Welfare and health aspects of free ranges for laying hens

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Welfare and health aspects of free ranges for laying hens

Welzijns- en gezondheidsaspecten van vrije uitlopen voor leghennen

(met een samenvatting in het Nederlands)

Proefschrift

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Chapter 1

General introduction

1.1 Organic and free-range egg production: market and regulations

Hens in organic and free-range production systems are given access to a free range because it is considered to contribute to their welfare (Pettersson et al., 2016) by means of providing more possibilities to perform natural behaviours such as foraging, eating plants, insects and worms, dust and sun bathing and having more space and fresh air, compared to an indoor environment. Consumers are willing to pay a higher price for free-range and organic eggs, compared to barn eggs. This resulted in an increase of organic and free-range laying hens in the Netherlands from 5.0 million (16% of all hens) in 2005 to 9.2 million (29% of all hens) in 2020 (AVINED, 2021). In the EU in 2021, 67.4 million (18%) of laying hens are kept in organic or free-range systems (EC Eggs market situation dashboard, 2021). These numbers may even further increase if the European Parliament adopts the recommendations of the 'End the cage' report (Rodenburg et al., 2020). The 2nd continent with a large market share of free-range egg production is Australia, with a grocery volume share of free-range eggs of 52% (Australian Eggs, 2021). In the USA, until 2011, over 95% of the eggs were produced in battery cages (Mench et al., 2011). In 2019, 76% of the hens were kept in cages, another 6% organic and the remaining 18% 'cage-free' (United Egg Producers, 2021). There are no federally regulated standards for free-range or pasture-raised (United Egg Producers, 2021; Brunnquell cited in Watson, 2021) and in practice not all organic hens have access to a free range (Brunnquell cited in Watson, 2021). Cage-free production in the USA, including free-range, is expected to grow to 36% in 2026 (United Egg Producers, 2021).

For organic laying hens the requirements for the housing, including the access to a free range, are described in the EU-regulation on 'organic production' (EU 2020/464). For free-range laying hens, they are described in the EU-directive on 'the minimum standards for the protection of laying hens' (1999/74/EC), the EU-regulation on 'marketing standards for eggs' (EC No 589/2008) and in additional criteria by the German KAT-Association (KAT, 2017). Table 1.1 gives an overview of the main features concerning maximum group size, indoor and outdoor stocking density and additional welfare requirements for the free range.

Table 1.1: Main features of organic and free-range production systems, compared to barn egg production

	Organic (EU 2020/464)	Free-range (1999/74/EC; KAT, 2017)	Barn (1999/74/EC; KAT, 2017)
Maximum number of hens per compartment	3,000	6,000	6,000
Maximum indoor stocking density (hens/m^2)	6	9	9
Outdoor space per hen at least (m^2)	4	4	0
Additional requirements of free range	Shall be covered mainly with vegetation	• Shelter against inclement weather and predators • Must be mainly covered with vegetation	Not applicable

1.2 Concepts of animal welfare

As mentioned above, a free range is provided to hens with the intention to contribute to their welfare. What is animal welfare and which aspects of a free range may contribute in which way to hen welfare? Besides a potential positive contribution to hen welfare, some aspects of a free range may entail risks, such as predation. This paragraph describes the different key elements of animal welfare and how they can be used to explain if and how free ranges may affect animal welfare.

1.2.1 Physical health & functioning

The report by the Brambell Committee (Brambell, 1965) is generally regarded as an important first step in defining animal welfare principles for the improvement of farm animal welfare. The committee states that an 'an animal should at least have sufficient freedom of movement to be able to turn around, groom itself, get up, lie down and stretch its limbs without difficulty'. Following the Brambell report, the Farm Animal Welfare Council (1993) describes the framework of 'ideal states', referred to as the 'Five Freedoms' that animals should have:

1. freedom from thirst, hunger or malnutrition by ready access to fresh water and a diet to maintain full health and vigour;
2. freedom from discomfort by providing an appropriate environment including shelter and a comfortable resting area;
3. freedom from pain, injury or disease by prevention or rapid diagnosis and treatment;
4. freedom to express normal behaviour by providing sufficient space, proper facilities and company of the animal's own kind;

5. freedom from fear and distress by ensuring conditions and treatment which avoid mental suffering.

The above approaches were later regarded as 'fairly traditional concerns of veterinarians and animal producers' (Fraser, 2008) and rather as prerequisites for `basic health and functioning' than as a concept of animal welfare. The basic or physical health and functioning approach mainly focuses on the absence of negative aspects. However, this ignores the biological function of a so-called 'negative' emotional reaction, which evolved specifically to protect an individual's overall welfare (Ohl and van der Staay, 2012).

1.2.2 Naturalness and natural living

The value of naturalness refers to how animals would behave in their (wild ancestors') natural environment (Bracke and Hopster, 2006). The assumption is that being able to live and behave as in nature (= perform species specific behaviour), for example made possible by the presence of natural elements, increases animal welfare. This requires natural elements in the animal's environment. Knowledge of the behavioural repertoire in a natural environment of a species helps to understand behaviour in a captive environment and can be used to improve their captive environment.

1.2.3 Behavioural needs, priorities and preferences

Behavioural needs are states which, if not attained, can result in signs of reduced welfare such as disturbed behaviour, an increased risk of pathology and/or a hormonal profile consistent with stress (Jensen and Toates, 1993). Examples of disturbed or currently called 'maladaptive' (i.e. apparently non-functional) behaviours are stereotypies, sham dustbathing by laying hens on a wire floor and feather pecking (Fijn et al., 2020). Feather pecking is the pulling out and often eating of feathers of flockmates, which seems to be a substitute for foraging behaviour (Rodenburg et al., 2013). Behavioural needs are also '(instinctive) behaviours that are performed even in the absence of an optimum environment or resource' (Weeks and Nicol, 2006). Weeks and Nicol (2006) review that behavioural needs for laying hens are foraging, nest-building prior to egg-laying, dustbathing and other comfort behaviours such as preening. Behavioural needs, priorities and preferences generally have their origin in the natural behaviour repertoire. They are performed by wild ancestors in their natural environment. Furthermore, behaviours and resources are priorities if 'experiments have shown that hens are prepared to work in order to perform or gain access to them' (Weeks and Nicol, 2006). For example, hens spent the same amount of time in a pen with woodchips if they had to enter it by squeezing through a narrow entrance, compared to a 'free' entrance condition (Bubier, 1996). In another study hens pushed doors with increasing resistance in order to get access to peat (Wichman and Keeling, 2008). This means that the hens regard the woodchips and the peat as a priority for respectively foraging and dustbathing. Preferences 'indicate the relative outcomes of choice experiments': they give information of how important the one option is, compared to another option (Weeks and Nicol, 2006).

1.2.4 Affective states and positive welfare

The term 'affective states' refers to emotions and moods that are experienced as pleasant or unpleasant (pleasure, fear, depression) and to the valenced component of sensations (pain, hunger, thirst) (Fraser, 2008; Mendl and Paul, 2020). When pleasant, they can be positive for an animal's welfare and when unpleasant, they can be negative for an animal's welfare. Besides being pleasant or unpleasant, affective states also vary in the level of activation or arousal. Being excited has a high arousal level and being relaxed a low one, but both are pleasant affective states. Absence of negative affective states does not automatically mean that an animal's welfare is good. Animal welfare is good when the balance of positive and negative experiences is strongly positive (Green and Mellor, 2011). And, a focus on neutralising negative affects 'could only lift a poor net welfare status to a neutral one' (Mellor, 2012). For example, the daily provision in a feed trough, could at best lead to a 'neutral state of welfare' or as necessary for physical health and functioning. A positive welfare state can be achieved by the provision of an environment that encourages 'diverting, enjoyable and varied exploratory and appetitive behaviours'. However, to a certain extent, negative affects can be considered necessary too, because they cause an animal to move away from a situation that may cause injury and reduce its fitness. A simple reasoning is that a positive stimulus is characterized by an animal voluntary moving towards it or make use of it, but this only addresses short term affect. More sustained states of affect can be measured with cognitive bias tests, of which many examples exist. Such tests are based on the theory that emotions affect cognitive processes (Mendl et al., 2009). Depending on the balance between an animal's positive and negative experiences, an animal will be in an optimistic or pessimistic mental state. In turn, the animal's mental state will strongly influence its response to ambiguous cues (judging a glass as half full or half empty). In humans, the appreciation of one's life as a whole, is called `happiness'. Delineated from this, Webb et al. (2019) introduce the concept of 'animal happiness' as a starting point for further investigation.

1.2.5 Mellor's five domains model

Mellor's five domains model has developed over the years and the current version (Mellor et al., 2020) distinguishes welfare compromises and welfare enhancements in five domains: three domains with survival-related factors (nutrition, physical environment and health), a domain with behavioural interactions (with the environment, other animals and humans) and the integrated fifth domain, the 'mental state'. It is very much like Fraser's concept of animal welfare (2008) and describes many examples of negative and positive conditions leading to negative or positive affect or emotions. It illustrates that many and diverse aspects are included when considering animal welfare. Mellor makes clear that positive conditions in all four conditional domains are needed for an overall good mental state. To be in good welfare, it is necessary for an animal to have varied food, feel safe and be healthy, but also to be able to perform the behaviours, which are important for it. Furthermore, Mellor et al. (2020) mention that being able to control or to adapt to undesirable conditions contributes to animal welfare. Essential for adaptation is that an animal is capable to freely `exercise agency'. Agency is 'the animals' ability to consciously engage in goal-directed behaviours [..] beyond its momentary needs, in order to gather knowledge and enhance its skills for future use in responding effectively to varied and novel challenges' (Mellor et al., 2020).

1.2.6 Integration of the above approaches into one concept of animal welfare

Fraser (2008) reasons that three approaches are a prerequisite for animal welfare: physical health and functioning, the possibility to perform natural behaviour and a positive balance between positive and negative affective states. Focus on only one of the approaches would lead to shortcomings in the domain of the other approaches. From the perspective of physical health and functioning, for example a hygienic rearing environment would be considered necessary for animal welfare. It prevents infectious diseases. Appropriate measures would include rearing young animals in single age groups, housed on wire mesh in the absence of occupational material, such as litter. From the perspective of the natural living approach, several shortcomings would be identified such as no contact with parent animals and limited possibilities or incentives for exploratory behaviour. From the perspective of affective states, frustration or boredom would be identified shortcomings. However, life does not consist of positive aspects alone. A 'variety of non-pleasurable states, such as fear- and defence-related states, are of biological relevance' (Ohl and van der Staay, 2012). Therefore, being able to adequately react to negative conditions is an essential part of animal welfare. 'Being able' refers to the conditions, that should not be too extreme, and to the animal, that should be capable to adapt. Ohl and Hellebrekers (2009) summarize it as follows: 'an animal is in a positive welfare state when it is able to actively adapt to its living conditions and therewith achieves a state that it perceives as positive'.

1.2.7 Conclusion: animal welfare concept when considering free ranges

The different concepts of animal welfare include a variety of opportunities to consider the different aspects of free ranges in terms of animal welfare. The most well-known approaches focus on physical health & functioning, naturalness and affective states, where naturalness is strongly linked to behavioural needs and species-specific behaviours. Elements of each of these approaches need to be taken into account when considering the contribution of a free range to laying hen welfare. From here on they are called the key elements of animal welfare. They are summarized in Figure 1.1. The next question to be answered is: which empirical evidence is there for the contribution of a free range to laying hen welfare?

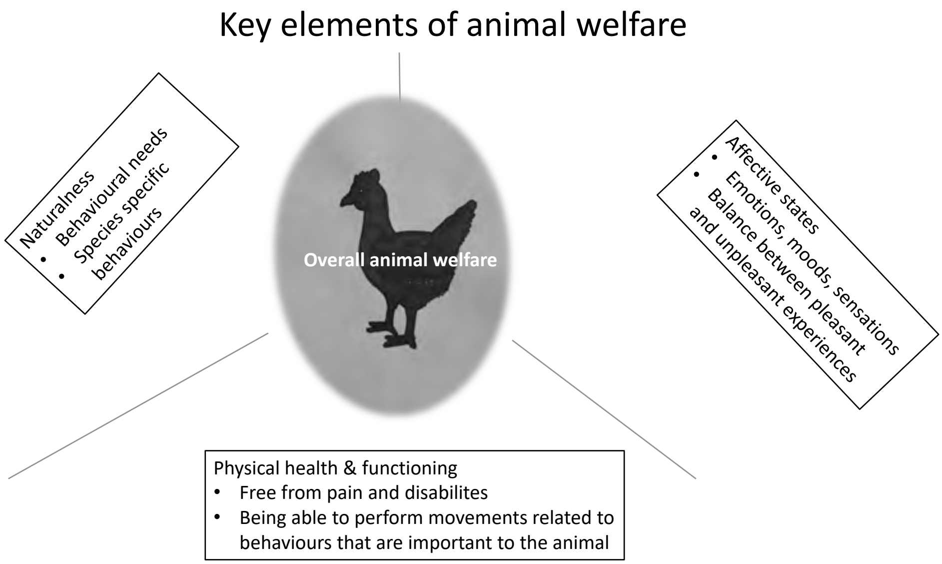


Figure 1.1: Representation of the key elements of animal welfare (based on Fraser, 2008). Aspects that influence animal welfare, can be divided into the key elements 'naturalness', 'physical health & functioning' and 'affective states'. When considering animal welfare, aspects of each of these elements need to be taken into account.

1.3 Evidence of a free range contributing to hen welfare

When assessing animal welfare, aspects of the above-mentioned key elements need to be included in order to get an indication of animal welfare that is as complete as possible. We cannot ask an animal to self-report like we can do in the case of human well-being. However, we can measure animal welfare indirectly by studying the animal's behaviour in terms of showing natural behaviour, maladaptive behaviour or cognitive bias. Furthermore, we can assess animal welfare by measuring animal based physical parameters and by assessing aspects of the animal's environment representing preconditions for physical health & functioning and for the performance of natural behaviour. This paragraph evaluates current findings of animal welfare including health aspects related to free ranges for laying hens.

1.3.1 Range use and natural living

When linking range use with naturalness and natural living, the habitat and way of life of the chicken's wild ancestor becomes relevant: the red jungle fowl (Fumihito et al., 1996). This species still occurs in large areas of Southeastern Asia, from India to Indonesia (BirdLife International, 2021). It lives in bamboo forests, interspersed with patches of small deciduous trees and shrubs (Johnson, 1962) and in forests with abandoned clearings that regrow with young trees (Collias and Saichuae, 1967).

Red junglefowl live in small harems of one dominant male with 2–5 females, with in their periphery some unaccompanied males living 'quietly and secretively' (Johnson, 1962). Observation of feral domestic chickens on an Australian island covered with dense forests, alternated with rocky parts with bushes reveals that domestic chickens largely behave like their wild ancestors (McBride et al., 1969). They live in harem flocks, spend large parts of the day on foraging behaviour, eating insects, several plant parts, fruits, carrion and chicks of other bird species. Whether or not a free range resembles a forest, it accommodates better or more opportunities for foraging behaviour and finding edible plants and animals, in addition to the compound feed provided inside the hen house. Schütz and Jensen (2001) compared the behaviour of red junglefowl, Swedish bantam (domesticated, but not selected) and white leghorn under semi-natural conditions. The hens were given the choice between 'freely available' food and food mixed with wood shavings, which takes effort to obtain. The white leghorn shows the same amount of foraging behaviour as the red junglefowl, namely 25 to 30 % of the observations, but by consuming a higher proportion of their daily intake from the freely available source, they ingest more food per time unit spent foraging. A free range may also provide more or better opportunities to perform other natural behaviour, such as dustbathing (Kruijt, 1964) and sun bathing (Huber, 1987). Some of these behaviours cannot be performed inside the hen house (sun bathing, eating small animals) or may be performed in a more rewarding manner in the free range (dustbathing, exploration in general). A behaviour is more rewarding if a reward follows the behaviour, for example finding something tasty or when experiencing a clean plumage after an undisturbed dust bath in fine sand. Knierim (2006) mentions the presence of natural light, which contains UV-radiation, to which hens are sensitive and in which they can use the full potential of their eyesight. Furthermore, a hen might also perceive a higher space allowance and freedom to change between different environments as positive (Knierim, 2006; Larsen et al., 2018).

However, a free range not only offers 'nice' opportunities for natural behaviour. For instance, outside, laying hens may become victims of predators like foxes, martens and birds of prey (Stahl et al., 2002; Bestman and Wagenaar, 2014). Exposure to a predator may induce severe stress. Therefore, staging such an exposure has become an animal model for biomedical research investigating human stress disorders (Clinchy et al., 2013).

Thesis topic 1: Predation of chickens in free ranges

Predation is reported by 40% of Dutch organic egg production farmers: 15% by birds of prey, 13% by foxes and 13% by both predators (Bestman and Wagenaar, 2014). Examples exist of predation related mortality from 0.5% (Moberly et al., 2004) to 14% (Hegelund et al., 2006) of laying hens being killed. Besides a welfare problem, predation is also an economic problem, because mortality leads to reduced production per hen housed. Although compensation exists for damage caused to crops by wild animals, predation of chickens is excluded from this. Predation by avian predators is more difficult to prevent than by foxes or martens. Bestman and van Liere (2011) evaluated several methods of repelling birds of prey from free-ranges and tested two methods themselves, but this did not result in an effective and practically feasible method. Also, questions remain about which birds of prey kill chickens. Another question is whether they target healthy chickens (= productive) or diseased (= less productive) and how the economic losses due to predation can be calculated. Answers to these questions can support farmers and authorities in thinking about compensation for losses by wild (and legally protected) predators. Chapter 5 describes a study in which live and camera observations are done in the free ranges of 11 farms, supplemented with a survey among organic and free-range poultry farmers.

1.3.2 Range use and indicators for positive affective states and positive welfare

A simple reasoning is that, if hens voluntary enter a free range, access to a free range thus results in positive affect. However, it is more interesting to know if range use causes a sustained positive affect. Kolakshyapati et al. (2020) found that time spent on the range during a hen's whole life is associated with increased curiosity (exploration), when tested in a novel arena at the end of the laying period. They also found that both range use and curiosity increase over time. They regard curiosity as an incentive for range use. They argue that if 'engagement with objects that are intrinsically rewarding' is characterizing a positive welfare state, then curious hens are likely to experience 'pleasure' during exploration, i.e. range use. That range use is related to positive affect, i.e. that hens like free ranges, is also demonstrated by studies that investigated hens during a longer time and found range use to increase with age or experience (Grigor et al., 1995a; Campbell et al., 2017a; Kolakshyapati et al., 2020). However, some studies found no effect of age (Rodriguez-Aurrekoetxea and Estevez, 2016) or that range use decreased with age (Hegelund et al., 2005).

Besides positive affective states, a lower prevalence of negative affective states also indicates a positive effect of a free range on hen welfare. Fear (a response to a specific threat) and anxiety (a response to a perceived or a potential threat, independent from a specific stimulus) are examples of a negative affective state (Ohl et al., 2008; Campbell et al., 2019a). Fear has a function in avoiding injury, pain or even death, for example seeking shelter when seeing a bird of prey. However, chronic fear is regarded as a negative affective state (Jones, 1996). Generally, outdoor-preferring hens seem to be less fearful than indoor-preferring hens (Campbell et al., 2016; Hartcher et al., 2016). Campbell et al. (2016) tracked laying hens with daily access to a free range and classified them as indoor-preferring, moderate-outdoor and outdoor-preferring. They tested them in an open field test and performed a tonic immobility test, looking at indicators of fearfulness. The indoor-preferring and moderate-outdoor hens are more fearful than the outdoor-preferring hens. They show longer latencies to move and cross fewer squares. However, the hens do not differ in the duration of the tonic immobility. Hartcher et al. (2016) compared the top 15 with the bottom 15 range users, based on their total time spent on the range, as assessed by tracking them individually for 13 days. The hens with longer tonic immobility, an indicator for fearfulness, spend less time outside.

A negative correlation between range use and fear or anxiety can be interpreted in two directions. One interpretation is that certain hens are ranging less because they are fearful/anxious. Another interpretation is that certain hens become less fearful/anxious because they spend more time in a stimulus rich environment. A stimulus rich environment during rearing is known to enhance the hen's ability to cope with environmental stressors (Campbell et al., 2018) and a stimulus rich environment during lay (a free range) might work the same. Does the direction of causality make a difference for the hens' welfare? Hens that dare not to go out because they are more fearful, might miss welfare enhancing stimuli in the outdoor environment, although they may still profit from the lower stocking density if their flockmates go out. Hens that become less fearful because they use the free range, might have a better welfare state, given that they still respond adequately fearful when needed. Although generally high ranging hens seem to be less fearful, Larsen et al. (2018) found that 'high rangers' are more fearful of humans but less fearful of a novel object. An explanation for this finding may be that during ranging, a certain level of vigilance is functional. Vigilance might be more developed in high-rangers compared to low-rangers or to indoor-preferring hens. If vigilance correlates to certain responses in fear tests, then it becomes difficult to interpret the results of fear tests in terms of animal welfare.

1.3.3 Range use and integrated welfare approach - Welfare Quality Assessment

The Welfare Quality Assessment protocol for poultry (Forkman and Keeling, 2009) describes animal-based measurements (both physical and behavioural) and environment-based measures (feeding place, aspects of furniture, hen house and the free range). The measurements are based on the five freedoms described by the Farm Animal Welfare Council (1993). Van Niekerk et al. (2012) analysed Welfare Quality assessments of 122 flocks, including 30 organic and free-range flocks. They estimated the proportion of hens using the free range and the proportion of the free range covered with artificial shelters or bushes. However, these measures are integrated with other observations of the same flocks into scores on flock level. Therefore, it is not possible to attribute welfare aspects specifically to the free range. For some flocks, however, they report observations of many hens dustbathing in the free range and not inside the hen house. This supports the assumptions mentioned above: preconditions for a behaviour, in this case the behavioural need dustbathing, might be better in a free range, compared to an indoor environment. However, besides these side comments, more about welfare and health aspects of free ranges cannot be deduced from the Welfare Quality protocol.

1.3.4 Range use and behavioural needs

There is no proof that having access to a free range is a behavioural need. No study has been performed yet, for example, investigating how much effort a hen is willing to display (i.e. 'consumer demand test' (Dawkins, 1983) in order to access a free range. But free ranges offer opportunities for performing behaviours that are regarded as behavioural needs, such as foraging and dustbathing. To a certain extent these behavioural needs can be satisfied in an indoor environment too. However, as mentioned above, these behaviours may be performed in a more rewarding manner in a free range compared to an indoor environment. Foraging might be more rewarding if something tasty can be found. Indoors the substrate for foraging mainly consists of dry manure, while outdoors there might be plants, snails, worms, insects, etc. This preference for performing exploratory behaviour outdoors over indoors is illustrated by Diep et al. (2018) and Ferreira et al. (2021). Diep et al. (2018) studied the behaviour of laying hens inside the shed and in the free range. They found that hens in the free range spend most of their time foraging and indoors they preen and rest more. They conclude that the hens prefer the free range for foraging. Ferreira et al. (2021) studied the behaviour of broiler chickens before they got range access, during the first weeks of range access and during the last weeks of range access. They found that chickens that spend more time foraging in the `before' period, later become 'high rangers', i.e. visit the range many times. When given the choice, high rangers also prefer working for food (obtaining mealworms mixed with a substrate) over free food (mealworms without substrate). They suggest that range use is probably linked to chickens' exploratory motivation. When looking at feather pecking as an example of maladaptive behaviour in relation to free-range use, significantly less feather pecking damage is seen in flocks and individual hens that show a high range use, compared to flocks (Green et al., 2000; Bestman and Wagenaar, 2003) or individuals (Chielo et al., 2016; Rodriguez-Aurrekoetxea and Estevez, 2016; Bari et al., 2020) that show a low range use.

Thesis topic 2: Feather pecking in organic laying hens

Feather pecking is the pulling out and often eating of feathers of flockmates (Rodenburg et al., 2013). It is an indicator for reduced welfare in both actor and victim (El-Lethey et al., 2000; Tahamtani et al., 2017). Feather pecking is also seen in flocks kept under conditions that are meant to enhance welfare, such as organic and free-range systems (Green et al., 2000; Bestman and Wagenaar, 2003; Coton et al., 2019). A question is: which factors within such a system are related to feather pecking damage? Answers to this question may help farmers to prevent feather pecking. Chapter 2 describes a study in which feather pecking damage and peck wounds are scored in 107 flocks with organic laying hens and are related to housing, feeding and free-range aspects.

1.3.5 Range use and Mellor's five domains model

Mellor's five domains model is described in terms that can be applied to many species, but no publication describes the use of the model for free-range egg production. A free range in Mellor's model may contribute to nutritional opportunities (variety of foods), to certain aspects of comfort (by providing space, substrate, fresh air, effective shelter and shade, absence of noise, provision of daylight, etc) and to promote the exercise of 'agency'. Agency is the animals' ability to consciously engage in goal-directed behaviours, such as exploring, hunting or self-care. It is promoted by a varied, novel environment, a variety of sensory inputs and available engaging choice (Mellor et al., 2020). A free range may meet those preconditions much better than the standard indoor environment of current poultry farms. However, whether these preconditions are met, depends on the design and management of the free range. A varied free range with trees resulting in a choice of shade and sunny places and with a variation of green edible vegetation and dry loose sand suitable for dustbathing, has more to offer than a sunny grassland without any shelter. Mellor et al. (2020) mention that an outdoor environment as well may contribute to negative affect, for example if animals are unable to access shelter in unfavourable weather conditions. This confirms the need for a well-designed and well-managed free range to which the hen has free access. Unfavourable weather conditions are of relative importance, as long as an animal can adapt to it, for example by seeking shelter.

1.3.6 Range use and physical health and functioning

A variety of physical parameters is investigated in relation to free-range use (summarized in Table 1.2): footpad dermatitis, toe nail length, keel bone fractures, bone strength, comb wounds, immune response, physiological stress response, infectious bacterial and viral diseases, internal parasites and mortality.

A lower incidence of footpad dermatitis is found in hens, classified as high rangers because of their 'heavy' use of the free range (Rodriguez-Aurrekoetxea and Estevez, 2016) and in flocks with access to a free range compared to flocks without (Heerkens et al., 2016), but Larsen et al. (2018) found no difference. Shorter (= better) toe nails are found in hens that spend more hours ranging per day (Campbell et al., 2017b; Bari et al., 2020). No difference in keel bone fractures are found between high and low ranging hens (Larsen et al., 2018; Bari et al., 2020) or flocks with and without access to a free range (Heerkens et al., 2016). Richards et al. (2012) however found that increased keel bone damage relates to a reduction of range use. They monitored range use and keel fractures of individually tagged hens throughout the laying period. They tried to compare range use before and after changes in keel bone score. However, due to the small number of hens that fulfilled the criteria for this test, the test does not reveal differences in range use before and after the keel bone break. The weakness of observational studies is that they can only reveal correlations. If a health problem like keel bone breaks is negatively correlated to free-range use, then cause and effect are not clear. Keel damage may cause hens to stay

Table 1.2: Summary of findings on health and disease in organic/free-range flocks with direction of alleged[1] effect of range access or range use being 0 (no effect), - (negative), + (positive) for welfare/health

Health aspect	Relation with free range access or use	Direction of alleged effect of range use on welfare	How many flocks included	Country	Reference
Footpad condition	No relationship between range score[2] and footpad condition	0	Total 285 hens from 2 flocks of 18,000 hens each	Australia	Larsen et al., 2018
	Lower incidence footpad dermatitis in hens with higher[3] frequency of use of outdoor area	+	Total 450 hens from 3 flocks of 6,000 hens each	Spain	Rodriguez-Aurrekoetxea & Estevez, 2016
	Footpad hyperkeratosis less prevalent in hens with access to free-range area	+	Total 2,350 hens from 47 flocks of 31,500 on average	Belgium	Heerkens et al., 2016
Toe nail length	High outdoor rangers[4] shortest (= best) toenails	+	Total 307 hens from 9 flocks of 154 hens each	Australia	Bari et al., 2020
	Toenail lenght negatively correlated with hours spent ranging, wheras shorter toenails are better	+	Total 450 hens from 6 flocks of 150 hens each	Australia	Campbell et al., 2017b
Keel bone fractures	Ranging no effect on keel bone damage	0	Total 307 hens from 9 flocks of 154 hens each	Australia	Bari et al., 2020
	No relationship between range score and keel bone condition	0	Total 291 hens from 2 flocks of 18,000 hens each	Australia	Larsen et al., 2018
	No relationship between access to a free-range and keel bone disorders	0	Total 2,350 hens from 47 flocks of 31,500 on average	Belgium	Heerkens et al., 2016
	Keel fractures resulted in a reduction of range use	0[5]	Total 611 hens from 4 flocks of 1,500 hens each	UK	Richards et al., 2012
Tibial bone strength	Range use[6] not associated with tibial bone strength	0	48 hens from 1 flock of 40,000 hens	Australia	Sibanda et al., 2020a

Comb wounds	High outdoor rangers fewer comb wounds than indoor hens	+	Total 307 hens from 9 flocks of 154 hens each	Australia	Bari et al., 2020
	No correlation between range use and comb peck wounds	0	Total 450 hens from 3 flocks of 6,000 hens each	Spain	Rodriguez-Aurrekoetxea & Estevez, 2016
Heterophil: lymphocyte ratio	No correlation between range use[7] and heterophil: lymphocyte ratio (immune response)	0	Total 141 hens from 1 flock of 18,000 hens	Australia	Rault et al., 2016
Plasma corticoster-one concentration	High rangers greater plasma corticosterone response (acute stress response) to handling and testing	-	Total 290 hens from 2 flocks of 18,000 hens each	Australia	Larsen et al., 2018
Faecal cortico-steroid metabolites	High rangers higher faecal (= basal) corticosterone concentration (chronic stress response)	-	Total 207 hens from 2 flocks of 18,000 hens each	Australia	Larsen et al., 2018
Albumen corticosterone concentrations	Higher (chronic and short-term stress response) in case of higher outdoor stocking density (2 compared to 1 or 0.2 hens/m^2)	+	270 eggs/day from 6 experimental flocks of 150 hens each	Australia	Campbell et al., 2017b
Plasma corticosterone	Plasma corticosterone level does not differ between indoor-preffering, moderate-outdoor and outdoor-preferring hens	0	Total 104 hens from 6 experimental flocks of 150 hens each	Australia	Campbell et al., 2016
Erysipelas bacteria	Free-range flocks higher prevalence compared with cage and indoor litter-based housing systems	-	4-10 hens from 129 commercial flocks	Sweden	Eriksson et al., 2013
Avian influenza	Outdoor-layer farms on average 6.3 x higher risk for low pathogenic avian influenza than indoor-layer farms	-	30 hens from 19,274 commercial farms	Netherlands	Bouwstra et al., 2017

[1] Effects are called 'alleged' because correlations only reveal relations and not cause and effect.
[2] Classification in non-rangers, low rangers, moderate rangers and high rangers is based based on the average duration that hens in the sample population spent in the outdoor range.
[3] Classification in never, light, medium or heavy rangers is based on proportion of observations that hens were observed in the outdoor area.
[4] Classification in indoor, low outdoor and high outdoor is based on number of hours spent outdoor per day.
[5] The authors report that keel fractures resulted in reduced range use, which is something different than stating that range use somehow affects keel fractures.
[6] Total time duration that an individual hen spent on the range.
[7] Range use expressed in number of days hens accessed the range, mean duration of daily visits and mean frequency of daily visits to outdoor range.

inside, because it is painful. However, indoor hens can have more accidents while moving between high and low locations. One thus needs to be careful in interpreting relationships between physical problems and free-range use. Tibial bone strength is not related to number of days on range in hens of 74 weeks of age from the same flock (Sibanda et al., 2020a). Comb wounds (caused by pecking) are seen less frequently in hens that use the free range intensively (Bari et al., 2020), but Rodriguez-Aurrekoetxea and Estevez (2016) did not find such a relation. Rault et al. (2016) investigated the heterophil: lymphocyte ratio, a higher ratio reflecting an immune response seen in stressful situations, in hens with different ranging patterns. They do not find a relation between immune response and range use. Campbell et al. (2016) found no difference in plasma corticosterone (representing a more reactive coping style) between indoor- and outdoor-preferring hens. Campbell et al. (2017b) found higher albumen corticosterone concentrations (chronic and short-term stress response) in eggs from hens with higher outdoor stocking densities (2 compared to 1 or 0.2 hens/m^2). Larsen et al. (2018) investigated blood plasma corticosterone concentration (acute stress response) and faecal corticosteroid metabolites (chronic stress response) and found that high rangers had a greater corticosterone response to handling and testing and a higher basal corticosterone concentration. Relations are found between corticosterone and range use, but they are not unambiguously. Some infectious diseases are seen more frequently in free-range hens compared to indoor kept hens, like the zoonotic *Erysipelas* bacteria (Eriksson et al., 2013), which survives in soil. Bouwstra et al. (2017) found that organic/free-range farms have on average a 6.3 times higher risk for low pathogenic Avian Influenza (another zoonosis) introductions than indoor-layer farms. It is expected that laying hens become infected by contact with wild bird faeces, contaminated water or contaminated soil in the free-range area (Elbers and Gonzales, 2019). Low pathogenic Avian Influenza can converse into a highly pathogenic virus. This conversion mostly happens in commercial poultry production systems (37 out of 39 events, since 1959) and less in wild birds (Dhingra et al., 2018).

