KBD. If farmers would like to assess keel bone fractures and deviations, they need to be trained, as investigations showed that the palpation on live birds needs to be done by well-trained assessors. Reliability values and accuracy differ substantially between investigations, highlighting the difficulty of KBD assessment. An automatic assessment of keel bone damage could support long time monitoring, and feedback about keel status could be given to the farmers. Recommendations for the prevention of KBD are urgently needed.

6 General conclusions

Feather pecking is a significant animal welfare problem and several scientific studies detected a number of possible influencing factors. Along with these findings, several recommendations for the prevention of FP exist. There are recommendations which are exclusively targeting the prevention of FP or give general advice for housing and management in laying hens. In this dissertation, it was checked whether practice recommendations for the prevention of FP are based on scientific evidence. The content of recommendations can differ to a large extent and sometimes only few prevention measures are stated. Recommendations need to be completed, as most of them contain only a fraction of the prevention measures which are scientifically confirmed. Besides, for some of the recommended factors, only contentious results were found. The influence of those factors should be investigated in future research, specifically under the aspects of lighting and feeding.

Also, the finding of the case-control study in Chapter 3, showing that a higher stocking density during lay increases the risk of FP in a flock, should be confirmed by further investigations, as this result is not confirmed by most previous studies. A new insight was the decreasing effect of the provision of a littered covered veranda on FP, which was not included in prior studies. Finally, a lower risk of a FP outbreak was found, when farmers managed their flocks in high compliance with recommendations. To strengthen this finding, a comparison of case and control flocks and their compliance with recommendations should be done again with data sets including all factors confirmed by studies. Efforts should be made in order to transfer the scientific knowledge to laying hen holders and to support the implementation of recommendations in their daily farm practice.

Keel bone damage is, like FP, a serious welfare problem with a high prevalence in laying hens. In this dissertation, newly identified risk factors for KBD are the absence of daylight and hens that are underweight. The already known influence of aviary systems was confirmed. The influence of laying performance is discussed controversially in different studies but was found to have an effect in the data set used in this dissertation. It is likely that, rather than the total egg number, the time frame in which a high number of eggs are laid by an individual plays a more important role. Differentiated analysis of egg laying may provide information about the physiological mechanisms involved. The study is based on data assessed in organic egg production, but it is likely, that the detected KBD increasing factors also play a role for the occurrence of KBD in conventional flocks. First prevention measures for a reduction of KBD are the provision of ramps in aviary systems, ensuring good light conditions and the fulfilment of breeder weight standards. It could also be preventive if hens come into lay in a later week of age. Further investigations are needed to substantiate this assumption.

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Publications and reports related to the research project in this PhD thesis in chronological order

Publications written in bold letters were used as basis for the PhD thesis, reference to the related Chapter is given in brackets. These publications have been written by me, taking into account inspiration and suggestions from all co-authors.

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Jung L and Knierim U (2018). Are practice recommendations for the prevention of feather pecking in laying hens in non-cage systems in line with the results of experimental and epidemiological studies? Applied Animal Behaviour Science 200, 1-12 http://dx.doi.org/10.1016/j.applanim.2017.10.005. (Chapter 2)

Jung L (2018). Automatic tracking systems for poultry activity, Scientific report. https://www.grouphousenet.eu/STSM%20REPORT_Lisa%20Jung.pdf

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Table 22: Assessment methods of keel bone damage in laying hens presented with assessment scales, age week, fracture (F) and/or deviation (D) or without consideration of damage with values for inter- and intra-observer reliability, accuracy, sensitivity and specificity as available from studies

Reference	Assessment method	Scale	Week of age	F	D	No differentiation	Tip extra	Inter - observer reliability	Intra - observer reliability	Accuracy %	Sensitivity %	Specificity %
Fleming et al. (2004)	Visual inspection, x-ray, histopathology	3	70	x	x		x					
Wilkins et al. (2004)	Palpation	2				x	x			83	87	76
Rodenburg et al. (2008)	Palpation, visual inspection of dissected bone	2, 5	54-82	x								
Scholz et al. (2008)	Histopathology	4	21, 42, 63, 84	x	x							
Sherwin et al. (2010)	Visual inspection of dissected bones	4	70	x	x							
Käppeli et al. (2011)	Palpation	4	6-65 (8x)		x				$\kappa_{weighted}$= 0.95			
Richards et al. (2011)	X-ray	3	30, 32, 35, 36	x						90	91	93
Wilkins et al. (2011)	Palpation, visual inspection	5								95		
Donaldson et al. (2012)	Palpation, visual inspection of dissected bone	2, 4	17-70			x						
Nasr et al. (2012b)	Palpation, Thermal camera	5	33-42	x								
Richards et al. (2012)	Palpation, visual inspection of dissected bones	3	25-70 (7x)			x						

