Universidade de Trás-os-Montes e Alto Douro

WELFARE OF POULTRY

Assessment of welfare indicators along the food chain

Ph.D. Thesis in Veterinary Sciences

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The statements presented in this thesis
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ABSTRACT

Several animal welfare indicators have been developed in research projects for farming industry to be assessed on various stages of production, transport and slaughter. For example, the Welfare Quality® protocols for layers and broilers include clinical scoring measures to be used on broilers and laying hens on farms and measures to be used at broilers' slaughterhouses. This assessment system was the basis for the conception and development of other studies.

Welfare assessment at slaughter has the potential to greatly improve welfare of broilers and laying hens at the farms. Slaughterhouses have been considered a relevant source of data to monitor animal health and welfare conditions.

With the present work we: i) propose strategies designed to minimize the effects of some of pre-slaughter factors impacting upon the welfare of broilers during transport; ii) study the relation between different welfare indicators collected at the slaughterhouse, and establish the most adequate welfare indicators according flocks of different average body weight (BW); iii) determine the effects of body weight and age on fear and welfare indicators and the relationships between fear and welfare indicators in laying hens from two similar barn systems; iv) investigate the effect of three housing system (furnished cages, barns and free range) on the prevalence, severity and morphology of keel bone deformations/fractures and on the prevalence and severity of keel bone protrusion; v) determine the prevalence of dead on arrival (DoA) birds and of carcass condemnation causes in end-of-lay hens flocks and investigate the effects of age, BW and housing system.

The welfare indicators collected at the broilers slaughterhouse included DoA, presence and locations of bruises and dehydrated carcasses. DoA rate increases with transport distance, the catching of birds after midnight and with longer lairage durations for birds caught after midnight, suggesting that short transport distances, catching the birds before midnight and doing the transport by night are advantageous.

Comparing flocks of broilers at the slaughterhouse accordingly to average BW, it was shown that absence of hock burns was more common in lighter flocks, mild hock burns and mild footpad dermatitis were more common in medium weight flocks, and severe hock burns and breast ulcer were more prevalent in heavier flocks.

Statistical models were performed to study in laying hens the influence of BW, age and welfare indicators on tonic immobility (TI) duration and number of TI inductions. It was

shown that the increase in BW, presence of skin injuries, high back, head and tail feather scores had impact in the increase of fear response.
During the analysis of condemnation causes for a laying hens it was observed that ascites and peritonitis lesions increased with hens' age, while emaciation and septicaemia were observed more frequently in younger hens. Regarding BW, it was shown that DoA birds, emaciation, and septicaemia were more prevalent in lighter hens, which can be related to the presence of infectious agents or poor management procedures that may lead to a low growth rate. The type of housing systems influenced the percentage of ascites, peritonitis, salpingitis and total condemnation rates, with hens from cages showing statistical differences from organic systems.

Keywords: poultry, welfare indicators, *post mortem* condemnation, fear, housing systems.

RESUMO

Vários indicadores de bem-estar animal têm sido alvo de estudo em projetos de investigação da indústria agropecuária, com vista à sua avaliação em várias fases de produção, transporte e abate. Por exemplo, os protolocos Welfare Quality® incluíram medidas de pontuação clínicas para aplicação em frangos e galinhas poedeiras na exploração e ainda medidas de avaliação para aplicação em frangos em matadouros. Este sistema de avaliação foi a base para a conceção e desenvolvimento de outros estudos.

A avaliação do bem-estar no abate tem o potencial de melhorar consideravelmente o bem-estar de frangos e galinhas poedeiras. Os matadouros foram considerados uma fonte relevante de dados para monitorizar as condições de saúde e bem-estar dos animais.

Com o presente trabalho, pretende-se: i) propor estratégias destinadas a minimizar os efeitos de alguns fatores pré-abate que afetam o bem-estar de frangos; ii) estudar a relação entre os diferentes indicadores de bem-estar avaliados no matadouro e estabelecer os indicadores de bem-estar mais adequados de acordo com os pesos vivos médios; iii) determinar os efeitos do peso vivo e idade nos indicadores de medo e bem-estar e as relações entre os indicadores de medo e bem-estar em galinhas poedeiras sob dois sistemas similares de produção em solo; iv) investigar o efeito de três sistemas de produção em galinhas poedeiras (gaiolas melhoradas, solo e ar livre) na prevalência, severidade e morfologia das deformações/fraturas ósseas da quilha e na prevalência e gravidade da protrusão óssea da quilha; v) determinar a prevalência de aves mortas à chegada e de causas de rejeição de carcaça em bandos de galinhas de poedeiras e investigar os efeitos da idade, do peso e do sistema de produção.

Os indicadores de bem-estar avaliados em matadouro de frangos incluíram aves mortas à chegada, presença de hematomas e carcaças desidratadas. A taxa de aves mortas à chegada aumentou com o aumento da distância de transporte, a captura de aves após a meia-noite e aumento das aves capturadas após a meia-noite, sugerindo que distâncias de transporte mais curtas, captura das aves antes da meia-noite e transporte noturno poderá ser vantajoso.

Comparando os bandos de frangos de acordo com o peso vivo médio, foi demonstrado que a ausência de queimaduras nas articulações tibiotársicas foi mais frequente em bandos com pesos inferiores, pododermatites e queimaduras na articulação tibiotársica ligeiras foram mais frequentes em bandos de peso vivo intermédio, e queimaduras graves nas articulações tibiotársicas e a presença de úlceras foram mais frequentes em bandos de frangos mais pesados.

Modelos estatísticos foram concebidos para estudar em galinhas poedeiras a influência do peso vivo médio, idade e bem-estar na duração da imobilidade tónica e no número de induções de TI. Foi demonstrado que o aumento do peso, presença de lesões de pele, perda de penas de elevado grau nas costas, cabeça e cauda teve impacto no aumento da resposta ao medo.

Durante a análise das causas de rejeição em galinhas poedeiras em matadouro verificou-se que a ascite e peritonite aumentaram com a idade, enquanto emaciação e septicémia foram observadas com maior frequência em galinhas mais jovens. Em relação ao peso, verificou-se uma maior frequência de aves mortas à chegada, assim como caquexia ou septicémia, em galinhas com peso vivo inferior, o que pode estar relacionado com a presença de agentes infeciosos ou mau maneio que pode levar a uma baixa taxa de crescimento. O efeito do sistema de produção nas rejeições foi significativo para ascite, peritonite e salpingite e taxa de rejeição total, com as galinhas provenientes de gaiolas a apresentar diferenças estatísticas relativamente aos sistemas de produção em modo biológico.

Palavras-chave: aves, indicadores de bem-estar, rejeição *post mortem*, medo, sistemas de produção.

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List of abbreviations

AIC - Akaike Information Criterion
B - Barns
BW - Body weight
CI - Confidence interval
CM - Communality
DF - Dirty feathers
DoA - Dead on arrival
EU - European Union
FAWC - Farm Animal Welfare Council
FC - Furnished cages
FL - Factor loadings
FPD - Footpad dermatitis
FR - Free range
GATT - General Agreement on Tariffs and Trade
GzLM - Generelized linear models
HB - Hock burns
KMO - Kaiser-Meyer-Olkin
MS - Member State
TI - Tonic immobility
WTO - World Trade Organization
OR - Odds ratio
PC - Principal component
QBA - Qualitative Behavioural Assessment
s - second
SD - Standard deviation
KMO - Kaiser-Meyer-Olkin
Wk - Week
χ^2 - Pearson's chi-squared test

CHAPTER I – INTRODUCTION

INTRODUCTION

Chickens (*Gallus gallus domesticus*) are used for both egg and meat production. Eggs are a primary source of animal protein in both developed and developing countries, and the European Union is the second largest producer of eggs in the world, behind China (Mench et al., 2011). In 2007, global egg production consisted of 65 tonnes of eggs (IEC, 2007). Current global production of eggs is approximately 101 tonnes and of broiler chickens is approaching 60 billion birds per year in the meat industry (FAOSTAT, 2017).

In European countries, with progressing industrialisation and urbanization, discussions about animal welfare have increased. In the middle of the last century most of the traditional livestock production systems were subjected to dramatic changes, particularly evident in laying hens (Fraser, 2005). Within a few years the prevailing small scale free range systems were replaced by large scale industrial battery cages. Consequently, caged laying hens became the focal point of the animal welfare debate (Harrison, 1964; Brambell, 1965). It ultimately led to sweeping legislative changes in the EU affecting multiple facets of farm animal production, including the housing systems of laying hens.

The concern for animal welfare, coupled with increasing scientific knowledge of the behavioural and physical needs of farm animals, has been guiding the legislation of several countries on how these animals should be farmed (Fraser, 2006; Veissier et al., 2008; Beaumont et al., 2010; Mench et al., 2011; Hemsworth, 2014). Moreover, labelling programs have been designed to differentiate products according to welfare standards or production methods (Eurobarometer, 2007; Martelli, 2009). In internationally standards agreements, the measures from Terrestrial Code are used by Member Countries as standards requirements for international trade which include for poultry some requirements related with welfare conditions during rearing (OIE, 2018). Welfare of farm animals is also important from the aspect of perception of the quality of product by consumers which consider that preservation of high welfare standards results in higher product quality (Koknaroglu & Akunal, 2013).

Several animal welfare indicators have been developed in research projects for farming industry to be assessed on various stages of production, transport and slaughter (RSPCA, 2008; Welfare Quality®, 2009). For example, the Welfare Quality® protocols for layers and broilers include clinical scoring measures to be used on farm but also measures to be used at broilers' slaughterhouses. This assessment system was the basis for the conception and development of other studies.

Welfare assessment at slaughter has the potential to greatly improve welfare of broilers and laying hens at farm level (Saraiva et al., 2016; Salines et al., 2017). Slaughterhouses have been considered a relevant source of data to monitor animal health and welfare conditions (Stärk et al., 2014; Huneau-Salaün et al., 2015). Numerical scoring systems have been applied at broilers' slaughterhouses to assess welfare conditions that occurred either during transport or on farms (Grandin, 2017). However, for laying hens there are very few published reports concerning the welfare status of flocks using measures collected before and during the slaughter and, therefore more investigation is needed.

Even though different housing systems offer access to perches, dustbathing opportunities and access to a nest box, there are large differences between the environment of alternative systems and the furnished cage environment, particularly in overall complexity (Brantsæter et al., 2016). Thus, with the present studies we tried to define and validate welfare indicators to be easily assessed on laying hens, on farms and at slaughterhouses. Pointing out the advantages and disadvantages of different production systems, could be an assert for future investigation and for the assessment of systems currently authorized for laying hens.

In summary, we suggest that a systematic evaluation of welfare parameters should be implemented for laying hens at the slaughterhouse.

CHAPTER II – SCIENTIFIC BACKGROUND

1. DIFFERENT VIEWS OF ANIMAL WELFARE

Animal welfare science has provided the rationale for diverse approaches in research involving animals (Fraser, 2008). Establishing animal welfare rules should be based on veterinary, ecological, ethical and ethological considerations and successful improvement of animal welfare is dependent on how different actors perceive it (Kauppinen et al., 2010). Furthermore, if efforts to improve animal welfare are to achieve widespread acceptance, they need to strike a balance among the different animal welfare objectives (Fraser, 2009). However, different philosophical views about what constitutes a good life are areas of disagreement among stakeholders.