Thesis topic 3: Presence of Avian Influenza risk birds in free ranges in relation to woody cover

Avian Influenza (AI) virus is transmitted from Eastern Asia to Europe by infected migrating waterfowl (Lycett et al., 2016). Migratory waterfowl such as ducks and geese are known to prefer areas including medium-sized waterways for foraging and resting. AI infection risk in commercial laying hens decreases with distance to such areas (Bouwstra et al., 2017). A feature of such 'wild bird areas' is openness, which refers to the presence of trees and bushes are present. It is conceivable that landscapes with trees or bushes are unattractive to waterfowl. At the same time, bushes and trees are known to enhance range use in laying hens (Bestman and Wagenaar, 2003; Zeltner and Hirt, 2003; Nagle and Glatz, 2012), whose ancestors are forest birds. The question is whether bushes and trees can be used to promote range use in laying hens and at the same time make free-ranges less attractive to waterfowl and other wild birds known as AI risk birds. Chapter 4 describes a study in which the presence of wild birds, categorized as high and low AI risk birds, is examined in the free ranges of 11 poultry farms in relation to the proportion the free-range surface being covered with trees or bushes.

Concerning internal parasites (summarized in Table 1.3), such as *Ascaridia galli* and *Heterakis gallinarum*, a free range is both described as a risk factor for hens to become infected by wild birds (Permin et al., 1999; Grafl et al., 2017), as well as a place to escape from high loads of parasite eggs in poultry faeces present inside the hen house (Sherwin et al., 2013; Thapa et al., 2015). The assumed risk of the free range is that hens can have contact with wild birds and their faeces (Permin et al., 1999). Parasites found in poultry are also found in wild birds, such as pheasants and partridges (Madsen, 1941). Some parasite species use earthworms or insects as intermediate hosts (Permin and Hansen, 1998), which might be more prevalent in the free range compared to the hen house. Another concern is that, despite a high mortality, some eggs of gut parasites may survive up to 2 years in the soil (Thapa et al., 2017). The number of methods for `cleaning' the free range from parasite eggs is assumed to be limited, compared to the possibilities for cleaning the hen house between two consecutive flocks (Permin et al., 1999). On the other hand, the number of parasite eggs (*A. galli* and *H. gallinarum*) is found to be much lower in soil samples (less than 5 eggs per gram; Heckendorn et al., 2009), compared to litter samples from inside the hens' house (on average 400 eggs per gram; Maurer et al., 2009). Jansson et al. (2010) and Bari et al. (2020) found no difference between respectively indoor and organic/free-range flocks and individual hens with different ranging patterns. Sibanda et al. (2020b) found that rangers (frequent range users) and roamers (intermittent range users) from the same flock are more likely to be infected with several species of gut parasites, compared to stayers (rare/no range users).

Thesis topic 4: Intestinal parasites in relation to range use

Intestinal parasites are widely present in organic/free-range laying hens (Jansson et al., 2010; Kaufmann et al., 2011; Thapa et al., 2015). Some of them may cause intestinal damage, transmit other pathogens and they can worsen the symptoms of concomitant infections and thus reduce the welfare of their host. Intestinal parasites in the Netherlands are generally treated with anthelmintics (Iepema et al., 2006; Bestman and Wagenaar, 2014). This may lead to unwanted residues in consumption eggs and via the manure in the environment of the free-range or arable land, when applied as fertilizer (Lahr et al., 2019). Furthermore, widespread use of anthelmintics may lead to resistance in the parasites, making anthelmintics less effective. The main risk factor for infestation with intestinal parasites is contact with faeces from flockmates or previous flocks (Jansson et al., 2010). There is no consensus yet whether the free-range is a risk factor too. Since a free range is an essential part of many poultry farms, a relevant question is: is there a relationship between parasite infections and range use? Knowledge of such relationships can be used in order to reduce parasite infections or to reduce the use of anthelmintics. Chapter 6 describes a study in which the presence of parasite eggs in faeces and soil is examined in samples from 40 organic poultry farms in relation to range use.

Average mortality of organic flocks ranges from 7 to 23% (Lambkin, 1997; Hegelund et al., 2006; Leenstra et al., 2012; Bestman and Wagenaar, 2014; Leenstra et al., 2014). Average mortality of free-range flocks ranges from 8 to 13% (Whay et al., 2007; Leenstra et al., 2012; Leenstra et al., 2014; Singh et al., 2017). However, several methods exist for

Table 1.3: Summary of findings on parasite infections in organic/free-range flocks with direction of alleged[1] effect of range access or range use being 0 (no effect), - (negative), + (positive) for welfare/health

Parasite species	Relation with free range access or use	Direction of alleged effect of range use on welfare	How many flocks included	Country	Reference
A. galli	No difference in *A. galli* burden between indoor and outdoor hens	0	307 hens in 9 experimental flocks of 154 hens each	Australia	Bari et al., 2020
A. galli	Frequent and intermittent range users higher prevalence of *A. galli* infection compared to rare/no range users	-	9,375 hens from 5 flocks of 40,000 hens each	Australia	Sibanda et al., 2020b
A. galli; H. gallinarum; Capillaria spp; Cestoda; *Eimeria* spp	Incidence *H. gallinarum*, *A. galli*, *Capillaria* spp. and Cestoda higher in org/free-range compared to indoor. Incidence *Eimeria* spp no difference between outdoor and indoor.	- 0	10 hens from 79 flocks of on average 5,900 hens	Austria	Grafl et al., 2017
A. galli	Pasture access time negatively associated with *A. galli* worm burden	+	892 hens from 55 commercial flocks	8 EU countries	Thapa et al., 2015
A. galli; H. gallinarum	*H. gallinarum* FEC[2] negatively correlated to % hens using range *A. galli* FEC higher in flocks with higher outdoor stocking density	+ +	20 hens from 19 flocks of 1,000 to 16,000 hens	UK	Sherwin et al., 2013
A. galli / H. gallinarum	No significant difference in ascarid infections between hens kept on litter indoors and free-range/organic hens	0	355 flocks from farms with 500 to 289,000 hens	Sweden	Jansson et al., 2010
A. galli; H. gallinarum, Capillaria anatis; Capillaria obsignata	Prevalence of *A. galli*, *H. gallinarum* and *Capillaria anatis* higher in org/free-range compared to deep-litter. Prevalence of *Capillaria obsignata* no difference between org/free-range and deep-litter.	- 0	268 hens from 16 farms	Denmark	Permin et al., 1999

[1] Effects are called 'alleged' because correlations only reveal relations and not cause and effect.
[2] FEC = Faecal egg count.

collecting information about mortality and causes of mortality. Cumulative mortality until for example 60 weeks cannot be compared to mortality over the complete lifetime of a flock. The lifetime may vary between flocks, lasting from 70 to 90 weeks. Figures based on actual farm records may differ from calculations, which include the feedback from the slaughterhouse on number of 'arrivals'. Not all hens that died are found by the farmer and included in the actual farm records (Hegelund et al., 2006). Their remains may have been overlooked or they may have been eaten by flock mates or predators before the farmer takes notice of them. Furthermore, the causes of death are not always categorized in a systematic and traceable way or may even not be clear. Therefore, it is difficult to estimate mortality related to the free range. In order to investigate this, a comparative study would be needed in which hens of the same genetic and rearing origin would be housed and managed in the same way with access to a free range as the only difference between treatment and control group, and with mortality records kept in the same systematic way. Something similar to this is done by Yilmaz-Dikmen et al. (2016). They compare 12 groups of 40 hens, allocated into three housing systems: conventional cages, enriched cages and a free-range system. At the end of the production cycle, at 66 weeks, they find 1.88% mortality in free-range hens and 1.25 and 6.25% in respectively conventional and enriched cages. They do not give details about causes of death. Another way of investigating the relationship between range use and mortality, would be by keeping records of individually tracked hens from the same flock, thus with a known degree of range use. This is done by Sibanda et al. (2020b). They assessed range use of 9,375 hens in five commercial flocks from 18 to 21 weeks of age and classified them as rangers (frequent range users), roamers (intermittent range users) and stayers (rare/no range users). They found that rangers and roamers were 2.4 times more likely to survive until 74 weeks of age than stayers. However, they give no details about causes of death. One could argue that in a free range more causes of death occur, compared to only indoor housing, such as predation and grass impaction in the crop. However, there is little information available about the mortality due to these causes. Another important aspect is that mortality related to free ranges may reduce with increasing experience of the farmer and its advisors. Schuck-Paim et al. (2021) did a meta-analysis on mortality data from 6,040 commercial flocks kept in different indoor housing systems and found that mortality reduces over the years, illustrating the importance of gaining experience with production systems. This may also count for production systems with access to a free range.

1.4 Low use of the free range

Despite the potential of a free range to offer opportunities for the welfare of laying hens, often only small proportions of a flock use the free range. Pettersson et al. (2016) review that in many flocks, less than 50% of the hens uses the range at any one time and in most of the flocks this is even less than 30%. Moreover, when looking at the range use of hens equipped with tracking devices, individual hens seem to differ in the time spend outside daily, how frequent they go out and how far they go from the pop-holes (Hartcher et al., 2016; Campbell et al., 2017a). Some hens never use the range. This is 2% of tagged hens in 6 flocks of 150 hens, observed between 22 and 36 weeks of age (Campbell et al., 2017a), 8% of tagged hens in 2 flocks of 18,000 hens, observed at 41 and 63 weeks of age (Larsen et al., 2018), 8% of tagged hens in 4 flocks of 1,500 hens, observed during varying periods between 38 and 69 weeks of age (Richards et al., 2011) and 50% of labelled hens in 3 flocks of 6,000 hens, observed from 20 to 69 weeks of age (Rodriguez-Aurrekoetxea and Estevez, 2016).

Whether or not and to what extent a hen uses the range, is influenced by her preferences and physical ability. Having a preference assumes well-informed decision making, i.e. that a hen knows the indoor and outside environment equally well and decides which one to use when. Grigor et al. (1995a) placed young hens, from 12 to 20 weeks, in a free range several times per week. When tested at 20 weeks of age, the hens with outdoor experience spend more time in the range and further away, compared to the hens that are only handled and compared to the hens that are not handled and not placed in a free range. When considering the situation on a farm, it is not clear whether all hens do know the indoor and outdoor environment equally well. Compared to a classic choice test, the farm situation has an incomplete design. One arm of the T-maze, a regular design for testing preferences in animals, is missing. In a regular T-maze, the animal is placed in the 'neutral' arm, from where it has the choice to enter either the left or the right arm, one of them representing the indoor and the other representing the outdoor environment. Staying in the neutral arm means that the animal expresses no preference. In a farm situation, the starting point or the 'neutral' arm is missing; all animals start in the indoor environment.

The decision to range or not is probably the 'sum' of expected attracting and repelling factors in the free range, attracting and repelling factors inside the hen house, physical ability of the hen and personality traits. Examples of attracting factors are a comfortable climate (fresh air, daylight), while strong wind or precipitation (Nicol et al., 2003) or absence of shelter might work as repellent (Bestman and Wagenaar, 2003; Nagle and Glatz, 2012). Furthermore, attractive factors might be the presence of flock mates, as reflected by a higher outdoor stocking density (> 1,000 hens/ha, compared to < 1,000; Sherwin et al., 2013), opportunities for dustbathing (Zeltner and Hirt, 2003) or feeders (Grigor et al., 1995b). Physical ability may be determined by pain or injury such as keel fractures (Richards et al., 2012). Locomotion disabilities or a bad plumage make hens vulnerable to cold, precipitation or wounds. An example of a personality trait is curiosity (Kolakshyapati et al., 2020).

Thesis topic 5: Factors related to range use

Differences in ranging behaviour are found on flock level and on the level of individual hens (Pettersson et al., 2016). If hens do not go out or to a lesser extent, it can be questioned to which degree they profit from the positive welfare aspects of a free-range. To make full use of the welfare opportunities of a free-range, it is necessary to know why hens are not using it and how they could be encouraged to use it. Chapter 3 describes a study in which proportion of hens using the range is related to several animal, housing and management factors in 169 flocks of organic and free-range laying hens.

1.5 Conceptual framework

As stated in 1.2.7, the main elements of animal welfare to consider in relation to the provision of a free range, are naturalness, affective states and physical health & functioning. When considering the outcomes of empirical studies that focused on aspects of animal welfare in relation to the provision of a free range, the below five topics of this thesis were introduced. In Figure 1.2 these topics are classified according to the key elements of animal welfare.

1. *Feather pecking in organic laying hens.* Feather pecking is regarded as a maladaptive behaviour in laying hens. One of its causes may be that conditions for behaviours that are important for laying hens, such as foraging, are not met. Therefore, this topic is classified under 'Naturalness'. Generally, less feather pecking (or its damage) is seen in relation to increased range use. Assuming this relationship to be causal, a free range is considered to have a positive effect on feather pecking damage and is thus shown in green in Figure 1.2.
2. *Factors related to range use.* Assuming that hens access the free range voluntarily, range use can be considered as an indicator of a positive affective state. It is therefore classified under 'Affective states'. However, when considering the meaning of the free range in terms of affective states for the hens that stay indoors, it cannot be said if they chose to stay inside or that they did not make a choice at all. Therefore, the effect of a free range on affective states is considered to be inconclusive and is thus shown in grey.
3. *Presence of Avian Influenza risk birds in relation to woody cover.* Avian influenza is a virus infection which may cause illness and death and is classified under 'Physical health & functioning'. Access to free ranges increases the risk of such an infection and is thus shown in red.
4. *Predation of chickens in free ranges.* Predation is considered to be a consequence of 'Naturalness', i.e. keeping the prey species laying hen in an environment where it is exposed to natural elements such as predators. Predation is considered to be detrimental to the welfare of both victim and bystander laying hens (witnesses) and is thus shown in red.

5. *Intestinal parasites in relation to range use.* Intestinal parasites may cause pain and other discomfort, which can be classified under 'Physical health & functioning'. So far, the role of the free range does not seem clear in terms of being a risk factor or an opportunity to escape from high loads of parasite eggs in the hen house. Gut parasites are thus shown in grey.

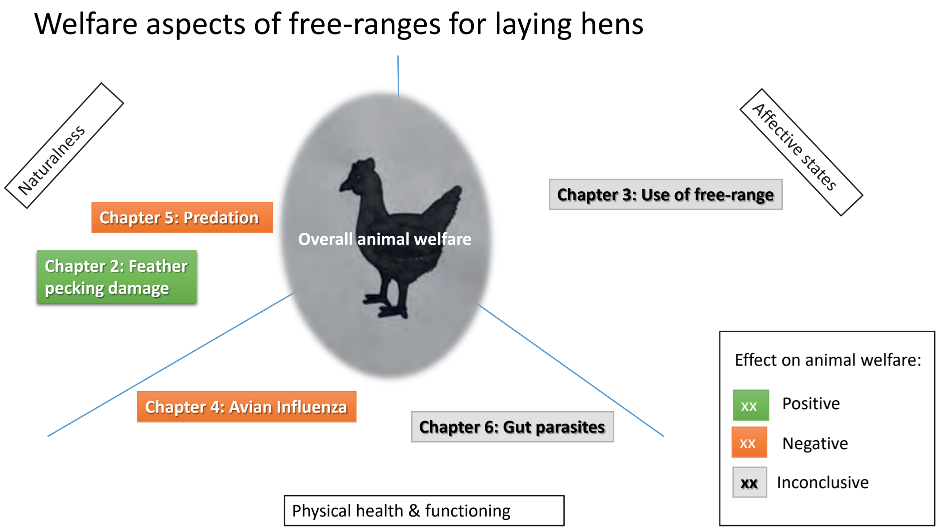


Figure 1.2: Conceptual framework (based on Fraser, 2008) illustrating the thesis topics: feather pecking damage, use of the free range, avian influenza, predation and intestinal parasites.
The topics are classified according to the key elements of animal welfare: naturalness, physical health & functioning and affective states. A free range can have a positive, a negative or an inconclusive effect on the topics, based on pre-existing scientific evidence.

1.6 Research objective and thesis outline

The overall objective of this thesis is to gain insight into the opportunities and risks of free ranges for animal welfare in laying hens, with the ultimate aim of optimizing hen welfare and health.

In **chapter 2** factors related to reduction of feather pecking in organic hens are discussed, one of them being daily access to a free range. In **chapter 3** it is discussed which features of farm management and of the hens are related to range use. In **chapter 4** the presence of avian influenza risk birds in and around poultry free-range areas is discussed in relation to cover with trees and bushes. In **chapter 5** the presence of predators and the consequent losses of hens are being discussed. In **chapter 6** the relationship between range use and intestinal parasites is discussed. **Chapter 7** contains the general discussion of the studies described in this thesis, as well as recommendations for farmers and further research.

Chapter 2

Feather pecking and injurious pecking in organic laying hens

Adapted from: Bestman M, Verwer C, Brenninkmeyer C, Willett A, Hinrichsen LK, Smajlhodzic F, Heerkens JLT, Gunnarsson S, Ferrante V. (2017). Feather pecking and injurious pecking in organic laying hens in 107 flocks from 8 European countries. Animal Welfare 26 (3): 355-363.
Doi: 10.7120/09627286.26.3.355

Abstract

Feather pecking and cannibalism may reduce the potential of organic husbandry to enhance the welfare of laying hens. We report risk factors for these issues based on a large survey of 107 commercial flocks in eight European countries. Information was collected regarding housing, management and flock characteristics (age, genotype). Near the end of lay, 50 hens per flock were assessed for plumage condition and wounds. Potential influencing factors were screened and submitted to a multivariate model. The majority of the flocks (81%) consisted of brown genotypes and were found in six countries. Since white genotypes (19%) were found only in the two Scandinavian countries, a country effect could not be excluded. Therefore, separate models were made for brown and white genotypes. Feather damage in brown hens could be explained by a model containing a lower dietary protein content and no daily access to the free range (30% of the variation explained). For feather damage in white hens no model could be made. Wounds in brown hens were associated with not having daily access to free range (14% of the variation explained). Wounds in white hens were explained by a model containing not topping up litter during the laying period (26% of the variation explained). These results suggest that better feeding management, daily access to the free-range area and improved litter management may reduce incidence of plumage damage and associated injurious pecking, hence enhancing the welfare of organic laying hens. Since this was an epidemiological study, further experimental studies are needed to investigate the causal relationships.

2.1 Introduction

Overall in Europe, 3.8% of all commercially farmed laying hens are kept on organic farms. In some northwestern European countries this percentage is higher: in Denmark for example 22% of the hens are organic (*Marktinfo Eier und Geflügel* 3/9/2015). One reason for this might be consumer expectation that organic production is more welfare friendly compared to cage, barn or free-range systems. The organic regulations aim for a higher level of animal welfare by giving the birds more space, access to outdoor areas and access to roughage. The European Regulation (EC 889/2008) prescribes a maximum group size of 3,000 hens per compartment, 6 hens per m^2 indoors, a free-range area of 4 m^2 per hen, 18 cm perch per hen and one third of indoor floor surface covered with litter. It also prohibits beak trimming, a widespread practice routinely performed in the conventional laying hen industry. Moreover, the hens should be fed organically grown feed, e.g. no synthetic amino acids are allowed. In the period 2012–2014, when the data presented were collected, the minimum requirement was that 95% of the feed should be from organic origin. In some countries additional regulations exist, for example concerning the rearing of organic hens or a free-range area of 10 m^2 per hen.

Despite these presumed welfare enhancing requirements in the organic regulations, welfare and health problems have been reported in flocks of organic laying hens (Bestman and Wagenaar 2003; Hegelund et al. 2006; van de Weerd et al. 2009; Leenstra et al. 2012; Bestman and Wagenaar 2014). Two major issues are feather pecking and injurious pecking. Feather pecking consists of forceful pecks and gripping/pulling of feathers, resulting in feather loss on the back, vent and tail area. Bald patches can be subjected to tissue pecking, which we regard as injurious pecking and which leads to wounds (Rodenburg et al. 2013). Injurious pecking may be considered a behavioural pathology, comparable to human psychopathological disorders (van Hierden et al. 2004) and it reflects reduced welfare in both the bird performing the feather pecking and the victimized bird (the latter because pulling out feathers is painful). The behaviour has strong relationships with stress (El-Lethey et al. 2000) and fear (Rodenburg et al. 2004). There is a reduced welfare in the victim because pulling out feathers is painful and hens with feather damage are more susceptible to further feather and injurious pecking (McAdie and Keeling 2000). The prevalent theory for feather pecking is that this maladaptive behaviour is redirected ground pecking that originates from insufficient foraging opportunities (Blokhuis 1986; Huber-Eicher and Wechsler 1997; Rodenburg et al. 2013). Feather pecking and injurious pecking may be caused by the same environmental risk factors (Pötzsch et al. 2001). Apart from being an animal welfare issue, feather pecking is also an economic problem: hens with feather/plumage damage may need up to 27% more feed in order to maintain their body temperature (Tauson and Svensson 1980). Another economic issue is that higher mortality, as caused by cannibalism, reduces egg production and thus farm income.

The aim of this epidemiological study was to identify risk factors for feather pecking and injurious pecking in commercial organic laying hens.

2.2 Animals, materials and methods

For this cross-sectional study, 114 organic layer farms were recruited across eight European countries: Austria, Belgium, Denmark, Germany, Italy, The Netherlands, Sweden and The United Kingdom. The inclusion criteria were that farms should have at least 500 hen places and that the housing should be permanent. Mobile houses relocated more frequent than every 14 days were excluded. Farms purchasing commercial rations were preferred in order to be able to use feed declarations as an information source. A random spatial distribution of farms within countries was not always feasible due to travelling distance and the willingness of organic farmers to participate in the study.

The studied flocks were visited twice during the laying period, namely at peak of lay and at end of lay. Management data were collected during the farm visit at the peak of lay around 36 weeks of age, by interviewing the farm manager or person responsible for hen care using predefined questions. Questions concerned general farm information (e.g. number of hen places), flock information (e.g. age at placement, hybrid), vaccinations and medical treatments, feeding (e.g. composition, phase feeds), housing and range management and specific problems (e.g. parasites, smothering). At the second visit, which took place around 62 weeks of age, there was a short interview covering changes made and any noticeable problems and treatments between both visits. Data on housing conditions were additionally recorded by taking measures of the hen house, covered veranda (if present) and free-range area, including the housing equipment (e.g. feeders, perches). Information on the feed composition was taken from the declarations from ready mixed rations or from standardized Near-Infrared (NIR) feed analysis where farms mixed their own feed.

The use of the free range and veranda was evaluated as follows. At each visit the total numbers of birds within the free-range area and the veranda were counted 3 times: 5h15min – 4h30min before sunset, 3h30min – 2h45min before sunset and 1h45min – 0h45min before sunset. With these numbers the proportions of hens using the veranda and the free-range area were calculated. In the statistical analysis only the highest percentage figures for bird use of the free-range area and the veranda were used.

The sampling and assessment of endoparasites in faeces and in guts is described in Thapa et al. (2015).

Ectoparasite burden was screened using 10 cardboard mite traps per flock at either the summer visit (all farms) or both visits (58 farms). The traps were fixed on the underside of the cross supports carrying the perches or the perches next to the cross supports in the evening and left in place for 7 days. After removing the traps in the morning they were transferred individually into zip-lock plastic bags and placed in a freezer at -20°C for at least 24 hours. Each sample was tapped out and distributed evenly in a petri-dish with a grid painted on. The grid served to estimate the number of mites by counting the number of mites within one square and multiplying this by the number of occupied squares. Based on this number, a score from 0 to 5 was assigned (0 = no mites, 1 = 1 to 10 mites, 2 = 11 to 100, 3 = 101 to 1,000, 4 = 1,001 to 10,000 and 5 = more than 10,000). In the statistical analysis the maximum score found for mites from every flock was used.

At the end of lay visit, a random sample of 50 hens per flock was caught and clinically scored regarding plumage condition and wounds at the neck, back, vent and tail using a modified four-point scoring scheme (Table 2.1), originally developed as a deliverable in the LayWel project (Tauson et al. 2005).

Table 2.1: Explanation of scores and definitions used for scoring feather damage and wounds

Score	Feather damage on neck, back and vent	Feather damage on tail	Wounds on back and vent
4	Very good plumage condition; no or very few feathers damaged	No or less than ≤ 5 tail feathers damaged	No wounds at all
3	Completely or almost completely feathered, few feathers damaged. Featherless areas < 5 cm^2	Tail feathers moderate to lightly damaged	Wound < 0.5 cm in diameter or a hematoma. Blood filled follicle after a feather was pulled out, is not regarded as wound.
2	Highly damaged feathers and/or featherless areas. Featherless areas ≥ 5 cm^2 (up to 75% featherless)	Tail feathers highly damaged	Wound < 2.2 cm
1	Very high graded damage of feathers with no or very few feather covered areas. Featherless area ≥ 5 cm^2 AND almost bare (75% featherless) up to completely featherless	Tail feathers highly damaged and almost bare quill.	Wound with diameter of > 2.2 cm (width of thumb)

The percentage of hens with feather damage was calculated per flock and a hen was regarded as having feather damage if the mean feather score of the 4 body parts was ≤ 3.00. The percentage of hens with wounds was calculated per flock and a hen was regarded as having a wound if the mean wound score of the two body parts was ≤ 3.50.

The data were analyzed with SPSS 19.0. Based on literature and other expert knowledge, a list of potential influencing factors was compiled for the dependent variables, percentage of hens with feather damage and percentage of hens with wounds. Independent categorical- and dichotomous variables were not taken into the analyses if one or more categories were not present in at least 20% of the sample. All continuous independent variables were transformed by means of ln (x + 1) to correct for zeros and to meet the assumptions of normality and homogeneity of variance. Potential factors were screened by means of partial correlation analyses for all continuous and categorical variables and controlled for country and genotype. Dichotomous independent variables were screened by means of linear regression. A p-value ≤0.07 was used as threshold for inclusion of the variable in a multivariate model (GLM). Associations, by means of regression analyses, between independent variables were calculated to avoid variance inflation. Models were built by means of automated stepwise backward selection (SPSS 19.0), by removing variables from the model with $p > 0.05$. Variables with $p \leq 0.05$ were retained in the model. Parameter estimates were back transformed for interpretation.

2.3 Results

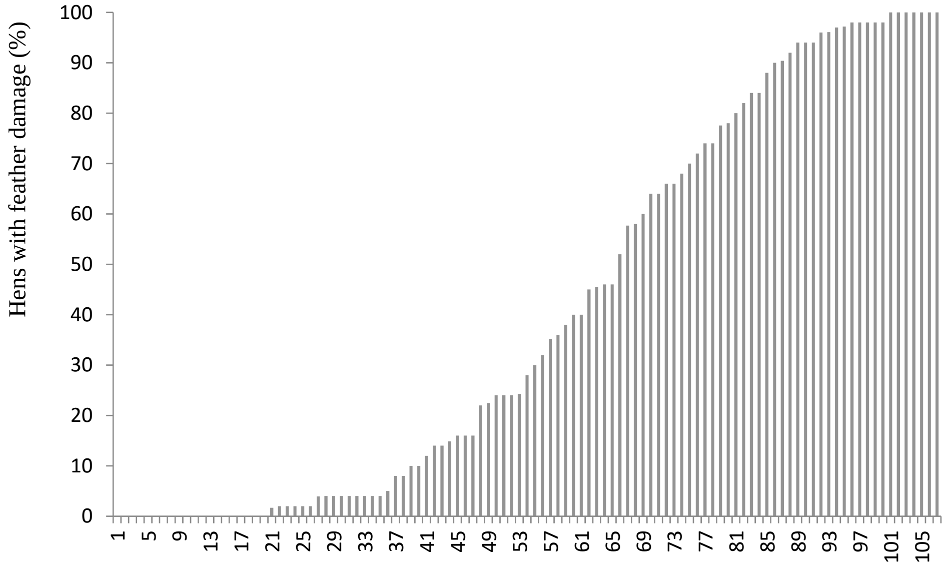
Data recording was performed between February 2012 and March 2014 on 114 organic laying hen farms at peak of lay (between 29 and 44 weeks of age), and on 110 farms a second time towards the end of lay (between 52 and 73 weeks of age). Thus, four farms dropped out before the 2nd visit because the hens were slaughtered earlier than originally planned or because induced molting was performed. Because of lack of essential information, data from another 3 farms could not be used. Due to missing values, for some of the calculations we had information from fewer than 107 farms.

2.3.1 Beak treatments

In total 14 flocks had treated beaks to varying degrees (in Italy, The United Kingdom and Belgium). The Italian flocks that were beak treated, were either treated with the infrared method on the first day of life at the hatchery or with a hot blade within the first 9 days of life at the farm. The UK flocks and Belgium flocks were mildly treated with infrared as day old chicks. Since no significant differences appeared in feather damage and wounds between flocks with or without beak treatment, the beak treated flocks were not excluded from statistical analysis.

2.3.2 Frequency of feather damage and wounds

Figure 2.1 shows that in 42 flocks (39%) more than half of the hens had feather damage. Figure 2.2 shows that in 17 flocks (16%) more than half of the hens had at least one wound.



2

Figure 2.1: Flocks (n = 107) in order of the proportion of hens with feather damage.

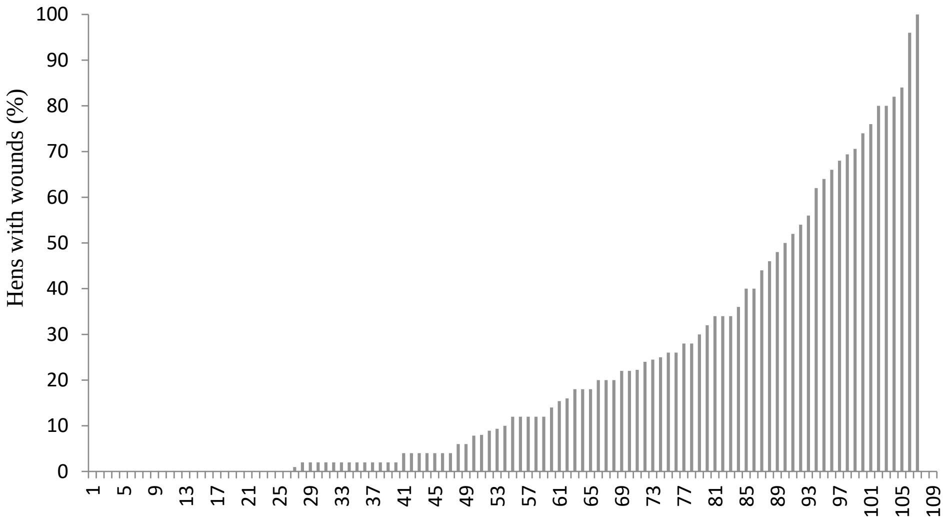


Figure 2.2: Flocks (n = 107) in order of the proportion of hens with wounds.

2.3.3 Genotype

Genotypes were categorized as white, brown or silver. The majority of the flocks (82 out of 107) were brown genotypes. In Austria, Belgium and Italy only brown flocks joined the study. White genotypes (20 out of 107) were only seen in Sweden and Denmark. Silvers (5 out of 107) were only seen in Germany, The United Kingdom and The Netherlands. The small number of silver flocks was included in the category of brown flocks. Although silver hens have a white appearance, they lay brown eggs and their body weight is closer to that of brown hens. For the remainder of this article 'brown hens', 'brown flocks' or 'brown genotypes', refer to both brown and silver hens, flocks or genotypes. White flocks had a significantly ($p \leq 0.001$) higher percentage of hens with feather damage (mean 72% (min – max 2–100); SD 32) than brown flocks (mean 33% (min – max 0–100); SD 36). Concerning the percentage of hens with wounds, no differences were found between white and brown flocks: mean 20% (min – max 0-64; SD = 17) in white hens and mean 22% (min – max 0–100); SD = 28) in brown hens. Since white flocks were only present in the 2 Scandinavian countries, a country effect could not be excluded when interpreting the results of the white genotypes. On the other hand, brown and silver genotypes were used in more countries and that were more different from each other. Therefore it was decided to discriminate between brown and white genotypes and thus build 4 models: Feather damage in brown hens, wounds in brown hens, feather damage in white hens and wounds in white hens.

2.3.4 Feather damage in flocks of brown hens

After screening and selecting the factors as described in the 'Animals, materials and methods' section, the variables as shown in Tables 2.2 and 2.3 were retained in the model for feather damage in brown hens.

Table 2.3 contains the univariate associations between percentage of brown hens with feather damage and a number of nutritional and management factors that showed to be significant and which were used in the final model.

Table 2.2: Univariate associations of continuous nutritional and management variables and percentage of hens with feather damage in brown genotypes

Factor	N flocks	Correlation coefficient	p-value	Mean (min–max)
Number of weeks pre-lay feed after placement	81	0.33	0.014	1.0 (0–7)
Dietary protein content at placement	70	-0.34	0.011	18.0 (16–22.3)
Dietary protein content at 55 weeks	73	-0.40	0.003	17.9 (14.6–22.2)
Methionine content at 55 weeks	65	-0.32	0.021	0.35 (0.28–0.40)
Hens in veranda at 35 weeks (%)	84	-0.24	0.046	30 (0–83)
Hens in free range area at 35 weeks (%)	84	-0.25	0.038	18 (0–64)
Number of deworming treatments	82	0.22	0.042	0.5 (0–3)
Number of alternative treatments[1]	82	0.20	0.062	0.5 (0–5)

[1] Alternative treatments include treatments with herbs, homeopathy, vitamins et cetera as a prevention or treatment of any health problem.