Table 22 continued: Assessment methods of keel bone damage in laying hens presented with assessment scales, age week, fracture (F) and/or deviation (D) or without consideration of damage with values for inter- and intra-observer reliability, accuracy, sensitivity and specificity as available from studies

Reference	Assessment method	No. of s	Week of age	F	D	No differentiation	Tip extra	Inter-observer reliability	Intra - observer reliability	Accuracy %	Sensitivity %	Specificity %
Nasr et al. (2013a)	Palpation, visual inspection of dissected bones	2	40	x								
Nasr et al. (2013b)	Palpation	3	35-38	x								
Petrik et al. (2013)	Palpation, visual inspection of dissected bones		68	x			x	κ=0.36-0.51		73.3-99.5	73.4-91.7	52.2-64.2
Tarlton et al (2013)	Palpation, visual inspection of dissected bones	4	30, 50, 70	x			x					
Bestman and Wagenaar (2014)	Palpation	2	50-60			x						
Casey-Trott (2015)	Palpation, visual inspection of dissected bones	2	68-81	x	x		x			84 (fractures) 91 (deviation)	81 (fractures) 84 (deviation)	87 (fractures) 97 (deviation)
Gebhardt-Henrich and Fröhlich (2015)	Palpation	4	18-65 (11x)	x	x							
Petrik et al. (2015)	Palpation	2	20, 35, 50, 65	x			x					
Stratmann et al. (2015a)	Palpation, visual inspection of dissected bones	2	18-60 (8x)	x	x				κ=0.86			
Stratmann et al. (2015b)	Palpation, visual inspection of dissected bones	2	18-64 (7x)	x	x			r=0.54	r=0.7			

Table 22 continued: Assessment methods of keel bone damage in laying hens presented with assessment scales, life week, fracture (F) and/or deviation (D) or without consideration of damage with values for inter- and intra-observer reliability, accuracy, sensitivity and specificity as available from studies

Reference	Assessment method	Scale	Week of age	F	D	No differentiation	Tip extra	Inter observer reliability	Intra observer reliability	Accuracy %	Sensitivity %	Specificity %
Blatchford et al. (2016)	Palpation, necropsy	2	19, 52, 72		x		x					
Heerkens e al. (2016a)	Palpation	3	58-64	x	x		x	*PABAK*= 0.84				
Heerkens et al. (2016b)	Palpation, visual inspection of dissected bones	2	19-52 (7x)	x	x		x			69.9 for deviation 87.8 fractures		
Hinrichsen et al. (2016)	Palpation	2	30-38, 62-77			x						
Regmi et al. (2016)	Computer tomography	5	78	x	x							
Riber and Hinrichsen (2016)	Palpation	2	32, 62	x	x			κ=0.82 (for deviations) *PABAK*=0.4 (for fractures)		86		
Stratmann et al. (2016)	Palpation, visual inspection of dissected bones	2	17-63 (8x)	x	x					83.6		
Andersson (2017)	Palpation	4	46, 70	x	x				*r*=0.82			
Candelotto et al. (2017)	Impact test	%	28-29	x								
Grafl et al. (2017)	Visual inspection on the slaughter line	3	64-109			x						

Table 22 continued: Assessment methods of keel bone damage in laying hens presented with assessment scales, life week, fracture (D) and/or deviation (D) or without consideration of damage with values for inter-and intra-observer reliability, accuracy, sensitivity and specificity as available from studies

Reference	Assessment method	Scale	Week of age	F	D	No differentiation	Tip extra	Inter-observer reliability	Intra-observer reliability	Accuracy %	Sensitivity %	Specificity %
Buijs et al. (2018)	Palpation	2	75	x	x		x	κ_{FC}= 0.39-0.65 Tip= 0.22-0.37	κ= 0.55-0.67 Tip= 0.36-0.44	0.68-0.85	0.65-0.91	0.40-0.88
Eusebio-Balcazar et al. (2018)	Palpation	2	16, 32, 53	x	x							
Eusemann et al. (2018)	X-ray	2	35, 51, 72	x	x							
Petow et al. (2018)	X-ray	2	12-60 (7x)	x								
Rufener et al. (2018b)	X-ray	6	22-61 (11x)	x			x	*ICC*= 0.985	*ICC*= 0.704-1.0			