Different attitudes of consumers (Frewer et al., 2005), veterinarians (Heleski et al., 2005; Sabuncuoglu & Coban, 2008), students (De Boo & Knight, 2005; Heleski & Zanella, 2006) and farmers (Hemsworth, 2003) concerning animal welfare have been discussed in several studies. Regarding production animals, the attitudes of farmers and caregivers have a vital influence on animal welfare, affecting its behaviour, welfare, health and production (Waiblinger et al., 2002; Boivin et al., 2003; Lund et al., 2004).

1.1. Biological functioning

All involved in modern animal production tend to emphasise the biological functioning of the animal as the key criterion for its welfare (Hemsworth et al., 2015). This concept aligns with Broom (1986) point of view, which defines welfare of an animal is "its state as regards its attempts to cope with its environment". Consequently, failure or difficulties in coping are indicators of poor welfare with biological costs to the animal, such as deterioration in growth efficiency, reproduction and health (Broom, 1991, 2000).

Proponents of this view consider that intensive production systems should be viewed as good for animal welfare as long as the animals are growing, producing well and healthy (Broom, 2000; Fraser, 2004). Consequently, more 'natural' systems with lower levels of health, growth and production are not viewed as promoting good welfare accordingly to biological functioning view (Fraser, 2004).

The biological functioning and affective state frameworks were initially seen as competing, but a recent more unified approach is that biological functioning is taken to include affective experiences which are recognised as products of biological functioning, and knowledge of the dynamic interactions between the two is considered fundamental to managing and improving animal welfare (Hemsworth et al., 2015). Since animals use a range of behavioural and physiological responses to assist them in coping with challenges, and because biological regulation in response to challenges should occur continuously, successful adaptation is not always possible (Barnett & Hemsworth, 2009; Hemsworth & Coleman, 2011). Conceptualised in these terms, it is the biological cost of stress that is the key to understanding the associated welfare implications (Moberg, 2000; Barnett, 2003). Therefore, common criticism of this conceptual framework for assessing animal welfare is that it does not adequately include emotions (Hemsworth et al., 2015).

1.2. Affective state

The second conceptual framework emphasises that the welfare of an animal derives from its capacity for affective experiences (Duncan & Fraser, 1997). Affective experiences are common in humanitarian thinking and among some animal welfare scientists whom highlight the ‘affective states’ of animals (Fraser, 2004). According with this view, animals should be allowed to enjoy normal pleasures of life, whether this occurs in intensive or non-intensive systems (Fraser & Duncan, 1998). The welfare state is likely to be positive when the predominant affects experienced spared them from unpleasant affective states as pain, distress and suffering. However, the affective experiences were considered inaccessible to scientific inquiry for many decades (Hemsworth et al., 2015).

In the last quarter of the 20^{th} Century, on one hand a grip of behaviourism slackened, and there was a growth of literature on the topic of feelings (Duncan, 2005). For example, Dawkins (2004) consider that animals may still suffer poor welfare while in good physical health, namely if they are deprived of activities and resources for which they are highly motivated.

Preference and motivation tests allowed making inferences about animal welfare based on the assumption that animals will avoid aversive stimuli and choose positive stimuli, making choices that are in their best interest (Duncan, 2005; Fraser & Nicol, 2011). Other approaches for assessing affective experiences include measures of behaviour, cognitive bias and physiology (Boissy et al., 2007; Mendl et al., 2009; Forkman et al., 2007), as well as employing the intuitive perception of human observers using an approach known as Qualitative Behavioural Assessment (QBA) (Wemelsfelder & Mullan, 2014).

1.3. Natural environment

A third view holds that animals should be allowed to lead reasonably natural lives by carrying out their normal behaviour in a reasonably natural environment, free from undue restraint (Fraser, 2008). This conceptual framework is predicated on the view that the welfare of animals is improved when they can express their normal behaviour and, in this sense, long term confinement of animals should be avoided. Most reviews of welfare nowadays start by listing the needs of animals, including needs to show certain behaviours. The notion that animals should perform their full repertoire of behaviour was common in early welfare research and is still common today, for example in material advocating so-called "welfare-friendly" production systems (Hemsworth & Coleman, 2011). Sophisticated studies has been developed to analyse what is important to animals and has replaced the earlier general guidelines described as freedoms (Broom, 2011). However, the concept of natural is usually too poorly defined to provide a sound basis for animal welfare assessment, and thus when applied uncritically it may lead to poorer welfare instead of an improvement (Mellor, 2015). The pursuit of more natural living conditions would arguably improve animal welfare in some respects but often introduces other problems such as increased exposure to predation and harsh weather (Lay et al., 2011). This view is common among consumers and among many critics of modern animal production (Velde et al., 2002) since they picture pastoral and non-confined systems when they picture farming which promotes good animal welfare (Siegford et al., 2008). Producers would likely prefer to adapt their current model of production to address sustainability and welfare problems while maintaining production yields, but the public prefer to see alternative production models (Petit & van der Werf, 2003). Each of these viewpoints makes valid claims and attracts valid criticisms and sometimes the different views do in fact agree.

People holding one or other of these views often assume that the three go hand in hand (Fraser, 2009). Boissy et al. (2007) considerations provide a context for investigating particular behaviours that may be accompanied by positive feelings. For example, allowing a laying hen to perform dust bathing in a hot day is good for her welfare by the natural living criteria because she can perform her natural behaviour, by the biological functioning criteria because helps prevent heat stress, and by the affective state criteria because the hen will be more comfortable.

Although recognising that neither sentiment nor economic factors can be entirely divorced from welfare, they should never be paramount in its consideration (Fraser, 2004).

2. EVOLUTION OF THE ANIMAL WELFARE CONCEPT

In 1964, Ruth Harrison published the book "Animal Machines" which drew public attention to how farm animals are housed and treated in industrialised agriculture (Harrison, 1964). As a response to this public interest, in 1965, the UK Ministry of Agriculture held an expert committee to look into the welfare of farm animals. The committee, chaired by Professor Brambell, presented a report entitled "Report of the Technical Committee to Enquire into the Welfare of Animals Kept under Intensive Livestock Husbandry Systems", which became known as the Brambell Report (Brambell, 1965). From this report came one of the first definitions of animal welfare: "Welfare is a wide term that embraces both the physical and the mental well-being of the animal". Any attempt to evaluate welfare, therefore, must take into account the scientific evidence available concerning the feelings of animals that can be derived from their structure and functions and also from their behaviour".

After this public exposure, several authors defined animal welfare in a variety of different ways. Hughes (1976) defined welfare as "a state of complete mental and physical health, where the animal is in harmony with its environment", while Carpenter (1980) proposed that "welfare of managed animals relates to the degree to which they can adapt without suffering to the environments designated by man." For Broom (1986) the welfare of an individual is "its state as regards its attempt to cope with its environment".

The majority of welfare definitions comprise physical, physiological and psychological aspects. There are, however, definitions which emphasise only one aspect. Manteca (1998), for example, highlighted another feature of farm animals' welfare regarding its emotional state prior to slaughter: "concern for animal welfare is a major consideration in meat production and is based upon the belief that animals can suffer." Moreover, Dawkins (1990) underlined the subjective feelings, while McGlone (1993) focused on the physiological systems. However, welfare should not be defined solely in terms of subjective experiences (Duncan, 1993) and should comprise all aspects of animal welfare more fully (Appleby & Hughes, 1997).

Welfare can be measured and, consequently, understood as a continuum from positive to negative impressions. This is clearly expressed by Spruijt et al. (2001) which defined welfare as “the positive net balance between positive and negative experiences and poor welfare represents the negative balance.”

The influence of diseases on the welfare conditions is generally acknowledged, however, “health” is explicitly addressed in just a few of the welfare definitions. Rushen (2003) expressed his concern about the underestimation of health aspects in the welfare discussion. Since health problems are closely related to the physical, physiological and psychological conditions of the animals, they are probably understood as a part of the other categories. However, some publications on animal health regularly associate the concepts of “fit” and “feeling good” (Webster et al., 2004) and Dawkins (2004) proposed that the assessment of animals be based on these two concepts.

Welfare definitions have become more complex with the development of scientific knowledge on behaviour, physiology, preferences and motivation of animals. Many scientists have emphasised the subjective feelings of animals as a key component in the scientific investigation of animal welfare (Phillips, 2009). Animals should not suffer from unpleasant mental states such as pain and discomfort which can be caused by presence of diseases, as well as fear and distress during predators’ attacks or antagonistic interactions with conspecifics, promoting further injury (Cockram & Hughes, 2011). It is also important that animals are able to express behaviours that are priorities in a captive environment (Weeks & Nicol, 2006).

Behaviour is the interface between the animal and the aspects of its environment and may therefore be both the source of some problems and a symptom of other problems, such disease (Appleby & Hughes, 1997).

Approach to animals’ feelings and emotions can be done experimentally. With this regard, tests for preferences, fear, avoidance and frustration and operant conditioning techniques, have been used extensively to uncover the emotional state and motivation of animals under welfare-related conditions (Forkman et al., 2007). When considering animal welfare as a whole, it is important to take many different components into consideration more to the physical welfare rather than to the mental to judge whether its welfare is good or bad.

3. EUROPEAN UNION POLICY APPROACH FOR POULTRY WELFARE

The concept of five freedoms originated in the Brambell (1965) report. In this report it is stated that farm animals should have freedom “to stand up, lie down, turn around, groom themselves and stretch their limbs.” The concept was subsequently refined by Farm Animal Welfare Council (FAWC, 1992) so that it actually took the form of five freedoms sometimes referred to as Brambell’s Five Freedoms. In July 1979, the British Government established the FAWC, which started to list the provisions that should be made for farm animals and whilst not mandatory intended to create the best possible standards for the welfare of animals in all systems of livestock husbandry. FAWC also published three reports concerning the welfare of hens: an Assessment of Egg Production Systems (1986); Advice to Ministers on the Handling and Transport of Poultry (1990); and The Welfare of Laying Hens in Colony Systems (1991). The first FAWC statement mentions that an animal's welfare, whether on farm, in transit, at market or at a place of slaughter should be considered in terms of five freedoms (FAWC, 1979). However, these freedoms define ideal states rather than standards for acceptable welfare, and the most commonly used requirements of welfare of any animal kept in captivity were published in 1992 by FAWC, which have come to be known as the “Five Freedoms for Animal Welfare”:

1. Freedom from Hunger and Thirst - by having 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 effective 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 (FAWC, 1992).

The first important attempt to introduce regulations on animal welfare in Europe occurred in 1997 with the implementation of the Amsterdam Treaty. Its goal regarding the protocol on protection and welfare of animals was to ensure improved protection and respect for the welfare of animals as sentient beings (Amsterdam Treaty, 1997).

In 1986 the minimum standards for the protection of laying hens kept in battery cages were established with the adoption of the Council Directive 86/113/EEC. However, the Court of Justice in Case 131/86 referred to minimum standards for the protection of laying hens kept in batter cages led to the annulment of Council Directive 86/113/EEC. In this sense, other measures were required to comply with the judgment of the Court of Justice, and therefore, the Council adopted in 1988 the Directive 88/166/EEC which complied with the decision of the Court of Justice and the provisions of minimum standards for the protection of laying hens kept in battery cages were adopted in the form given in the Annex of the Directive.