Table 2.3: Univariate associations of categorical and dichotomous nutritional and management variables for percentage of hens with feather damage in brown genotypes

Variable	Correlation coefficient	p-value	No Mean (min–max)	No N	Yes Mean (min–max)	Yes N
Only 1 diet till 55 weeks	-0.31	0.004	45 (0–100)	38	23 (0–100)	47
Litter replacement	-0.33	0.020	39 (0–100)	50	15 (0–84)	30
Litter topping	-0.39	0.001	47 (0–98)	30	20 (0–100)	50
Daily access to free range	-0.28	0.012	36 (0–100)	56	16 (0–98)	24
Roughage during rearing	0.32	0.022	20 (0–84)	33	42 (0–100)	19
Daylight	-0.20	0.063	48 (0–100)	16	30 (0–100)	71
Needle vaccination after rearing	0.37	0.001	23 (0–84)	51	50 (0–100)	33

Since several of the variables were correlated with each other, some of them were not taken into the multivariate analyses. This was the case for dietary protein content at weeks 25, 35 and 55. Dietary protein content at week 55 was used, as this was the closest to hen assessment. Number of weeks pre-lay diet was fed was included in the model, while the age until pre-lay diet was fed and the presence or absence of pre-lay diet after placement was left out. The multivariate analysis reveals that the outcome variable 'percentage of hens with feather damage' for brown genotypes can be explained by the 'protein content at 55 weeks of age' (p = 0.004) and by 'daily access to free range' (p = 0.001), together explaining 30% of the variation based on a sample size of 53 flocks. This means that an increased percentage of brown hens with feather damage was related to decreased dietary protein content at 55 weeks of age and to the absence of daily access to the free range. The model is as follows:

Percentage of brown hens with feather damage =
134 - 6.8 * (dietary protein content at week 55) + 21.6 * (daily access free range = 0)

Dietary protein content of the feed at 55 weeks of age varied between 14.6 and 22.2%.

2.3.5 Feather damage in flocks of white hens

After screening and selecting the factors as described in the `Animals, materials and methods` section, the variables as shown in Table 2.4 and in the paragraph below were used to make the final model.

If there was no needle vaccination at placement (n = 8), then a mean of 93% (min 70, max 100) of the hens had feather damage. If there was a needle vaccination (n = 9), then a mean of 66% (min 15, max 100) of the hens had feather damage. Correlation coefficient -0.48; p = 0.049; n = 17). The outcome variable 'percentage of hens with feather damage' for white genotypes could not be further explained if the above mentioned continuous and dichotomous variables were submitted in the GLM.

Table 2.4: Univariate associations of continuous nutritional and management variables and percentage of white hens with feather damage

Factor	N	Correlation coefficient	p-value	Mean (min–max)
No of feed phases till end of lay	20	0.52	0.033	2.3 (1–6)
Phosphorous content at 35 weeks	18	-0.53	0.050	0.55 (0.49–0.65)
Sodium content at 55 weeks	16	-0.52	0.058	0.16 (0.15–0.17)
Viability at 70 weeks	8	-0.78	0.040	93 (84–97)

2.3.6 Wounds in flocks of brown hens

After screening and selecting the factors as described in the 'Animals, materials and methods' section, the variables as shown in Tables 2.5 and 2.6 were retained in the model for brown hens with wounds.

Table 2.6 gives an overview of the correlations found between percentage of brown hens with wounds and the presence or absence of a number of nutritional and management factors.

Table 2.5: Univariate associations of continuous nutritional and management variables and percentage of hens with wounds in brown genotypes

Factor	N	Correlation coefficient	p-value	Mean (min–max)
Dietary protein content at placement	70	-0.33	0.066	18.0 (16–22.3)
Degree of presence of red mites[1]	82	0.22	0.050	2.3 (0–5)

[1] The highest score of 2 visits was used.

Table 2.6: Univariate categorical and dichotomous associations of the presence or absence of nutritional and management variables and percentage of brown hens with wounds

			No		Yes	
Variable	Correlation coefficient	p-value	Mean (min–max)	N	Mean (min–max)	N
Needle vaccination at placement	-0.24	0.026	26 (0–100)	61	11 (0–68)	23
Daily access to free range	-0.21	0.063	22 (0–100)	56	11 (0–80)	24
Access to range restricted in poor weather	0.23	0.042	11 (0–80)	29	23 (0–100)	51

The presence or absence of pre-lay diet after placement was correlated with the number of weeks this diet was given. The number of weeks pre-lay feed after placement was not taken into the multivariate model, as its association was weaker than the presence or absence of pre-lay diet after placement. Only one diet till 55 weeks was exchangeable with the number of feed phases till end of lay. The latter was taken into the analysis as a stronger association was found for this variable. The outcome variable 'percentage of brown hens with wounds' could be explained by 'daily access to free range' ($p = 0.001$), explaining 14.4% of the variation. An increased percentage of brown hens with wounds was seen if there was no daily access to the free range. The model is as follows:

Percentage of brown hens with wounds = 10.9 + 11.5 * (daily access free range = 0)

2.3.7 Wounds in flocks of white hens

Univariate analyses on the percentage of white hens with wounds revealed that an increased calcium content at 25 weeks of age ($r = -0.49$; $p = 0.053$; mean 3.64; min. 3.50; max. 3.90) was related to a decreased percentage of white hens with wounds. The topping up of litter during the laying period was correlated with a decreased percentage of white hens with wounds ($r = -0.48$; $p = 0.021$; litter topping = 0: mean 94% (min. 84, max.100); litter topping = 1: mean 65% (min. 2, max. 100)).

The outcome variable 'percentage of white hens with wounds' could be explained by 'litter topping' ($p = 0.022$), explaining 26% of the variation. An increased percentage of white hens with wounds was associated with farms that did not top up litter. The model was as follows:

Percentage of white hens with wounds = 14.9 + 19.1 * (litter topping = 0)

2.4 Discussion

Beak trimming is prohibited in organic animal husbandry (EC No 889/2008), but at least in the UK there is a derogation that allows non-organic chicks to be converted to organic. Therefore, farmers can buy conventional chicks that have been beak trimmed. Beak trimmed flocks were included in the statistical analysis, because no differences were found in feather damage between beak trimmed and non-beak trimmed flocks. Whay et al. (2007) also found in a study in 25 free range flocks in the UK that neither feather pecking nor feather loss was affected by the severity of beak trimming.

Our data show that feather damage and wounding is a serious issue for organic egg production.

Through the application of best practice, managers can reduce the risk of feather pecking and cannibalism to facilitate good welfare in the hens. It was difficult to compare the frequency and degree of damage we found with other studies, because we determined the degree of feather damage in a flock as % of hens with a certain degree of feather damage, while other studies that used the same Laywel/Tauson scoring method, expressed it in a mean flock score. Moreover, the studies differ in the characteristics of the study flocks, such as number of countries involved (country effect being an important factor; see below), genotype, housing, and beak treatments.

The majority of the flocks, 81%, were brown or silver hens and 19% were white hens. The white hens were found in only Sweden and Denmark. We found significant differences between brown and white genotypes concerning the percentages of hens with feather damage, the mean for brown and white flocks being 33% and 72%, respectively. The differences found between white and brown flocks in the present study could also be explained by other factors than genotype, e.g. geographical location and its consequence for the availability of the free-range area. In Scandinavian countries the hens are usually kept indoors for a longer period because of snow or other unfavorable winter conditions. In the present study no daily access to the free-range area was significantly associated to an increased percentage of hens with feather damage. Leenstra et al. (2012) investigated the performance of commercial laying hen genotypes on free range and organic farms in three European countries and found differences between genotypes: white genotypes in organic systems showed less feather pecking. However, in that study a country effect could have explained the results as well.

As in all epidemiological studies, associations found do not imply a causal relation between the factors studied. Associations found were used for practical recommendations as we attempted to test an existing hypothesis and explain some of our findings. However, confounding factors cannot be ruled out completely.

2.4.1 Feather damage irrespective of genotype

A higher dietary protein content of the feed at 55 weeks of age contributed to the multivariate model explaining feather damage in brown hens. Inappropriate or insufficient protein and amino acid levels are well known risk factors for feather pecking (van Krimpen et al. 2005). Another motivation for feather pecking, may be to increase the fibre content of the diet, as most commercial laying hen diets have a relatively low fibre content. The consumption of feathers may be related to their positive effect on gut motility, which may be similar to the effect of fibre, illustrating that hens may indeed eat feathers to increase satiety (Harlander-Matauschek et al. 2006).

Also, daily access to the free-range area was significantly correlated with a decreased percentage of brown hens with feather damage. Daily access contributed to a multivariate model explaining feather damage and wounds in brown hens. Lack of association for white flocks in the present study could be related to the fact that all the flocks with white hens were kept in Denmark and Sweden. Short day length in northern latitudes means that during the winter hens have restricted access to the outdoors, especially if the weather is inclement. In Sweden the hens are allowed to be kept inside for the whole winter. A relation between higher percentage of hens using the free-range area and less feather damage has been found in several other studies (Bestman and Wagenaar

2003; Green et al. 2000; Lambton et al. 2010; Mahboub et al. 2004; Nicol et al. 2003). A free-range area can be considered as environmental enrichment. Another explanation is that if a flock is distributed over a larger area, the stocking density decreases. Lower stocking density (in combination with a smaller group size) is also associated with less feather pecking (Huber-Eicher and Audigé 1999; Nicol et al. 1999; Savory et al. 1999). An increased percentage of hens using the free-range area could be achieved by providing shelter (Zeltner and Hirt 2003; Bestman and Wagenaar 2003).

2.4.2 Wounds irrespective of genotype

No daily access to the free-range area was related to an increased percentage of hens with wounds. Possible explanations and similar findings have been discussed in the paragraph above. Moreover, this variable was also related to percentage of brown hens with feather damage. Pötzsch et al. (2001) stated that vent pecking and feather pecking damage could be caused by common risk factors. The second variable contributing to the percentage of (white) hens with wounds, is the topping of litter during the laying period. Rodenburg et al. (2013) reviewed underlying principles of feather pecking and stated that early (i.e. during rearing) access to litter is an important factor in the reduction of feather pecking. The importance of litter in the prevention of feather pecking has been recognized for some time (Blokhuis and van der Haar 1992). Also, in commercial flocks the importance of litter for the reduction of feather pecking has been shown. Green et al. (2000) found that absence of loose litter at the end of lay increased the risk for feather pecking. Nicol et al. (2003) found a relation between feather pecking and the restriction of hen access to the litter area in their case-control study of 100 commercial farms in the UK.

For most statistically significant variables the correlation coefficients were relatively low. Thus the proportion of variation explained by its associations is also low. For example, 30% of the flock's plumage damage was explained in the model for brown birds by lower dietary protein content and no daily access to the free range. However, another 70% needs to be accounted for, reinforcing the complex and multifactorial nature of this problem.

2.5 Conclusion and animal welfare implications

This study identified risk factors for plumage damage and, or wounds in organic laying hens. These findings could also apply to conventional laying hens, whereas some risk factors are more specific for organic or free-range systems. Measures that could be recommended are feeding enough protein, providing daily access to the free-range area and improved litter quality. Further research is needed for determining differences between white and brown genotypes.

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Chapter 3

Factors related to free-range use in laying hens

Adapted from: Bestman M, Verwer C, Niekerk T van, Leenstra F, Reuvekamp B, Amsler-Kepalaite Z, Maurer V. (2019). Factors related to free-range use in commercial laying hens. Applied Animal Behaviour Science 214: 57-63. Doi: 10.1016/j.applanim.2019.02.015.

Abstract

Free-range use is expected to contribute to the welfare of laying hens, and more so if a high proportion of the hens in a flock uses the range. In the Netherlands and Switzerland, data were collected about free-range use, genotype, rearing conditions, housing system, management, performance, health, welfare and behaviour in 169 free-range and organic layer flocks by farm visits at an age between 45 and 66 weeks. The aim of this study was to identify which factors are related to free-range use. We analysed the % of hens seen outside when conditions for ranging were optimal (% Hens Out). Based on literature and expert knowledge, 26 potential correlating factors were subjected to preselection. Pearson correlation, independent samples t-tests and one-way ANOVA's were performed to investigate correlation between the factors and % Hens Out one by one. Twelve factors appeared to be related significantly to % Hens Out and these were entered in a linear regression model: country, production system, genotype, flock size, stocking density, presence of roosters, feather damage, keel bone damage, health at 60 weeks, outside access during rearing, type of ventilation, and amount of daylight in the house. The final model for the total sample explained 47% of the variation in % Hens Out and contained 5 variables. A higher % Hens Out was associated with brown genotype, smaller flock size, roosters in the flock, better feather cover and natural ventilation. Further analyses were done with subsets of the database for either free-range or organic flocks in either the Netherlands or Switzerland. No factors could be found that explained % Hens Out in Dutch free-range flocks. A better feather score and higher amount of daylight explained 44% of the variation in % Hens Out in Dutch organic flocks. Roosters and rearing on the laying farm explained 41% of the variation in % Hens Out in Swiss free-range flocks. Les fearfulness and brown genotypes or more than one genotype per flock explained 33% of the variation in % Hens Out in Swiss organic flocks. The results may contribute to improving range use by laying hens.

3.1 Introduction

Free-range access is associated with animal welfare in laying hens, when welfare is measured in terms of less feather pecking damage (Green et al., 2000; Bestman and Wagenaar, 2003; Nicol et al., 2003; Mahboub et al., 2004; Lambton et al., 2010; Bestman and Wagenaar, 2014; Bestman et al., 2017). These studies show that the welfare opportunities of range access are dependent on the proportion of hens using the range. The higher the proportion, the less feather pecking damage is seen. However, often free ranges are available but only poorly used. Pettersson et al. (2016) summarized ranging percentages from 14 studies, nearly all relating to commercial flocks. The mean % of hens on range per study varied from 9 to 38%. Bestman and Wagenaar (2003) reported flocks with more than 75% of hens seen outside at the same time. Several factors are related to the proportion of hens using the free-range area.

Rearing conditions like early experience with a free range (Grigor et al., 1995) or a younger age when moved to the laying farm (Bestman and Wagenaar, 2003) were found to be related to a higher proportion of hens seen outside at later age. Social factors, like flock mates being out already, attract other hens to go out too (Sherwin et al., 2013) or go further from the stable (Keeling et al., 1988 cited in: Pettersson et al., 2016). A higher proportion of hens is seen outside in case of smaller flock size (Bestman and Wagenaar, 2003; Hegelund et al., 2005; Whay et al., 2007; Gebhardt-Henrich et al., 2014; Gilani et al., 2014). Furthermore, a lower stocking density (Gilani et al., 2014) and roosters in the flock (Bestman and Wagenaar, 2003) are related to higher range use. Bubier and Bradsaw (1998) suggested feed systems with limited feed or limited feeding time being a risk for less hens seen outside, compared to ad libitum feed. An explanation is that modern hybrids with a high egg production cannot afford to spend much time in a free-range area where no highly nutritious feed is available in sufficient quantities. Larger pop-holes (expressed in cm/hen) and more pop-holes (expressed in number/hen) were found to be positively related to range use (respectively Gilani et al., 2014 and Sherwin et al., 2013). In the free range the presence of cover, for example trees, bushes or artificial structures was related to more hens in the free range (Bestman and Wagenaar, 2003; Nicol et al., 2003; Zeltner and Hirt, 2003; Nagle and Glatz, 2012). Weather conditions have shown to be relevant. For example, Nicol et al. (2003) found that a higher percentage of birds went out when the weather was 'calm and dull', compared to when it was 'wet', 'cold' or 'sunny'. Richards et al. (2011) found more hens using the pop-holes in case of lower wind speed, lower rainfall and below 20 degrees Celsius. Finally, the hens have to be physically comfortable enough to go out. Richards et al. (2012) found that hens with keel fractures showed less range use.

At commercial farms all these and probably more factors, play a role in the proportion of hens that go outside. Our question was which factors are associated with a higher proportion of a flock using their range, when those factors have an interactive effect.

3.2 Materials and methods

Important elements of the regulations with regard to organic and free-range egg production in the Netherlands and Switzerland at the time of the data collection (2011–2013) are presented in Table 3.1.

Table 3.1: Legal requirements on housing, beak trimming and outside access during rearing for free-range and organic production systems in the Netherlands and Switzerland

Country/system	Maximum group size	Maximum stocking density	Beak trimming	Outside access during rearing
NL free-range (EC 589/2008)	6,000	9	Yes	No
NL organic (EC 889/2008; SKAL, 2018)	3,000	6	No	Yes
CH free-range (BLW, 2013)	6,000	6 (7 in case hens < 2 kg)	Depends on label**	Pasture not mandatory; winter garden is mandatory
CH organic (Bio Suisse, 2018)	2,000*	5	No	Yes

* In practice, flocks are often divided into groups of 500, which used to be mandatory.
** All our study flocks belonged to a label that did not allow beak trimming.

3.2.1 Recruitment of flocks

Flocks were recruited by sending an invitation explaining the aim of the study to all Dutch organic and free-range farmers and by contacting Swiss poultry farmers through their egg traders. All farmers that reacted positively were involved in the study.

3.2.2 Data collection

A questionnaire was made, covering housing and management during rearing and lay and flock characteristics such as genotype, flock size, number of roosters, stocking density, mortality and production.

Flocks were visited at around the age of 50 weeks. The visit started with an interview with the farmer. Range use in this study was estimated by farmers based on the definition: '% of hens of this flock seen outside when conditions for ranging were optimal'.

After the interview, in one compartment the researchers collected data from the hens and their environment. Air quality was estimated by smelling and scored in terms of bad, moderate or good. Amount of daylight was visually estimated and scored in terms of no, little, sufficient or much. Amount of total light was scored in terms of little, sufficient or much. In the same compartment they caught 50 hens, weighed and scored them for feather damage (neck, breast, belly, back, tail, wings), wounds (comb, back+belly), keel bone deformations, footpad infections and missing toes. See Table 3.2 for scoring methods. Feathers, wounds on combs, belly, back and foot pads were scored visually according to the method of Tauson et al. (2005). Keel bones were scored by palpation as described by Scholz et al. (2008). Fear score was based on the hens' behaviour while working between them. Before the start of data collection, the one Swiss and the two Dutch observers scored 2 flocks together. After data collection was finished, the one Swiss and one Dutch observer separately scored the same 50 hens of one flock and they agreed in 96% of cases (50 hens x 11 body scores mentioned above). This revealed that the scoring system was robust and that there were no systematic differences between the teams.

Table 3.2: Methods for scoring body condition and fear in laying hens

Characteristic	Method	Meaning of scores
Feather damage*	Visually on neck, breast, belly, back, tail and wings. A 4 -point scale is used for each body part.	1 = bald; 2 = several to considerable feathers broken or missing; 3 = few feathers broken or missing; 4 = intact
Skin wounds*	Visually on belly and back	1 = wound > 2.2 cm diameter (= width of thumb); 2 = wound 0.52.2 cm; 3 = wound < 0.5 cm diameter; 4 = no wounds
Comb wounds*	Visually	1 = considerable (number of) wound(s); 2 = several small wounds; 3 = very few small wound(s); 4 = no wounds
Footpad infections*	Visually	1 = severely swollen bumble foot, wound or scab; 2 = slightly enlarged foot or small wound or scab; 3 = skin irregularity; 4 = no infection or wound
Keel bone deformations**	Palpation by running 2 fingers down the edge of the keel bone, feeling for deformities	1 = severe deformation; 2 = moderate deformation; 3 = slight deformation; 4 = no deformity
Fear	Impression of observers after finishing catching and scoring 50 animals for the above mentioned parameters	1= extreme calm, no fear, curious, relaxed, no alarm calls, not aroused if observer moves; 5 = generally calm, but a bit on guard, some alarm calls, slightly aroused when observer moves; 10 = extremely fearful, crowding at the end of the house, tense, alarm calls, small movements of observer causes large panic reactions

* Protocol described by Tauson (2005).
** Method described by Scholz et al. (2008).

Based on literature and the authors' knowledge, 26 variables potentially related to the proportion of hens seen outside, were selected from the available farm data: country, production system (organic/free-range), genotype, flock size, stocking density, roosters in the flock, feather damage, keel bone damage, footpad infections, comb wounds, fearfulness, health status as judged by the farmer, mortality till 60 weeks, egg production till 60 weeks, housing system during lay, winter garden at how many sides of the barn, relative size of winter garden, time of opening pop-holes, daily outside access, type of ventilation system in the barn, air quality in the barn, amount of light and daylight in the barn, rearing on the laying farm, rearing system and outside access during rearing.

3.2.3 Statistics

Statistics were performed with IBM SPSS 25. The output variable was the '% of hens seen outside when conditions for ranging were optimal', from hereon called '% Hens Out'. First, the below described analyses were performed with the total sample and after that the analyses were performed on the four subsets: Dutch free-range, Dutch organic, Swiss free-range and Swiss organic. The output variable was visually judged for normality by a histogram and Q-Q plot. Preselection of variables to enter in the regression analysis, was done by univariate analyses: Pearson correlation analyses for continuous variables, independent samples t-test for dichotomous variables and one-way ANOVA for categorical variables. For the variables that correlated significantly with the output variable, flocks with > 5% of missing values were excluded. Categories with < 10% of observations were combined together to one new category and if this was not possible in a logical way, this variable was excluded from further analyses. Subsequently, the correlations between % Hens Out and the possible explanatory factors with a $p < 0.01$ and $R \geq 0.2$ were entered in a linear regression model. In case of multicollinearity (Variance Inflation Factor > 5), regression analysis was done again, but without the variables with VIF > 5. The final model was made by entering only the variables that contributed significantly to the model in order to avoid complex models with many variables that did not contribute significantly.

3.3 Results

3.3.1 All flocks

A total of 169 flocks were visited: 85 flocks in Switzerland and 84 flocks in the Netherlands. From these flocks, 69 were free-range and 100 were organic. Flocks were visited in 2011–2013, mostly at an age between 45 and 55 weeks, ten flocks were between 57–66 weeks. Five flocks were excluded from analysis because the '% Hens Out' was missing. The variables 'rearing on the laying farm', 'winter garden at how many sides of the stable' and 'amount of light' were excluded, because one or more categories of these variables contained less than 10% of the flocks and could not be combined with another category into one new category in a logical way. Concerning the variable 'amount of daylight' the categories 'no' and 'little' were combined to the new category 'no/little daylight'. Furthermore, the variable 'Winter garden % of floor' was excluded because of too many missing values (13%).

Table 3.3, 3.4 and 3.5 contain the variables that were hypothesised to be related to the output variable '% Hens Out' and their value for the complete dataset and the different subsets of Dutch free-range, Dutch organic, Swiss free-range and Swiss organic flocks. The highest % Hens Out was seen in Swiss organic flocks (57%), followed by Dutch organic (52%) and Swiss free-range (48%). The least % Hens Out was seen in Dutch free-range flocks (23%).

Table 3.3: Mean and standard deviation (between brackets) of factors potentially correlated with % Hens Out

	All	NL		CH	
		Free-range	Organic	Free-range	Organic
N	169	32	52	37	48
% Hens Out	47 (24)	23 (15)[a]	52 (25)[b]	48 (19)[b]	57 (21)[b]
Flock size	8,450 (9,345)	23,879 (9,639)[a]	8,562 (4,540)[b]	3,504 (1,817)[c]	1,854 (442)[c]
Stocking density[1]	6.5 (1.4)	9.0 (0.1)[a]	5.9 (0.4)[b]	7.0 (0.0)[c]	5.1 (0.5)[d]
Winter garden (% of floor)	41 (12)	31 (13)[a]	30 (7)[a]	50 (0.0)[b]	50 (0.0)[b]
Time pop-holes open[2]	10.9 (0.8)	10.9 (1.0)	10.6 (0.9)[a]	11.1 (0.6)[b]	11.0 (0.6)
Eggs/hen 60 wks	249 (14)	250 (10)[a]	240 (15)[b]	256 (9)[ac]	249 (14)[ad]
Mortality % 60 wks	5.8 (3.7)	5.8 (3.8)	6.3 (3.5)	5.7 (3.0)	5.3 (4.4)
Health 60 wks[3]	7.7 (1.6)	7.3 (1.9)	7.6 (1.3)	8.0 (0.9)	7.9 (2.0)
Comb wounds[4]	3.33 (0.25)	3.45 (0.20)[a]	3.15 (0.19)[b]	3.35 (0.20)[a]	3.43 (0.25)[a]
Keel bone damage[5]	3.32 (0.42)	2.99 (0.43)[a]	3.07 (0.35)[a]	3.51 (0.25)[b]	3.66 (0.17)[b]
Footpad infections[6]	3.39 (0.42)	3.52 (0.33)[a]	3.47 (0.31)[a]	3.21 (0.51)[bc]	3.36 (0.46)[ac]
Fear[7]	3.9 (1.7)	3.4 (1.0)	4.2 (1.8)	4.2 (2.0)	3.6 (1.6)
Feather score[8]	18.18 (3.77)	15.14 (2.80)[a]	16.67 (4.24)[a]	19.60 (1.96)[b]	20.74(2.45)[b]

[1] In hens/m^2 accessible area.
[2] Time in hours is presented here as continuous variable, as it was analysed too.
[3] Health status of the flock, estimated by the farmer and expressed on a scale of 1 (= extremely bad) to 10 (= extremely good).
[4] According to method Tauson et al. (2005), value ranging from 1 (= considerable (number of) wound(s) to 4 (= no wounds).
[5] According to method Scholz et al. (2008), value ranging from 1 (= severe deformity) to 4 (= no deformity).
[6] According to method Tauson et al. (2005), value ranging from 1 (= severely infected) to 4 (= no infection or wound).
[7] Estimated by the observers, value ranging from 1 (= no fear at all) to 10 (= extremely fearful).
[8] Sum of 6 body parts. According to method Tauson et al. (2005), value ranging from 1 (= bald) to 4 (= intact).

Table 3.4: Dichotomous variables potentially correlated with % Hens Out

	All	NL		CH	
		Free-range	Organic	Free-range	Organic
Rearing on laying farm[1]					
No	93	100	90	86	96
Yes	7	0	10	14	4
Outside access rearing					
No	42	100	8	89	2
Yes	58	0	92	11	98
Roosters					
No	43	78	65	38	0
Yes	57	22	35	62	100
Daily outside access					
No	2	6	2	0	0
Yes	98	94	98	100	100

[1] If rearing took place on the laying farm, it is unknown if this was in the future laying barn.

Preselection of continuous variables showed that % Hens Out was higher in case of smaller flock size ($r = -0.476$; $p < 0.001$), lower stocking density ($r = -0.498$; $p < 0.001$), less keel bone damage ($r = 0.308$; $p < 0.001$) and better feather score ($r = 0.460$; $p < 0.001$). Preselection of dichotomous variables showed that % Hens Out was higher in Switzerland (compared to the Netherlands; $p = 0.001$), in organic production systems (compared to free-range; $p < 0.001$), if hens had outside access during rearing ($p < 0.001$) and if roosters were present ($p < 0.001$). Preselection of categorical variables showed that % Hens Out was higher when mixed flocks, brown or silver genotypes instead of white hens were kept ($p = 0.001$), natural ventilation was applied ($p < 0.001$) and amount of daylight in the barn was higher ($p < 0.001$).

The following variables were entered in the initial regression model: country, production system, genotype, flock size, stocking density, roosters, feather score, keel bone damage, health at 60 weeks, amount of daylight in barn, ventilation type and outside access during rearing. Variance Inflation Factor of the variables production system, country and stocking density was > 5. The regression model was run again without these variables in order to avoid multicollinearity. The final regression model ($F(5\ 158) = 28.084$; $p < 0.001$; $R^2 = 0.471$) explained 47% of the variation in % Hens Out (Table 3.6). Percentage Hens Out is higher in case of brown genotype, smaller flock size, roosters in the flock, a better feather cover and natural ventilation applied.

As several factors differed significantly between the countries and/or the systems, the data were also analysed within country and system. As preselection, the same variables were explored in their relationship with % Hens Out as for the total sample.

Table 3.5: Categorical variables potentially correlated with % Hens Out; % of farms per category

	All	NL		CH	
		Free-range	Organic	Free-range	Organic
Genotype					
Brown	51	75	56	41	38
White	22	22	0	46	27
Silver	12	3	37	0	0
Mixed[1]	15	0	8	14	35
Rearing system					
Floor	3	6	6	0	0
Aviary	63	47	12	100	100
Variable floors	30	47	71	0	0
Other	4	0	12	0	0
Winter garden how many sides of stable					
No WG	8	3	25	0	0
One side	66	47	48	86	83
Two sides	25	50	27	14	17
Housing system					
Floor	14	19	33	0	2
Aviary	76	81	35	100	98
Floor+aviary	9	0	29	0	0
Other	1	0	4	0	0
Ventilation					
Natural	13	6	33	0	6
Mechanical	79	94	50	95	90
Both	8	0	17	5	4
Air quality stable					
Bad	2	0	0	5	4
Moderate	27	34	25	19	29
Good	71	66	75	76	67
Amount of light					
Little	8	3	10	14	6
Sufficient	54	88	60	54	25
Much	38	9	31	32	69
Amount of daylight					
No	4	16	2	0	0
Little	42	63	52	43	17
Sufficient	30	22	21	41	35
Much	25	0	25	16	48

[1] Mixed genotype means hens of 2 or more different genotypes in one flock, for example white and brown hens.

3.3.2 Percentage Hens Out in Dutch free-range flocks

Preselection did not yield any variable as significantly correlated to % Hens Out in Dutch free-range flocks (n = 32).

3

Table 3.6: Variables explaining % Hens Out in total sample

Variable	Bèta	p
Constant	19.293	0.028
Roosters	8.696	0.009
Feather score	1.663	0.000
Flock size	-0.001	0.000
Genotype = White	-14.870	0.000
Ventilation = Natural	18.065	0.000

3.3.3 Percentage Hens Out in Dutch organic flocks

Preselection yielded flock size, feather score, presence of roosters, housing system, ventilation type and amount of daylight as significantly related to % Hens Out. Feather score and amount of daylight contributed significantly to the final model (F(2 49) = 19.027; $p < 0.001$; $R^2 = 0.437$), that explained 44% of the variation in % Hens Out (Table 3.7). This means that in Dutch organic flocks the % Hens Out is higher in flocks with higher (better) feather score and if there is more daylight in the hen house.

Table 3.7: Variables explaining % Hens Out in Dutch organic

Variable	Bèta	p
(Constant)	-8.003	0.475
Feather score	2.312	0.001
Amount Daylight	12.726	0.000

3.3.4 Percentage Hens Out in Swiss free-range flocks

Preselection yielded feather score, roosters, rearing on the laying farm, genotype and amount of daylight in the barn as significantly related to % Hens Out. The final model (F(2 31) = 10.541; $p < 0.001$; $R^2 = 0.405$) explained 41% of the variation in % Hens Out (Table 3.8). In Swiss free-range flocks % Hens Out was higher in flocks that were reared on the laying farm and where roosters were present.

Table 3.8: Variables explaining % Hens Out in Swiss free-range

Variable	Bèta	p
(Constant)	37.143	0.000
Roosters	12.190	0.037
Rearing Laying	23.667	0.005

3.3.5 Percentage Hens Out in Swiss organic flocks

Preselection yielded mortality till 60 weeks of age, fear and genotype as significantly correlated to % Hens Out. The final model ($F(2\ 43) = 10.665$; $p < 0.001$; $R^2 = 0.332$) explained 33% of the variation in % Hens Out (Table 3.9). This means that in Swiss organic flocks the % Hens Out was higher in flocks of brown genotypes or 2 or more genotypes being kept together, compared to white genotypes and in case of less fear.

Table 3.9: Variables explaining % Hens Out in Swiss organic flocks

Variable	Bèta	p
(Constant)	77.830	0.000
Fear	-4.494	0.014
Genotype = White	-15.243	0.024

3.4 Discussion

Higher % Hens Out were seen in organic flocks, compared to free-range flocks. Gilani et al. (2014) also found a higher proportion of hens seen outside in organic flocks compared to free-range flocks. Several explanations for this difference between free-range and organic flocks may be possible. Free-range flocks are generally larger (Table 3.3), and flock size is in general found to be negatively correlated to the proportion of hens that go outside (Bubier and Bradshaw, 1998; Bestman and Wagenaar, 2003; Gebhardt-Heinrich et al., 2014; Gilani et al., 2014; Harlander-Matauschek et al., 2003; Musslick et al., 2004; Zeltner et al., 2004; Hegelund et al., 2005; Chielo et al., 2016). Free-range flocks have higher stocking densities than organic flocks (Table 3.3), which is related to a smaller proportion of hens seen outside (Gilani et al., 2014; Campbell et al., 2017). Also, organic hens more often had free-range access during rearing (Table 3.4). This is obligatory from 8 weeks of age for organic hens in the Netherlands (SKAL, 2018) and from 7 weeks of age in Switzerland (Schürmann et al., 2018), while it is not obligatory and therefore not practiced in free-range rearing hens. Outdoor access during rearing is known to be related to free-range use (Grigor et al., 1995). All together these factors may have contributed to the difference found in % Hens out between free-range and organic flocks.

Factors that explained % Hens Out in the total sample significantly were presence of roosters, feather score, flock size, genotype and natural ventilation. Factors that explained % Hens Out additionally in the 'Country x Production system' subsets were amount of daylight, rearing on the laying farm and fear.

3.4.1 Genotype

A higher % Hens Out was seen in mixed flocks or flocks of brown or silver genotypes, compared to flocks of white hens. Nearly all studies on range use by laying hens refer to brown genotypes. Only Mahboub et al. (2004) compared white and brown genotypes. In their study the brown hens spent more time outside than the white hens. The white hens in their study were also more fearful, as measured in tonic immobility tests. White hens were also found to be more fearful than brown hens by de Haas et al. (2013). The differences in ranging behaviour between white and brown genotypes may thus be caused by differences in fearfulness.

3.4.2 Flock size

A smaller flock size is related to higher proportion of hens seen outside. This is also found in other studies (Bestman and Wagenaar, 2003; Hegelund et al., 2005; Whay et al., 2007; Gebhardt-Henrich et al., 2014; Gilani et al., 2014). An explanation may be that most hens tend to stay within the vicinity of the majority of their group. In larger flocks many hens have to go out before such a majority outside is reached.

3.4.3 Roosters

A higher proportion of hens is seen outside if roosters are present. This was also seen by Bestman and Wagenaar (2003). An explanation may be that roosters may act as pacemaker and thus stimulate the hens to go out too (Bestman and Wagenaar, 2003). Furthermore, roosters may provide safety to the hens by acting as watcher, i.e. by keeping an eye on potential dangers. While roosters keep an eye on the surroundings, the hens can generally perform other behaviour, such as foraging. Finally, roosters are seen to defend hens against predators (Bestman and Wagenaar, 2003).

3.4.4 Quality of feather cover

Better feather cover is associated with a higher % Hens Out, but which is cause and which is effect, is unclear. One explanation for the feather cover being the cause for poor range use, could be that hens with feather damage, especially when they have bald patches, may be more vulnerable to sunburn or get wounded when walking through vegetation. Maybe this makes them more cautious and therefore tend to stay inside. Another explanation might be that the quality of feather cover can be regarded as an indicator for a certain level of stress in the flock (El-Lethey et al., 2000). Hens from flocks with a lower stress level, which may have better feather cover, may be more confident and therefore may dare to go out more easily. The other direction is that range use leads to better feather quality. Several studies found a negative relation between range use and feather damage (Green et al., 2000; Bestman and Wagenaar, 2003; Nicol et al., 2003; Mahboub et al., 2004; Whay et al., 2007; Lambton et al., 2010; Bestman and Wagenaar, 2014; Chielo et al., 2016; Bestman et al., 2017). Range use can function as environmental enrichment, which is known to result in less feather damage. Range use can also lead to a lower stocking density in the house, which is also known to be related to less feather damage (Nicol et al., 1999). The uncertainty of how to explain the relation in terms of cause and effect, is inherent to the design of our study. A more experimental set up in which range use and feather cover of individual hens are followed during a period or in which test and control groups composed of hens with better or worse feather cover are compared, may give a more decisive answer.

3.4.5 Fear

A higher % Hens Out is correlated with less fearfulness measured in the flock. This correlation between range use and fearfulness is also found in other studies (Grigor et al., 1995; Mahboub et al., 2004). The explanation for this relation can be that hens with lower levels of fearfulness are more likely to go outside, but the explanation can also be that range use functions as environmental enrichment and decreases fearfulness. In the latter case fearfulness declines with increased familiarity with the environment. This would only be valid in individual hens that actually use the outdoor range, but in our study this was not checked. Campbell et al. (2016) found that indoor-preferring hens were more fearful than outdoor-preferring hens. However, Whay et al. (2007) found a positive correlation between range use and arousal (flightiness). They explain this 'counter intuitive association' by the fact that their low ranging flocks were large flocks (up to 16,000 hens), which in their study also showed lower levels of arousal.

3.4.6 Ventilation

The % Hens Out was higher where natural ventilation was applied, compared to a combination of natural and mechanical ventilation or mechanical ventilation alone. Most flocks with natural ventilation were Dutch organic flocks (Table 3.5). No other studies found a relation between natural ventilation and range use. In natural ventilated stables there is more daylight, because of the open parts along the roof top and along the sides (Corts and Ellen, both personal information). Another feature of natural ventilation is the absence of mechanical noise and strong air patterns in the stable (Hassing cited in Burgers, 2017). These features might make the transition from inside to outside less drastic. Furthermore, if a farm uses mechanical ventilation based on negative pressure (exhaust), this may lead to draughts as soon as the pop-holes are opened. This means that if the hens want to go out, they have to move against crosswind, which may discourage them to go out (Borren and Ellen, both personal information). Another explanation may be that the group of organic farmers with only natural ventilation consisted of farmers that chose for organic farming from a more holistic point of view and did more effort to provide an attractive range for their hens (Borren and Ellen, both personal information). Nowadays mechanical systems may have improved, especially in newly built stables, compared to the period of data collection, when a part of the stables originally was built for indoor housing only (Ellen, personal information).

3.4.7 Amount of daylight

Gilani et al. (2014) found that more hens went out when light intensity in the house was higher. They did not distinguish daylight from artificial light. However, their explanation for light intensity can also be applied to daylight: a higher light intensity inside means a smaller difference between inside and outdoor area, which make the transition from inside to outside less drastic. Hens kept under higher light intensity are more active and less fearful, compared to lower light intensity (Hughes and Black, 1974). This may also explain our findings. Furthermore, in rabbits the diurnal variation in the 'colour temperature' of light can synchronise behaviour patterns, independent of changes in illuminance (Nuboer et al., 1983). Synchronised ranging behaviour would result in a higher % Hens Out.

3.4.8 Rearing on laying farm

The % Hens Out is higher in flocks that are reared on the laying farm, irrespective whether it is in the later laying barn, compared to being reared on a separate rearing farm. This finding does not say anything about the rearing conditions as such, only that rearing conditions or moving from one farm to another matter in relation to free-range use at later age. Janczak and Riber (2015) wrote that hens should be reared in an environment similar to that in which they will live as adults in order to guarantee a certain level of welfare during their adult life. In their review paper they cited many examples in which rearing conditions were of importance for behaviour and well-being at later age. Regarding rearing conditions and range use at later age, Grigor et al. (1995) found that regular exposure to an outdoor area during rearing was related to increased readiness to utilise the outdoor area as adults. Bestman and Wagenaar (2003) found a higher % Hens Out when the hens arrived at the laying farm on an age younger than the regular 16–18 weeks. This also indicates an association between free-range use and rearing conditions. At the age of 16 to 18 weeks, hens are becoming sexually mature. This means a lot of changes: start of egg laying, hormonal changes and conspecifics becoming sexually mature. Another 'life changing event', such as moving to another farm on top of that, may have a drawback on their confidence to use the free-range area in their later life. Such a movement may be stressful because of the movement as such (catching, transport) and because of change of conditions between rearing and laying house. Stress can have long term consequences for the hens' development and maybe also for their tendency to use the range.

3.5 Conclusions

In conclusion, we found that range use is higher in mixed, brown or silver (compared to white) genotypes, smaller flocks that contained roosters with the hens, flocks with a better feather score, that were less fearful, were kept in barns with natural ventilation, more daylight and that were reared on the laying farm. These findings can be used for recommendations for further research or application in poultry farming practice in order to help improve range use.

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Chapter 4

Presence of avian influenza risk birds in and around free ranges

Adapted from: Bestman M, Jong W de, Wagenaar J-P, Weerts T. (2018). Presence of avian influenza risk birds in and around poultry free-range areas in relation to range vegetation and openness of surrounding landscape. Agroforestry Systems 92: 1001-1008. Doi: 10.1007/s10457-017-0117-2.

Abstract

Free-range areas contribute to the welfare of poultry. Chickens are most likely to use these areas if there is sufficient cover by trees. However, wild birds in free-range areas may infect the chickens with avian influenza (AI). This study aimed to investigate the relation between the presence of AI risk birds and woody vegetation within the range areas as well as in the landscape surrounding the range areas. During two seasons all wild birds were counted in the free-range areas of 11 poultry farms and their immediate surroundings. More high-risk birds were observed in free-range areas with less than 5% woody cover, compared to free-range areas with more woody cover. Furthermore, more high-risk birds were observed in the surroundings of free-range areas in open landscapes, compared to half-open landscapes. As for low-risk birds, no relation was found between woody cover or openness of the landscape and the presence of these birds in free-range areas or surroundings. However, interpretation of the results was hampered by the incomplete factorial design, which did not allow to differentiate between the effect of woody cover within the range area and openness of the surrounding landscape. The results of this pilot study need to be confirmed with further experimental research on the relation between the presence of AI risk birds and woody vegetation in and around poultry free-range areas.

4.1 Introduction

Free-range areas contribute to the welfare of laying hens. Feather-pecking, a generally recognized indicator of chicken welfare (Rodenburg et al. 2013), is less prevalent if more hens of a flock go outside (Bestman and Wagenaar 2003; Green et al. 2000). The number of chickens going outside has been found to depend on the degree of cover provided by trees or artificial structures in the range area (Bestman and Wagenaar 2003; Zeltner and Hirt 2003). These findings were corroborated by Bright et al. (2016), who found less feather-pecking damage in chickens that had more trees in their free-range area. Furthermore, range use also depends on the relative number of cockerels, the age of the hens on arrival at the laying farm (Bestman and Wagenaar 2003), and flock size (Bubier and Bradshaw 1998; Hirt et al. 2000; Appleby and Hughes 1991). Besides reduced feather-pecking, an additional benefit of (tree) cover is a more even distribution of chickens across the range area, which may reduce the risk of parasitic contamination (Bray and Lancaster 1992) and local accumulation of nitrogen and phosphate (Dekker et al. 2012). Thus, outdoor areas with (tree) cover contribute not only to the welfare, but also to the health of free-range chickens, and may also have environmental benefits.

Chickens ranging outside can come into contact with wild birds and their faeces. If these wild birds are infected with the avian influenza (AI) virus, the virus may be transmitted to the chickens. Among wild birds, prevalence of AI-virus is highest in migratory water birds, which are therefore regarded as the most probable transmitters of the AI virus to poultry. The prevalence of AI-virus is much lower in birds of prey, which therefore are considered of low risk with regard to AI transmission (Verhagen et al. 2015; van der Goot et al. 2015). However, it is not certain whether wild birds transmit the virus to poultry directly, or whether intermediate hosts, such as pigeons or rats, (also) play a role (EFSA 2006).

Our first research question was whether there is a relation between the degree of tree cover in free-range areas ('woody cover' hereafter) and the number of AI high-risk and low-risk birds observed within these areas. Another question was whether there is a relation between the openness of the landscape surrounding the poultry farm and the number of AI high-risk and low-risk birds in the immediate surroundings of the free-range areas.

4.2 Methods

4.2.1 Farm selection

At the start of the project we selected ten poultry farms from our network, based on the varying degree of woody cover in their free-range areas. The woody cover on these farms consisted of fruit trees, biomass willows, or Miscanthus (*Miscanthus giganteus*).

Although Miscanthus is a grass, we regarded it as 'woody cover' since it grows up to 4 meters high and has a density comparable to biomass willows. The degree of woody cover on the selected farms ranged from 0 to 90% (Table 4.1). The landscape surrounding the ranges was classified either as 'open' or 'half-open', with the open landscapes mainly consisting of grassland and only very few trees or bushes, and the half-open landscapes containing woodland strips or tree plots within 500 meters from the border of the free-range area. After analyzing the results of the first season, we added an eleventh farm to our study. This was a farm with a high degree of woody cover in the range area and which was located in an open landscape, a combination of characteristics that was missing from our initial farm sample. Table 4.1 shows the characteristics of the 11 farms. The farms varied in the type of vegetation and were unevenly distributed across open and half-open landscapes. The degree of woody cover (% of free-range area) was estimated after walking around and through the entire range area during an initial visit.

4.2.2 Bird observations

The first observation period (hereafter called 'spring') ran from February 4 to April 23, 2014. The second observation period (hereafter called 'autumn/winter') ran from October 10, 2014 to February 2, 2015. Bird observations were carried out on 10 farms in spring, and on 11 farms in autumn/winter. In each season, we visited each farm four times.

At the first visit of each farm, maps of the free-range area, farm buildings, farmyard, and direct surroundings up to a distance of 500 meter were made. For bird counts in the surroundings, we selected two plots bordering (or close to) the range area, which could be observed from a car from the public road. On each farm, bird observations started with observing the surrounding plots from the car for 30 minutes, after which the observer continued on foot to visit the range area and farmyard. The range area was observed while walking around or standing at predefined spots. A range area of 5–10 ha took approximately 60 minutes, a range area of 10–15 ha 75 minutes and a range area of 15–20 ha 90 minutes. The mean observation time for a range area, farmyard and surrounding plots together was 90 minutes.

On most farms, pop-holes opened at 11:00 am and chickens would be outside until dark. Since we expected less disturbance and better visibility of wild birds if no chickens were present in the outdoor ranges, we started our observations at 9:00 am, when the chickens were still inside. Per day, one farm was visited. In the seldom case of 'extreme' weather, such as heavy rain or fog, the farm visit was postponed for an hour or moved to another day. Birds were considered present in the free-range area if they touched the ground or the vegetation within the free-range area. Birds were considered present in the surroundings if they flew over the free-range area, the surrounding plots or the farmyard, or if they were sitting in the surroundings or the farmyard. Although we could not do observations inside dense vegetation such as Miscanthus, we did not expect

Table 4.1: Characteristics of the study farms

	No of hens, rounded to 1,000	Size of free-range area in hectares	Woody cover in % of free-range area	Cover category*	Type of vegetation in free-range area	Vegetation of surrounding landscape	Openness of surrounding landscape
1	24,000	12	0	1	Grass	Grassland	Open
2	18,000	8	35	3	Grass, fruit, Miscanthus	Agriculture**, woodland strips, forest	Half-open
3	30,000	17	8	2	Grass, trees, bushes	Agriculture, woodland strips	Half-open
4	15,000	6	75	4	Miscanthus, grass	Agriculture, woodland strips, forest	Half-open
5	12,000	5	90	4	Fruit, grass	Agriculture	Half-open
6	17,000	8	0	1	Grass	Grassland	Open
7	16,000	6	35	3	Grass, fruit	Agriculture, woodland strips	Half-open
8	15,000	8	50	3	Fruit, biomass willows, grass	Agriculture, woodland strips	Half-open
9	15,000	7	10	2	Grass, fruit	Agriculture, woodland strips, forest	Half-open
10	24,000	10	10	2	Grass, fruit	Agriculture, woodland strips, forest	Half-open
11	6,000	2	90	4	Fruit, diverse bushes	Grassland	Open

* See methods.
** 'Agriculture': maize or wheat (= arable crops related to livestock farms).

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this to be a problem, since the most important species of our study (waterfowl and birds of prey) were not expected to be present there.

4.2.3 Risk categories

Wild birds were classified into three categories (high-risk, low-risk, and no/unknown-risk species), based on large-scale wild bird monitoring for prevalence of AI (Breed et al. 2011), a categorization made by Veen et al. (2007), and expert judgement (personal communication, Roy Slaterus, Sovon, Dutch center for field ornithology). High-risk species, i.e. species with a high prevalence of infection with the AI virus, are water birds and (long-legged) wading birds, such as geese, ducks, swans, gulls, oystercatchers and lapwings. Low-risk birds are not as vulnerable to influenza infection as the high-risk birds, but can still carry the virus after contact with infected birds. This category includes all birds of prey and carrion-feeding birds as corvids, for example, hawks, buzzards, crows and ravens. The no/unknown-risk birds include all other birds (mainly songbirds). Birds in this category are rarely or never found to be infected with AI and are no longer monitored in the European AI monitoring program (Breed et al. 2011).

4.2.4 Data analysis

For data analysis, the counted wild birds were categorized into three risk classes, as described above. Furthermore, depending on where the birds were observed, their location was classified as either “free-range area” or “surroundings”. Free-range areas were categorized according to their degree of woody cover: cover category 1: < 5%; cover category 2: 5–20%; cover category 3: 20–50%; and cover category 4: >50%. The surrounding landscape was classified either as open or half-open (see Farm selection, above). Per farm, the total number of birds observed per season (summed over 4 observations per season) were used for the analysis: (1) all high-risk birds in the free-range area, (2) all low-risk birds in the free-range area, (3) all high-risk birds in surroundings and (4) all low-risk birds in surroundings. The no/unknown-risk birds were not included in the statistical analysis. Hence, for each combination of bird category and location (range area versus surroundings), a total of 21 observations were available for analysis (10 farms in spring, 11 farms in autumn/winter). Data were natural-log transformed to normalize distributions, and analyzed using the General Linear Models procedure in Genstat, using the following model:

> Ln (total number of birds observed per farm and per season) = season + woody cover + openness landscape

where

> Season = block (spring or autumn/winter); woody cover = woody vegetation within range area (four levels); openness landscape = absence or presence of woody vegetation in the immediate surroundings of the range area (two levels: open or half-open).

4.3 Results

In total, 24,103 wild birds were counted during this study, of which 5,706 were either high-risk or low-risk birds (Table 4.2). The complete list can be obtained from the main author.

Table 4.2: Results used for the statistical analysis, categorized as either high-risk or low-risk and observed either within the free-range areas or the surrounding landscape

	Order	Free-range area	Surroundings	Total
High-risk birds	Ducks	28	308	
	Geese	108	1,443	
	Charadriiformes	85	1,435	
	Other	47	186	
	Subtotal	268	3,372	3,640
Low-risk birds	Bird of prey	24	194	
	Corvid	403	1,445	
	Subtotal	427	1,639	2,066
	Total	695	5,011	5,706

4.3.1 High-risk birds in free-range areas

If either woody cover or openness of the landscape was used in the model, this resulted in models with significant factors (Table 4.3). Due to the incomplete factorial design it was not possible to run models with both factors together. Hence it was not possible to differentiate the effect of woody cover within the range area from the effect of openness of the landscape surrounding the range area.

Regression analysis with woody cover as fixed factor (Table 4.3, model 1) resulted in the following model ($p = 0.026$; $R^2 = 35$; se = 15.8):

Ln (total number of high-risk birds in free-range area) = 2.55 + (2.47 * Season Spring) + (-5.10 * Cover Category 2 / -5.12 * Cover Category 3 / -4.62 * Cover Category 4).

In this model, the number of high-risk birds in free-range areas was weakly related to season ($p = 0.052$) and significantly related to woody cover ($p = 0.042$). More high-risk birds were observed in spring than in autumn/winter, and more high-risk birds were observed if woody cover was low. The number of high-risk birds in the woody cover category < 5% differed significantly ($p < 0.02$) from the other cover categories (5–20, 20–50 and > 50% woody cover).

Using openness of the landscape as a fixed factor (Table 4.3, model 2) resulted in the following model ($p = 0.029$; $R^2 = 25$; se = 19.3):

Ln (total number of high-risk birds in free-range area) = -2.38 + (2.7 * Season Spring) + (3.37 * Open Landscape).

In this model, the number of high-risk birds was weakly related to season (p = 0.066) and significantly related to openness of the landscape (p = 0.040), with more high-risk birds observed in free-range areas surrounded by open landscapes, compared to free-range areas surrounded by half-open landscapes.

Table 4.3: Results from the General Linear Model analysis

Model	Response variable	Fixed factor	Model R^2	se	p	Variables (p)		
						Season	Cover	Openness
1	Ln (total HRB in FR)	Woody cover	35	15.8	0.026	NS (0.052)	0.042	Excluded
2	Ln (total HRB in FR)	Openness landscape	25	19.3	0.042	NS (0.066)	Excluded	0.040
3	Ln (total LRB in FR)	Woody cover	-	2.5	0.613	NS	NS	Excluded
4	Ln (total LRB in FR)	Openness landscape	-	2.5	0.701	NS	Excluded	NS
5	Ln (total HRB in SUR)	Openness landscape	39	1.3	0.005	0.013	0.016	Excluded
6	Ln (total HRB in SUR)	Woody cover	52	1.3	0.016	0.007	Excluded	0.010
7	Ln (total LRB in SUR)	Openness landscape	-	1.3	0.580	NS	NS	Excluded
8	Ln (total LRB in SUR)	Woody cover	-	1.3	0.924	NS	Excluded	NS

Ln = natural logarithm; HRB = high-risk birds; FR = free-range area; LRB = low-risk birds; SUR = surroundings; NS = not significant.

4.3.2 Low-risk birds in free-range areas

The number of birds of prey and corvids in free-range areas was not significantly related to woody cover within the range area (Table 4.3, model 3: p = 0.613; se = 2.5) nor to the openness of the surrounding landscape (model 4: p = 0.701; se = 2.48). Furthermore, no effect of season was found.

4.3.3 High-risk birds in surroundings of free-range areas

If either openness of the landscape or woody cover in the range area was used in the model, this resulted in models with significant factors (Table 4.3). Due to the incomplete factorial design it was not possible to run models with both factors together. Hence it was not possible to differentiate the effect of openness of the surrounding landscape from the effect of woody cover within the range.

Regression analysis with openness of the landscape as a fixed factor (Table 4.3, model 5) resulted in the following model (p = 0.005; R^2 = 39; se = 1.3):

Ln (total number of high-risk birds in surroundings) = 2.10 + (-0.37 * Season Spring) + (0.456 * Open Landscape)

In this model, the number of high-risk birds in the surroundings of free-range areas was significantly related to season ($p = 0.013$) and openness of the landscape ($p = 0.016$). More high-risk species were observed in the surroundings of free-range areas if these surroundings were open landscapes, and more high-risk birds were observed in autumn/winter than in spring.

Regression analysis with woody cover as a fixed factor (Table 4.3, model 6) resulted in the following model ($p = 0.003$; $R^2 = 52.1$; se = 1.34):

Ln (total number of high-risk birds in surroundings) = 2.55 + (-0.40 * Season Spring) + (-0.72 * Cover Category 2 / -0.24 Cover Category 3 / -0.27 * Cover Category 4)

In this model, the number of high-risk birds in the surroundings of free-range areas was significantly related to season ($p = 0.007$) and woody cover within the range area ($p = 0.01$). More high-risk species were observed in the surroundings of free-range areas with low woody cover, and more birds were observed in autumn/winter than in spring. The difference between Cover Category 1 and 2 was significant, as was the differences between Cover Category 2 and Cover Categories 3 and 4. The lowest numbers of high-risk birds were observed in the surroundings of the free-range areas, if these had a woody cover of 5–20%.

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4.3.4 Low-risk birds in surroundings of free-range areas

Neither openness of the landscape (Table 4.3, model 7: $p = 0.58$; se = 1.3) nor woody cover as fixed factor (model 8: $p = 0.924$; se = 1.34) were significantly related to the numbers of birds of prey and corvids observed in the surroundings of free-range areas. Furthermore, no effect of season was found.

4.4 Discussion

4.4.1 High-risk birds in free-range areas

The largest numbers of high-risk birds were observed in free-range areas with a minimum (< 5%) of woody cover. As soon as woody cover was more than 5%, numbers of high-risk birds were significantly lower. A likely explanation for this result is that geese and ducks (the main groups of high-risk birds) prefer areas with short grass and without bushes, where they can spot their predators in time (Loonen and Bos, 2003). Moreover, these species move around in large groups, for which they need space (Stahl, personal information; Sovon, Dutch center for field ornithology). However, interpretation of our results is hampered by the incomplete design of this pilot study: in our farm sample, the two farms with 0% woody cover were both located in an open landscape. Hence, it is not possible to say whether the larger number of high-risk birds on these farms was related to the absence of woody cover in their free-range areas or to the openness of the surrounding landscape (or both). In these cases, the larger number of water birds present in range areas with minimal woody cover may be partly due to the larger 'reservoir' of water birds in the open landscape surrounding the range areas in question.

4.4.2 Low-risk birds in free-range areas

No relation was found between the number of birds of prey or corvids in free-range areas and woody cover within these areas, nor between the number of these birds and the openness of the surrounding landscape. In general, the presence of birds of prey was low compared to other birds. If more observations had been done on a larger number of farms, a relation with woody cover or openness of the landscape might have emerged, but it is also possible that other factors played a stronger role in attracting (or keeping off) birds of prey. In another study (Bestman, unpublished results) a buzzard nest was found in a tree a few hundred meters from an organic poultry house, where the entire buzzard family was observed hunting in the free-range area. This observation indicates that a relation with woody vegetation (trees for nesting) within and outside the range area is conceivable. While the number of corvids was much larger than the number of birds of prey, no relation between corvid abundance and woody cover or openness of the landscape was found, either. This group of birds may have been attracted to carrion in the free-range areas or feed spills in the farmyard.

4.4.3 High-risk birds in the surroundings of free-range areas

More high-risk birds were observed in the surroundings of free-range areas if these surroundings were an open landscape, rather than a half-open landscape. However, interpretation of the results was hampered by the incomplete design of our pilot study: of the three farms in an open landscape, two had 0% woody cover and one had 90% woody cover in the range area. The largest numbers of high-risk birds were observed in the surroundings of the farms with 0% woody cover, but it is not possible to say whether this was due to the absence of woody cover in the free-range area or to the openness of the surrounding landscape (or both). In general, the presence of geese and ducks in open landscapes can be explained by the fact that these species prefer large open spaces, where they can spot their predators in time (Loonen and Bos 2003) and where they can move around in large groups (Stahl, personal communication).

4.4.4 Low-risk birds in the surroundings of free-range areas

No relation was found between the numbers of birds of prey and corvids in the surroundings of free-range areas and openness of the landscape or degree of woody cover within the free-range area. As argued above, the numbers of birds of prey were low and relationships between their abundance and woody vegetation might have emerged if more observations had been made on a larger number of farms. As for the corvids, it is possible that they feel at home in both open and half-open landscapes.

4.4.5 Limitations of the study

Ideally, this pilot study would have covered two sets of poultry farms with a varying degree of woody cover, one complete set located in open landscapes and one complete set in half-open landscapes. However, our choice of farms was limited, in particular of farms with substantial woody cover within the range area. While poultry farmers show a growing interest in planting trees in free-range areas, the number of poultry farms with substantial woody vegetation is still limited. This is due, most importantly, to the high costs of planting trees, and to the legal restrictions on cutting trees once they are grown. This limits their uses and makes agricultural land with trees less valuable. In addition, when interviewed about agroforestry, farmers tended to emphasize the general complexity of working with trees and difficulties with mechanization (Graves et al. 2009). Although we did find farms with differing degrees of woody cover, the vegetation varied from fruit trees and biomass willows to Miscanthus. Furthermore, our farm sample size and number of observations was limited by time; we had only one observer available and only one farm could be visited per day because of farm-hygienic reasons. An additional limitation was that, for practical reasons, we did our observations only during the daytime. However, some high-risk birds such as mallards are known to be nocturnal (Kleyheeg et al. 2015): during the day they are observed (resting) in other places than during the night (foraging). Hence, birds visiting poultry free-range areas at night are not covered by our study.

4.4.6 Implications of findings for further research

The results of this pilot study need to be confirmed with further research to investigate the effect of planting woody vegetation in poultry free-range areas as a measure to reduce the presence of AI high-risk birds. Such follow-up studies could be based on an experimental approach, where the number of high-risk and low-risk birds is followed in free-range areas that previously had no woody cover but where trees are planted as part of the experiment. Such “before-and after” experiments should be carried out in both open and half-open landscapes. Similarly, experiments could be set up by planting vegetation in the surroundings of free-range areas and observing the effects on the presence of the various bird risk categories within and outside the range areas. Additionally, further studies could compare bird presence in planted ranges versus open ranges within the same season, as season and year may also affect the number of birds present in the range areas. As soon as more is known about what animal species serve as intermediate AI hosts, these should be included in the study with appropriate (if necessary), nocturnal observations.

4.5 Conclusions

- In poultry free-range areas with a higher degree of woody cover, fewer AI high-risk birds were observed than in free-range areas without or with very limited (< 5%) woody cover. However, due to the incomplete design of this pilot study, the effect of woody cover within the range areas could not be differentiated from the effect of the surrounding landscape: most of the investigated free-range areas with higher degrees of woody cover were located in half-open landscapes, where the presence of woody vegetation was statistically related to lower abundances of high-risk birds within the free-range areas.
- In open landscapes, more high-risk birds were observed in the surroundings of free-range areas than in half-open landscapes. However, due to the incomplete design of this study, the effect of openness of the landscape could not be differentiated from the effect of woody cover within the range areas, which was statistically related to lower abundances of high-risk birds in the surroundings.
- No relation was found between the numbers of low-risk birds in free-range areas or their surroundings and the degree of woody cover or openness of the landscape.
- Our results need to be confirmed with further studies to investigate the relations between woody cover in and around poultry free-range areas and the presence of AI high-risk birds.

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Chapter 5

Predation in organic and free-range egg production

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Abstract

On organic and free-range poultry farms, a free-range is provided for animal welfare reasons. However, farmers report sightings of birds of prey and sometimes foxes or other predators within the free-range areas. In addition to seeing actual attacks, they also find chicken carcasses in the free-range, the deaths of which they attribute to predators. In addition, and in contrast to indoor poultry farmers, organic/free-range farmers report hundreds of chickens missing, per flock, when comparing the slaughterhouse arrival numbers with farm mortality records. The farmers assume these missing animals are hens that vanished from the free-range area and that predation is the major cause for their disappearance. If so, predation may impact farm yields. This study investigated whether birds of prey kill chickens on organic/free-range egg production farms and the impact, in terms of numbers of chickens and yield losses. This study was to provide qualitative and quantitative information in support of chicken mortality caused by birds of prey. Data were collected through field observations on organic/free-range farms (n = 11) and an online survey among organic/free-range farmers. Seventy-nine field observations on 11 farms resulted in 141 sightings of birds of prey, mostly common buzzards (*Buteo buteo*) and northern goshawks (*Accipiter gentilis*). Forty-four dead hens were found, 36 of them were very likely killed by either birds of prey or foxes. Sixteen attacks on hens by goshawk or buzzard were seen. There were no reasons to assume the attacked hens were in a poor condition prior to the attack. From responses to the online survey (n = 27 farms experiencing predation), it was estimated that on average 3.7% of hens of organic/free-range flocks were killed by predators, while total mortality was 12.2%. After calculating missed yield per killed hen, it was roughly estimated that per flock, predation caused yield losses of EUR 5,700 on an average organic farm (size 12,700 hens) and EUR 6700 on an average free-range farm (size 25,000 hens).

5.1 Introduction

In the Netherlands, on 1 January 2019 [1], 6.3 million free-range hens and 2.4 million organic hens were being kept, each of them having 4 m^2 of free-range area at their disposal, on 252 and 190 farms, respectively.

The poultry are kept in free-range areas for animal welfare reasons. However, predation may cast a shadow on the welfare of chickens and cause economic losses. Predator-related deaths were reported by 40% of Dutch flocks of organic laying hens [2]. A similar situation applies to the free-range poultry in other countries. Predation was estimated to be the cause of death for 0.5% (up to 12%) of laying hens and geese in the United Kingdom [3], 6.3% (up to 34%) of broilers in France [4], 6.7% of laying hens in Switzerland [5] cited in [6], up to 14.2% of laying hens in Denmark [7] and 9.5% (up to 23.5%) of laying hens in Germany [8] cited in [6].

Poultry farmers regard chicken mortality as an economic loss, especially because they believe the predators also, or even mainly, kill healthy, productive hens. Generally speaking, Dutch authorities provide compensation to farmers for the damage caused by protected wildlife (i.e., that cannot be hunted), such as birds of prey, but predation of free-range chickens is not officially recognized as wildlife damage. Prevention of predation is only possible to a limited extent. Predation by foxes can be prevented by fencing in the free-range area and by ensuring that all chickens spend the night inside a fox-proof hen house. In the Netherlands, under certain conditions, hunting licenses are issued to local hunters to kill foxes. Prevention of predation by birds of prey is much more difficult: these birds hunt in the daytime when the hens have access to the free-range. Although netting a range might appear a solution to protect the hens, covering ranges of 5 or 10 hectares (sizes are based on average Dutch organic and free-range farms, respectively [1]) is considered impractical, also because they sometimes contain trees, ditches or large grazing animals. Moreover, farmers applying for municipal environmental permits for such large 'roofs' would meet with legal difficulties, and face regulations concerning the aesthetic aspects of large structures within the countryside. Finally, farmers consider such large covers to be too expensive.

The main subject of this study concerned whether birds of prey cause damage on organic/free-range egg production farms and to what extent this damage can be estimated. This study was to provide qualitative and quantitative information in support of chicken mortality caused by birds of prey.

We formulated the following three research questions, in consultation with poultry farmers, people from the wildlife damage commission (BIJ12-Faunafonds) and a birds of prey expert [9]:

1. Which bird of prey species kill hens?
2. Are there any particularities perceptible concerning the condition of hens prior to the attack that may give an impression of their health status?
3. What is the impact of predation, in terms of numbers of hens being killed and the related estimated yield loss?

5.2 Materials and methods

We addressed these questions by conducting field observations in free-range areas, an online survey among poultry farmers and model calculations. Field observations and video recordings were made to provide qualitative data used to answer questions 1 and 2. The aim of the online survey was to provide quantitative data (e.g., estimations of the numbers of hens killed by predators) for the calculations used in answering question 3. Other sources of such quantitative data consist of key figures and prices published biennially by Wageningen Livestock Research [10].

5.2.1 Field observations and video recordings

Poultry farms with bird-of-prey-related mortality were approached to participate by 'Pluimveehouderij', a Dutch magazine for poultry farmers. This resulted in 11 farms, experiencing bird-of-prey-related mortality and that were keeping hens in the period the field observations were planned to take place, namely in July to November 2015.

An observation protocol was created based on a farm visit, together with representatives from the organic poultry farmers union, the wildlife damage commission (BIJ12-Faunafonds) and a bird of prey expert [9]. The observations were done on 11 farms in total. Per observation day, two farms were visited in succession. On the first farm, the free-range was inspected on foot, looking for dead, visibly ill or otherwise impaired hens. The check for the presence of visibly ill or otherwise impaired hens would help to say something about the condition of hens prior to an attack, in case during the following observation an attack would take place. The most commonly observed behaviour in free-range hens includes standing still, pecking, walking and foraging [11]. Hens were considered 'healthy' when, in addition to these alternating behaviours, no other peculiarities were seen in behaviour or appearance that would suggest the hens were somehow impaired or diseased. For all carcasses or their remains found, the cause of death was determined on the basis of three categories: fox, bird of prey, or other/unknown. A killing was attributed to a fox when the hen had been decapitated and/or if feathers were gnawed [4,12]. A bird of prey was deemed responsible if parts of the hen had been eaten and feathers were pulled out [4,12]. The third category, 'other/unknown', contained any other cause of death. The dead hens were photographed for documentation and evidence. After the inspection of the area on foot, 90-min observations were conducted from under a camouflage net, at a location (inside or outside the free-range) with a clear view either of as much as possible of the free-range area or of a specific spot in the free-range; for example, an area with regular evidence of predation, such as carcasses. On the second farm of the day, the free-range was not entered for biosecurity reasons, in order to prevent diseases from being transmitted. The 90-min observations on this second farm were conducted from outside the free-range; if possible, from a car. After a couple of days, the same farms were visited but in reverse order, in order to observe each free-range at different times of the day. From July to November, as many observations were done as possible; several times on two days close to one another. The final number of observations per farm would depend on the age of the hens and whether there was indeed predation. All observations took place when the pop holes were open; from 8:30 to 20:30, depending on the time of the year. During the 90-min observations, all sightings of birds of prey and their behaviour were noted down in a semi structured way: bird of prey species and behaviour, behaviour of the hen during an attack, and condition of the hen prior to the attack (dead, visibly ill/impaired, healthy), based on the criteria mentioned above. Where possible, photographs were made of birds of prey and their attacks.