On the other hand, Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes, based of the European Convention for the Protection of Animals kept for Farming Purposes, instated Community provisions designed to give effect to the principles laid down in the Convention. These requirements included the provision of housing, food, water and care appropriate to the physiological and ethological needs of the animals. Moreover, article 3 refers that Member States (MS) shall make provision to ensure that the owners and keepers take all reasonable steps to ensure the welfare of animal under their care and to ensure that those animals are not subjected to any unnecessary pain, suffering or injury.

It should also be noted that in 1995, the Standing Committee of the European Convention for the Protection of Animals kept for Farming Purposes adopted a detailed recommendation, which included laying hens. The Scientific Veterinary Committee concluded that the welfare conditions of hens kept in current battery cages and in other systems of rearing were inadequate and that certain needs could not be met in such conditions. The Scientific Veterinary Committee opinion was the support for the Commission report. This report represented the turning point to the current legislation and stated that the highest possible standards should be introduced in order to improve the conditions of hens reared in different housing systems. Thus, Directive 88/166/EEC was repealed with effect from 1 January 2003 and replaced by Council Directive 1999/74/EC establishing that all MS should ensure from 1 January 2002 that all newly built or rebuilt alternative systems, as well as all the enriched cages comply respectively, with requirements from chapter I and III of Directive. Rearing hens in unenriched cage systems referred to in chapter II was prohibited from 2012 onwards. In addition, with effect from 1 January 2003, unenriched cage were built or brought into service for the first time.

Moreover, Regulation (EC) No 854/2004 of the European Parliament and of the Council laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption and Regulation (EC) No 882/2004 of the European Parliament and of the Council on official controls performed to ensure the verification of compliance with feed and food law, animal health and welfare rules already establish a framework for official controls including compliance with some animal welfare rules.

Additionally, slaughterhouses were recognised as a relevant source of data for monitoring welfare conditions of birds (EFSA, 2004) and the Directive 2007/43/EC was the first regulation defining specific welfare parameters to be controlled by the official veterinarian at slaughterhouses. This official assessment was implemented to ensure the protection of broilers during intensive production (Saraiva et al., 2016).

Regarding the transport regulation, the Council Regulation 1/2005 specified that suitable food and water supplies should be available in adequate quantities in the case of a journey lasting more than 12h, although this is hardly feasible for birds. Pre-slaughter factors should also be controlled and measured at the slaughterhouse through animal-based indicators (injuries, haemorrhages, fractures) and dead on arrival prevalence, as well as by evaluating transport and weather conditions (EFSA, 2004).

3.1. Broilers

Farm animal welfare has been a major issue in Europe, which resulted in regulations and development of research dedicated to animal welfare, especially in broilers frequently considered as having very poor welfare (Beaumont et al., 2010).

Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes layed down minimum standards for the protection of animals bred or kept for farming purposes including provisions on housing, food, water and care suitable to the physiological and ethological needs. Specific recommendation concerning domestic fowl (*Gallus gallus*) which includes additional provisions for poultry kept for meat production was adopted within the framework of the convention. Moreover, several reports on the welfare of chickens kept for meat production (broilers) concluded that the fast growth rate of chicken strains currently used for this purpose is not accompanied by a satisfactory level of animal welfare and health, and that the negative effects of high stocking rates are reduced in buildings where good indoor climatic conditions can be sustained (EFSA, 2004; Beaumont et al., 2010).

Further research and practical experience to improve welfare of chickens kept for meat production are objectives of the Commission. Under Directive 2007/43/EC, which provided minimum standards to ensure the protection of broilers during intensive production, the official controls include monitoring and follow-up welfare parameters at slaughterhouses. Following these requirements, and taking into account the results of studies carried out in this field, European level thresholds for some welfare parameters have been defined and can be easily assessed at the slaughterhouse (EFSA, 2004; Saraiva et al., 2016). In the case of stocking densities higher than 33 kg/m^2, the documents accompanying the flock shall include the daily mortality rate and the cumulative daily mortality rate. Data on stocking densities, daily and cumulative mortality, as well as the number of broilers DoA shall be recorded under the supervision of the official veterinarian.

In the context of the controls defined by Regulation (EC) No 854/2004 the official veterinarian shall evaluate the results of the *post mortem* inspection to identify other possible indications of poor welfare conditions such as abnormal levels of contact dermatitis, parasitism and systemic illness in the holding or the unit of the house of the holding of origin (European Union, 2007.).

In case mortality rates and/or the results of the *post mortem* inspection indicates poor animal welfare conditions, the official veterinarian need to communicate the data to the owner or keeper of the animals and to the competent authority. Appropriate actions should be taken by the owner or the keeper of the animals and by the competent authority (European Union, 2007.).

3.2. Laying hens

According to the Directive 98/58/EC, some of requirements cover the inspection of hens for at least once a day and dead hens must be removed every day; constant or sudden noise should be avoided; ventilation fans, feeding machinery or other equipment should be constructed, placed, operated and maintained in such a way that they cause the least possible noise. All buildings should have light levels sufficient to allow all hens to see one another and be seen clearly, to investigate their surroundings visually and to show normal levels of activity. Accommodation comprising two or more tiers of cages must have devices or appropriate measures must be taken to allow inspection of all tiers without difficulty and facilitate the removal of hens. After the first days of conditioning, the lighting regime shall be such as to prevent health and behavioural problems. Accordingly, it must follow a 24-hour rhythm and include an adequate uninterrupted period of darkness lasting, by way of indication, about one third of the day, so that the hens may rest and to avoid problems such as immunodepression and ocular anomalies. In order to prevent feather pecking and cannibalism the member state may authorise beak trimming carried out by qualified staff on chickens intended for laying that are less than 10 days old (European Communities, 1999). Over the years, cages for laying hens have been criticized primarily for the limited space and the lack of opportunities for hens to perform their natural behavior (Odén et al., 2002). Scientific studies have found that hens use more than 450cm² when performing certain normal behaviours. For example, it has been shown that hens use between 1000 and 2000cm² when turning, wing flapping and preening (Lay et al., 2011). On the other hand, studies have demonstrated that providing space at 800-1200cm²/hen in cages may increase levels of aggression (Rodenburg et al., 2008). In response to these welfare concerns, the EU Council Directive 1999/74/EC established that, from 2012 onwards, laying hens may only be housed in either furnished cages or in alternative non-cage systems. Birds in alternative systems are likely to benefit from such factors as more space and access to perches, nest boxes and littered areas (Ali et al., 2016; Lay et al., 2011).

4. OTHER APPROACHES TO POULTRY WELFARE

Several European countries have opted to regulate farm animal protection through their own legislation (Veissier et al., 2008). However, various animal welfare assurance programs are being used to encourage the adoption of animal welfare standards in food production (Fraser, 2006; Sørensen & Fraser, 2010). Welfare Quality® was the largest European research project on animal welfare. The “Welfare Quality®” project aims for the production of reliable schemes by including animal-based measures which indicate direct effects on animals (Veissier et al., 2008). In this project the principles and criteria of good welfare were defined. Indicators, primarily animal-based measures, for each welfare criterion were developed and then integrated in an overall assessment model. Other options include internationally agreed standards (OIE, 2010) and certification schemes designed to distinguish products according to welfare standards or production methods (Martelli, 2009).

General agreement on tariffs and trade (GATT) and world trade organization (WTO) arrangements should be established to prevent imports of eggs and egg products into the European Union (EU) from countries in which conventional battery cages are still in use.

5. APPLYING SCIENCE TO ANIMAL WELFARE STANDARDS

Welfare refers to the state of an animal, and therefore we should be able to use measurements of that state to grade welfare (Broom, 1991). Many aspects of an individual's biology can reflect its attempts to cope with its environment, because there are various ways of trying to cope as well as numerous indicators of failure to cope (Broom & Johnson, 1993). In practice, measurements of poor welfare are more common than those of good welfare, since poor welfare is associated with more obvious behavioural, physiological and pathological signs (Broom & Johnson, 1993). Currently, different standards are being proposed, all claiming to ensure a high level of animal welfare and all claiming to be science-based (Fraser, 2004).

5.1. Indicators for poultry (broilers and laying hens) welfare assessment

The present study aims to identify reliable ways to assess the welfare of broilers and laying hens. We studied welfare measures during transport and at slaughter of birds from three main types of production system: enriched cages, free range and other alternative namely, aviary and deep litter systems.

The general requirements, whether on-farm, in transit or at slaughter, were considered with reference to the "Five Freedoms" (FAWC, 1992) which formed the background to our study.

A logical and comprehensive framework for the analysis of welfare within each system, together with the steps and compromises necessary to safeguard and improve welfare within the proper constraints of an efficient livestock industry, were evaluated.

The main criteria which are addressed by the definitions of welfare are listed in the above categories.

5.1.1. Freedom from hunger and thirst

Feeding and nutrition

Broilers and hens must be fed in sufficient quantity to maintain them in good health and to satisfy their nutritional needs. Hens must also have access to an adequate supply of fresh drinking water at all times.

All present systems can provide these fundamental requirements when operated to proper standards. Most studies on nutrition suggest that lack of animal protein in the diet predisposes to injurious pecking leading to cannibalism and death; plumage quality is also adversely affected (Albentosa et al., 2003; Kjaer et al., 2001; Ramadan & Von Borell, 2008). It is important, therefore, that any possibility of a nutritional cause for injurious behaviour be clarified immediately. Certain other fractions of diets, e.g. wheat, normally fed to laying hens may alter injurious behaviour (Wahlström et al., 2001).

5.1.2. Freedom from discomfort

Broilers and hens must be provided with acceptable environmental conditions namely, adequate temperature, humidity, ventilation, dust and gas levels, litter quality and light levels (Rodenburg et al., 2008; Allain et al., 2009; Shepherd & Fairchild, 2010).

Temperature

Hens are more biologically energy efficient at temperatures in the range of 18ºC to 24ºC. However, hens with ad libitum access to feed can withstand a very wide range of temperatures. In cool conditions good insulation is provided by clean, dry feathers,

unruffled by outdoor wind or indoor draughts. Free range birds benefit from shelter from wind and rain and need access to clean pasture which will not dirty the plumage.
Broilers suffer discomfort at temperatures high enough to cause heat stress, as indicated by deep and prolonged panting; this should be avoided. The temperature above which such panting occurs varies with several factors including body weight, standard of feather cover, acclimatization, stocking density, air speed, humidity, radiant environment, level of performance and breed (Rodenburg et al., 2005; 2012).

Light

Current practice, which does not appear to cause welfare problems, usually incorporates the use of daylengths within the range of 8 to 17 hours of light per day but the intensity, colour and source of light required for optimum hen welfare still uncertain (Bright, 2007). It is important that houses have sufficient light level to allow birds to see and be seen. However, high intensity and uneven light intensity within a building are undesirable because they may increase the risk of pecking (Kjaer et al., 2001).
There is also a need for a balance between providing sufficient light at lower levels and avoiding excessive light intensity at levels closest to the light source which might cause injurious behavior (Bright, 2007). More information is required to determine the minimum light levels required for hens to perform normal investigative behaviour and how visual acuity is affected by lighting conditions (Mohammed et al., 2010).