On the free-range of one farm, attacks took place repeatedly at the same spot. A wireless surveillance camera (RDI Technology (Shenzhen, China), type CM812732) was installed to make continuous recordings over the course of 16 days. The recordings were in full colour during the day and in black and white between sunset and sunrise. Attacks recorded by this camera were described in the same semi-structured way as described above for the live observations.

5.2.2 Online survey

The questions were formulated in consultation with representatives of the organic poultry farmers union, three bird of prey experts, a representative from the Dutch Ministry of Economic Affairs, and a communications expert. The questions included ones about production systems (organic/free-range), number of hens, percentage of hens observed on the range under optimum conditions, whether farmers detected mortality caused by predators; and some figures from their last culled flock—number of hens at start, number of hens who died from disease, those found dead on the range who were not killed by predators, those found dead on the range who were killed by predators, the number of hens missing after the count at the slaughterhouse, and the suspected reasons for their absence. Poultry farmers were approached by agricultural magazines 'Pluimveehouderij' and 'Boerderij' to fill in the online survey about predation, and, in an email, the approximately 50 members of the organic poultry farmers union were asked to do the same. Also, farmers without predation were invited, and we mentioned that we were curious as to why they had no predation. The survey was set up by MWM2 (https://www.mwm2.nl/) and remained available online for 50 days. MWM2 subsequently presented the answers in MS Excel format. The main criterion for including participants' responses in our analyses was that they answered all quantitative questions about their last culled flock.

5.2.3 Calculation of yield losses

To calculate the yield losses due to predation, yields and costs were compared between hens slaughtered at the end of the laying period and those killed by predators exactly halfway through the laying period. Because there was no information about when during the laying period (beginning, middle, end) predation occurred, we assumed the deaths were evenly distributed over the laying period, meaning the same numbers would be killed before the middle of the laying period as after it. Therefore, we calculated 'hens killed by predators' to all have been killed halfway through the laying period. Since costs related to young hens and feed differed between organic and free-range farms, the yield losses were calculated for both production systems. Key figures and prices were obtained from the manual 'Quantitative information animal production 2018–2019' [10], average farm sizes for organic and free-range farms were used [1], and the percentage of hens assumed to be killed by predators was derived from our own online survey. The calculations were done for brown hens, since these were the only genotypes for which key figures and prices were available. This was the most kept genotype on organic/free-range farms.

5.3 Results

5.3.1 Characteristics of the farms included in the study

From July to November 2015, 79 field observations were conducted on 11 farms. Table 5.1 shows some of the farm characteristics.

Table 5.2 shows date and times of the observations.

5.3.2 Observed Birds of Prey, Killed Hens, and Attacks

During these 79 observations, there were 141 sightings of birds of prey. Buzzards were regularly seen in groups, the maximum was a family of 5 members on farm 4, but goshawks were only observed to be solitary. Table 5.3 summarises the numbers of sightings per bird of prey species per farm.

During the 79 farm visits, a total of 41 inspections of free-range areas were carried out on foot, resulting in the discovery of 44 dead hens (Table 5.4).

Table 5.1: Farms where field observations took place

Farm	Number of hens on the farm (rounded)	Genotype	Age of hens at first and last observation (in weeks)	Percentage (%) of hens using free-range area under favourable conditions [1]	Roosters	Number of 90-minute observations	Size of free-range area in hectares	Tree cover as % of free-range surface [2]	Vegetation and shelters in free-range area	Openness of surrounding landscape
1	17,000	Brown	66–73	45	No	5	6.9	< 5	Grass, willow trees, maize	Half open
2	6,000	Brown	57–64	90	No	4	2.4	5	Grass, adult oaks	Half open
3	12,000	Brown	52–66	25	No	10	4.8	< 5	Grass, small shelters	Half open
4	19,000	Brown	45–60	50	No	10	7.5	< 5	Grass, young fruit trees, small shelters	Half open
5	9,000	Brown	35–49	90	Yes	9	3.4	75	Trees, grass	Open
6	15,000	Brown	55–70	45	No	8	6.0	75	Trees, shrubs, grass	Open
7	6,000	Silver [3]	66	80	No	2	2.4	< 5	Grass, young trees	Half open
8	12,000	Brown	37–51	65	Yes	10	4.9	90	Young fruit trees, grass	Half open
9	13,660	Brown	26–38	75	No	9	5.4	< 5	Grass, young trees, shrubs	Half open
10	11,760	Brown	32–37	50	No	6	4.7	< 5	Grass, young trees	Open
11	9,000	Brown	68–73	33	No	6	3.6	5	Grass, young trees, small shelters	Open

[1] This was an estimate by the farmer, who was asked what percentage of this flock he generally sees outside under favourable conditions: before sunset with cloudy and calm weather.
[2] Estimation based on Google Maps satellite images and photographs made in the free-range areas.
[3] Silver hens are a reverse-cross white layer breed; they have a weight comparable to brown hens, have mostly white and a few brown feathers and lay brown eggs [13].

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Table 5.2: Date and start times * (M(orning); A(fternoon); E(vening)) of 90-minutes observation periods (n = 79)

Farm	July	August	September	October	November
1	20M, 21A, 30M		7E, 9M		
2	22M, 23M		7A, 8A		
3	28A, 29M		16M, 17M	6A, 7M, 21A, 22A, 28M	3A
4	20A, 21M	5M	8M, 9M, 28M, 29A	23A [7], 26A	5A [9]
5	24A, 27M		11A [3], 15A	1E, 5A, 19A, 29A, 30A	
6	24M, 27A		11A, 15M	1A, 2M, 29A	2M
7	22A, 23M				
8	28A, 29A		10E [2], 16A	6A [5], 8A, 15A, 22A, 27A	3A
9		6M	10A [1], 17A	7A, 8A [6], 15A, 21A, 27A, 28A	
10			28A, 29A [4]	23A, 26A [8]	4A, 5A [10]
11				2A, 5A, 16A, 20A, 30M	2A

* Morning < 12:00 h; Afternoon 12:00–18:00 h; Evening > 18:00 h. [1–10] Attacks observed; numbers correspond with attack numbers in Table 5.5.

Table 5.3: Sightings of birds of prey during field observations

Farm	Number of 90-min observations	Common buzzard *Buteo buteo*	Northern goshawk *Accipiter gentilis*	Common kestrel *Falco tinnunculus*	Eurasian hobby *Falco subbuteo*	White-tailed eagle *Haliaeetus albicilla*	Total number of birds of prey
1	5	8	1	0	0	0	9
2	4	2	0	0	0	0	2
3	10	6	0	4	2	0	12
4	10	23	1	6	3	0	33
5	9	9	1	0	1	0	11
6	8	11	0	1	0	1	13
7	2	2	0	0	0	0	2
8	10	19	0	4	0	0	23
9	9	10	2	0	0	0	12
10	6	11	0	2	0	0	13
11	6	8	0	3	0	0	11
Total	79	109	5	20	6	1	141

During the 79 observations, a total of 10 attacks on 12 hens by birds of prey were observed, resulting in 3 hens being killed by birds of prey and 1 severely injured hen was killed by the farmer in order to prevent further suffering (Table 5.5).

After the manager of farm 9 reported that he repeatedly found carcasses of hens killed by birds of prey in the same spot, a video camera was installed that made continuous recordings. Another 6 attacks were filmed with this camera (Table 5.6 and Figure 5.1).

Table 5.4: Sightings of birds of prey during field observations

Farm	Number of inspections	Suspected predation		Other/unknown	Total
		Bird of prey	Fox		
1	3	3	0	4	7
2	2	1	0	0	1
3	5	3	0	0	3
4	5	8	2	2	12
5	5	5	1	0	6
6	4	2	0	1	3
7	1	0	0	1	1
8	5	0	1	0	1
9	5	4	0	0	4
10	3	4	0	0	4
11	3	2	0	0	2
Total	41	32	4	8	44

5.3.3 Features and behaviour of attacked hens and bystander hens

None of the hens attacked in the 16 described attacks were visibly ill, impaired, weakened or already dead. In other words: birds of prey attacked hens that were healthy (attacks 1–16). Sometimes, the initial response of hens was to drop down (attacks 4–8, 10), but most hens tried to escape or fought back (attacks 4–7, 10). Bystander hens ran away (attack 8) but were also seen trying to chase away the bird of prey (attacks 14, 15). In several instances, while the bird of prey was eating from its prey, other hens came closer and closer (attacks 11–14), sometimes to less than one metre from the scene. When a bird of prey, for example, was sitting on a pole (which was part of the fencing), hens walked underneath it and did not seem to be scared (Figure 5.2). Two farms (5 and 8) kept roosters and hens at a ratio of 1:30. Roosters were seen to attack and chase away birds of prey (attacks 2, 3), but they were not always in the right spot at the right moment (attack 5). Generally, bystander hens started cannibalising the killed hen as soon as the bird of prey left (attacks 1, 8, 11–14). Sometimes hens were eating from the carcass at the same time as the bird of prey (attacks 13, 14).

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5.3.4 Scavengers eating the remains of killed hens

Video recordings on farm 9 revealed that a killed hen was eaten within 2 to 3 days until a clean skeleton remained. Scavengers seen were the common buzzard (*Buteo buteo*) and hens. Some of the video recordings made in the free-range area of farm 9 showed carrion crow (*Corvus corone*), Eurasian magpie (*Pica pica*), red fox (*Vulpes vulpes*) and a domestic cat (*Felis silvestris catus*) to be present. In most cases, the clean skeletons were laying on the ground, but on farm 6 they were twice seen hanging from an electric fence (Figure 5.3). The common buzzard was mentioned in a comparable case of the remains of prey hanging on barbed wire [12].

Table 5.5: Attacks on hens by birds of prey (BOP) seen during field observations, numbered in chronological order

Attack	Date & time [1]	Farm	Flock age (weeks)	Bird of prey species	Condition [2] of hen prior to attack	Predator killed hen	Observations
1	10 September 16:01–17:31	9	31	*Accipiter gentilis*	Healthy	Yes	Hen was attacked next to fence. Immediately after the BOP left, other hens ran to the killed hen and cannibalized it.
2	10 September 19:09–20:29	8	43	*Buteo buteo*	Healthy	No	Hen was attacked in open field, next to the fence. Immediately at the start of the attack, roosters ran to BOP and chased it away. Hen survived.
3	11 September 16:22–17:57	5	42	*Accipiter gentilis*	Healthy	No	Hens were attacked under the trees. BOP attacked 3 hens, and was chased away by roosters. Hens survived. While flying away, the BOP was also chased by *Buteo buteo*.
4	29 September 17:00–18:30	10	32	*Buteo buteo*	Healthy	Yes	BOP1 attacked hen in open field, next to fence. Hen first dropped down, but then resisted. BOP2 flew over BOP1 during attack. BOPs ate together from the hen. BOP3 flew over and disappeared.
5	6 October 17:52–19:22	8	47	*Buteo buteo*	Healthy	No	BOP attacked solitary hen in open field, next to fence, while other hens and roosters had gone inside. Hen first dropped down, but then resisted. BOP scared off by the observer and fled. Hen was euthanised by the farmer because of a severe breast wound.

6	8 October 12:55–14:52	9	35	*Accipiter gentilis*	Healthy	No	BOP attacked hen in open field, next to fence. Hen first dropped down, but then resisted, fled and got attacked again. Fled again and BOP flew into tree. Hen ran away.
7	23 October 15:30–17:00	4	58	*Accipiter gentilis*	Healthy	No	BOP attacked hen under a tree, next to fence. Hen first dropped down, but then resisted and fled, right into an electric fence. BOP flew away, flew back over hen and disappeared.
8	26 October 16:00–7:27	10	36	*Buteo buteo*	Healthy	Yes	BOP attacked hen in open field, next to fence. Hen dropped down, screamed and did not resist. Other hens ran towards hen house. BOP left hen after 15 min eating breast. Hens ran to killed hen and cannibalised it.
9	5 November 13:27–15:00	4	60	*Buteo buteo*	Healthy	No	BOP attacked hen in open field, next to artificial shelter. Hen ran under shelter. BOP disappeared.
10	5 November 15:27–17:27	10	37	*Buteo buteo*	Healthy	No	BOP attacked hen in open field. Hen first dropped down, but then resisted and after 5 min BOP disappeared. Hen ran towards hen house.

[1] Start and end times of observations.

[2] A hen was considered 'healthy' if she was displaying normal free-range behaviour (alternating standing, pecking, walking and foraging [11]) and no other behaviour or particularities were seen that would suggest the hen to be somehow impaired, diseased or weakened.

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Table 5.6: Attacks on hens by birds of prey (BOP) filmed with camera, numbered in chronological order

Attack	Date & time	Farm	Flock age (weeks)	Bird of prey killing the hen	Condition [1] of hen prior to attack	Observations
11	14 November 12:16–13:17	9	40	*Accipiter gentilis*	Healthy	BOP killed resisting hen in open field, close to fence. While feeding on hen, other hens were getting closer, < 1 m. After 1 h, BOP left. Other hens immediately started cannibalising the killed hen.
12	21 November 12:23–13:44	9	41	*Accipiter gentilis*	Healthy	BOP killed resisting hen in open field, close to fence. After feeding on the hen for 10 min, BOP was chased away by a *Buteo buteo*. Within 1 h, 3 *Buteo buteo* were seen with the hen. More and more hens came closer to *Buteo buteo* feeding on the hen. After BOP left, other hens started cannibalising the killed hen.
13	22 November 14:37–15:28	9	41	*Accipiter gentilis*	Healthy	BOP killed resisting hen in open field, close to fence. After feeding on hen for 20 min, BOP was chased away by a *Buteo buteo*. In total, 2 *Buteo buteo* were seen with the hen. While *Buteo buteo* were feeding, other hens and magpie (*Pica pica*) also fed on the hen. BOPs left 1 h after the attack and other hens moved in and cannibalised the killed hen.
14	23 November 13:11–14:28	9	42	*Accipiter gentilis*	Healthy	BOP killed resisting hen in open field, close to fence. After feeding on the hen for 20 min, BOP was chased away by a *Buteo buteo*. While BOP was feeding, 2 hens unsuccessfully tried to chase it away, then stayed and ate blowing down feathers from the scene. More hens approached. After 1 h of feeding, BOP left and hens started cannibalising the dead hen.
15	28 November 12:53–13:23	9	42	*Accipiter gentilis*	Healthy	BOP killed resisting hen in open field, close to fence. While it was feeding from the hen, another hen tried to chase it away BOP without success. After 40 min, BOP was chased away by a *Buteo buteo*.
16	29 November 13:05–13:53	9	42	*Accipiter gentilis*	Healthy	BOP killed resisting hen in open field, close to fence. After feeding on the hen for approx. 1 h, BOP left on its own initiative.

[1] A hen was considered 'healthy' if she was displaying normal free-range behaviour (alternating standing, pecking, walking and foraging [11]) and no other behaviour or particularities were seen that would suggest the hen to be somehow impaired, diseased or weakened.

Figure 5.1: Northern goshawk (*Accipiter gentiles*) during attack 11.

Figure 5.2: Hens on farm 8 performed normal foraging behaviour ('walking with pecking and scratching' [11]) while being watched by 2 common buzzards (*Buteo buteo*).

Figure 5.3: Chicken skeleton left on electric fence (farm 6).

5.3.5 Vegetation on the free-range areas and artificial shelters

The free-range areas varied from being sparsely to largely covered with trees. Farms 3, 4 and 11 had a few small shelters on their free-range areas. Attacks took place in an open field close to a fence (attacks 1, 2, 4–6, 8, 10–16), under trees (attacks 3, 7) or close to a shelter (attack 9). In one case, an attack was observed to stop after the hen had run under a shelter (attack 9). In some of the attacks, the hen was able to escape alive (attacks 2, 3, 6, 7, 9, 10), but, because of our sample size, we cannot say whether this was related to the presence of trees or an artificial shelter.

5.3.6 Online survey about mortality caused by predation

Although a total of 61 farmers partly filled out the online survey, only 27 completed the quantitative questions about their last culled flock. Table 5.7 shows the contribution of several causes of death and disappearance to the mortality of hens during the laying period. The number of hens killed by predators were assumed to be the sum of the hens found dead in the free-range areas that were recognisably killed by a predator, plus the number of hens that seemed to be missing when comparing the number of hens that arrived at the slaughterhouse with the farm's mortality records. It was calculated that, on average, 3.7% of the hens in organic/free-range flocks were killed by predators. The average mortality in organic/free-range flocks was 12.2%; 8.1% died because of disease and 0.3% died from other causes.

Flock size was positively correlated with the number of hens killed by predators ($n = 27$; $R = 0.42$; $p = 0.031$). However, flock size was not correlated with percentage (%) of hens killed by predators ($n = 27$; $R = -0.24$; $p = 0.220$).

Table 5.7: Causes of death of chickens in the last culled flock on organic/free-range farms

Initial number of hens and causes of death and disappearance	Mean number of hens (minimum–maximum)	Percentage (%) of hens, relative to initial number (minimum–maximum)
Initial number of hens	17,868 (200–46,000)	100
Killed by disease	1543 (3–10,371)	8.1 (1.5–41.9 [2])
Found dead on free-range, death caused by predator	172 (0–1,400)	1.2 (0.0–5.4)
Found dead on free-range, cause of death other than by predator	29 (0–300)	0.3 (0.0–6.0)
Birds missing after comparing arrivals at slaughterhouse with farm records	406 (0–1,817)	2.5 (0.0–10.0)
Mortality caused by predation [1]	579 (5–2,600)	3.7 (0.2–12.0)
Total mortality	2150 (9–12,588)	12.2 (3.3–50.8)

[1] Mortality caused by predation is the total number of animals found dead in the free-range area after having been killed by a predator, plus those that are missing, after comparing the numbers arriving at the slaughterhouse with farm mortality records.
[2] In one flock, mortality was extremely high due to an infection with *Pasteurella multocida*.

5.3.7 Yield losses due to predation

Table 5.8 shows the costs and yields per organic and per free-range hen, comparing results for hens who had completed the laying period (aged 78 weeks for organic and 82 for free-range hens) with those killed halfway the laying period (49 and 51 weeks, respectively). The laying period starts at 20 weeks of age.

Table 5.8: Financial result (margin) per hen, under scenarios with and without predation, both for an organic (ORG) and a free-range (FR) production system

Key figure	ORG hen scenario NO predation	ORG hen scenario WITH predation [1]	FR hen scenario NO predation	FR hen scenario WITH predation [1]
Length of laying period (days)	406	203	434	217
Eggs/housed hen	338	169	360	180
Price/egg (€)	0.135	0.135	0.075	0.075
Feed intake (grams/hen/day)	126	126	121	121
Feed intake (kg/hen)	48.3	24.15	49.8	24.9
Feed conversion	2.33	2.33	2.25	2.25
Price/kg feed (€)	0.46	0.46	0.265	0.265
Yields (€)				
Eggs	45.63	22.82	27.00	13.50
Carcass after slaughter	0.40	0.00	0.36	0.00
Total yield	46.03	22.82	27.36	13.50
Costs (€)				
Purchase young hen	7.50	7.50	4.44	4.44
Feed	22.22	11.11	13.20	6.60
Other production costs [2]	1.56	1.56	1.56	1.56
Interest costs [3]	0.23	0.23	0.14	0.14
Total costs	31.51	20.40	19.34	12.74
Margin (€)	14.52	2.42	8.03	0.76
Yield reduction (€)	-	12.11	-	7.26

[1] The predation was assumed to have taken place distributed evenly over the laying period. Thus, the same numbers of hens were assumed to be killed before and after the middle of the laying period, which meant that calculations could be done with all predation taking place 'halfway' through the laying period.
[2] Other production costs include those of electricity, water, health care and hygiene, litter, monitoring, catching, and cadaver pick-up.
[3] Interest costs were based on long-term investments related to egg production (housing, land).

5

On 1 January 2019, 190 organic and 252 free-range farms were registered in the Netherlands [1], with 2,411,548 and 6,293,531 hens, respectively. The mean farm size was thus 12,692 for organic farms and 24,974 for free-range farms. Assuming an average 3.7% of hens killed by predators (Table 5.7), this results in yield losses of (0.037 × 12,692 × 12.11 =) EUR 5,687 for an average organic farm, and (0.037 × 24,974 × 7.26 =) EUR 6,709 for an average free-range farm.

5.4 Discussion

5.4.1 What are the bird of prey species that kill hens?

During the field observations, both common buzzards (*Buteo buteo*, hereafter referred to as 'buzzards') and northern goshawk (*Accipiter gentilis*, hereafter referred to as 'goshawks') were seen to attack and kill hens. Buzzards are known to catch small mammals, sometimes an adult rabbit, amphibians and young birds, and they also eat carrion [14]. Buzzards mostly hunt from a position high above the ground [14]. An adult hen of around 2 kg is substantial heavier than most of the prey normally caught by buzzards. However, chickens may represent easy prey, since they seem unafraid of a buzzard sitting on a fence pole or in a tree, which are some of its regular hunting positions. Goshawks are known to catch small- to medium-sized birds, but sometime also larger ones, up to the size of small geese [15]. Goshawks hunt from the air [15]. They generally do not eat carrion, although there is some evidence to the contrary [16]. An adult laying hen corresponds well to the average prey size of goshawks. In our observations, a laying hen was attacked and killed by a goshawk, but subsequently eaten by one or more buzzards, after they chased away the goshawk. Stealing or scrounging other animals' food or prey (i.e., kleptoparasitism) is described for several animal species, including buzzards [17]. Buzzards are described as both the robber and the robbed, while goshawk is described only as the one being robbed.

We did our field observations in July to November. We cannot exclude that at other times of the year (breeding season, wintering birds from Nordic countries, variation in abundancy of alternative prey animals, possibly fewer chickens outside during rainy and windy season) birds of prey and mammalian predators might behave differently. For the answers to qualitative research questions 1 and 2, we do not expect a difference, since buzzard and goshawk, the species that attacked hens, are species that are present here year-round, with additional Nordic buzzards in winter. There is anecdotical information from farmers that foxes kill more chickens during the breeding season compared to the rest of the year. Thus, depending on the season, the number of chickens killed by avian or mammalian predators may vary.

We did not see any attacks on hens in the morning. An explanation for this may be the fact that the pop-holes on many farms opened at 10 or 11 a.m. This made the morning observation period considerably shorter compared to the afternoon and evening observation periods.

5.4.2 Condition of hens prior to attack

If predated hens were healthy and, therefore, would likely still have been producing sellable eggs, their predation would result in a yield loss. It was not possible to check the health or productive state of hens prior to being attacked by birds of prey. However, the

16 documented attacks showed no irregularities that would indicate health problems in the hens involved. Moreover, in most of the attacks, the hens were observed to struggle in order to escape from the bird of prey, which probably would have been less the case in diseased or weakened hens. We have no reason to assume that predated hens were in poorer health or lesser productive state prior to the attack, compared to non-predated hens.

5.4.3 Impact of predation

The impact of predation is expressed in terms of numbers of hens per flock being killed and in terms of yield losses (Euros). Since our own field data were from a few months in summer and autumn and not collected continuously ('24/7'), they were not representative for year-round predation. Therefore, to determine the number of hens being killed per flock, the results of the online survey were used. Those results were based on culled flocks. Since the productive life of a flock of laying hens generally lasts for a minimum of one year [10], the mortality figures from the online survey covered all seasons. When necessary to interpret qualitative aspects of those mortality figures, however, we used our findings from the field observations.

Numbers of hens killed

We used farm records, collected by means of an online survey, to estimate the numbers of hens being killed by predators. It was clear to the responders that the survey was about predation. In addition, although farmers without a predation problem were explicitly invited as well, it was possible that farmers who had experienced predation-related mortality, were overrepresented in the responses. All farmers that responded had experienced predation, either because they found hens killed by predators, or hens seemed to be missing after comparing the counts at the slaughterhouse with the farm mortality records. Based on our results, we cannot say what proportion of the total population of organic/free-range poultry farmers experience predation-related mortality. In a survey in the UK [3], 81% of the responding poultry and geese farmers had experienced predation. The farmers responding to that survey knew the survey was about fox predation, so an over-representation of farmers experiencing predation could not be excluded. A Dutch study on health and welfare [2] reported 40% of organic egg producers to have experienced predation-related mortality. Since this study was about health and welfare and the questions about predation constituted only a minor part of it, this Dutch study may better reflect the actual proportion of farmers experiencing predation. A French study [4], however, reported that 70% of the respondents reported predation, while when visiting the farms that did not report predation, field evidence nevertheless indicated predation on some of those farms. It remains difficult to conclude what proportion of farms experience predation.

Various comments can be made about the results of our own field observations, which are also the case for the farmers response 'death caused by predator' in the online survey. Concerning cadavers found in the free-range, even when showing signs of predation, it cannot always completely be excluded that the hen had died from another cause and was subsequently fed on (i.e., secondary predation). Also, there are reasons to assume that the real number of hens killed by predators is higher than the number of those observed to be killed or found dead. Observers, but also farmers, are not able to oversee the whole free-range continuously, so attacks will very likely be missed. Carcasses of killed hens seemed to disappear fast, which also makes it likely that not all of them will be detected when inspecting the free-range.

It is generally assumed that the majority of hens that seem to be missing after comparing the number of hens counted that arrived at the slaughterhouse with those in the farm mortality records are killed by predators. However, how reliable is this? First, how reliable is the number of young hens that arrived on the laying farm? This was verified by asking a representative from a rearing company that delivers young hens to organic egg production farms. He explained [18] that, from the moment of putting fertilised eggs into the hatching machine to the moment of delivering the young hens to the egg production farm, the eggs/hens will be counted several times; some of the counts are performed automatically, and they are believed to be very precise. This results in deviations in the number of delivered hens that are 'closer to 10 than to 50'.

Second, how certain is it that farmers will find all of the hens that died on their farms? This was verified by asking a farmer who was producing both barn eggs and free-range eggs with a total of 50,000 hens. He explained [19] that, from his free-range flocks, he generally lost more than a thousand hens per flock and 'none' from his barn flock. Another free-range farmer, who was keeping 38,000 hens, said that, inside the hen house, he 'rarely overlooked a dead hen' [20]. The experiences of these 2 farmers suggest that if hens were missing, they had not disappeared from the hen house, nor were their carcasses overlooked inside the hen house.

Third, how precise is the count of the number of arrivals at the slaughterhouse? This was verified by asking the manager of a slaughterhouse processing the majority of Dutch organic hens. He explained that their automatic counting system was precise, resulting in a 'closed count' [21].

Fourth, assuming the missing hens disappeared from the free-range area, how likely is this to be due to predation? Other causes of disappearances from or death in the free-range areas could be disease, hens being locked out of the hen house because of pop holes closing before all hens were inside, smothering, and drowning. Concerning disease, hens who are 'close to death' or in pain would not be expected to be physically able or willing to leave the hen house to go outside, as was found in hens with keel fractures

[22]. The risk of hens becoming locked out is generally avoided by installing automatic timers, and, if it were to happen, those dead hens would probably have been found by the farmer. Hens who died because of smothering or drowning would probably have been found by the farmer, too. Hens found dead by the farmer would be included in the farm mortality records and thus not end up as 'missing' after the slaughterhouse count. These considerations still suggest predation as the main cause of death in the free-range areas.

Fifth, assuming the hens had disappeared because of predation, how many of them were killed by birds of prey? Our observations of hens found dead during the inspections of the free-range areas suggested that the majority were killed by birds of prey (73%), rather than foxes (9%). A higher proportion killed by birds of prey was also found for French broiler production: 52% were killed by birds of prey and 28% were killed by 'mammals' [4]. A German experimental farm only described birds of prey causing predation [8] cited in [6]. Two farmers joining our study who had kept detailed records of causes of death mentioned 15% (farm 9) and 25% (farm 8) of kills having been caused by foxes and 85% or 75% by birds of prey. In contrast, in English egg production, nearly all killings were by foxes [3]. In summary, in the flocks included in our study, the most likely predators seemed to be birds of prey. This may be related to our message while recruiting farms; we were specifically looking for farms with mortality caused by birds of prey.

One of the results from our online survey was that farmers reported that 1.2% of the hens in their free-range flocks were found dead within the range area; according to the farmer, they were killed by a predator. Another 2.5% of the hens in free-range flocks seemed to be missing according to a count on their arrival at the slaughterhouse—as stated above, probably killed by a predator, in most cases a bird of prey. Taking into account the above considerations, and assuming there were no other substantial causes of death in the free-range areas, it was estimated that, on average, 3.7% (0.2 to 12.0) of the hens in free-range flocks that were included in our online survey had died because of predation, and most of them were considered to be killed by birds of prey. These numbers correspond to what other researchers found: 0.5% (up to 12%) of laying hens and geese in the United Kingdom [3], 6.3% (up to 34%) of broilers in France [4], 6.7% of laying hens in Switzerland [5] cited in [6], up to 14.2% of laying hens in Denmark [7] and 9.5 (up to 23.5%) of laying hens in Germany [8] cited in [6].

Yield losses

Yield losses were calculated as the difference in yield between a hen living a productive life until the day of slaughter (78 and 82 weeks for organic and free-range hens, respectively) and one living only half of its productive life. The yield losses were calculated to be EUR 12.11 per killed organic and EUR 7.26 per killed free-range hen. Furthermore, we calculated the yield losses to be roughly EUR 5,700 for an average organic farm and EUR 6,700 for an average free-range farm. We used average key figures, standardised prices for young hens, eggs, feed and other costs. However, the average mortality caused by predators may vary per farm; we found a mean of 3.7% of predation in a 'population' of 27 farms, which was likely overrepresented by farmers with predation-related mortality. The percentage of hens killed by predators in the complete population of organic/free-range farms would possibly include farms without or with less predation, as well. Calculating a mean for the complete population would then result in < 3.7% of predation-related mortality. On top of that, egg price, feed costs and other costs vary from farm to farm as well.

Starting points for preventive measures

If, on average, 4% or up to 12% of the hens of a flock is being killed by predators, in our study mostly birds of prey, taking measures becomes an obvious next step. As mentioned in the introduction section, prevention of predation by birds of prey seems possible to only a limited extent. Roosters, which we observed to chase away birds of prey, were not always in the right place at the right time and could not prevent the killing of hens. Trees that could function as shelter were also used by birds of prey as a starting point for attacks. Fence poles were also used as a starting point, but they are a necessary part of the fence. In addition to attacks from starting points, attacks were also seen from open air; therefore, removing 'physical starting points' is not expected to be able to prevent attacks. One attack was aborted after the attacked hen ran under a shelter. To what extent such structures can be used as preventive measures is doubtful; just like the roosters, they would not always be in the right place at the right time. Although we recommend further research into preventive measures, our results do not suggest starting points for doing so for the size of farms (up to 19,000 in the 'field study group' and up to 46,000 in the 'survey group') in our study.

5.5 Conclusions

Both northern goshawks (*Accipiter gentilis*) and common buzzards (*Buteo buteo*) killed laying hens. Common buzzards were also observed to scavenge, after having chased away the northern goshawk, who had killed the particular hen. Hens that were attacked, did not show symptoms of disease or weakness prior to the attack. Moreover, in most cases, they tried to escape from their attacker. There were no reasons to assume that predated hens were in poorer health than non-predated hens. Predation was estimated to have been the cause of, on average, one third of the mortality in the organic/free-range flocks that were included in our survey; total mortality was reported to be 12.2%, of which 3.7% was estimated to be due to predators. Combining these findings with average key figures gives a rough estimate of yield losses of EUR 5,700 on an organic farm (size: 12,700 hens) and EUR 6,700 on a free-range farm (size: 25,000 hens) experiencing predation-related mortality.

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Chapter 6

Free-range use and intestinal parasites in organic laying hens

Adapted from: Bestman M, Niekerk T van, Göransson L, Ferrante V, Gunnarsson S, Grilli G, Arndt SS, Rodenburg TB. (submitted). Free-range use and parasite infections in organic laying hens.

Abstract

Intestinal parasites are commonly found in non-cage laying hens. Parasites may reduce welfare and performance. Anthelmintics are not always effective and may lead to residues in eggs and in the environment. Aim of this study was to evaluate the relationship between free-range use and infections with intestinal parasites in organic laying hens, in order to identify directions for preventive measures. The study included 40 farms in three countries. Per farm 6 pooled soil and 14 pooled faecal samples were analysed using the McMaster method. Of the faecal samples, 71% (median) contained ascarid eggs, with a median of 143 eggs/gram (EPG). *Capillaria* eggs were found in 7% (median) of the faecal samples (median EPG = 5). Of the soil samples, 0% (median) contained ascarids eggs. *Capillaria* eggs were only detected in Italian soil samples. No relationship was found between parasite eggs in faeces and range use or flock performance. The low number of ascarid eggs in free-range soil suggest to focus further investigations on the conditions inside the hen house rather than in the free range.

6.1 Introduction

Intestinal worm infections in poultry are found in all housing systems, especially in systems where the hens come into contact with their faeces. In non-cage systems, a large amount of litter is available to the hens in order to meet their behavioural needs for foraging and dustbathing. In organic/free-range egg production, a free-range area is mandatory to provide hens with even more possibilities for dustbathing, foraging and sunbathing. A study by Jansson et al. (2010) on 169 Swedish flocks in 2008, before anthelmintics became available there, found 4.3% infections in caged flocks, compared with 29% and 52% in flocks in single-tiered and multi-tiered indoor systems, respectively, and 77% in free-range/organic systems. In a study including 55 organic flocks in eight European countries, Thapa et al. (2015) found *A. galli* in 70% of the flocks, Heterakis spp. in 29% and Raillietinae spp. in 14%, which is in agreement with findings by Jansson et al. (2010). In German and Italian studies, it has been found that organic layers have a high worm burden, with up to 100% in 18 and four flocks investigated, respectively (Kaufmann et al., 2011; Wuthijaree et al., 2017).

The effect of the mandatory free-range area on helminth (parasitic worm) infections in laying hens is not always clear. Swedish studies on 169 flocks (Jansson et al., 2010) and six flocks (Hoglund and Jansson, 2011) found no significant differences in ascarid infections between barn and free-range systems. However, a Danish study of 16 farms found that free-range/organic hens had a higher prevalence of *A. galli* and *H. gallinarum* infections than hens in indoor systems (Permin et al., 1999). An Austrian study on 79 flocks found a higher *A. galli* and *H. gallinarum* infection rate in organic/free-range hens than in indoor hens (Grafl et al., 2017). On the other hand, a study on 50 flocks in eight European countries found that flocks which were able to spend more time ranging had lower levels of *A. galli* (Thapa et al., 2015). A British survey of 19 flocks found lower faecal egg counts for *A. galli* and *H. gallinarum* in flocks with a higher proportion of hens using a free-range area and lower faecal egg counts for *A. galli* when more outdoor space was available per hen (Sherwin et al., 2013). All studies cited above were based on measures at flock level but in recent studies hens have been tracked individually, making it possible to link individual ranging patterns to health and welfare aspects. An Australian study that classified 307 of experimental hens (all with access to a free-range area) into 'indoor hens', 'low outdoor hens' or 'high outdoor hens', depending on the frequency and duration of their individual use of the free-range area, found no differences in the number of *A. galli* nematodes in hens from the three different groups (Bari et al., 2020). Another Australian study found that 'rangers' were more often infected with *A. galli* and cestodes than 'stayers', i.e. hens that rarely or never went outside (Sibanda et al., 2020).

Suggested reasons for higher levels of parasite infection in free-range/organic hens include contact with faeces of wild birds, earthworms as intermediate hosts (for *H. gal-*

linarum and some *Capillaria* species) or residual contamination from previous flocks (Permin et al., 1999). It has been found that embryonated ascarid eggs can survive and remain viable for at least two years in Danish pasture soil (Thapa et al., 2017). Transmission of parasites from wildlife to domestic species via soil cannot be ruled out, while transmission from domestic to wild species is also gaining attention (Walker and Morgan, 2014). The initial introduction of parasite (eggs) into the free-range soil might come from wild birds, but also from young hens. After the initial introduction, successive flocks of hens may infect each other. Permin et al. (1999) suggest that lack of disinfection of the hen house could also be a risk factor. Reported risk factors for parasite infection, other than the free range, are absence of a hygiene barrier at the farmers entrance of the hen house or unit and age of the equipment used in the hen house (Jansson et al., 2010). An explanation for lower levels of parasite infections in hens using a free-range area might be a lower risk of contact with parasite eggs, as infected faeces may potentially be spread over a larger area than in an indoor system (Thapa et al., 2015). Another explanation might be that free-range use decreases the density of faeces indoors, and therefore lowers the risk of infection indoors (Sherwin et al., 2013).

In 2006, 50% of organic flocks in the Netherlands were treated with the anthelmintic flubendazole (Iepema et al., 2006). Discussions about the use of anthelmintics highlight the adverse side-effects caused by residues ending up in products intended for human consumption (Kan et al., 1998; De Ruyck et al., 2004) or in the environment (Wagil et al., 2015; Lahr et al., 2018). Moreover, use of anthelmintic products does not prevent reinfection (Tarbiat et al., 2016a). So to keep infections low, deworming is done at regular intervals. Anthelmintics are also known to have an adverse effect on poultry, e.g. Levkut et al. (2019) found a potential inflammatory effect of flubendazole on broiler chicken intestines. Another risk of widespread use of anthelmintics is that nematodes can develop resistance, as seen for nematode parasites in cattle (Sutherland and Leathwick, 2011). One option for reducing the use of anthelmintics is to extend the interval between treatments according to a tailor-made management plan (Tarbiat et al., 2016b). However, when devising such a plan it is necessary to have good insights into the dynamics of intestinal parasites and the effect of each housing and management component on the level of infection. The aim of this study was to investigate the relationships between free-range use and parasite infection in organic laying hens.

6.2 Materials and methods

6.2.1 Recruitment of flocks

A total of 40 organic flocks of laying hens were recruited in Sweden, the Netherlands and Italy. These countries were expected to differ in climate. In Sweden, a set of 16 farms was provided by an advisor on organic poultry production. Of these, farmers who were successfully contacted and willing to participate, did so. In the Netherlands, organic poultry farmers were invited to join the study by a letter (103 Dutch farmers) and a call in a Dutch poultry farmers' journal. Italian farmers were invited to join the study through veterinarians and organic producer associations. The following criteria were set for participation: free range already in use for poultry for at least 8–10 years, and no other animals (e.g. sheep and horses) on the free-range area in the past five years. Farm visits were planned when the hens were at least 45 weeks of age and had had outdoor access continuously for the past two months (e.g. no interruption because of avian influenza). If the farmer was planning to treat the hens for parasites, the visit was planned as soon before the treatment as possible. All flocks sampled from October to March were regarded as winter flocks, while all flocks sampled from April to September were regarded as summer flocks (Kaufmann et al., 2011).

6.2.2 Data collection

In accordance with the EU legislation on organic production, the farms kept their hens in groups of no more than 3,000 birds. If a farm had multiple groups, only one of these was sampled. Free-range use was measured as number of years that the free-range area was in use for poultry, the number of weeks the sampled flock had uninterrupted access to the free-range area, the percentage of hens seen outside by the farmer under optimum conditions (before sunset, in calm, dry weather), the mean number of signs of hen presence in the six soil sampling locations and the mean proportion of soil covered with grass/herbs (Heckendorn et al., 2009) in the six soil sampling locations (see 'soil sample' section).

6.2.3 Questionnaire

A questionnaire was prepared in order to collect data about the hens participating in the study (date of birth, brand name), farm (age of the free-range area, number of hens on the farm, number of hens per compartment), hen performance (laying percentage at 60 weeks of age, mortality percentage to 60 weeks of age, health (see below), free-range use (see below), treatments for parasites (name of treatment, age of the hens when treated), and management of the soil (new layer of soil or litter added, ploughing or other inversion of the upper soil layer, rotational use) in the zones sampled. The farmers were asked to estimate the health (including production) of their hens by giving a score on a scale of 1 (very bad) to 10 (perfect). The farmers were also asked to estimate the proportion of hens seen outside. All questions were asked and all responses were recorded by the researcher or technical assistant. If the hens were younger than 60 weeks at the time of the visit, the farmer was contacted again when the birds had reached 60 weeks of age, for information on production and mortality percentages.

6.2.4 Soil samples

Figure 6.1 shows a schematic representation of the free-range area and sampling locations. A total of six soil samples were taken from the free-range soil at three different distances from the popholes (or as close to this distance as possible), two samples at 5 m, two at 20 m and two at 50 m. At each distance from the popholes, two different plots of 1 m^2 each were chosen. These plots differed in distance from the fence and from trees. All 1 m^2 plots were described on a recording sheet in terms of proportion of soil visible, proportion of cover by grass/herbs, proportion of cover by shade (tree/bushes canopy, artificial cover), soil cultivation since removal of last flock, depth of soil cultivation, cover with litter or stones (yes/no, type, proportion of surface covered). Furthermore, the presence (yes/no) of six different signs of hens was recorded: hen(s), dropping(s), dust bath or scratching pit, scratching, feathers and footprints. In every 1 m^2 plot, 10 samples to 0–10 cm depth were taken using a soil sampling device. All 10 samples from each 1 m^2 plot were pooled to one sample and stored in a refrigerator at the end of the day, until further processing for McMaster worm egg counts (see 'McMaster counts' section).

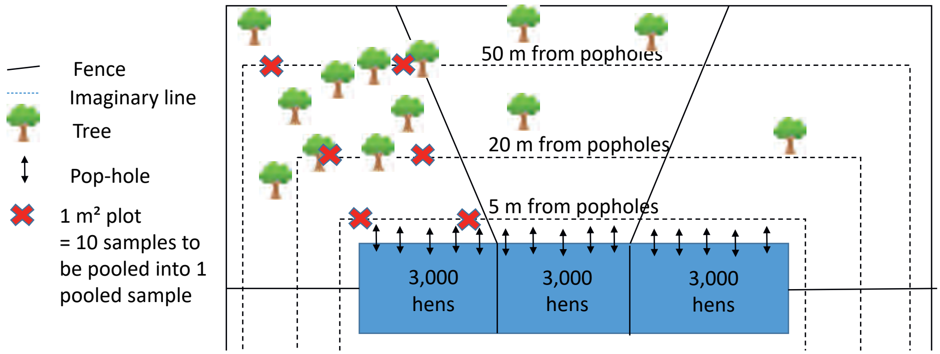

Figure 6.1: Schematic diagram of free-range area layout and soil sampling locations.

6.2.5 Faecal samples

Faecal samples and soil samples were collected from the same group of hens. In total, seven mixed samples were collected outside and seven were collected inside. Each mixed sample contained 10 fresh droppings pooled together. Outdoor faeces were collected at a minimum of 50 m distance from the popholes. Inside the hen house, as far away from the popholes as possible, another seven mixed samples were collected. Droppings were considered to be fresh if they had a shiny (moist) appearance and were soft. They were collected with gloves and a spoon, with a new set for each mixed sample, scooping without touching the ground or litter. Only intestinal droppings were collected, as cecal droppings seemed not to be sufficiently abundant to compile all mixed samples with the same ratio of caeca and intestinal droppings. The faecal samples were stored in a refrigerator at the end of the day, until further processing for worm egg counts.

6.2.6 McMaster counts

Counts of eggs of *A. galli*, *H. gallinarum* and *Capillaria* were made on all samples, using the McMaster method (Permin and Hansen, 1998). Eggs of *A. galli* and *H. gallinarum* were all counted as coming from the group 'ascarids'. Hereafter, 'ascarids' refers to both *A. galli* and *H. gallinarum*. The *Capillaria* genus contains different species, but eggs from the genus *Capillaria* were all counted as *Capillaria* eggs. The sample (3 g faeces or 3–10 g soil) was placed in a disposable container, to which 42 mL of flotation fluid was added. The contents were stirred with a spatula and the suspension was filtered through a tea strainer into another container. While the filtrate was being stirred, a sample was taken and the first compartment of the McMaster counting chamber was filled. The fluid was stirred again and a second compartment was filled. The counting compartments were allowed to stand for five minutes and then the samples were examined under a microscope at 10x10 magnification. All eggs and oocysts within the engraved area of both chambers were identified and counted. In Italy, 3 g of both faeces and soil were used and the number of eggs per g (EPG) of faeces or soil was calculated by multiplying the sum of eggs in the engraved areas of both chambers by 50. In the Netherlands, 6 g of soil was used and the sum of eggs in the two McMaster chambers was multiplied by 50/2 = 25 to give the parasite egg content in EPG. In Sweden, 10 g of soil was used and all ascarid and *Capillaria* eggs were counted, not only those in the two McMaster chambers. The Dutch and Swedish soil results were transformed into the same 'system' of EPG (0 or a multiple of 50) by rounding the raw data to 0, 50, 100, etc.

6.2.7 Statistics

All data were entered into a MS Excel spreadsheet and all descriptive and analytical statistics were produced using SPSS 26 (IBM, 2019).

Mean ascarid EPG and mean *Capillaria* EPG at flock level were calculated as a mean of ascarid or *Capillaria* EPGs for all samples within a flock. These means were calculated separately for faecal and soil samples. Per flock, the proportions of faecal and soil samples testing positive for either ascarids or *Capillaria* were also calculated. Thus, parasite infection was expressed in two sets of four different variables, one set for faecal samples and one set for soil samples. These variables were: proportion of samples testing positive for ascarids, mean number of ascarid eggs/g sample material, proportion of samples testing positive for *Capillaria* and mean number of *Capillaria* eggs/g sample material. Parasitological variables were checked for normality and variables that were not normally distributed were subjected to non-parametric tests. The means of normally distributed descriptive flock and farm variables were compared between countries with one-way independent ANOVA, using post-hoc Bonferroni tests. In the case of non-normally distributed variables, this was done with the Kruskal-Wallis test with pairwise comparisons adjusted for Bonferroni. Correlations between parasitological parameters in soil and faeces were calculated with Spearman's rho. Correlations between faeces collected outdoors and indoors were calculated with the Wilcoxon signed ranks test.

Three variables were used to reflect the degree of free-range use by a flock: 'Percentage of hens outside', 'Mean soil vegetation percentage' and 'Mean number of hen signs'. Mutual Pearson correlations were calculated to assess whether use of just one of these three variables was justified, which would be the case if they were highly correlated. The relationship between parasitological parameters for faeces and free-range use, farm and flock characteristics were analysed using linear mixed models. The selection of variables that were expected to influence the parasitological variables, was based on literature and other expert knowledge. Variables that were not normally distributed were log10-transformed to obtain a normal distribution. Variables that could not be transformed into a normal distribution were rank transformed. Rank transformations are a bridge between parametric and non-parametric statistics (Conover and Iman, 1981). Dependent variables were: ranked proportion of faecal samples containing ascarid eggs, log10-transformed ascarid EPG, ranked proportion of faecal samples containing *Capillaria* eggs and ranked *Capillaria* EPG. Fixed effects were country, season, number of hens per flock, number of hens per farm, 'age' of the free-range area, number of weeks in which the hens had had uninterrupted access to the free-range area at the time of sampling, age of the hens, proportion of hens using the free-range area, mean number of signs of hen presence, flock health estimated by the farmer, laying percentage at 60 weeks of age and mortality percentage by 60 weeks of age. The next selection step was based on univariate analyses: all variables with $p < 0.1$ in the univariate analysis were entered together in a model. Variables with $p > 0.05$ were removed from the model, to obtain a reduced model. The final model was selected based on Akaike information criterion and p-values ≤ 0.05 for explanatory variables.

6.3 Results

In total, 40 flocks (10 in Sweden, 20 in the Netherlands and 10 in Italy) were visited from October 2018 to October 2020, when the hens were aged between 45 and 94 (mean 62) weeks. If the flock had been treated with anthelmintics, the visit was made as long as possible after the last treatment (i.e. shortly before the next treatment), resulting in 21 to 68 (mean 41) days after treatment. The flocks had uninterrupted free-range access for at least 17 to 64 (mean 31) weeks.

6.3.1 Prevalence of parasite infections in free-range soil and faeces

Table 6.1 shows the variables investigated for the 40 flocks. In all three countries, the free-range area had been in use for a similar period (on average 16 years), there were high scores for hen health (on average 8.1) and there was relatively low mortality (on average 5.1% to 60 weeks of age). However, flocks and farms differed in size and genotype between the countries. Italian farmers estimated range use of their flocks highest ($F(2, 35) = 12.9$; $p < 0.001$). Based on the observers estimates of proportion of soil covered with grass/weeds ($F(2, 37) = 25.1$; $p < 0.001$) and mean number of signs of hen presence ($F(2, 37) = 17.5$; $p < 0.001$), range use was highest in the Netherlands. Italian flocks were rated as healthy as Dutch and Swedish flocks by farmers, but they showed a lower actual production level than Dutch and Swedish flocks ($F(2, 36) = 26.9$; $p < 0.001$) (Table 6.1). Only Dutch flocks (19 out of 20 studied) were treated with anthelmintics (mostly flubendazole, sometimes fenbendazole), on average five times by 60 weeks of age.

Table 6.1: Mean value (standard deviation) of variables characterising the flocks studied

Variable	All	Sweden	Netherlands	Italy
No. of flocks	40	10	20	10
Hens/farm	11,714 (10,433)	19,435 (17,062)[a]	11,496 (3,971)[ab]	4,430 (4,687)[b]
Hens/flock	3,384 (3,322)	6,540[1] (5,918)[a]	2,771 (519)[b]	1,770 (1,098)[b]
Years free range in use	16 (5.6)	18 (7.3)	15 (4.1)	14 (6.2)
Genotype				
Brown		1	17	6
White		9	3	1
Mixed/other		0	0	3
Age of hens in weeks	62 (12)	66 (11)	62 (11)	60 (16)
Access to free range in weeks	31 (9)	28 (10)	32 (5)	34 (13)
% Hens out	51 (25)	31 (20)[a]	48 (22)[a]	76 (10)[b]
% Soil covered by grass/ weeds	30 (33)	61 (26)[a]	5.5 (11)[b]	47 (33)[a]
Mean number of signs of hen presence	4.2 (1.5)	3.4 (1.8)[a]	5.2 (0.6)[b]	2.9 (1.0)[a]
Health at 60 wks[2]	8.0 (1.3)	8.0 (1.5)	7.9 (1.1)	8.5 (1.4)
Laying % 60 wks	86 (7.5)	88 (3.1)[a]	89 (3.3)[a]	76 (8.1)[b]
Mortality % by 60 wks	5.1 (4.6)	4.3 (2.4)	4.5 (1.9)	7.2 (8.5)
Number of flocks treated with anthelmintics	19/40	0/10	19/20	0/10
Number of anthelmintic treatments by 60 wks	2.5 (2.8)	0 (0)[a]	4.8 (1.9)[b]	0 (0)[a]
Days since last anthelmintic treatment		Not applicable	41 (14)	Not applicable

[1] Because the winter gardens/free ranges on the Swedish farms did not contain physical structures to separate hens, the hens from different compartments could thus come into contact with each other's faeces. This was a relevant aspect in this study and therefore these hens were considered as one flock. Indoors, the groups were separated and included no more than 3,000 hens.
[2] Health status of the flock, estimated by the farmer and expressed on a scale of 1 (= extremely bad) to 10 (= extremely good).

The parasite levels found in faecal and soil samples are shown in Table 6.2. In general, the range of values (minimum to maximum) was rather high. A similarly high percentage (median 71%) of faecal samples was found to be infected with ascarid eggs (median 143 eggs/g faeces) in all three countries. However, *Capillaria* was rarely found in Swedish faecal samples, while the numbers were similar for Dutch and Italian faeces (proportion of samples with *Capillaria* $H(2) = 11.9$; $p = 0.003$; mean number of *Capillaria* eggs $H(2) = 11.3$; $p = 0.003$). Among soil samples, ascarid eggs were only found in Dutch and Italian soil (proportion of samples with ascarid eggs $H(2) = 10.3$; $p = 0.006$); mean number of ascarid eggs $H(2) = 9.7$; $p = 0.008$) and *Capillaria* eggs were only found in Italian soil (proportion of soil samples with *Capillaria* $H(2) = 33.4$; $p < 0.001$; mean number of *Capillaria* eggs $H(2) = 33.3$; $p < 0.001$).

Concerning the presence of ascarid eggs, no significant correlation was found between proportion of positive faecal samples and proportion of positive soil samples (Spearman's rho = -0.239; $p = 0.138$; $n = 40$) or between ascarid EPG in faecal and soil samples (Spearman's rho = -0.035; $p = 0.828$; $n = 40$). Concerning the presence of *Capillaria* eggs, the proportion of positive faecal samples was correlated to the proportion of positive soil samples (Spearman's rho = 0.410; $p = 0.009$; $n = 40$). The number of *Capillaria* eggs in faeces was also related to the mean number of *Capillaria* eggs in soil (Spearman's rho = 0.336; $p = 0.034$; $n = 40$).

Table 6.2: Median value (minimum–maximum) of parasitological parameters in faecal and soil samples

Variable	All	Sweden	Netherlands	Italy
No. of flocks	40	10	20	10
No. of faecal samples	524	132	280	112
Median % of samples containing ascarid eggs	71 (0–100)	96 (21–100)	71 (21–100)	50 (0–100)
Median number of ascarid eggs/g faeces	143 (0–1,936)	379 (22–1,471)	141 (14–1,936)	136 (0–300)
Median % of samples containing *Capillaria* eggs	7 (0–71)	0 (0–7)[a]	14 (0–71)[b]	18 (0–71)[b]
Median number of *Capillaria* eggs/g faeces	5 (0–150)	0 (0–0)[a]	14 (0–150)[b]	12 (0–79)[b]
No. of soil samples	240	60	120	60
Median % of samples containing ascarid eggs	0 (0–67)	0 (0–0)[a]	17 (0–50)[b]	17 (0–67)[b]
Median number of ascarid eggs/g soil	0 (0–100)	0 (0–0)[a]	8 (0–100)[b]	8 (0–75)[b]
Median % of samples containing *Capillaria* eggs	0 (0–100)	0 (0–0)[a]	0 (0–0)[a]	83 (0–100)[b]
Median number of *Capillaria* eggs/g soil	0 (0–283)	0 (0–0)[a]	0 (0–0)[a]	83 (0–283)[b]

Regarding the four variables for parasite eggs in faeces, no differences were found between samples collected outside at distances more than 50 m from the popholes and samples collected inside the hen house (Wilcoxon signed rank test; $n = 38$; $-1.441 < Z < -1.034$; $0.301 < p < 0.150$).

6.3.2 Choice of variables reflecting free-range use

Table 6.3 shows the correlations between three different variables for use of the free-range area. No correlation was found between the proportion of hens seen outside by the farmer and that estimated by the observer based on cover of grass/weeds ($r = -0.02$; $p = 0.91$) and signs of hen presence at the six sampling locations ($r = 0.09$; $p = 0.59$), when corrected for country and season. However, a strong negative correlation was found between the two types of estimates made (vegetation cover, hen presence) by the observer ($r = -0.83$; $p < 0.001$) (Table 6.3). Of these, mean number of signs of hen presence was chosen for further calculations, since this estimate directly represented use of the free-range area, while absence of vegetation cover was an indirect indicator.

Table 6.3: Pearson correlation between the different variables for use of the free range, controlled for country and season

	% of hens seen outside by the farmer	Mean % of soil sampling locations covered with grass/weeds	Mean number of signs of hen presence at soil sampling locations
% of hens seen outside by the farmer	1.0	-0.019	0.094
Mean % of soil sampling locations covered with grass/weeds	-0.019	1.0	-0.834***
Mean number of signs of hen presence at soil sampling locations	0.094	-0.834***	1.0

*** $p < 0.001$.

No model could be fitted for ascarid or *Capillaria* EPG in faeces or for proportion of faecal samples containing ascarid or *Capillaria* eggs. A minority of soil samples contained ascarid eggs (median 0%) and these were found only in the Netherlands and Italy. *Capillaria* eggs were only found in Italian soil samples.

6.4 Discussion

6.4.1 Prevalence of parasite eggs in free-range soil and faeces

This study confirmed that ascarid infections are widespread in organic laying hen flocks. A median of 71% of faecal samples analysed contained ascarid eggs. Other studies have also found high prevalence of ascarid infections in organic and/or free-range laying hens (Thapa et al., 2015; Permin et al., 1999; Sherwin et al., 2013; Grafl et al., 2017). The prevalence of *Capillaria* infections showed lower variation than the prevalence of ascarid infections. A median of 7% of the faecal samples contained *Capillaria* eggs. This is within the range found in other studies (Jansson et al., 2010; Grafl et al., 2017; Permin et al., 1999; Wuthijaree et al., 2017).

The median proportion of soil samples per flock infected with ascarid eggs was 0%, but it ranged from 0% in Sweden to 17% in the Netherlands and Italy. Heckendorn et al. (2009) found ascarid eggs in 100% of the soil samples they analysed, but with fewer EPG (at most 2.5, compared with 18 in our study). However, the results of these studies are not directly comparable, since we expressed EPG in multiplies of 50 instead of an exact number of eggs (e.g. a sample containing 2.5 EPG was reported as EPG = 0). A median of 0% of soil samples per flock were infected with *Capillaria*, but the value ranged from 0% in Swedish and Dutch soil up to 83% in Italian soil. This is the first study to detect *Capillaria* eggs in soil from free-range areas. A previous analysis of litter samples from hen houses revealed that 91% contained ascarid eggs and 13% contained *Capillaria* eggs. The mean number of ascarid eggs per gram litter material was 400 and the number of *Capillaria* eggs ranged between 0 and 28 (Maurer et al., 2009). This indicates that, for ascarids and depending on the region also for *Capillaria*, litter inside the hen house may carry a higher risk of parasite infections than soil in the free-range area. In the present study, we found a positive correlation between *Capillaria* eggs in soil and in faeces, but we could not determine causality. Maurer et al. (2009) did not find a correlation between parasitological parameters in litter and faeces.

6.4.2 Use of free-range area

We addressed use of the free-range area at flock and individual level in several ways: as farmers' estimates of free-range use by the current flock, as observers' estimates of signs of hen presence and cover with grass/weeds, and 'age' of the free range. However, none of these indicators for use of the free-range area was found to be associated with parasitological parameters. Other studies investigating the relationship between parasite infections and use of the free-range area have found a positive relationship, i.e. more parasites with more free-range use (Permin et al., 1999; Sibanda et al., 2020), a negative relationship (Sherwin et al., 2013; Thapa et al., 2015) or no relationship (Bari et al., 2020). It is possible that our sample size of 40 flocks was not large enough, when taking into account the ratio between median number of eggs/g and the range between minimum and maximum values.

6.4.3 Anthelmintic treatments

Ascarid infections did not differ between Dutch and Italian flocks, even though 19 out 20 Dutch flocks studied were treated on average five times up to 60 weeks of age with flubendazole or fenbendazole, whereas the Italian flocks were not treated at all. Because all treated flocks were Dutch and all untreated flocks except one were Swedish or Italian, we could not assess the effect of anthelmintic treatment on parasite eggs in soil or faecal samples or determine whether this difference was caused by other differences between the three countries. However, the parasitological parameters for Dutch flocks raise questions about the effectiveness of anthelmintic treatment. This confirms findings in experimental studies in which laying hens were treated with flubendazole and found to be parasite-free only one for week (Tarbiat et al., 2016a) or 2–4 weeks (Höglund and Jansson, 2011) post-treatment. An experiment with a more tailor-made approach, consisting of measuring faecal egg count every two weeks and treatment in cases of > 200 eggs/g, found that the number of eggs/g was lower in the experimentally treated flocks than in untreated or standard-treated flocks (Tarbiat et al., 2016b). Together, these findings suggest to reflect on the use of anthelmintic treatments 'by calendar'.

6.4.4 Health and productivity

We did not find a relationship between any of the parasitological parameters studied and hen health or production parameters. Other studies have also generally found no relationship between parasitological parameters and mortality (Gauly et al., 2008; Sherwin et al., 2013; Wongrak et al., 2015). However, Stehr et al. (2019) found a lower laying rate and lower egg weight in experimentally infected hens. Mortality has sometimes been found to be higher in hens with an *A. galli* infection, but with other factors also playing a part, e.g. too low protein content in the feed (Ikeme, 1971) or a bacterial infection (Dahl et al., 2002; Eigaard et al., 2006; Permin et al., 2006). However, Hinrichsen et al. (2016) found higher mortality in peak-of-lay hens on highly infected organic farms in summer. The lack of relationship we found between parasitological parameters and egg production is in line with other studies (Gauly et al., 2007; Sherwin et al., 2013), with decreased egg production reported only in a case with bacterial co-infection (Dahl et al., 2002). Generally, ascarid infection alone does not seem to be associated with higher mortality or lower egg production, but under commercial conditions bacterial co-infections can be expected (Sharma et al., 2019).

6.4.5 Management of the free-range area

Because of the regionally low prevalence of parasite eggs in soil samples analysed in this study, it was not possible to test relationships between soil treatment, presence of shade provided by tree canopies or artificial structures, and parasite eggs in soil samples. Heckendorn et al. (2009) investigated naturally 'infected' soil and counted absolute numbers of parasite eggs, whereas we counted multiplies of 50. They found ascarid eggs in all soil samples, but observed no effect of mowing the free-range area. An experimental study by Maurer et al. (2020) found that ascarid eggs disappeared faster from gravel and wood chips than from soil. However, they mixed poultry faeces with pea gravel, wood chips and soil, resulting in ≥ 350 ascarid eggs/g of chips/gravel/soil, i.e. much more than the EPG found by Heckendorn et al. (2009) and in the present study. In order to investigate the effect of management on parasite eggs in soil samples, an experimental set-up would be more adequate than studies on commercial flocks.

6.4.6 Limitations of the study

Including flocks from at least 45 weeks of age in this study posed a risk of substantial age differences between the sampled flocks. However, this risk had to be accepted because the alternative, choosing a shorter age period, posed the risk of us being unable to sample numerous flocks because of statutory confinement due to avian influenza, which was imposed regularly (almost yearly) by national authorities during the study period. Another of our criteria was that flocks should have had access to the free-range area for at least two months. Introduction of a confinement period would have seriously delayed sampling (by months of confinement + 2 months) and flocks might have been at slaughter age before this delay period was over.

6.5 Conclusions

Ascarid and *Capillaria* infections were widely present in faecal samples from laying hens in all three countries studied, including samples from flocks treated repeatedly with anthelmintics. In Sweden and the Netherlands (almost) no parasite eggs were found in soil, while the majority of Italian soil samples contained *Capillaria* eggs. No associations were found between indicators of use of the free-range area and parasite eggs in faecal samples, or between parasite eggs in faecal samples and hen health and productivity. Intensity of use of the free-range area did not seem to be related to ascarid or *Capillaria* infections. The low number of ascarid and, depending on the region, *Capillaria* eggs in soil suggest to focus further investigations on the conditions inside the hen house rather than in the free range. Thereby, regional differences should be taken into account.

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Chapter 7

General discussion and conclusions

7.1 Introduction

There is an increasing number of laying hens with access to a free range in the Netherlands, Europe, Australia and recently also in the United States of America. A free range is offered to contribute to the welfare of laying hens in terms of more space and more and better opportunities for performing natural behaviours, such as exploration, foraging, dust- and sun bathing. However, free ranges are also associated with aspects that can be detrimental to animal welfare, such as predation, avian influenza and intestinal parasites. Moreover, although they are expected to contribute to animal welfare, free ranges are not automatically used by the hens. Probably certain demands or quality aspects play a role that are not always met. The potential net effect of a free range on the welfare of laying hens is the balance between opportunities and risks for animal welfare.

The overall objective of this thesis is to gain insight into the opportunities and risks of free ranges for animal welfare in laying hens, with the ultimate aim of optimizing hen welfare, including health.

In chapters 2 to 6 the results of five studies carried out on commercial organic and free-range farms are presented and discussed. In this chapter, the main findings are discussed and analyzed further: which novel insights do they provide for safeguarding animal welfare, how can these be implemented in practice and which suggestions can be done for future research?

7.2 Significance of the thesis findings for animal welfare

In chapter 1 several theoretical approaches of animal welfare are discussed and how they can explain the potential positive or negative contribution of a free range to the welfare of laying hens. It is concluded that animal welfare encompasses three key elements: 1. physical health & functioning, 2. naturalness and 3. affective states. Overall, animal welfare can only be good if positive conditions are reached in all three domains.

Physical health means that the animal has the physical ability to perform the activities that are important to it and that it is not hindered by pain or otherwise discomfort. For example, keel bone fractures are related to a reduction in free range use (Richards et al., 2012). Also, breeding and selection for desired traits should not be at the expense of the physical abilities mentioned above.

Naturalness refers to natural behaviour, i.e. the behaviour of wild ancestors, the red junglefowl, in their natural environment. Several similarities in behaviour still exist between chickens and their ancestors. Some of them are known as behavioural needs: foraging, dustbathing, nest-building prior to egg laying (Weeks and Nicol, 2006) and to how animals would behave in their (wild ancestors') natural environment (Bracke and Hopster, 2006). An example of naturalness in hens is the preference that hens have for peat for dustbathing, over sand, sawdust or wood shavings (Petherick and Duncan, 1989) and sand over wood shavings (van Liere et al., 1990). Another example is daylight, including UV-light, which is preferred over artificial light (Rana et al., 2021). UV-light added to LED-light, compared to LED-light only, also reduces fear and stress responses in laying hens (Sobotik et al., 2020). 'Affective states' relates to a balance between positive and negative experiences that make an animal's life sufficiently pleasurable and thus perceived as positive or as a 'life worth living', a term currently used by animal welfare scientists (Mellor, 2016). Being able to experience negative affective states however, such as hunger and pain, remains necessary. They are 'survival-critical affects', i.e. essential to survive. They are part of action-oriented systems: they make an animal that for example experiences hunger, starts to look for food (Mellor, 2012).

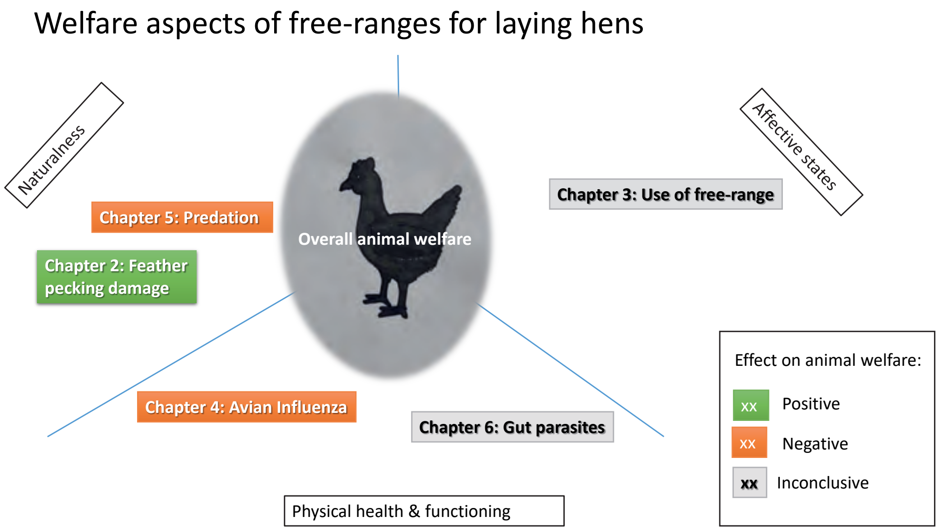

Figure 7.1: Conceptual framework (based on Fraser, 2008) illustrating the thesis topics: feather pecking damage, use of the free range, avian influenza, predation and intestinal parasites.
The topics are classified according to the key elements of animal welfare: naturalness, physical health & functioning and affective states. A free range can have a positive, a negative or an inconclusive effect on the topics.