Dust and gases

Hens require a supply of fresh air, either by access to an outdoor environment or by provision of ventilation. In indoor systems, the latter is normally specified and designed to provide sufficient oxygen and adequately dilute and disperse metabolic heat, moisture, carbon dioxide, dust, ammonia and odour (Rodenburg et al., 2005; Allain et al., 2009).
Concentrations of dust vary widely within and across systems but they are generally highest in litter based systems, though litter is not the only source of dust. High levels of ammonia tend to occur in systems where undried manure accumulates within the house. High concentrations of dust and ammonia are respiratory irritants, which increase the risk of respiratory disease. Rates of carcasses condemnation have been found to reflect certain housing conditions on farm, including litter quality and atmospheric ammonia concentrations (Xin et al., 1996; Haslam et al., 2008).

The dirty feathers measure is easy to assess at the slaughterhouse and could be an asset in gaining information regarding the birds' living conditions, namely management quality and litter humidity (Arnould et al., 2009; Saraiva et al., 2016).

5.1.3. Freedom from pain, injury and disease

Broilers and hens must be maintained in good health to ensure good welfare. In all systems, equipment should be designed, sited and installed so as to minimise the risk of birds becoming injured (Allain et al., 2009; Butterworth & Weeks, 2010; Saraiva et al., 2019).

Diseases

In general, the infectious diseases of laying hens are well controlled by vaccination. Routine vaccination is used for Marek's disease, Newcastle disease, infectious bronchitis, infectious bursal disease, avian rhinotracheitis, infectious avian encephalomyelitis and more commonly now vaccination for infectious laryngotracheitis is required (Salines, 2017).

Mortality from disease tends to be lower in well-designed and managed cage systems compared to non-cage systems (Lay et al., 2011). In free-range systems birds may be exposed to organisms carried by free flying birds such as *Pasteurella* spp., *Salmonella* spp. and avian tuberculosis (Bremner & Johnston, 1996; Collins & Huey, 2015).

Parasites

Mite control is becoming more of a problem in most systems and regular spraying of equipment is needed while the birds are in situ. In cases of exposure to litter and pasture the control of coccidiosis and worms is essential (Rodenburg et al., 2005; 2012).

Bone weakness and damage

Breakage of bones mainly wings, legs and keel (Figure 1) due to osteoporosis is a common problem in the laying hen (Hester et al., 2013; Harlander-Matauschek et al., 2015). Skeletal weakness, which contributes to these fractures, is exacerbated by high levels of egg production (Pickel et al., 2011). Evidence has demonstrated that bone strength varies with the hens' strain and also with the rearing system (Saraiva et al., 2019). There is clear evidence that lack of exercise of birds in conventional cages causes poor bone strength (Fleming et al., 2004; Nasr et al., 2012; 2013). On the other hand, hens which have more exercise, changing levels from floor to raised perches, tend to have better foot condition and greater bone strength but may still suffer some damage by flying into furniture and fittings (Stratmann et al., 2015; 2016).

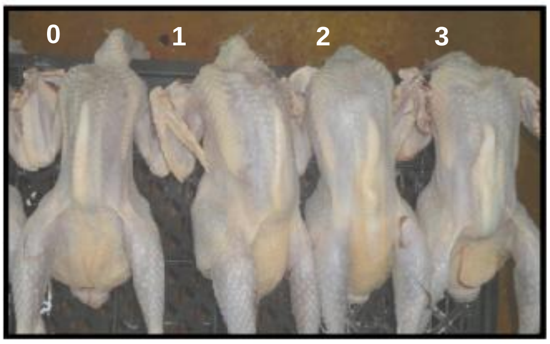

Figure 1. Keel bone lesions: 0=absence; 1=slight; 2=moderate; 3=severe.

Beak trimming and injurious pecking

A very important consideration in relation to pain is beak trimming which is used extensively, particularly in alternative systems to limit injurious feather pecking and cannibalism (Lay et al., 2011). Where the operation is performed correctly, it can help to avoid worse problems. The avoidance of injurious pecking is a major difficulty and research workers and the poultry industry must continue to address the problems of feather pecking and cannibalism to find satisfactory solutions (Rodenburg et al., 2012).

There appears to be a great potential for genetic selection to overcome, either partly or wholly, the problem of feather pecking and cannibalism and hence the need for beak trimming. There are various degrees of severity of beak trimming but all trimming must be carried out in agreement with Council Directive 1999/74/EC and, when necessary, only one third of the upper and lower beak may be removed. The literature demonstrates quite clearly that beak trimming of older birds causes acute pain at the time of the operation and also chronic pain resulting from neuromas.

Injuries

Contact dermatitis is characterized by an inflammation of the skin affecting: the plantar surface of the feet (footpad dermatitis); the hock (hock burns); or the breast (breast burns) (Allain et al., 2009). In broilers the contact dermatitis is thought to be caused by a combination of moisture, high ammonia content and the presence of other chemicals in the litter (Berg, 2004; Saraiva et al., 2016).

Figure 2. Foot pad dermatitis graded in three classes: 0=no lesions (superficial lesions/little discoloration); 1=mild lesions (black papillae less invasive, single or multiple/ hyperkeratosis) and 2=severe lesions (ulcers/haemorrhage).

The footpads are most commonly affected, followed by the hocks and breast, although all conditions may occur together in a single bird (Greene et al., 1985). These lesions are a direct source of pain and reflect many aspects of rearing conditions, being considered valid welfare indicators (Haslam et al., 2007; Meluzzi et al., 2008). The presence of hock burns may also be a useful indicator of broilers' health, influencing the welfare and profitability of affected flocks. In severe cases, breast ulcers can appear covered by necrotic tissue and subcutaneous oedema (Greene et al., 1985).

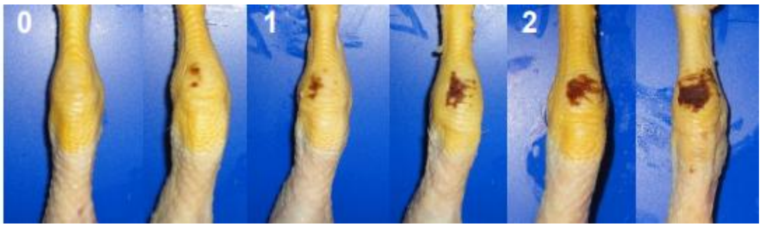

Figure 3. Hock burns graded in three classes: 0=no lesions; 1= mild lesions (moderate discoloration, superficial lesions/dermatitis) and 2=severe lesions (dermatitis/ulcers/deep haemorrhage).

Breast blisters are characterized by fluid-containing swellings of the sternal bursa and in severe cases skin can be damaged adding to the discomfort of birds (Allain et al., 2009; Arnould et al., 2009).

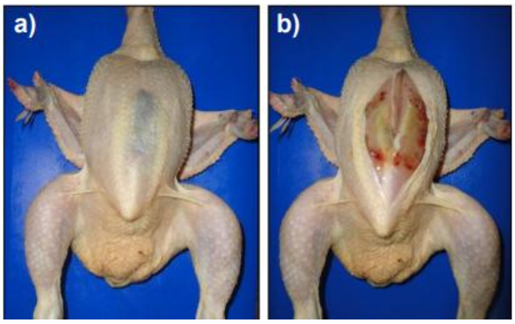

Figure 4. Breast blister is a fluid-containing swelling of the sternal burse: a) before incision; b) after incision.

5.1.4. Freedom to express normal behaviour

Conditions should be provided in which the birds can show those behaviours which are natural to them (Broom, 2016). Systems providing an enriched and varied environment offer more scope for hens to express normal behaviour and the problem has been how to assess the significance of restrictions to this freedom experienced by birds in certain systems (Casey-Trott & Widowski, 2016). Several studies show evidence which suggest that nesting, foraging, dust bathing, perching and other activities are all of importance. Opportunity to express these behaviours is largely prevented in certain systems, yet it is clearly possible for hens to survive and perform well, though with some restriction or modification of patterns of behaviour (Lay et al., 2011; Kappeli et al., 2011).

Space allowances

Definition of space allowances must include both horizontal surface area as well as height, both of which affect bird behaviour. Beside, scientific literature provides relatively little information on the use of space by hens (Donaldson et al., 2012). There is a need for a clearer understanding of how hens share space and the effect of this on their individual space requirements, and for more information upon the effects of group sizes on hen behaviour and their use of space. Whenever possible, they should have sufficient space to allow them to walk from one resource to another, investigate their surroundings, flap their wings and have safe access to perches (Saraiva et al., 2019).

Group size

It is difficult to find clear experimental evidence to support an optimum size for groups of laying hens. Over a wide range of conditions, it appears that large group sizes may be detrimental in respect of a number of behavioural factors including: injurious feather pecking and cannibalism, fearfulness, hysteria and mortality (Vits et al., 2005).

Behavioural activities

Whilst we would like to see all hens have the opportunity to exhibit the widest possible range of behaviour patterns, we find it difficult to quantify the degree of frustration or suffering experienced by birds restricted by lack of space or provision of any particular facilities. Several studies showed that the behavioural activities which are most important for laying hens are nesting, perching and using litter for scratching, pecking and dust bathing. Hens are strongly motivated to nest and may be frustrated if deprived of the opportunity to perform pre-nesting behaviour and to lay in a nest (Ali et al., 2016). In their natural surroundings, hens make considerable use of perches, particularly to roost at night. Hens are strongly motivated to perch, reducing foot problems and improving bone strength. Perching opportunities are important to the hen but it remains uncertain how much frustration and suffering is caused by deprivation (Lay et al., 2011).

Where a friable litter substrate is provided, it is intensively used by hens for scratching, dust bathing and pecking. There is experimental evidence to show that hens, when given the choice, strongly prefer litter to a wire mesh floor. Hens which are deprived of litter may have a greater tendency towards injurious pecking. It is not clear how difficult it is for hens to cope without litter but ideally hens should have daily access to litter in order to dust bathe and forage (Rodenburg et al., 2013).

5.1.5. Freedom from fear and distress

Birds may display fear in response to different types of stimuli in all systems. For birds exposed to a relatively unvarying indoor environment, any sudden visual or auditory stimulus may be frightening (El-Lethey et al., 2000, 2001). The almost universal practice of knocking on the door or speaking before entering a hen house indicates the likelihood of panic reaction that the unexpected appearance of a person might induce in a flock. Particularly in non-cages housing systems, such panic reactions can lead to injury and, in extreme cases, suffocation if large numbers of hens rush to the far end of a building or pen in alarm. In the more varied outdoor environment, hens tend to display different types of fear reaction.

Feather pecking and cannibalism have been associated with increased fear and stress in laying hens (Freire & Cowling, 201; Sherwin et al., 2010; Shimmura et al., 2010). However, the relationship between fear and feather pecking is not consensual among studies, neither the effect of age on fear response (Alm et al., 2015) needing to be further investigated.

The close contact with humans (Campler et al., 2009) and environmental complexity (Rodenburg et al., 2005) can be perceived by hens as potentially dangerous.