The conceptual framework in the general introduction of this thesis and repeated in Figure 7.1 positions the five thesis topics in relation to the three key elements of animal welfare. Less feather pecking (chapter 2) and an increased risk of predation (chapter 5) are presented as a respectively positive (shown in green) and negative (shown in red) potential outcome of a free range when looking at the 'Naturalness' aspect. Voluntary use of the free range (chapter 3) is presented as an inconclusive (shown in grey) outcome of the 'Affective states' aspect. Hens that go out voluntarily, have positive expectations of the free range, but the expectations of hens that stay indoors, are not clear. Risk of infection with avian influenza virus (chapter 4) is positioned as a potential negative (shown in red) outcome of the free range in the 'Physical health & functioning' aspect, because free-range access increases the risk of contact with wild birds that may be infected with avian influenza virus. Intestinal parasites, being part of the 'Physical health & functioning' part of animal welfare (chapter 6) are found to have both a higher and a lower prevalence in relation to free-range access. Therefore, they are considered to be inconclusive (shown in grey). Below the findings from chapters 2 to 6 are discussed in relation to the conceptual framework.

7.2.1 Feather pecking and range use

The 1st research objective of this thesis is to assess which factors are related to feather pecking in organic laying hens. Feather pecking is pulling out (mostly followed by eating) of feathers, resulting in feather damage and bald patches on the back, vent and tail area of the victim. The bald patches may be subject to further pecking, resulting in wounds (Rodenburg et al., 2013). Various factors can trigger the onset of feather pecking, but the common denominator is stress in the hen that pulls out the feathers. The inability or inadequacy of fulfilling one or more behavioural needs, e.g. foraging or dustbathing, is probably one of the most important causes (Blokhuis and Arkes, 1984; Vestergaard et al., 1993; Huber-Eicher and Wechsler, 1997). However, the underlying mechanisms that links feather pecking to eg. behavioural needs and affective states are not clear yet (Fijn et al., 2020).

Chapter 2 is a study based on 107 organic flocks in the Netherlands and 7 other European countries. It shows that if a free range is part of the production system, daily access compared to less frequent access, contributes to the welfare of laying hens in terms of less feather pecking damage and less pecking wounds. Such a relationship, namely less feather pecking in case of increased (possibilities for) range use, has been found in almost all studies that looked at this relationship. This has been found on flock level: flocks with higher range use are in better feather condition (Green et al., 2000; Bestman and Wagenaar, 2003; Nicol et al., 2003; Mahboub et al., 2004; Lambton et al., 2010; Pettersson et al., 2017). This has been found at individual hen level too: hens that range more frequently have a better feather condition, compared to hens that range less frequently from the same flock (Rodriguez-Aurrekoetxea and Estevez, 2016; Sibanda et al., 2020b). Hens scored outside have a better plumage score than hens scored inside the hen house (de Koning et al., 2019). Our study was an observational one, i.e. we observed hens without intervention in an existing situation. Therefore we cannot prove which is cause and which is effect; plumage condition may influence ranging behaviour and ranging behaviour may influence plumage condition. Hens with worse plumage condition might be vulnerable to low temperatures and therefore be reluctant to go out. Vice versa, ranging behaviour may reduce the risk of feather pecking behaviour and thus plumage damage.

One explanation for range use leading to less feather pecking, might be that less ranging and more feather pecking both are characteristics of more fearful hens. Several authors find that low ranging hens indeed are more fearful than high ranging hens (Campbell et al., 2016; Hartcher et al., 2016; Campbell et al., 2019b). The study described in chapter 3 also shows that less fearful flocks range more. Incidentally, some studies find less clear-cut relationship between ranging behaviour and fearfulness. Larsen et al. (2018) for example find high rangers to be more fearful of humans but less fearful of a novel object. Feather pecking too is related to fearfulness (Rodenburg et al., 2013), i.e. feather peckers are more fearful than non-feather peckers (Vestergaard et al., 1993) and feather pecking and cannibalism might lead to increased fearfulness in victims (Campo et al., 2008; Uitdehaag et al., 2008).

Another explanation might be that feather pecking is an indicator of behavioural needs not being fulfilled. A behavioural need (Jensen and Toates, 1993) is a species-specific behaviour that is of such importance to an animal, that if it cannot be performed in a satisfying way, the animal may show maladaptive behaviour, such as feather pecking. Examples of behavioural needs in laying hens are foraging and dustbathing (Weeks and Nicol, 2006). Not being able to forage, because the hens are kept on slatted floors instead of on litter floors, has been shown to cause feather pecking in laying hens (Blokhuis and Arkes, 1984; Blokhuis, 1986; Huber-Eicher and Wechsler, 1997; Nicol et al., 2001; Bestman et al., 2009). Being able to forage, but without a reward in the form of edible items, is also found to cause feather pecking (Blokhuis and van der Haar, 1992). Not being able to dustbathe, because the hens are kept on wire mesh instead of sand with sods, results in higher feather pecking too (Vestergaard et al., 1993), which however is not confirmed in other experiments. Behavioural observations of hens both on the range and indoors show that more foraging (Campbell et al., 2017b) and more dustbathing (van Niekerk et al., 2012; Campbell et al., 2017b) is performed outdoors.

A third explanation for less plumage damage in relation to range use, might be that inactive hens are more likely to become target of feather pecking than hens performing dustbathing behaviour or otherwise being active (Riber and Forkman, 2007). Hens in the free range, especially in the outer range are found to walk and forage more, i.e. are more active, compared to the hens close to or inside the hen house (Chielo et al., 2016; Rodriguez-Aurrekoetxea and Estevez, 2016; Campbell et al., 2017b).

A fourth explanation may be that stocking density and group size inside the hen house decrease as soon as a proportion of the flock enters the free range. Both are known to reduce feather pecking (Huber-Eicher and Audigé, 1999; Nicol et al., 1999).

Irrespective of the relationship between range use and feather pecking, causal or not, the welfare of victims of feather pecking and of the peckers themselves seems worse than that of hens with an intact plumage. El-Lethey et al. (2001) fed corticosterone to laying hens with the intention to mimic the effect of stress. The hens in this experiment developed high rates of feather pecking and were more fearful. Feather pecking behaviour can therefore be regarded as an indicator of stress. Furthermore, Tahamtani et al. (2017) compare feather peckers, victims and control hens and looked at characteristics that are presumed to be bilaterally symmetric, such as length of ulna, tarsus and middle toe and width of tarsus and hock. Control hens are more symmetric than feather peckers and victims and the authors conclude that feather peckers and victims are exposed to 'similar levels of negative experiences, causing developmental instability, whereas control hens are less negatively affected.' Furthermore, damaged plumage or bare skin make hens prone to further feather pecking, getting wounds or other physical discomfort. In short, feather pecking and its damage are an indicator of reduced welfare. Since less feather pecking (damage) is found in flocks and in individuals that range more, one can conclude that increased range use is associated with better welfare. The results described in chapter 2 support the assumption that a free range has a positive effect on animal welfare from the perspective of naturalness.

7.2.2 Factors related to free-range use

The 2nd research objective of this thesis is to identify which factors make hens use their free range. When given access to a free range, some flocks make more use of it than others (Green et al., 2000; Bestman and Wagenaar, 2003). Also differences exist between individual hens from the same flock. Some hens use the free range not or sparsely, while their flockmates use it daily and for a longer period of time per day (Campbell et al., 2016; Richards et al., 2011; Rodriguez-Aurrekoetxea and Estevez, 2016).

Chapter 3 is a study based on 169 Dutch and Swiss organic and free-range flocks. It describes that the mean proportion of hens of a flock using the range at any one time, as estimated by the farmer, is 47% and varies between the four subsets (Dutch/Swiss and Organic/Free-range) from 23% (Dutch free-range) to 57% (Swiss organic). The results also show that a higher proportion of the flock is seen out in case of brown genotypes (compared to white, silver or two or more genotypes in one flock), when kept in smaller flocks, if roosters are kept with the hens, in case of natural ventilation (compared to combined natural and mechanical ventilation) and in flocks with better plumage. Results per subgroup classified per production system (organic or free-range) and country (Netherlands or Switzerland), show a higher range use in flocks that contain more than one genotype, that are reared on the laying farm (compared to rearing on a separate farm), have more daylight inside the hen house and are less afraid of the observers. Factors related to the physical environment (rearing location, amount of daylight, type of ventilation) or to the composition of the flock (in terms of genotype, number of hens, presence of roosters) are the direct result of a famer's choice; the majority of these factors can be influenced by the farmer. Another factor under the influence of the farmer, but not included in the study described in chapter

3, is the presence of shelter in the free range in the form of trees, bushes or artificial, for example lean-to's. Shelter, especially 'woody vegetation' fits very well in the image of a hen as a forest bird (Petterson et al., 2016; Larsen et al., 2017) and several studies found that its presence is related to a higher proportion of the hens using the free range (Bestman and Wagenaar, 2003; Nicol et al., 2003; Zeltner and Hirt, 2003; Hegelund et al., 2005; Gilani et al., 2014) and to reduced feather pecking damage (Bright et al., 2016). The latter might be an indirect effect, caused by increased range use. These positive aspects of shelter and especially woody vegetation is a recurring topic in the next paragraphs.

When thinking of a free range providing opportunities for animal welfare, the thoughts mainly go out to effects on the hens that do range or range the most. However, hens that do not range or range less, may also profit from the free range. For example because of the lower stocking density, smaller group size or more daylight in the hen house when the pop-holes are open. Larsen et al., (2018) find fewer differences in welfare (measured as plumage, footpad and beak condition, keel bone deformations or fear) between low, moderate and high ranging laying hens than they expected. They suggest that a free range provides hens 'with adequate choice to cope with their environment', which in itself could influence the welfare of the hens, irrespective of their actual range use. Furthermore, they suggest looking at other welfare aspects too, such as possibilities for performing natural behaviour and the effect of range use on the hens' affective states. The results of chapter 3 support the assumption that the effects of a free range on affective states are inconclusive.

When considering the variety between flocks in range use, the potential to increase range use, the welfare aspects that have not yet been investigated and that the provision of a free range may also improve the welfare of hens that use it to a lesser extent, one can conclude that the potential of free ranges for animal welfare may be greater than currently thought.

7.2.3 Avian influenza risk birds in the free-range area

The 3rd research objective of this thesis is to assess the relationship between the presence of trees and bushes in and around free ranges and the presence of 'avian influenza risk birds', i.e. species with a high prevalence of infection with avian influenza virus. These are water birds and wading birds, such as geese, ducks, swans, gulls, oystercathers and lapwings (Veen et al., 2007; Breed et al., 2011; Slaterus, 2014). Organic/free-range flocks have a 6.3 times higher chance to become infected with avian influenza compared to flocks kept indoors, which is related to distance to waterways and areas with wild waterfowl (Bouwstra et al., 2017).

Chapter 4 is a study based on 11 Dutch organic and free-range farms. It shows that in free ranges with at least 8% of cover with woody vegetation, fewer wild birds are seen from known risk species, compared to free ranges with no woody cover at all. In case of a half-open landscape, i.e. with woody elements, also fewer wild birds are seen in the surroundings of the free range, compared to open landscapes.

Possible explanations for wild birds staying away from woody vegetation might be that they either prefer open spaces, for example to see approaching predators (Loonen and Bos, 2003) or that they need grassland (which are often open spaces), on which they forage in large groups (Stahl, 2015). However, our study has an observational design: there is no comparison of a before and after situation or of a case and control situation. Therefore, a causal relationship between woody vegetation and avian influenza risk birds cannot be established. One important aspect that needs further investigation, is whether the chance of contact between a hen and a wild bird (or its droppings) changes with increasing woody vegetation. If more hens of a flock use the free range for a longer time period per day, there might be more contact moments with wild birds or with their droppings, even if these wild birds use the free range in smaller numbers.

The finding that woody vegetation may help keep away avian influenza risk birds, does not change the perception of an avian influenza infection in relation to the conceptual framework of welfare of laying hens as presented in the general introduction of this thesis. Avian influenza influences the welfare of laying hens in several ways. Depending on the virus lineage and its pathogenicity (low or high), an infection with avian influenza virus can have a large impact on animal health: chickens may become severely ill and die. Furthermore, preventive confinement of organic/free-range laying hens, a measure imposed by the national authorities when wild bird surveillance indicates an increased prevalence of AI-infection, reduces the welfare of hens. Confinement from one day to the next, of hens that are used to outdoor access, aggravates feather pecking (Kijlstra and van der Werf, 2006). Depopulation of flocks, i.e. on-farm killing with carbon dioxide, as a measure to prevent further outbreaks within a zone of one kilometre around an infected farm, is considered to reduce animal welfare (Anonymous, 2020). The 2020–2021 avian influenza epidemic affected 22,9 million poultry birds in Europe (EFSA, 2021) and which resulted in the Netherlands in an eight months period of confinement of poultry, is reason to (again) raise the question to explore the possibilities of vaccinating poultry against avian influenza. However, this is a measure that requires various technical and legal obstacles to be taken, also on an international level, and thus a long time before it can be implemented. Moreover, there is no vaccine yet that protects against all avian influenza virus strains. Therefore, besides vaccination other measures, such as bird repellents, are still necessary. The potential of the finding that woody vegetation may help keep away avian influenza risk birds, gives a new angle of view for the prevention of avian influenza. A 'side effect' of woody vegetation, is that it makes the free range more attractive to its intended residents, who are descendants of red junglefowl, a forest bird (Fumihito et al., 1996). As long as woody vegetation does not lead to an increase in interactions between chickens and wild birds, it may thus serve multiple purposes. Another measure found to chase away waterfowl from a free range, is an automated laser installation (Elbers and Gonzales, 2021). At night, when the hens are inside the hen house, the free range was lasered and in daytime, when the hens had access to the free range, the surroundings of the free range were lasered. The laser reduces the rate of wild birds visiting the study area for 98%. Automated lasers do not damage wild bird eyes, because the lasers continuously move. The chance to hit the bird eye is minimal (Henskes, 2021a). A laser may disturb also non target birds, which is especially not wanted in nature areas. Both the planting of trees and the use of a laser are bound by laws and regulations, but they both can be part of the poultry farmers' toolbox against avian influenza risk birds in the free range.

7.2.4 Predation of free-range laying hens

The 4th research objective of this thesis is to assess the extent of predation of hens in the free range, including clues for prevention. Organic and free-range farmers report predation by foxes and/or birds of prey (Bestman and Wagenaar, 2014). Predation is a problem from an economic point of view and maybe also be a problem from an animal welfare point of view. Predation by land predators can be prevented to some extent by adequate fencing and making sure all hens sleep inside at night. Predation by air predators, however, is difficult or even impossible to prevent, when considering the size of regular organic and free-range farms (Bestman and van Liere, 2011). The costs of covering the free range with nets, e.g. as in cherry production, are too high for free ranges measuring up to 10 hectares. Moreover, large scale netting is expected to require a permit from the municipality. Furthermore, there is no system of compensation, as exists for damage by protected wild animals to certain crops.

Chapter 5 is a study based on observations on 11 Dutch organic/free-range farms. It shows that 32 out of 44 (73%) hens found dead during observations are suspected to be killed by a bird of prey and 4 (9%) by a fox. In 109 out of 141 sightings (77%) common buzzards (*Buteo buteo*) are seen in or close to the free ranges. Live observations and video recordings show that both common buzzard and northern goshawk (*Accipiter gentilis*) attack and kill hens, which are assumed to be healthy and thus productive prior to the attack. Being productive is relevant for determining the economic aspects of predation. An additional survey among 27 organic/free-range farmers shows that per flock on average they lose 3.7% of the hens to predation, while total mortality (mainly caused by diseases) is 12.2%. Economic losses per flock (in production for appr. 60 weeks) are calculated to be EUR 5,700 for an average organic farm (12,700 hens) and EUR 6700 for an average free-range farm (25,000 hens). The behaviour of birds of prey, victim hens and bystander hens and roosters were reported for 16 attacks. A general course of events is that most victim hens first drop down and then try to resist the bird of prey. Bystander hens or roosters, if present, try to chase away the bird of prey. After a hen is caught, bystander hens are seen standing within a few meters from the eating bird of prey and some are seen cannibalising their flock mate as soon as the bird of prey leaves. Animals exposed to predators or predator cues are considered to experience acute stress during an attack. However, despite birds of prey being present in the free range, the proportion of the flocks seen out was nearly 60% on average. This is rather high when taking into account the flock size of 11,800 hens on average. Range use is negatively correlated to flock size (chapter 3; Pettersson et al., 2016). The mean proportion of 60% of hens using the range is not in accordance with the assumption that presence of birds of prey causes fear in the hens. Observations of a flock of (initially) 100 hens in a mobile house in a tree nursery show that the hens did not leave their house anymore after repeated attacks by birds of prey (Bestman, 2017), while such a flock size normally is related to a high range use (Petterson et al., 2016). This raises the question whether, in terms of fearfulness, birds of prey have a larger impact in small flocks (100 hens), compared to commercial size flocks (up to 19,000 hens). An explanation might be that in small flocks, with small free ranges, a higher proportion of the hens witnesses an attack on flockmates. If witnessing an attack leads to increased fearfulness in bystander hens, then in small flocks a higher proportion of the hens becomes fearful. If this reasoning is right, a larger flock size has an advantage for animal welfare within the context of predation. Although not proven in an experimental set up, there is no reason to think that the presence of trees leads to less predation. In fact, birds of prey were seen to use trees (and fence poles) to sit on and watch the hens. While trees seem to have advantages for other welfare aspects (making the range attractive for the hens and less attractive for wild birds from avian influenza risk species), their role within the context of predation is not sure. However, even if trees and bushes do not hinder or facilitate attacks of chickens by birds of prey, as a shelter they help make chickens feel protected. The answer to the question how to deal with potential negative welfare aspects of predation, might be that the highest attainable is to let hens FEEL safe, in contrast to let hens BE safe. For the contribution of predation to the conceptual framework as presented in the general introduction of this thesis, the reasoning here above means that it might be more appropriate to categorize predation from 'negative' for animal welfare and health to 'inconclusive'.

7.2.5 Range use and intestinal parasites

The 5th research objective of this thesis is to assess the relationship between range use and intestinal parasites. Free ranges are described as risks for intestinal parasite infections in laying hens. More intestinal parasites are found in organic/free-range hens, compared to barn hens (Permin et al., 1999). Sibanda et al. (2020b) found that high ranging hens are more likely to be infected with *Ascaridia galli* and cestodes, compared to low ranging hens. Other studies found no relationship (Jansson et al., 2010; Bari et al., 2020) or found the opposite; fewer intestinal parasites seen in case of a higher proportion of hens of a flock using the free range (Sherwin et al., 2013; Thapa et al., 2015). Since an increasing number of hens is getting access to a free range, not only in the Netherlands and Europe, but also worldwide, more insight in the relationship between free-range use and intestinal parasites is required. More insight is needed in terms of size and direction of the relationship and in terms of how management of the free range can reduce health risks.

Chapter 6 is a study based on 40 organic flocks in the Netherlands, Sweden and Italy. It investigated the relationship between range use and the most common intestinal parasites: *A. galli* and *H. gallinarum* (hereafter called 'ascarids') and *Capillaria*. The study shows that, with the exception of *Capillaria* in Italy, only very few or no eggs of the most common intestinal parasites are found in soil samples from free ranges, even when in use for 15 years or longer. This suggests, at least for ascarids and for *Capillaria* in Sweden and the Netherlands, to focus further investigations for measures against accumulation of parasite eggs on the conditions inside the hen house rather than in the free range. No relationship is found between parasites and several indicators of range use: number of weeks the hens have uninterrupted access to the free range, proportion of hens using the free range and the number of years the free range being in use as such. Furthermore, no relationship is found between infection with parasites and health, mortality and production parameters. Flocks in the only country with widespread use of anthelmintics (the Netherlands), do not score better on intestinal parasite infections than flocks in countries where no such treatments are used. Free-range use does not seem to be a (major) risk factor for parasite infections and it can be questioned how effective the current regular use of anthelmintic treatments is. As in general no acute risk for health, welfare or production seems to be related to ascarids and *Capillaria*, the results leave room for exploring strategies for reducing the use of anthelmintics. For example a more tailor-made approach, as described by Tarbiat et al., (2016a). Their approach consists of measuring faecal egg count every two weeks and anthelmintic treatments (fenbendazole) are applied in case the faecal egg count exceeds 200 eggs/gram. This approach led to a lower parasite infection, compared to untreated flocks and to standard treated flocks (i.e. treated once with fenbendazole at a certain age).

If the increase of *Capillaria* eggs in soil from north (Sweden) to south (Italy) is related to temperature, then an increase in temperature because of climate change may increase the numbers of parasite eggs in soil.

The results described in chapter 6 are no reason to change the meaning of a free range, described as inconclusive in the conceptual framework of this thesis, into either positive or negative for intestinal parasites. This has mainly to do with the standard deviation of faecal egg counts within and between flocks. They often surpass the mean value. If a relationship between range use and parasites is present, with this variation it cannot be detected. In other words, the sensitivity of our study is too low. However, the number of ascarid eggs we found in soil, are much smaller compared to the numbers found by Maurer et al. (2009) in litter from hen houses. This was different for *Capillaria*. We did not find *Capillaria* eggs in soil in Sweden and the Netherlands, but the number of *Capillaria* eggs in Italian soil surpassed the number found in litter inside the hen house (Maurer et al., 2009). For ascarids and for *Capillaria* in Sweden and the Netherlands, the risk of infection seems higher inside the hen house, compared to outside in the free range.

Taken together, the results from chapters 5 and 6 support the assumption that a free range has a negative effect on animal welfare from the perspective of physical health and functioning with regards to the increased risk of avian influenza. The effects of a free range on intestinal parasites are inconclusive.

7.2.6 Implications of the thesis findings for the conceptual framework

The conceptual framework presented in the general introduction of this thesis, positions the five thesis topics in relation to three key elements of animal welfare. When taking into account the findings described in chapters 2 to 6 and discussed in the general discussion of this thesis, only the findings from the predation study (chapter 5) lead to a new insight with regard to the conceptual framework. Although at first predation was considered to be detrimental to the welfare of free-range laying hens, in the general discussion of this thesis, it is concluded that this is inconclusive. Figure 7.2 reflects the revised conceptual framework, with predation shown in grey instead of red.

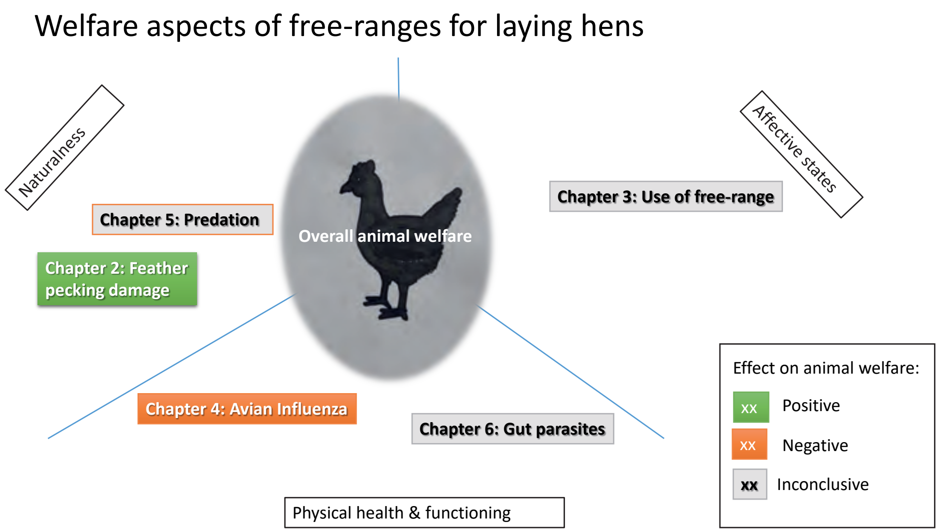

Figure 7.2: Revised conceptual framework (based on Fraser, 2008) illustrating the thesis topics: feather pecking damage, use of the free range, avian influenza, predation and intestinal parasites. The topics are classified according to the key elements of animal welfare: naturalness, physical health & functioning and affective states. A free range can have a positive, a negative or an inconclusive effect on the topics.

7.3 Practical implications – towards a higher welfare potential of free ranges

7.3.1 The value and limitations of observational on-farm research

This thesis consists of five studies, which are all carried out on farms and not in experimental facilities. Several reasons exist for this choice. The main reason is that the research questions relate to specific production conditions, which cannot or only with great difficulty be simulated experimentally. Another reason is that farmers are more willing to apply practical recommendations on their own farm, when they are based on results from a recognizable study environment. Moreover, this type of research does not require animal testing, which is a benefit from an ethical point of view. Finally, observational research on farms is cheaper than research in experimental facilities. However, a drawback of observational research is that it depends on what is available. Therefore, a perfect factorial design, including all combinations of the investigated variables and corresponding robust statistical analysis is not always possible. Furthermore, findings in observational studies are often correlations. Although such findings sometimes seem to have a very logical explanation, strictly speaking they cannot be regarded as a proof of cause and effect. To obtain causal information, (many) repeated measures over time or a case control set up are necessary, but this is often not possible in an on-farm situation. On the other hand, experimental studies often need to be followed by more practical investigations in order to formulate practical recommendations. Therefore, results of both kind of studies, observational and experimental, are needed.

7.3.2 Range use and feather pecking

This thesis demonstrates that daily range use is associated with less feather pecking damage and less peck wounds in flocks of laying hens, when compared to flocks that had less frequent access than daily (chapter 2). Assuming a causal relationship, i.e. daily free-range use reducing maladaptive pecking behaviour, it is recommended to stimulate range use in laying hens by providing daily access to the free range.

7.3.3 Encouraging range use by the hens

This thesis demonstrates several factors, on which the farmer has influence and that are associated with range use (chapter 3). Assuming the relationships to be causal, smaller flock sizes are recommended instead of larger, as well as keeping roosters with the hens and providing more daylight inside the hen house. 'Smaller flock size' needs to be interpreted in terms of 'smaller' within the order of magnitude of 1,900 (smallest mean flock size) to 23,900 (largest mean flock size). For smaller flocks mobile housing could be considered. Every time a mobile house is moved to a new place, fresh edible vegetation is available. Mobile houses exist for flocks up to 2000 hens (Farmermobil, 2021), but the most used mobile houses in the Netherlands house up to 250 chickens.

The number of 250 chickens is the lower limit for the distinction between 'hobby' and 'commercial farm'; above the owner has to comply with more regulations (RVO, 2021). 'Roosters in the flock' needs to be interpreted as the 'presence as such', because it is neither the number of roosters nor the proportion of roosters that is calculated with. 'More daylight in the hen house' needs to be interpreted as 'sufficient or much' within the range of 'no/little' to 'much', as visually estimated by an observer.

7.3.4 Discourage avian influenza risk birds to be present in the free range or its surroundings

This thesis demonstrates that less high-risk birds (belonging to species with a high prevalence of infection with avian influenza virus) are seen in free ranges, of which the surface is covered with at least 8% of woody vegetation (chapter 4). Assuming a causal relationship, i.e. high-risk bird species avoiding woody vegetation, it is recommended to plant woody vegetation such as trees, bushes or miscanthus in the free range, covering at least 8% of the surface. Furthermore, less high-risk birds are seen in the surroundings of free ranges when located in half-open landscapes (containing woodland strips and/or forest), compared to open landscapes (only grassland). Assuming again that high risk bird species avoid woody vegetation also on landscape level, it is recommended not to establish a free range in such landscapes, unless woody vegetation could be established inside the free range or within 500 meter from the border of the free range.

7.3.5 Predation of free-range laying hens

This thesis demonstrates that on average 3.7 or up to 12% of the hens of an organic/free-range flock disappears and that the most likely explanation for this is predation, mostly by birds of prey (chapter 5). The corresponding economic losses are estimated to be EUR 5,700 and 6,700 per flock (being in production for appr. 60 weeks), depending on the production system; organic or free-range respectively. Concerning the financial aspects of predation, it is recommended to opt for a system of compensation, as exists for damage by other protected wild animals to crops. Although not experimentally investigated, attacks by birds of prey are seen in free ranges with and without trees. It is reasoned that the welfare implications of predation, at least in flocks of 6,000 to 19,000 hens, may be limited. Insofar as one would dare to deduce a sound practical recommendation from this, it would be that there is no evidence that planting of trees and bushes, as recommended in order to keep away avian influenza risk birds, forms a risk in terms of predation.

7.3.6 Range use and intestinal parasites

It was not possible to find a relationship between range use and infections with intestinal parasites (chapter 6). Moreover, the numbers of ascarid eggs in soil samples from free ranges are much lower compared to the numbers found by others in litter inside the hen house. *Capillaria* eggs are not found in soil from Sweden and the Netherlands, but the numbers in soil from Italy exceed the numbers found by others in litter. This means that for ascarid and depending on the region for *Capillaria*, there is no evidence that encouraging range use, as recommended in 7.3.2 as a preventive measure against feather pecking and cannibalism, forms a risk in terms of intestinal parasites.

7.4 Future research possibilities

7.4.1 How much range use is needed to safeguard animal welfare?

Regarding the relationship between range use and animal welfare, a free range seems to have the potential to increase animal welfare. The strongest clue for this is the decrease of feather pecking in relation to range use (this thesis; Green et al., 2000; Bestman and Wagenaar, 2003; Nicol et al., 2003; Mahboub et al., 2004; Lambton et al., 2010; Rodriguez-Aurekoetxea and Estevez, 2016; Pettersson et al., 2017; de Koning et al., 2019; Sibanda et al., 2020b). Assuming feather pecking as a measure of animal welfare, how much range use is needed to prevent the onset of this behaviour in commercial flocks? The answer to this question may help to estimate the effort needed for encouraging range use. A rough estimate can be made by means of a meta-analysis of studies investigating range use and feather pecking.

Another way of investigating the contribution of a free range for hen welfare, is by somehow asking the hens, according to the 'consumer demand' principles (Dawkins, 1983). This can be done in an experiment in which hens express their 'will' to enter the free range by showing effort or by how much time they want to spend in the free range. This can be compared to the effort she is willing to do for a resource like food or the time spent on other behaviours.

7.4.2 How much effort is needed to encourage range use?

In continuation of the previous question, what effort is needed to encourage range use? A measure with proven positive effect on range use, is the presence of shelter, especially trees (Bestman and Wagenaar, 2003; Nicol et al., 2003; Zeltner and Hirt, 2003; Gilani et al., 2014; Stadig et al., 2017). Which proportion of the range needs to be covered in order to increase range use to the desired degree? Is there a relationship between size of trees and range use? Young trees are smaller, cheaper and easier to handle than older trees. If this measure should have effect year-round, does it then matter which tree species (conifers, evergreen, leaf-losing) are chosen? The answers to these questions may help farmers to encourage the hens to use the range. Furthermore, when translated into regulatory standards, such as the EU-regulation for organic production (EU 2020/464) and the EU-regulation for marketing standards for eggs (EC 589/2008), this measure can be applied on a large scale. A rough estimate of how much cover would be needed and which features of the woody vegetation is of importance, can be made by means of a meta-analysis of studies investigating design of free ranges and range use. However, an experimental setup is needed to investigate the relationship between qualitative tree properties and range use.

Concerning the 2 to 50% (Richards et al., 2011; Rodriguez-Aurrekoetxea and Estevez, 2016; Campbell et al., 2017a; Larsen et al., 2018) of the hens of a flock that never use the range, it is questioned whether this is the outcome of a choice. A choice requires that hens know both options (indoor and outdoor environment) equally well. If a hen has never been outside, she does not know both options equally well. The question arises how to 'inform' hens about the free range or train them to use it. Organic regulation requires range access from 8 weeks of life (Skal, 2019), while conventional free-range flocks get range access only at the laying farm. Organic flocks range better than conventional free-range flocks (Leenstra et al., 2012; chapter 3). Several explanations are possible and early range access is one of them. Early range access is positively correlated to range use at a later age (Grigor et al., 1995a; Bestman and Wagenaar, 2003) although Gilani et al. (2014) found no relationship. However, range access during rearing, as practiced in organic flocks, is on voluntary basis; it is still possible that some hens never use the range. In an experimental setup, the effect of voluntary range use versus 'somehow range use for all' during rearing, on voluntary range use in adult life should be investigated. Depending on the results, a more adequate information or training program for hens can be developed.

7.4.3 Woody vegetation as a measure against avian influenza risk birds

Besides being related to higher range use by laying hens, woody vegetation in free ranges is related to lower numbers of wild bird species, known for their susceptibility to avian influenza, present in the free range. The study described in chapter 4 is the first that found a relationship between woody range vegetation and presence of avian influenza risk birds. However, due to an incomplete factorial setup, not all possible combinations between categories of proportion of woody cover with openness of landscape could be investigated. Most of the free ranges with a higher degree of woody cover were located in half-open landscapes. An experimental setup with a complete design and comparing the before and after situation or comparing a case with a control situation is recommended to further find out whether woody cover can be applied in order to reduce the number of unwanted visitors in the free range. It is recommended to include other animals and nocturnal observations too, in order to study the effect on nocturnal animals (Kleyheeg et al., 2015; Elbers and Gonzales, 2019).

Assuming woody cover reduces the number of unwanted wild birds and at the same time increases the number of chickens being present in the free range, the next question is: what will be the net result of woody vegetation on the chance of contact between a hen and an avian influenza risk bird? This question is essential, because the answer determines the effectiveness of woody cover as 'bird repellent'. In fact, a worst-case scenario would be if the net number of contacts between hens and wild birds would increase.