6. OBJECTIVES

The objectives of this study consisted in:

- analyzing the effect of pre-slaughter factors on welfare of broilers, based on the following welfare indicators -DoA rate, presence of bruises on wings, legs and breast and of dehydrated carcasses' - collected in commercial flocks of broilers;

- studying the relation between different welfare indicators of broilers collected at the slaughterhouse, such as clinical scoring and cleanliness of feathers, and to analyse if these welfare measures differed between flocks of different average BW;

- establishing for broilers the most adequate welfare indicators according to flocks of different BW;

- highlighting the relationships between fear and welfare indicators in laying hens by measuring a variety of different variables to identify behavioral issues, fear, injuries and health problems which can have significant impact on hen welfare;

- testing the influence of BW, age and welfare indicators on TI duration and number of TI induction trails in laying hens;

- investigating the effect of three housing system (furnished cages - FC, barns - B and free range - FR) on the prevalence, severity and morphology of keel bone lesions;

- determining the prevalence of DoA birds and of carcass condemnation causes in end-of-lay hens flocks and investigate the effects of age, BW and housing system.

CHAPTER III – WELFARE ASSESSMENT IN BROILERS

III.1. IMPACT OF PRE-SLAUGHTER FACTORS ON WELFARE OF BROILERS

III.1. IMPACT OF PRE-SLAUGHTER FACTORS ON WELFARE OF BROILERS

Abstract

Pre-slaughter factors adversely affecting bird welfare were studied at the slaughterhouse. The incidence of DoA, bruises and dehydration was investigated in 64 different mixed-sex batches of broilers coming from 64 different farms rearing fast-growing genotypes (Ross or Cobb).

The effects of catching team, method of catching, time of day for catching and transport, density per cage, transport duration, transport distance, lairage duration and withdrawal were considered. The average of birds found DoA was 0.29%, ranging from 0.02% to 1.89% per batch. DoA rate has a higher probability of increase with the increase in transport distance (t=2.142; *P*=0.037; estimate=0.009), the catching of birds after midnight, and with longer lairage durations for birds caught after midnight (t=2.998; *P*=0.004; estimate=0.007), suggesting that short transport distances, catching the birds before midnight and doing the transport by night are advantageous. Bruises were observed in 3.37% of birds, ranging from 0.43% to 8.29% per batch. Bruises occurred mostly on wings (3.06%), followed by legs (0.19%) and breast (0.12%). A higher percentage of bruises occurred in batches with more birds per transport crate (t=2.185; *P*=0.029; estimate=0.001). Dehydrated carcasses were observed in 22 out of 64 batches, accounting for 2.68% of condemnations. Signs of dehydration on carcasses were more frequently observed in batches subjected to longer withdrawal durations. It is proposed that strategies and management practices can be designed to minimize the effects of pre-slaughter factors impacting upon the welfare of broilers during loading, transport, and lairage.

Key words: broiler, bruising, handling, mortality, pre-slaughter transport, welfare indicator

1.1. INTRODUCTION

Current global production of broiler chickens is approaching 60 billion birds per annum (FAOSTAT, 2017). These birds are transported for slaughtering from their geographically dispersed farms. Prior to transportation, birds are subjected to fasting periods of varying duration. They are then caught and placed into transport crates, which are subsequently loaded on to vehicles and transported to slaughterhouses (Nijdam et al., 2004). Upon arrival, the crates are unloaded from the vehicles and held in lairage for periods of differing durations (Tinker et al., 2005; Petracci et al., 2006).

The pre-slaughter procedures and practices impose varying degrees of stress upon the birds which will compromise their welfare status (Mitchell & Kettlewell, 2009; Jabobs et al., 2017). Catching and loading may be the most important moments because if birds are injured during this process it will have a profound effect on their response to the rest of their journey to the slaughterhouse (Whiting et al., 2007). Several reports indicate that appropriate handling procedure is essential in the reduction of mortality and trauma, such as haemorrhages, bruises and fractures (Nijdam et al., 2004; Caffrey et al., 2017). Broilers can be caught by hand wherein multiple birds are grasped by the legs, inverted, and carried by the catcher in both hands (Nijdam et al., 2005). Although birds appear to be more restless during one leg catching, the cautious handling of broilers to reduce stress seems to be more important than holding them by both legs (Langkabel et al., 2015).

Transport represents a brief period in the total lifespan of birds, however, there are indications that it is a time when both mental and physical suffering can be high (Knowles et al., 1990; Knezacek et al., 2010; Siegel et al., 2014). During transportation the combination of stressors, rather than a single cause, is responsible for the decrease in welfare (Mitchell & Kettlewell, 1998; 2004; 2009). Although, it is generally accepted that animal transport of long duration is more likely to compromise animal welfare than short journeys, it is important to recognise that it is not journey duration *per se*, but the conditions of transport and the associated stress imposed, that are the source of welfare issues (Vecerek et al., 2006; Nielsen et al., 2011; Weeks et al., 2012). The microclimate within the trailer can be the most important factor affecting broiler welfare, as heat and cold stresses are two major contributors to both death and overall transportation stress in broilers (Mitchell & Kettlewell, 2004; Dadgar et al., 2010; Cockram & Dulal, 2018). Factors such as lack of feed, water and rest are all exacerbated by the length of exposure to challenging conditions, and thus, journey duration (Nielsen et al., 2011). The time of day for catching and transport, as well as density per crate are also important factors to be considered (Nijdam et al., 2004; Caffrey et al., 2017). Moreover, the period between catching and transport to the slaughterhouse is considered by several authors as one of the most critical periods with regards to the risk of dehydration (Vanderhasselt et al., 2013).

After transport, a suitable lairage period in proper holding areas, with environmental control, is necessary to reduce thermal stress of live birds (Vosmerova et al., 2010). However, short lairage times are recommended for poultry due to low energy availability in metabolically active birds, who may have suffered physiological changes and body weight loss due to fasting before transport handling (Nijdam et al., 2004; Delezie et al., 2007; Mitchell & Kettlewell, 2008). Pre-slaughter factors can also affect the process of converting muscle to meat and meat quality parameters muscle to meat conversion and meat quality parameters which can have a negative impact on consumer acceptability (Schwartzkopf-Genswein et al., 2012).

Slaughterhouses have been recognized as a relevant source of data for monitoring welfare conditions of birds (Grandin, 2017; Saraiva et al., 2016). Under the Directive 2007/43/EC which provided minimum standards to ensure the protection of broilers during intensive production the official veterinarian controls include monitoring and follow-up welfare parameters at the slaughterhouse (European Union, 2007). Following these requirements, and taking into account the results of studies carried out in this field, European level thresholds for some welfare parameters have been defined and can be easily assessed at the slaughterhouse (Saraiva et al., 2016; EFSA, 2004). For example, indicators of poor welfare in transit may include dead on arrival (DoA) greater than 0.5%.

The aim of this study was to analyse the effect of pre-slaughter factors on DoA rate, presence of bruises on wings, legs and breast and of dehydrated carcasses in commercial flocks of broilers.

1.2. MATERIAL AND METHODS

1.2.1. Sample characterisation

The welfare indicators were collected in one of the largest broilers slaughterhouses in Portugal and the study took place during springtime. The incidence of DoA, bruises and dehydration was investigated in 64 different mixed-sex batches of broilers coming from 64 different farms with intensive system production rearing fast-growing genotypes (Ross or Cobb). The study did not include any long distance export or intra-community trade journeys. Birds had a mean age of 36 days (range 30 to 45d) with body weight of 1.85 ± 0.26kg (range 1.43 to 2.41kg). The average number of birds in each bach of transported was 5.110 ± 745 birds (range 2.360 to 6.804 birds).

1.2.2. Pre-slaughter procedure

Each vehicle, per slaughter day, undertook two journeys from two distinct farms. The first journey from farm to the slaughter occurred before midnight (00h) and the second after midnight (some of them already in daylight) on the slaughter day. The method of catching was manual in all flocks and each vehicle transported broilers in 486 crates made from LCS plastic. Once at the slaughterhouse, the vehicle was unloaded and the crates with the broilers were placed in a holding area equipped with fans and sprinkler systems. According to the established slaughter schedule, the slaughter started at 5 a.m. when the crates were tipped over automatically and the broilers were dropped onto a conveyor and transferred to a carousel table from where they were hung on a shackle line.

1.2.3. Data collection

Dead broilers were removed at the carousel table and accounted by batch or consignment (load) immediately after slaughter and checked by the official veterinarian. The same official veterinarian recorded from 700 carcasses per batch (total 44.800) the number of bruises on wings, legs and breast and from 1.400 carcasses per batch (total 89.600) the number of carcasses condemned by dehydration. Bruises were classified according to the approximate age using visual and objective color assessment standards (Northcutt et al., 2000) so as to consider only those which had occurred during the pre-slaughter period. Dehydrated carcasses were recognised during *post mortem* inspection by being dry, tacky and badly bled (Butterworth & Niebuhr, 2009).

Information regarding age at slaughter (d); mean body weight (Kg); batch size (number of broilers/vehicle); time of catching; team for catching and vehicle; time of arrival at the slaughterhouse; duration of transport (min); distance of transport (km); crate floor area (cm^2/kg); density per crate (kg/m^2); number of birds/crate; lairage duration (min) and feed/water withdrawal duration (min) was collected for the 64 batches.

1.2.4. Statistical analysis

Generalized linear models (GzLM) were conducted to study the effects of pre-slaughter factors (explanatory variables) on DoA rate (model I), percentage of bruises (≤ 4% or > 4%; model II) and percentage of dehydrated carcasses (model III), using stepwise procedures to select significant predictors on dependent variables (Mccullaghn and Nelder, 1989).

Gaussian errors and log link function was applied for DoA rate (model I) using the initial explanatory variables: body weight, transport duration, transport distance, lairage duration, withdrawal duration, stocking density and catching period. The interaction effects were evaluated for catching period and lairage duration, as well for catching period and transport duration. All of explanatory variables mentioned above were numerical except the catching period which was categorical in a binary scale (before/after 00h).

The binomial errors and logit link function was applied for bruises (model II) using the initial explanatory variables: batch size, team for catching, transport duration, transport distance, catching period in a binary scale (before/after 00h), as well as lairage duration and withdrawal duration using 3-point grading scales (< 8h; > 8h and < 12h; > 12h). In the stepwise GzLM analysis, variables and their first-level interaction were integrated into the final model if they significantly ($P < 0.05$) changed the deviance. The final models were recalculated with the heterogeneity factor and Akaike Information Criterion (AIC) was measured for goodness of fit. The effect of each factor in the final model was expressed as an odds ratio (OR) and this value is the equivalent to the relative risk, assessing each specific factor relative to its reference class.

Gaussian errors and log link function was applied for dehydration (model III) using the initial explanatory variables: body weight, transport duration, lairage duration, withdrawal duration and catching period. GzLM model was not found for dehydration and data was analysed thought univariate chi-square for categorical variables and Mann-Whitney for numeric variables.

1.3. RESULTS

The mean percentage of birds DoA was 0.29 ± 0.21%, ranging from 0.02% to 1.89% per batch. Eleven batches presented DoA higher than 0.5% and two batches presented DoA higher than 1.0%. The batches with DoA rate lower than 0.5%, between 0.5% and 1.0%, and higher than 1.0% were subjected to an average transport distance of 63km, 89km and 171km, respectively.

The mean prevalence of bruises on wings, legs and breast was 3.37 ± 0.02%, ranging from 0.43% to 8.29% per batch. Twenty five batches presented bruises in more than 4.0% of birds. Bruises were much frequent on wings (3.06%) comparatively to the legs (0.19%) and breast (0.12%). Dehydrated carcasses were observed in 22 out of 64 batches, representing 2.68% of condemnations.

The characterisation of pre-slaughter period is described in Table 1.

Table 1. Descriptive statistic of pre-slaughter period (n=64).

Pre-slaughter factors	Mean	SD	Minimum	Median	Maximum
Transport duration (h:min)	1:12	0:05	0:22	0:59	3:04
Velocity of transport (km/h)	55.44	1.83	32.0	52.50	93.00
Transport distance (km)	70.92	6.60	15.00	45.00	196.00
Lairage duration (h:min)	6:52	0:32	0:17	6:25	14:39
Withdrawal duration (h:min)	9:38	0:30	2:40	8:45	17:30
Crate floor area (cm^2/kg)	199.50	1.96	172.46	200.83	232.54
Birds per crate (number)	11.08	0.22	8	11	15

The transport duration (1h12 ± 0h05) and transport distance (70.92 ± 6.60km) were, on average, short. The average withdrawal duration was 09h38 ± 0h30 with a maximum of 17h30. Batches with dehydrated birds presented higher average lairage durations (8h00 vs. 6h26) and higher withdrawal durations (10h45 vs. 9h22), in comparison with those without dehydrated birds.

The GzLM model I analysing the effects of pre-slaughter factors (explanatory variables) on DoA rate are presented in Table 2. The significant effects are represented in Figure 1.

Table 2. Effect of explanatory variables on model I for dead on arrival (DoA) rate.

Model for DoA (AIC = 8.573)						
Explanatory variables	**Estimate**	**Std. Error.**	**t**	**P>|t|**	**exp**	**Conf. interval (95%)**
(Intercept)	-1.649	0.853	-1.932	0.058	0.192	(0.030-0.823)
Catch. per. (+00h)	-3.813	1.301	-2.931	0.005**	0.022	(0.001-0.282)
Transp. dur. (min)	-0.014	0.008	-1.043	0.087	0.986	(0.970-1.001)
Transp. dist. (km)	0.009	0.004	2.142	0.037*	1.009	(1.000-1.019)
Lair. dur. (min)	0.001	0.001	0.724	0.472	1.001	(0.999-1.003)
Catch. per. (±00h)*Transp. dur. (km)	0.031	0.007	4.109	0.000***	1.031	(1.018-1.049)
Catch. per. (±00h)*Lair.dur. (min)	0.007	0.002	2.998	0.004**	1.007	(1.003-1.013)

Significant differences: *P < 0.05, **P < 0.01, ***P < 0.001.

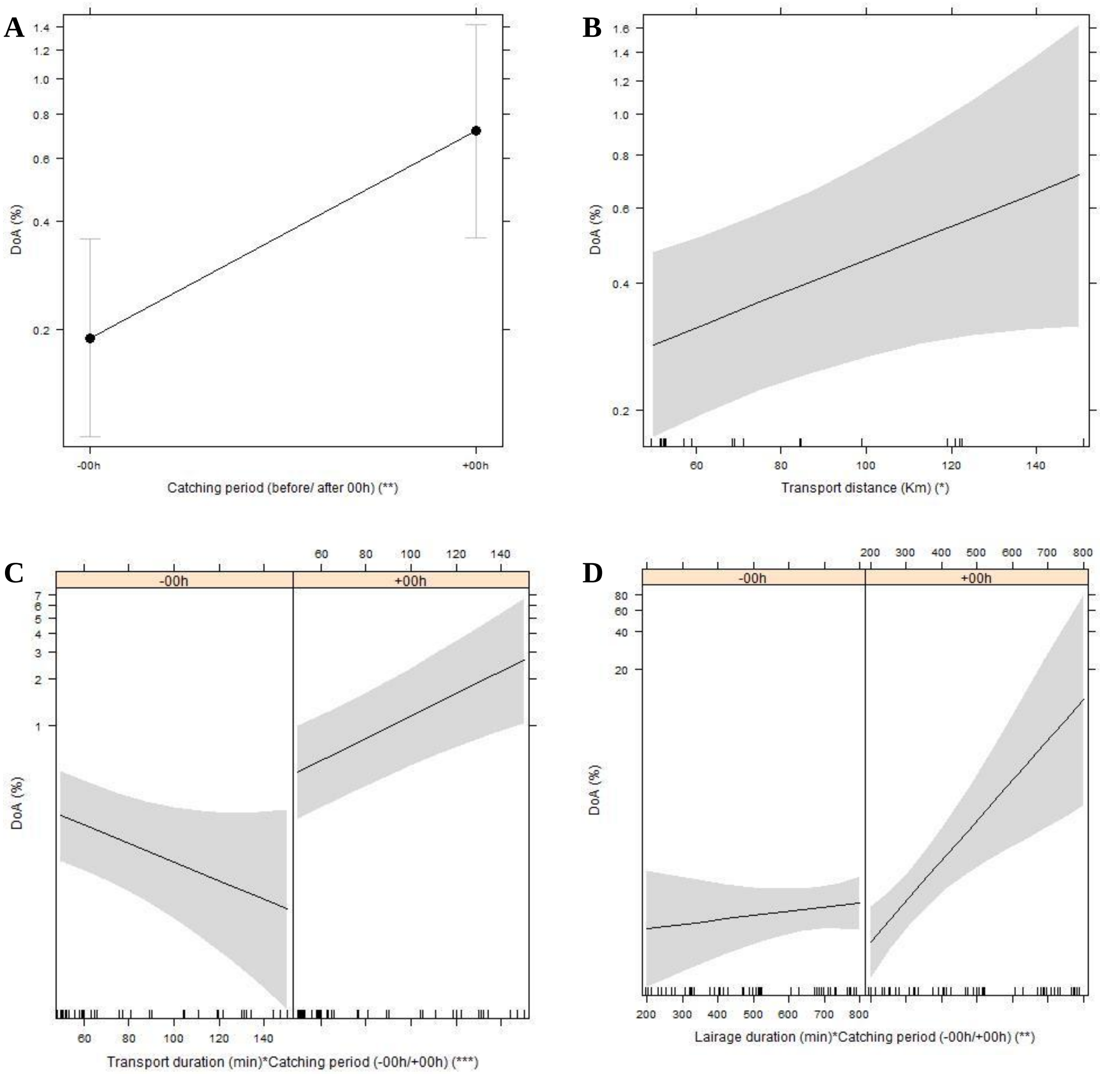

Figure 1. Effects of transport distance (km) (A), catching period (before/after 00h) (B), transport duration and catching period interaction (C) and lairage duration and catching period interaction (D) on DoA rate. Values are significantly different at *P < 0.05, **P < 0.01, ***P < 0.001.

The percentage of birds found DoA increased with transport distance (t = 2.142; P = 0.037; estimate = 0.009). The time of catching also had a significant effect on DoA. For birds caught after midnight, the increase of transport duration increased the birds found DoA. For birds caught after midnight, the increase of lairage duration increased the DoA rate and for birds caught before midnight, the increase of lairage duration did not increased the DoA.

The model II analyzed the effects of pre-slaughter factors (explanatory variables) on percentage of bruises (≤ 4% or > 4%) and data is presented in Table 3. The significant effects are represented in Figure 2.

Table 3. Effect of explanatory variables on model II for bruises.

Model for bruises (AIC = 81.299)						
Explanatory variables	**Estimate**	**Std. Error.**	**t**	**P>|t|**	**exp**	**Conf. interval (95%)**
(Intercept)	-8.933	5.502	-1.624	0.104	0.000	(0.000-4.106)
Batch size (No. of birds)	0.001	0.000	2.185	0.029*	1.001	(1.000-1.002)
Catch. per. (+00h)	-2.616	1.101	-2.376	0.017*	0.067	(0.006-0.539)
Transp. dist. (km)	-0.025	0.009	-2.881	0.004**	0.982	(0.942-1.017)
Lair. dur. (min)	-0.006	0.002	-2.542	0.011*	0.994	(0.989-0.998)
Crate floor area (cm^2/kg)	0.044	0.022	1.962	0.049*	1.045	(1.002-1.096)

Significant differences: *P < 0.05, **P < 0.01, ***P < 0.001.

A higher percentage of bruises (> 4%) occurred in larger batch sizes. Batches with lower stocking density and therefore with a higher floor area of crate per kilogram of body weight (cm^2/kg) also presented a higher percentage of bruises (> 4%). Batches with lower lairage duration and lower transport distance showed higher probability of having bruises' prevalence above 4%. Catching the birds before midnight increased the probability of birds having higher percentage of bruises (> 4%).

A positive association was found between batches with dehydrated carcasses and withdrawal durations (χ^2 = 7.273, df = 2, P = 0.026). A positive association was also found between catching period (before/after 00h) and dehydration (χ^2 = 4.403, df = 1, P = 0.036). Birds subjected to longer withdrawal durations (> 12h) and caught before midnight were more likely to become dehydrated.

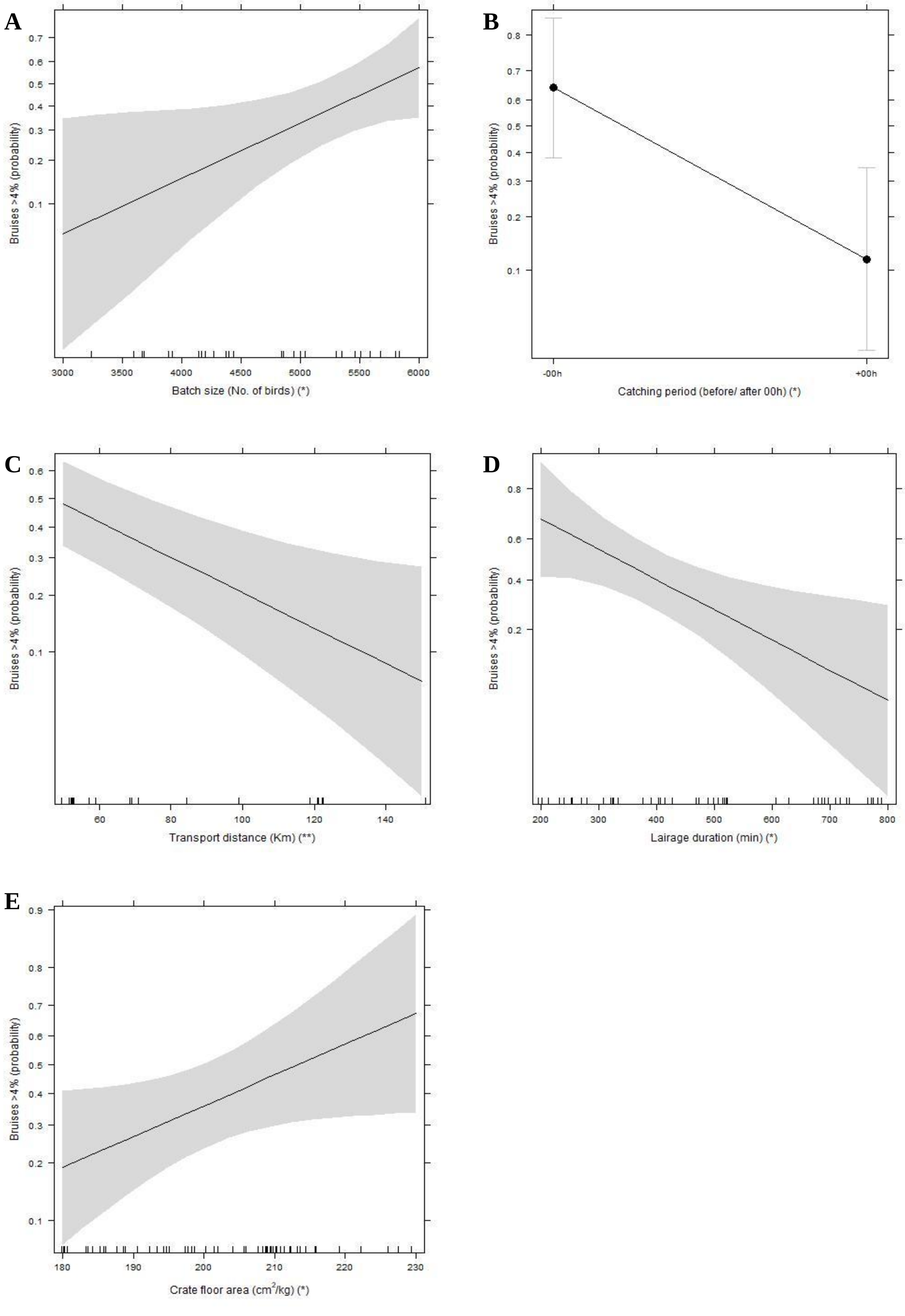

Figure 2. Effects of batch size (number of birds) (A), catching period (before/after 00h) (B), transport distance (km) (C), lairage duration (min) (D) and crate floor area (cm^2/kg) (E) on percentage of bruises. Values are significantly different at **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