7.4.4 Measures against (costs of) predation

This thesis estimates economic damage caused by predation to be 5,700 and 6,700 euros per organic and free-range flock respectively. The study described in chapter 5 also suggests how to estimate the numbers of chickens lost per flock, based on the numerous counts of the hens done in the successive links of the production chain and the farm records. A recommendation is to develop and test in practice a 'block chain like procedure', in which hatchery, rearer, laying hen farmer and slaughterhouse provide the results of their consecutive counts of the hens.

Previous research showed that there was little that could be done against predation of free-range hens (Bestman and van Liere, 2011). However, since then new insights arose and given the extent of the damage, some more expensive measures are also in the picture. There are promising results with an automatic laser system chasing away wild waterfowl from free ranges (Elbers and Gonzales, 2021). Although birds of prey seem to respond differently to the lasers (Henskes, 2021b), it is recommended to test such a system against birds of prey. Maybe further development of such a system may be effective against birds of prey. A system with drones can also be considered instead of lasers. Another measure may be livestock guardian dogs. A Dutch farm with two Pyrenese mountain dogs fenced in with a mobile house homing 150 laying hens already for six years, reports to have no mortality by predators (Bijleveld, 2019). The use of livestock guardian dogs, who protect other animals independently (i.e. without continuous human supervision), is rather unusual for the Netherlands. They are more common in countries with more expansive rural areas, such as the outback of Australia. Examples exist of a couple of Maremma sheep dogs, sometimes including their offspring, kept together with chickens (ABC, 2021; Anonymous, 2021). Their owners say they are effective in keeping away dog-type predators from poultry. The costs of purchasing and maintaining a dog for free-range poultry was fully offset by the values of stock saved within 3 years (Bommel and Johnsen, 2012).

7.4.5 Intestinal parasites

Free-range soil seems to contain far less eggs of ascarids and, depending on the region also for *Capillaria*, than litter inside the hen house. However, this is based on soil samples in one study (chapter 6) and litter samples in another study (Maurer et al., 2009). Moreover, regional differences are found in the prevalence of *Capillaria* eggs in soil. It is recommended to investigate soil and litter samples from the same set of farms, starting after providing clean litter at the start of a flock, take repeated samples throughout the laying period and count parasite eggs in the same way. If it turns out that litter might be a greater source of parasite eggs than soil, this helps to focus further investigations on preventive measures. Caution should be exercised in extrapolating results from one region to another.

The majority of organic egg production flocks in the Netherlands is treated with the anthelmintics flubendazole and/or fenbendazole, on average 4.8 times till the age of 60 weeks (chapter 6). This is assumed to be comparable to other egg production systems in the Netherlands. Parasites may become resistant against anthelmintics, as seen for nematode parasites in cattle (Sutherland and Leathwick, 2011). Via direct excretion of faeces in the free range and by using manure as fertilizer on arable land, residues of anthelmintics end up in the environment (Lahr et al., 2018). For several reasons this is a cause for concern (Lahr et al., 2019). Together these aspects argue for investigation whether the use of anthelmintics can be reduced. It is therefore recommended to get further insight into the course of infections with intestinal parasites from young to end-of-lay hens on commercial farms and its relationships with health and production parameters and deworming. This is investigated in Sweden (Höglund and Jansson, 2011; Tarbiat et al., 2016b), but a repeat on Dutch farms may be necessary to obtain insight into the Dutch situation. A next step may be a more tailor-made approach. An example of this is analyzing faecal egg counts every two weeks and apply anthelmintics only when a chosen threshold value has been exceeded repeatedly (Tarbiat et al., 2016a).

7.4.6 A free range for every laying hen?

This thesis is only about welfare aspects of free ranges for laying hens. However, other aspects relating to sustainability should be considered as well: emissions to the environment, feed conversion of hens with more exercise, food safety (van Asselt et al., 2015), land availability and some areas may have increased veterinary risks (Bouwstra et al., 2017; Velkers et al., 2020).

Several studies found an emission of nitrogen and phosphate via poultry faeces to the soil in poultry free ranges (Aarnink, et al., 2006; Dekker et al., 2012). Especially within 20 meters from the hen house, with up to 2,845 kg N and up to 709 kg P_2O_5/ha/year (Aarnink et al., 2006), this exceeds the European fertilisation standard of 170 kg N/ha/year (EU-directive 91/676/EEC) and the Dutch fertilisation standard of 75 P_2O_5 (Fosfaatgebruiksnormen, 2020). The crop with the highest nutrient uptake is grass, with a yearly uptake of 90–118 kg P_2O_5/ha/year (Ehlert et al., 2009), but as long as at the same time the parcel is in use as poultry free range, the crop will be eaten and does not take up enough P_2O_5. Trees, for example apple and pear trees yearly take up 20 kg P_2O_5/ha/year and hemp 79 kg (Ehlert et al., 2009). It goes beyond the topic of this thesis to review all possible remedies to this, but to date no satisfying methods are available to exploit free ranges in a nutrient neutral way.

Another sustainability aspect may be the increased feed conversion and thus the increased carbon footprint of hens with more exercise that consequently have a higher energy intake. The daily feed intake is 110 grams in white cage hens (KWIN, 2018–2019), 118 in brown barn hens (KWIN, 2018-2019), 121 and 125 in brown free-range hens (KWIN, 2018–2019; Yilmaz-Dikmen et al., 2016) and 126 and 131 gram in brown organic hens (KWIN, 2018–2019; Hermansen et al., 2004). Feed conversion increases from 1.99 kg feed/kg egg in battery cages via 2.28 in barn systems and 2.33 in free-range systems to 2.59 in organic systems (Dekker et al., 2011). Feed conversion can be decreased by choosing other genotypes, i.e. white layers instead of brown layers. However, white layers differ in their ranging behaviour from brown layers; white layers use the range less compared to brown layers (Mahboub et al., 2004; chapter 3). White hens may be less ideal from an animal welfare perspective. Feed conversion can also be decreased relatively by a different feed composition, for example by designing a more circular food system. In such a system, the role of laying hens would be to eat feed ingredients that are unsuitable or undesirable for human consumption (van Hal et al., 2019; van Hal, 2020). However, it is not clear yet which consequences such changes in animal feed have for animal welfare (Meijboom et al., 2021).

Furthermore, agricultural land in the Netherlands is limited and associated with it, land prices are (after Malta) the highest in Europe (Eurostat, 2021). Currently in the Netherlands, there is already more than 3700 hectares (37 km^2) of free range available for the 9.2 million organic/free-range laying hens (AVINED, 2021), each of whom has 4 m^2 to her disposal. If all 32.4 million laying hens would get 4 m^2 of free range to her disposal, then appr. 13,000 hectares (130 km^2) are needed. Within the light of scarce land and high land prices, this is not feasible. A way to cope with this, would be by combining land use functions. A free range for chickens could at the same time be used for ecosystem services as livestock feed production (permanent grassland, low density maize cultivation), human food production (fruit, nuts), carbon sequestration (trees), wood production (trees), tree nurseries (young trees intended for planting elsewhere), litter production (miscanthus) or energy production (solar panels, miscanthus). More research is needed to match the combinations of free range with the other examples of land use practically and economically or to even create synergies, as some authors suggest. Hermansen et al. (2004) suggest to keep poultry in orchards to reduce pests. Broilers kept in an apple orchard are related to a significantly reduction of sawflies (Pedersen et al., 2002 cited in Hermansen et al., 2004). The presence of chickens and geese in an apple orchard is related to fewer insect pests (Clark and Gage, 1996, cited in Hermansen et al., 2004). Sheep and laying hens grazing in a grain and pasture rotation system is suggested as a measure to reduce weed, eaten by the sheep, and their seeds, eaten by the hens (Miao et al., 2006). However, even when combined with other functions, 13,000 hectares of free range would be a lot.

There are locations where the realization of (large-scale) free ranges is not feasible. For example, in areas close to large waterways, known to be risky from the perspective of avian influenza (Bouwstra et al., 2017). Moreover, the periods of confinement due to increased detections in both wild birds and poultry, may last very long and strongly limit the welfare potential of free ranges. The last period of confinement in the Netherlands lasted eight months, from October 2020 (NVWA, 2021) to July 2021 (Rijksoverheid, 2021). Too much emphasis on the outdoor part of a production system for animal welfare is risky. The indoor part of the production system needs to be sufficient for animal welfare too, possibly with additional measures in times of confinement.

In the past there were incidents with dioxin residues in eggs from free-range or organic hens. Soil contamination after big fires or burning waste seems to be important causes (Kan, 2005). Since then dioxin levels in eggs decreased (EFSA, 2012), probably because of measures taken and because a more intensified monitoring became part of the quality schemes, especially in organic and free-range eggs (IKB Ei, 2021). Because hens may ingest soil while foraging outside (Kan, 2005), residues in soil are a point of attention on new locations for egg production.

The above-mentioned considerations regarding (other than animal welfare) sustainability aspects of free ranges, indicate that there are restrictions on offering all 32.4 million Dutch laying hens a free range, let alone other poultry species. There are, so to say, several limits to the growth of the total area in use as poultry free range. Some of the drawbacks can be overcome with mobile poultry housing, innovative production systems with covered 'free ranges', such as Kipster (www.kipster.farm/) or Rondeel (www.rondeeleieren.nl/) or higher outdoor stocking density than the current 4 m^2 per animal. Even these approaches ultimately have limits to growth. This touches on the question regarding the sustainable size of livestock production. Besides what is feasible from a geographical and land price perspective, it is relevant to question what is necessary for human food production. From different perspectives, i.e. human health and the impact of food production on the environment and ecosystems, it is emphasized that switching to a more plant-based human diet is inevitable (Tilman and Clark, 2014; Muller et al., 2017; Poux and Aubert, 2018; de Boer et al., 2020).

The answer to the question in the title of this paragraph, 'a free range for every laying hen?' is 'yes' from an animal welfare perspective. However, when taking into account the above-mentioned considerations on other sustainability aspects, it does not seem possible to provide free-range access to the total number of hens that is currently kept.

7.5 Conclusions

Provision of daily access to a free-range area is related to less feather pecking damage and less peck wounds in organic laying hens. Because feather pecking and its damage are an indicator of reduced welfare in both actor and victim, this means that increased range use is associated with better welfare. Range use is higher in mixed, brown or silver (compared to white) genotypes, in smaller flocks, in flocks with a better plumage condition, in flocks that were less fearful, that were kept in barns with natural ventilation (compared to a combination of natural and mechanical ventilation), with more daylight and that are reared on the laying farm.

Fewer wild 'avian influenza high-risk birds' (i.e. wild ducks, geese, etc.), are seen in free ranges with at least 8% of their surface covered with trees or bushes, compared to free ranges without such cover. Moreover, when located in half-open landscapes, fewer avian influenza high-risk birds are seen in the surroundings of free ranges, compared to free ranges in open landscapes.

Mortality among organic/free-range laying hens due to both avian predators and foxes, is estimated to be 3.7% and total mortality (including also disease and accidents) to be 12.2%. Daytime attacks are mainly done by Northern goshawk (*Accipiter gentilis*) and Common buzzard (*Buteo buteo*). The attacked hens do not show symptoms of disease or weakness prior to the attack. Thus they are assumed to be healthy and thus productive. Combined with average key figures, yield losses per flock due to predation are estimated to be EUR 5,700 on an average organic farm and EUR 6,700 on an average free-range farm, compared to culling at on average 80 weeks of age.

Infections with the intestinal parasites *A. galli*, *H. gallinarum* and *Capillaria*, are widely present in faecal samples from organic laying hens in Sweden, the Netherlands and Italy, including samples from flocks treated repeatedly with anthelmintics. No relationships are found or could not be found between indicators of range use and parasite eggs in faeces. No relationships are found or could not be found between parasite eggs in faeces and hen health, as rated by the farmer, laying percentage and cumulative mortality till 60 weeks of age. Ascarid eggs are not or in very low quantities found in soil. *Capillaria* eggs are not found in soil in Sweden and the Netherlands, while the majority of Italian soil samples do contain *Capillaria* eggs. The low number of ascarid eggs and, depending on the region, of *Capillaria* eggs in soil of free ranges suggest to focus further investigations on the conditions inside the hen house rather than in the free range. Regional differences should be taken into account.

Overall, this thesis supports the assumption that a free range contributes to the welfare of laying hens. This is most clear for the 'Naturalness' part of animal welfare. For the 'Affective states' and 'Physical health & functioning' parts, inconclusive or detrimental relationships are found. Concerning some risks for animal welfare, directions are given for how to address them in terms of practical recommendations or further research.

Addendum

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Summary

Laying hens in organic and free-range production systems are given access to a free range because it is considered to contribute to their welfare. Compared to an indoor environment, a free range provides more opportunities to perform natural behaviours such as foraging, eating plants, insects and worms, dust and sun bathing. Outside also more space and fresh air are available. Consumers are willing to pay a higher price for free-range and organic eggs, compared to barn eggs. This resulted in an increase of organic and free-range laying hens in the EU, Australia and the USA. In the Netherlands the number of hens with access to a free range increased from 5.0 million in 2005 to 9.2 million in 2020.

The overall objective of this thesis was to gain insight into the opportunities and risks of a free range for the welfare of laying hens, with the ultimate aim of optimizing hen welfare including health.

Welfare aspects of free ranges explained using three key elements
Overall animal welfare consists of three key elements: 1) physical health & functioning, 2) naturalness and 3) affective states. 'Physical health & functioning' implies that animals are in good welfare if they are in good health, have sufficient space to take different poses and are free from thirst, hunger, discomfort and pain. Furthermore, they should be free to express normal behaviour and be free from fear and distress. 'Naturalness' implies that being able to live and behave like their ancestors do in nature, safeguards welfare, for example because of the presence of natural elements. Some natural behaviours are considered 'behavioural needs'. Not being able to perform such needs can result in signs of reduced welfare, such as feather pecking, an increased risk of pathology and/or a hormonal profile consistent with stress. Examples of behavioural needs for laying hens are foraging, nest-building prior to egg-laying, dustbathing and preening. 'Affective states' implies that animals have emotions, moods and sensations. They can be pleasant or unpleasant and they can differ in their level of activation or arousal. A free range can have a positive, a negative or no (conclusive) effect on the different key elements. Overall animal welfare can only be safeguarded if positive conditions are reached in all three key elements.

This thesis contains studies into the relationship between range use and feather pecking damage (chapter 2), factors related to range use (chapter 3), presence of `avian influenza risk birds' in the free range (chapter 4), predation of laying hens (chapter 5) and intestinal parasites (chapter 6). These topics can be classified according to the three key elements of animal welfare: naturalness (feather pecking damage and predation), physical health & functioning (avian influenza and intestinal parasites) and affective states (range use).

Risk factors for feather pecking

Chapter 2 is a study based on 107 organic flocks in the Netherlands and 7 other European countries. This study shows that if a free range is part of the production system, daily access (compared to less frequent access), contributes to the welfare of laying hens in terms of less feather pecking damage and less pecking wounds. Such a relationship, namely less feather pecking in case of increased (opportunities for) range use, is found in almost all studies that looked at this relationship. The welfare of victims of feather pecking and of the peckers themselves is worse than that of hens with an intact plumage or hens not showing feather pecking. Since less feather pecking (damage) is found in flocks that have more range access, it can be concluded that increased range use is associated with better welfare.

Factors related to range use

Chapter 3 is a study based on 169 Dutch and Swiss organic and free-range flocks. The study describes that the mean proportion of hens of a flock using the range, as estimated by the farmer, is 47% and varies between the four subsets (Dutch/Swiss and Organic/Free-range) from 23 (Dutch free-range) to 57% (Swiss organic). The results also show that a higher proportion of the flock is seen out in case of brown genotypes (compared to white, silver or two or more genotypes in one flock), when kept in smaller flocks, if roosters are kept with the hens, in case of natural ventilation (compared to combined natural and mechanical ventilation) and in flocks with better plumage condition. Results per subgroup classified per production system (organic or free-range) and country (Netherlands or Switzerland), show a higher range use in flocks that contain more than one genotype, are reared on the laying farm (compared to rearing on a separate farm), have more daylight inside the house and are less afraid of the observers. Factors related to the physical environment (rearing location, amount of daylight, type of ventilation) or to the composition of the flock (in terms of genotype, number of hens, presence of roosters) are the direct result of a famer's choice; they can be influenced by the farmer. When considering the variety between flocks in range use and the potential to increase range use, one can conclude that the opportunities of free ranges for animal welfare may be greater than currently thought.

Avian influenza risk birds in relation to woody vegetation in free ranges

Chapter 4 is a study based on 11 Dutch organic or free-range farms. It shows that in free ranges with at least 8% of their surface covered with woody vegetation, fewer wild birds, from species known to pose a risk regarding avian influenza, are seen, compared to free ranges with no woody cover at all. In case of a half-open landscape, i.e. with woody vegetation, also fewer wild birds are seen in the close surroundings of the free range, compared to open landscapes. The planting of trees or bushes may be part of the poultry farmers' toolbox against avian influenza risk birds visiting the free range.

An important aspect that needs further investigation, is whether the risk of contact between a hen and a wild bird (or its droppings) changes with increasing woody vegetation. If more hens of a flock use the free range for a longer time period per day, there might be more contact moments with wild birds or with their droppings, even if these wild birds use the free range in smaller numbers.

Predation of hens in free ranges

Chapter 5 is a study based on observations on 11 Dutch organic and free-range farms. It shows that 32 out of 44 (73%) hens found dead during observations are suspected to be killed by a bird of prey and 4 (9%) by a fox. In 109 out of 141 sightings (77%) common buzzards are seen in or close to the free ranges. Live observations and video recordings show that both common buzzard and northern goshawk attack and kill hens. These hens are assumed to be healthy and thus productive prior to the attack. Being productive is relevant for determining the economic aspects of predation. An additional survey among 27 organic/free-range farmers shows that per flock on average they lose 3.7% of the hens to predation, while total mortality (mainly caused by diseases) is 12.2% on average. Economic losses per flock (in production for appr. 60 weeks) are calculated to be EUR 5700 on an average organic farm (12,700 hens) and EUR 6,700 on an average free-range farm (25,000 hens). A general course of events in 16 attacks reported in detail, is that most victim hens first drop down and then try to resist the bird of prey. Bystander hens or roosters, if present, try to chase away the bird of prey. After a hen is caught, bystander hens are seen standing within a few meters from the eating bird of prey. Some of these bystander hens are seen cannibalising their dead flock mate as soon as the bird of prey leaves.

Intestinal parasites and range use

Chapter 6 is a study based on 40 organic flocks in the Netherlands, Sweden and Italy. It investigates the presence of the intestinal parasites *Ascaridia galli*, *Heterakis gallinarum* (hereafter called 'ascarids') and *Capillaria*. This study shows that, except for *Capillaria* in Italy, only very few or no eggs of the most common intestinal parasites are found in soil samples from free ranges, even when in use for 15 years or longer. This suggests that the conditions inside the hen house may be more relevant to consider for measures against accumulation of parasite eggs than conditions in the free range, at least for ascarids and for *Capillaria* in Sweden and the Netherlands. No relationship is found between parasite infections and several indicators of range use: number of weeks the hens have uninterrupted access to the free range, proportion of hens using the free range and the number of years the free range being in use as such. Furthermore, no relationship is found between infection with parasites and health, mortality and production parameters. Flocks in the only country with widespread use of anthelmintics (the Netherlands), do not score better on intestinal parasite infections than flocks in countries where no such treatments are used. Free-range use does not seem to be a (major) risk factor for ascarid infections and, depending on the region also not for Capillaria infections. It can be questioned how effective the current regular use of anthelmintic treatments is. The standard deviation of the number of parasite eggs per gram faeces often surpasses the mean value. This demands a larger sample size than our study provided.

Conclusions

From this thesis is can be concluded that daily access to a free-range area is related to less feather pecking damage and less pecking wounds (topic categorized in the 'Naturalness' part of animal welfare). Because feather pecking and cannibalism are indicators of reduced welfare in both actor and victim, this means that increased range use is associated with better welfare. Flocks differ in the proportion of hens using the free range (topic categorized in the 'Affective states' part of animal welfare). This was related to genotype, flock size, roosters, provision of daylight. Fewer avian influenza risk birds are seen in free ranges with at least 8% of the surface covered with trees or bushes (topic categorized in the 'Physical health & functioning' part of animal welfare). Mortality caused by avian predators and possibly foxes ranges from 4 to 12% (topic categorized in the 'Naturalnes' part of animal welfare). Infections with intestinal parasites are widely present, but no relationship could be found with range use (topic categorized in the 'Physical health & functioning' part of animal welfare). Overall, this thesis supports the assumption that a free range contributes to the welfare of laying hens, especially for the 'Naturalness' part of animal welfare. Concerning the investigated risks for animal welfare, directions are given for how to address them in terms of practical recommendations or further research.

Samenvatting

Biologische en vrije uitloopkippen krijgen uitloop naar buiten omdat dit naar verwachting bijdraagt aan hun welzijn. In vergelijking met een binnen-omgeving biedt een uitloop meer mogelijkheden voor natuurlijk gedrag, zoals foerageren, het eten van groen, insecten en wormen en voor stof- en zonnebaden. Ook is er meer ruimte en frisse lucht. Consumenten zijn bereid een hogere prijs te betalen voor eieren van vrije uitloop en biologische kippen dan voor scharreleieren. Dit leidde tot een toename van biologische en vrije uitloop leghennen in Nederland van 5,0 miljoen in 2005 naar 9,2 miljoen in 2020.

Het doel van dit proefschrift was om meer inzicht te krijgen in de mogelijkheden en risico's van vrije uitloop voor het welzijn van leghennen, met als uiteindelijk doel het optimaliseren van dierenwelzijn en -gezondheid.

Welzijnsaspecten van uitlopen verklaard aan de hand van drie benaderingen

Dierenwelzijn bestaat uit drie onderdelen: 1) fysieke gezondheid & algemeen functioneren, 2) natuurlijkheid en 3) emoties & gemoedstoestand. 'Fysieke gezondheid & algemeen functioneren' houdt in dat voor een goed welzijn een goede gezondheid nodig is, voldoende bewegingsruimte en dieren mogen geen dorst, honger, ongemak of pijn ervaren. Ook zouden dieren hun normale gedrag moeten kunnen vertonen en geen angst of paniek ervaren. 'Natuurlijkheid' houdt in, dat voor het waarborgen van een goed welzijn, het nodig is dat dieren kunnen leven en zich gedragen zoals hun voorouders dat doen in de natuur, bijvoorbeeld door de aanwezigheid van natuurlijke elementen. Sommige natuurlijke gedragingen worden beschouwd als 'essentiële gedragsbehoeften'. Als een dier dergelijke gedragingen niet kan uitvoeren, leidt dat tot tekenen van verminderd welzijn, zoals verenpikken, een hogere kans op gezondheidsproblemen en/of een hormonaal profiel dat kenmerkend is voor stress. Voorbeelden van essentiële gedragsbehoeften bij leghennen zijn foerageren, een nest maken voor het leggen van eieren, stofbaden en verenpoetsen. 'Emoties & gemoedstoestanden' houdt in dat dieren emoties, gemoedstoestanden en sensaties hebben. Die kunnen aangenaam of onaangenaam zijn en ze verschillen in hun mate van heftigheid. Een vrije uitloop kan een positieve, een negatieve of geen (eenduidig) effect hebben op de bovengenoemde onderdelen van dierenwelzijn. Voor het waarborgen van dierenwelzijn is op alle drie de onderdelen een positief resultaat nodig.

Dit proefschrift bevat onderzoeken naar de relatie tussen uitloopgebruik en verenpikschade (hoofdstuk 2), factoren gerelateerd aan uitloopgebruik (hoofdstuk 3), aanwezigheid van wilde vogels in de uitloop die een risico vormen voor vogelgriep (hoofdstuk 4), predatie van leghennen (hoofdstuk 5) en darmparasieten (hoofdstuk 6). Deze onderwerpen kunnen worden ingedeeld in de drie onderdelen van dieren-

welzijn: natuurlijkheid (verenpikschade en predatie), fysieke gezondheid & algemeen functioneren (vogelgriep en darmparasieten) en emoties & gemoedstoestanden (uitloopgebruik). Een vrije uitloop kan een positief, een negatief of geen effect hebben op deze onderwerpen.

Risicofactoren voor verenpikken

Hoofdstuk 2 beschrijft een onderzoek aan 107 biologische koppels in Nederland en 7 andere Europese landen. Dit onderzoek toont aan dat dagelijks toegang tot de uitloop gerelateerd is aan minder verenpikschade en minder pikwonden. Een dergelijk verband, namelijk minder verenpikken bij meer (mogelijkheden voor) uitloopgebruik, is gevonden in bijna alle onderzoeken die dit verband bekeken hebben. Verenpikken en verenpikschade zijn tekenen van minder welzijn bij resp. daders en slachtoffers. Aangezien er minder verenpikschade is gevonden in koppels die vaker naar buiten kunnen, is de conclusie dat meer uitloopgebruik gerelateerd is aan een beter dierenwelzijn.

Factoren die uitloopgebruik beïnvloeden

Hoofdstuk 3 beschrijft een onderzoek aan 169 Nederlandse en Zwitserse biologische en vrije uitloop koppels. Het laat zien dat, naar schatting door de pluimveehouders, gemiddeld hooguit 47% van de kippen uit een koppel naar buiten gaat. Dit varieert tussen de vier subgroepen van 23% bij Nederlandse vrije uitloop tot 57% bij Zwitserse biologische koppels. De resultaten van alle koppels samen laten zien dat een groter deel van de koppel naar buiten gaat in geval van bruine hennen (vergeleken met witte of gemengde koppels), kleinere koppels, met hanen erbij, in stallen met natuurlijke ventilatie (vergeleken met een combinatie van natuurlijke en mechanische ventilatie) en bij koppels met een beter verenkleed. Bij indeling o.b.v. productiesysteem en land, wordt een hoger uitloopgebruik gezien in gemengde koppels, bij opfok op het legbedrijf (vergeleken met opfok op een opfokbedrijf), bij meer daglicht in de stal en als de kippen minder bang zijn voor de onderzoekers. Het merendeel van deze factoren is te beïnvloeden door de pluimveehouder. De conclusie is dat maatregelen het uitloopgebruik van veel koppels kunnen verbeteren en daarmee ook het welzijn van de kippen. De potentie van een uitloop voor dierenwelzijn is mogelijk groter dan wordt gedacht.

'Vogelgrieprisicovogels' in relatie tot houtige vegetatie in de uitloop

Hoofdstuk 4 beschrijft een onderzoek op 11 biologische en vrije uitloopbedrijven. Het laat zien dat in uitlopen, waarvan tenminste 8% van het oppervlak begroeid is met houtige beplanting (bomen, struiken of miscanthus), minder vogels worden gezien van soorten die een risico vormen voor vogelgriep. Dit in vergelijking met uitlopen zonder houtige beplanting. In halfopen landschappen, bijv. met houtwallen of -singels, worden ook minder risicovogels gezien in de nabije omgeving van kippenuitlopen, dan in open landschappen. Het planten van bomen of struiken past in de set maatregelen van pluimveehouders tegen aanwezigheid van risicovogels in de vrije uitloop. Overigens is nader onderzoek nodig naar de kans op contact tussen een kip en een wilde vogel (of zijn uitwerpselen) bij een toename van de houtige beplanting in uitlopen. Immers, als de hennen hun uitloop beter gaan gebruiken, kunnen er meer contactmomenten ontstaan met wilde vogels of met hun uitwerpselen, ook als die wilde vogels de uitloop minder gebruiken.

Predatie van hennen in de uitloop
Hoofdstuk 5 beschrijft een onderzoek op 11 biologische en vrije uitloopbedrijven. Het laat zien dat 32 van de 44 (73%) dood gevonden kippen, vermoedelijk gedood is door een roofvogel en 4 (9%) door een vos. Bij 109 van de 141 waarnemingen (77%) zijn buizerds gezien in of bij de uitloop. Live-observaties en video-opnames laten zien dat zowel de buizerd als de havik kippen aanvalt en doodt. De kippen zijn voorafgaand aan de aanval `op het oog' gezond en dus productief. Dat ze nog productief zijn, is een belangrijk aspect voor het bepalen van de economische schade door predatie. Uit een aanvullende enquête onder 27 biologische/vrije uitloop pluimveehouders blijkt dat per koppel gemiddeld 3,7% van de hennen verloren gaat door predatie, op een totale sterfte (verder voornamelijk veroorzaakt door ziekten) van 12,2%. De economische schade per koppel, dat ca. 60 weken in productie is, is berekend op 5700 euro voor een gemiddeld biologisch bedrijf (12.700 hennen) en op 6.700 euro voor een gemiddeld vrije uitloopbedrijf (25.000 hennen). Een algemeen verloop bij 16 beschreven aanvallen is dat de meeste slachtoffers eerst neervallen en zich vervolgens proberen te verzetten tegen de roofvogel. Omstanderkippen of hanen, indien aanwezig, proberen de roofvogel weg te jagen. Nadat een hen gevangen is, staan omstanderkippen binnen enkele meters van de etende roofvogel. Van sommige van deze omstanderkippen is gezien dat ze van de slachtoffers eten nadat de roofvogel vertrokken is.

Darmparasieten en uitloopgebruik
Hoofdstuk 6 beschrijft een onderzoek aan 40 biologische koppels in Nederland, Zweden en Italië. De onderzochte parasieten zijn grote en kleine spoelworm en haarworm. Het onderzoek laat zien dat er, met uitzondering van haarworm in Italië, nauwelijks of geen wormeitjes in de grond van kippenuitlopen aanwezig zijn. Zelfs niet als die 15 jaar of langer in gebruik zijn. Dit pleit ervoor, in ieder geval voor spoelworm en voor haarworm in Zweden en Nederland, om verder onderzoek naar maatregelen tegen wormeitjes te richten op de omstandigheden in de stal, i.p.v. in de vrije uitloop. Er is geen relatie gevonden tussen worminfectie en de volgende indicatoren voor uitloopgebruik: aantal weken dat de hennen ononderbroken toegang hebben tot de uitloop, het percentage hennen van een koppel dat de uitloop gebruikt en het aantal jaren dat de uitloop als zodanig in gebruik is. Er is geen relatie gevonden tussen worminfectie en gezondheid,

uitval en productie. Nederlandse koppels, de enige koppels die ontwormd werden in deze studie, scoren niet beter qua worminfecties dan de Zweedse en Italiaanse koppels, die geen van allen ontwormd werden. Uitloopgebruik lijkt geen grote risicofactor voor infecties met spoelworm en afhankelijk van de regio, ook niet met haarworm. Het is de vraag hoe effectief het huidige gebruik van ontwormingsmiddelen is. Overigens is de variatie in wormeitjes per gram tussen mestmonsters vaak groter dan het gemiddelde. Dat vereist een veel grotere steekproef van bedrijven dan die in dit onderzoek.

Conclusies

Op basis van dit proefschrift kan worden geconcludeerd dat dagelijkse toegang tot een uitloop gerelateerd is aan minder verenpikschade en minder pikwonden (onderdeel 'Natuurlijkheid' van dierenwelzijn). Dit zijn indicatoren van verminderd welzijn bij zowel de pikker als het slachtoffer. Dit betekent dat een hoger uitloopgebruik gerelateerd is aan een beter welzijn. Koppels verschillen in hun mate van uitloopgebruik (onderdeel 'Emoties & gemoedstoestanden' van dierenwelzijn). Dit heeft o.a. te maken met genotype, koppelgrootte, hanen en hoeveelheid daglicht in de stal. Er worden minder risicovogels gezien in uitlopen waarvan ten minste 8% van het oppervlak begroeid is met bomen of struiken (onderdeel 'Fysieke gezondheid & algemeen functioneren' van dierenwelzijn). De uitval door roofvogels en mogelijk vossen, varieert van 4 tot 12% (onderdeel 'Natuurlijkheid' van dierenwelzijn). Infecties met darmparasieten zijn wijdverbreid aanwezig, maar een relatie met uitloopgebruik kan niet worden aangetoond (onderdeel 'Fysieke gezondheid & algemeen functioneren' van dierenwelzijn). Over het algemeen ondersteunt dit proefschrift de aanname dat uitloopgebruik bijdraagt aan het welzijn van leghennen, vooral aan het onderdeel 'Natuurlijkheid'. Voor de onderzochte risico's voor dierenwelzijn worden praktische aanbevelingen gedaan of suggesties voor vervolgonderzoek.

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About the author

Monique Bestman was born in 1970 in Bruinisse, the Netherlands. Because of her interest in animals she studied biology at Wageningen University (1988–1996). By chosing the 'free orientation' she was able to follow courses about behaviour and welfare of captive animals, life and ecology of wild animals and even human psychology. In 1999 she started at the Louis Bolk Institute on a project on feather pecking in organic laying hens. From then on she did research and demonstration projects on welfare and health aspects of organic and free-range laying hens, sometimes including also the rearing period. All investigations were done on commercial farms, which helped to learn from farmers and share the results with them. So they could apply the results in the care for their animals. Some of the projects were carried out in cooperation with other national and international institutes and universities. The international projects made it possible to visit several farms in other countries. After about 10 years of doing nearly only chicken projects, she gradually broadened her work and started doing projects on agroforestry (mainly trees in poultry free ranges), welfare of dairy cows and even paludiculture crops and agrobiodiversity (the latter because of the combination of nice field work and societal relevance). For the duration of her PhD trajectory (2020–2022) she had a guest agreement with the Faculty of Veterinary Medicine of the Utrecht University. Her strongest motivation in her working life is improving and safeguarding the welfare of animals.

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Monique Bestman is also (co-)author of conference papers, brochures, reports and articles in farmers journals.

For an actual list, see https://www.louisbolk.nl/over-ons/medewerkers/monique-wp-bestman.

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