1.4. DISCUSSION

The transport of domestic fowl (*Gallus gallus*) is the largest translocation of a single class of livestock in the world. Any problems with transport tend therefore to be important in terms of the number of individuals whose welfare may be affected (EFSA, 2004). In the present study, several pre-slaughter factors were investigated in 64 batches of broilers transported from farms to slaughter to determine potential causes of poor welfare based on indicators collected at the slaughterhouse namely, DoA rate, presence of bruises and of dehydrated carcasses.

The percentage of birds found DoA was 0.29 ± 0.21%, from which 11 batches presented DoA higher than 0.5% and two batches presented DoA higher than 1.0%. The lowest DoA rates were observed for the shortest transport distances. In case of short distances (<50km) the average mortality was of 0.23 ± 0.07% and in case of longer distances (>150km) the average mortality was 0.60 ± 0.10%. In an extensive study comparing mortality rates in poultry species during transport to slaughter was observed for broilers a DoA rate of 0.15% for a transport distance up to 50km and for transport distances over 200km a DoA rate of 0.54% (Vecerek et al., 2016). Another study found an average mortality for broilers during transport of 0.25%. However, the DoA rates varied according to transport distances to the processing plant, ranging from 0.15% for shorter distances (<50 km) to 0.86% for longer distances (>300 km) (Nielsen et al., 2011).

A significant effect of transport distance (t = 2.142; P = 0.037; estimate = 0.009) was found for model I on DoA rate, indicating that DoA will increase 0.9% with the increase of transport distance in 100km, a result that is in line with findings obtained by Vecerek et al. (2016), showing average mortality to be very dependent on the distance of travel (Kittelsen et al., 2015). Other large surveys in several countries have found for all species a significant risk of increased mortality rates with journey length (Vecerek et al., 2006; Weeks et al., 2012; Voslářová et al., 2007).

The percentage of birds found DoA was higher when the catching period occurred after midnight (t = -2.931; P = 0.005; estimate = -3.813). This result shows that DoA would decrease by a factor of 0:022 for catching period before midnight when compared with catching after midnight. The interactions between catching period and transport duration (t = 4.109; P < 0.001) and between catching period and lairage duration (t = 2.998; P = 0.004) indicates that DoA increases with the increase of transport and lairage durations for birds caught after midnight. Nijdam et al. (2004) in a work that sought to determine factors influencing mortality showed that the risk of dying during transport or waiting increases considerably as time increases (Nijdam et al., 2004). According to Cockram and Dulal (2018) the duration of loading, transport, and lairage increases the mortality risk. However, the present study shows that there are other factors which can influence the increase of DoA rate, particularly the time of catching. Therefore, the increased risk of dying with longer time in transport or waiting could be problematic in case of a late catching period. According to the present study, a late catching must occur in farms near the slaughterhouse and have short lairage durations so as to reduce DoA. After nocturnal transportation, birds show less change of body temperature compared to morning transportation indicating that birds experienced less stress at night than in the morning (Jacobs et al., 2017). Comparing *post mortem* findings in dead-on-farm and DoA broilers indicated that the transportation process caused the majority of pathological lesions, such as, lung congestion and trauma which were responsible for the mortalities registered (Kittelsen et al., 2015). Furthermore, Lund et al. (2013) underlined the importance of increased focus on handling based on the chronicity of the lesions found on DoA broilers, which were primarily related to management and handling procedures.

Moreover, climatic conditions have been found to be associated with DoA (Chauvin et al., 2011), being heat stress recognised as a major risk factor (Petracci et al., 2006; Whiting et al., 2007; Mitchell & Kettlewell, 2004). Seasonal variation was not relevant in this study since it was carried out in spring, but a higher DoA rate would be expected in summer and winter months, as confirmed by several reports (Vecerek et al., 2006; Vieira et al., 2011).

The average percentage of birds with bruises was 3.37 ± 0.02%, ranging from 0.43% to 8.29% per batch. Batches with more birds ($t = 2.185$; $P = 0.029$) presented a higher prevalence of bruises. Furthermore, batches with a lower stocking density and more space per crate ($t = 1.962$; $P = 0.049$) presented more bruises, which is in line with findings obtained by Knowles and Broom (1990), indicating that transport systems with lesser space per bird can be more suitable in preventing bruising. This may be explained by the fact that birds sustain each other's body, reducing falling or the need to spread the wings and legs to keep balance. However, the present study demonstrated that bruises did not increase with transport duration indicating that bruises were more likely to have occurred on farms during catching, crating and loading. According to Vosmerova et al. (2010), pre-transport handling procedures may be more stressful for broilers than the transport itself. The main hazard responsible for occurrence of bruises is inadequate handlers (Marahrens et al., 2011) and individual features of the catching teams' elements might explain different degrees of lesions (Langkabel et al., 2015). Stocking density can also be managed to lessen negative influences on animal welfare (Fisher et al., 2009). Birds may benefit from slightly higher densities in spring, or if weather conditions are anticipated to be cold (EFSA, 2004; Marahrens et al., 2011). The prevalence of bruises was much higher in the wings (3.06%) when compared to the legs (0.19%) and breast (0.12%). Catching the birds before midnight also contributed to the increase of bruises (> 4%). Lesions on the wings usually occur during crating because of an increase in wing flapping, and when a large number of birds are squeezed into the same crate. Birds should be handled cautiously, tranquility of the flock should be maintained as to avoid wing flapping, and modular containers should be positioned as close to the animals as possible (Langkabel et al., 2015; Fisher et al., 2009). The setting up of standard operating procedures might help to attain a situation within which the welfare of the animals is maintained at all stages of catching and crating (Langkabel et al., 2015).

The maximum transport duration of 3h04 and distance of 196km were not very high. In contrast, the lairage duration was high with mean value of 06h52 and maximum of 14h39, as well as the withdrawal duration with mean value of 09h38 and maximum of 17h30. Frequently, upon arrival at the slaughter plant, a variable amount of time passes before the birds are slaughtered (Delezie, 2006). Twenty one batches (32.81%) were subjected to more than 12h of fasting and even with good weather, dehydrated carcasses were observed in 22 out of 64 batches. In commercial practice, water is usually withdrawn just before the first bird of a flock is caught and crated for transport to the slaughterhouse. This depopulation process often takes several hours and water deprivation continues as the birds are transported to the slaughter plant (Delezie, 2006). According to the Council Regulation 1/2005, suitable food and water shall be available in adequate quantities in the case of a journey lasting more than 12h (European Union, 2004), although this is hardly feasible for birds. This is particularly important in hot weather, due to the increased risk of birds restricted from access to water easily became dehydrated, and depending on their age and physiological state, birds vary in the ability to cope with periods of feed and water withdrawal (Fisher et al., 2009). Moreover, the duration of the pre-slaughter stages, the thermal environment, fasting, ill-health, and injury can reduce the physiological capacity of the birds to maintain homoeostasis which can result in exhaustion and death (Caffrey et al., 2017; Cockram & Dulal, 2018). The welfare of broilers during the pre-slaughter can be greatly improved through changes in the human approach, by the implementation of standard operating procedures and by ensuring adequate planning. Training is essential for those involved in handling animals and driving vehicles (Northcutt et al., 2000). Planning the catching, loading and transport from the farm in coordination with planned slaughter times is essential and it will ensure that birds that have been the longest without feed and water are the first to be killed. All those involved should consider carefully the weather conditions, the transport distances and the transport time (night/day), as well as, the body weight and the health state of birds. Similarly, it is important to ensure that the total period of feed withdrawal, including the time at the farm plus the journey and lairage times, does not exceed the 12h maximum feed withdrawal time limit. It is emphasised that when a broiler transport vehicle is stationary, or when a modular load is stacked in lairage, the ventilation of the transport containers is entirely passive and dependent upon buoyancy forces. The reduced ventilation of the load in these circumstances may not be adequate to fully dissipate the heat and moisture loads produced by the birds. This in turn will increase the risk of thermal (heat) stress and possibly, an increase in mortality. It should be stressed, perhaps that in many circumstances the term DoA is inappropriate as birds may die as a result of the factors described above rather than on the journey, and often mortality in transport is not estimated until the birds are hung on the shackle line. Thus, birds should be unloaded from vehicles as soon as possible after arrival, avoiding unnecessary delay. Broilers should be placed immediately in environmentally controlled lairage areas, as this will reduce stress and reduced welfare during standing and lairage and may decrease apparent DoA in addition to avoiding negative impacts on carcass and meat quality (e.g. live shrink).

1.5. CONCLUSION

Important risk factors affecting broiler welfare during transport from farms to slaughterhouses can be identified by assessing welfare indicators such as DoA rate, presence of bruises and dehydration. In the present study it was shown that pre-slaughter operations should be adequate planned and carried out for short transport and lairage durations, catching the birds by night or before midnight and ensure adequate catching and crating procedures. Close attention to, and control of, all of these factors is essential to ensure high standards of animal welfare in the transportation of broiler chickens.

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III.2. FEATHER CONDITIONS AND CLINICAL SCORES AS INDICATORS OF BROILERS WELFARE AT THE SLAUGHTERHOUSE

III.2. FEATHER CONDITIONS AND CLINICAL SCORES AS INDICATORS OF BROILERS WELFARE AT THE SLAUGHTERHOUSE

Abstract

The objective of this study was to evaluate the welfare of 64 different broiler farms on the basis of feather conditions and clinical scores measures collected at the slaughterhouse. A 3-point scale (0, 1 or 2) was used to classify dirty feathers, footpad dermatitis and hock burns measures, and a 2-point scale (present or absent) was used to classify breast burns, breast blisters and breast ulcer measures. Flocks were allocated into three body weight (BW) classes (A, B, C): class A (light) ≥1.43 and ≤1.68kg, class B (medium) ≥1.69 and ≤1.93kg; class C (heavy) ≥1.94 and ≤2.41kg. The absence of hock burns was more common in class A, while mild hock burns was more common in class B flocks. Breast ulcer was observed in class C flocks. The association observed for mild hock burns, breast burns and severe footpad dermatitis can indicate a simultaneous occurrence of these painful lesions. Very dirty feathers and severe footpad dermatitis relationship suggest litter humidity to be the common underlying cause. In conclusion, it was shown that clinical indicators can be used at the slaughterhouse to identify welfare problems. In the flocks studied footpad dermatitis, feather conditions and hock burns were the main restrictions to good welfare and should be considered significant welfare indicators of the on-farm rearing conditions.

Key words: animal welfare, broiler, clinical score, feather conditions, slaughterhouse, welfare measure

2.1. INTRODUCION

Animal welfare has been a major issue in Europe over the last decades, which resulted in regulations and research being devoted to farm animal welfare and especially to standard poultry production, frequently considered as having very poor welfare (Beaumont et al., 2010). The Council Directive 2007/43/EC (EU, 2007) stipulated the minimum rules for the protection of chickens kept for meat production and also established a systematic welfare evaluation system at the slaughterhouse. Veterinarians should evaluate the results of the *post mortem* inspection in order to identify indicators of poor welfare conditions on farms, such as abnormal levels of contact dermatitis, parasitism or systemic illness (Council Directive 2007/43/EC). In addition to legislation, internationally agreed standards (OIE, 2010) and voluntary labelling programs have been designed to distinguish products according to welfare standards (Martelli, 2009). Currently, different animal welfare assurance schemes are being used to encourage the adoption of animal welfare standards in food production (Fraser, 2006; Sørensen and Fraser, 2010). These developments have been created a rapid changing environment for production, marketing and international trade of animal products, with potential opportunities and constraints for the animal-based industries (Fraser, 2006; Xavier et al., 2010). The “Welfare Quality®” project developed welfare assessment schemes for broilers that include animal-based measures which indicate direct effects on animals, such as footpad lesions (Veissier et al., 2008; Butterworth and Niebuhr, 2009).

For this study, measures were selected based on their relevance for the evaluation of broiler welfare and their easiness of identification at the slaughterhouse. Contact dermatitis is characterized by an inflammation of the skin affecting the plantar surface of the feet - footpad dermatitis; the hock - hock burns; and the breast - breast burns (Allain et al., 2009). In contact dermatitis syndrome, the footpads are most commonly affected, followed by the hocks and breast, although all conditions may occur together in a single bird (Greene et al., 1985). These lesions are a direct source of pain and reflect many aspects of rearing conditions, being considered valid welfare indicators (Haslam et al., 2007; Meluzzi et al., 2008). The presence of hock burns may also be a useful indicator of broilers' health, influencing the welfare and profitability of affected flocks (Hepworth et al., 2011). In severe cases, breast ulcers can appear covered by necrotic tissue and subcutaneous oedema (Greene et al., 1985). Breast blisters are characterized by fluid containing swellings of the sternal bursa and in severe cases, skin can be damaged adding to the discomfort of birds (Allain et al., 2009; Arnould et al., 2009). The dirty feathers measure is easy to assess at the slaughterhouse and could be an asset in gaining information regarding the birds' living conditions. The validity of this measure used at broilers slaughterhouse has not been adequately evaluated (Wilkins et al., 2003), though it may be a good indicator of the management quality and litter humidity (Arnould et al., 2009).

Several studies have used welfare parameters at the slaughterhouse to assess the welfare conditions on farms (Haslam et al., 2008; Allain et al., 2009; Arnould et al., 2009; Butterworth and Niebuhr, 2009; Xavier et al., 2010). However, there are very few published reports concerning the welfare status of flocks using all the measures selected for this study and, therefore relations between them have been poorly explored.

The objective of this study was to study the relation between different welfare indicators collected at the slaughterhouse, such as clinical scoring and cleanliness of feathers, and to analyse if these welfare measures differed between flocks of different average bodyweight (BW). A second objective was to establish the most adequate welfare indicators according to flocks of different BW.

2.2. MATERIAL AND METHODS

This study was performed at a poultry slaughterhouse in Portugal. The welfare of 64 different mixed-sex flocks from 64 different farms with an intensive system production and a fast-growing genotype (Ross or Cobb) was evaluated in 900 birds per flock from a total of 5600 birds. Flocks had on average 36 days old ranging from 30 to 45 days, with an average body weight of 1.854 ± 0.26 kg. According to the BW, flocks were allocated into one of three BW classes (A = light, B = medium, C = heavy). Class A ≥ 1.43 and ≤1.68 kg (n = 21); class B ≥ 1.69 and ≤1.93 kg (n = 22); class C ≥ 1.94 and ≤2.41 kg (n = 21). Class A flocks were on average 34 days old (30 to 40 days); class B were on average 38 days old (32 to 45 days) and class C were on average 40 days old (36 to 43 days).

The removal of a proportion of the birds from the farm, referred to as thinning, is commonly carried out at the end of the growing period, providing the birds with more space and reducing environmental pressure. The majority of these farms, which resorted frequently to thinning during the growth, were dedicated to producing “barbecue chicken”, while other farms, where thinning was rare, produced mainly heavier broilers. Class A flocks had never been subjected to thinning before. Due to the market demand for “barbecue chicken” class A flocks represented their “first thinning” for slaughter. However, 22.7% of class B flocks and 61.9% of class C flocks had been previously thinned during growth. The other 38.1% of class C flocks came from smaller farms (3000 to 6000 birds per rearing) with lower stocking densities.

For each flock, data were collected at the slaughter-line. Immediately after electrical stunning, 100 birds were randomly selected and scored for dirty feathers. After defeathering, 100 feet were randomly selected and scored for footpad dermatitis, and 100 hocks were randomly selected and scored for hock burns. These measures were classified using a 3-point scale, ranging from 0 to 2, and the means were calculated per flocks. Footpad dermatitis and hock burns scoring were done on the right foot and right hock of each carcass, as it was assumed that all flocks would present the same percentage of lesions on both legs (Ekstrand et al., 1997). Seven hundred broilers per flock were checked for breast injuries, namely ulcers, blisters or burns.

The classification of the measures was completed according to the following description:

Dirty feathers: 0 = clean feathers (white feathers with absence of dirt); 1 = moderately dirty feathers (soiling feathers localized in the breast and abdominal areas without caked dirt) and 2 = very dirty feathers (generalized dirty brown feathers sometimes with dirt adhered or caked to feathers) (adapted from Welfare Quality®, 2009).

Footpad dermatitis: 0 = no lesions (no visible lesions: smooth epidermis, no discoloration), 1 = mild lesions (papillae only with hyperkeratosis and/or mild/superficial lesions with discoloration or erosions in the epidermal layer up to 5 mm) and 2 = severe lesions (severe papillae and ulcerations: discoloration, hyperkeratosis, ulcers and signs of inflammatory reactions with more than 5 mm) (Ekstrand et al., 1997; Dawkins et al., 2004).

Hock burns: 0 = no lesions (no visible lesions), 1 = mild lesions (brown lesion up to 5 mm) and 2 = severe lesions (black lesion with more than 5 mm) (adapted from Allain et al., 2009).

Breast blisters were defined as present when this was equal to or larger than 0.5 cm^2 (Allain et al., 2009).

Breast burn was defined as present when one or more breast burns were observed as having a brownish-coloured scab (erosion) (Greene et al., 1985).

Breast ulcer was defined when the breast skin was covered with black exudates (Greene et al., 1985).

All of the measurements were carried out by an official experienced veterinarian. Information concerning the identification of farms, bird age, and BW was collected from the slaughter records.

2.2.1. Statistical analysis

The effect ($P < 0.05$) of flock weight class was studied using non-parametric tests (Mann-Whitney-Wilcoxon test and Kruskal-Wallis test) for twelve variables (clean feathers, moderately dirty feathers, very dirty feathers, absence of footpad dermatitis, mild footpad dermatitis, severe footpad dermatitis, absence of hock burns, mild hock burns, severe hock burns, breast burns, breast blister and breast ulcer). Spearman's correlation coefficients' ($P < 0.01$) were calculated in order to study the relationships between variables. To get a broader view from the results, the variables were subjected to Principal Components Analysis (PCA). The appropriateness to perform PCA was confirmed by Bartlett's sphericity test ($P < 0.0001$). The number of components retained in the final solution was based on the Kaiser-Meyer-Olkin criterion for the analysis of eigenvalues (>1) and the proportion of variance retained (>70%), usually seen as the minimum needed to make the model suitable for explaining the original data (Reis, 1997). The components of lesser significance were ignored since they did not have significant impact on the outcome. Variables were finally selected (clean feathers, moderately dirty feathers, absence of footpad dermatitis, severe footpad dermatitis, absence of hock burns, mild hock burns and breast burns) in order to calculate the first two principal components (PC) on the basis of factor loadings (FL) modulus higher than 0.50 in absolute values and communalities (CM) higher than 0.5. Data analysis was carried out using XLStat (release 2011, Addinsoft).

2.3. RESULTS

Table 1 shows the effects of flock weight classes (A, B and C) on the welfare measures.

Table 1. Level of significance of percentages (Means ± standard deviation) for dirty feathers (DF), footpad dermatitis (FPD), hock burns (HB), breast injuries (burn, blister and ulcer), according to flock weight classes (Class A, n= 21; Class B, n= 22; Class C, n= 21).

Variables (%)	Class A	Class B	Class C	Total	*P-value*
DF0	47.48 ± 40.10	15.18 ± 29.64	30.71 ± 40.01	30.88±38.59	ns
DF1	37.62 ± 34.37	70.73 ± 32.23	56.00 ± 39.04	55.03±37.32	ns
DF2	15.86 ± 31.33	14.09 ± 24.18	13.29 ± 27.85	14.39±27.46	ns
FPD0	52.10 ± 41.18	42.73 ± 31.99	58.81 ± 31.07	51.08±35.08	ns
FPD1	17.57 ± 18.15	22.18 ± 16.37	20.76 ± 12.65	20.20±15.76	ns
FPD2	30.33 ± 36.52	35.09 ± 33.50	20.43 ± 31.44	28.72±33.90	ns
HB0	92.90 ± 20.35[a]	88.09 ± 12.92[b]	90.10 ± 12.92[a]	90.33±18.64	**0.04**
HB1	6.05 ± 16.09[a]	10.86 ± 11.03[b]	4.95 ± 11.03[ab]	7.34±12.05	**0.03**
HB2	1.05 ± 4.36	1.05 ± 2.59	4.95 ± 2.59	2.33±11.67	ns
Beast burn	1.32 ± 4.20	0.11 ± 0.46	0.29 ± 1.28	0.56±2.54	ns
Breast blister	0.93 ± 0.69	0.97 ± 0.82	0.86 ± 0.79	0.93±0.76	ns
Breast ulcer	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.03 ± 0.10[b]	0.01±0.06	**0.04**

DF0 = clean feathers; DF1 = moderately dirty feathers; DF2 = very dirty feathers.
FPD0 and HB0=no lesions; FPD1 and HB1=mild lesions; FPD2 and HB2=severe lesions.
ns - not significant ($P \geq 0.05$); In each row, means with different letters differs significantly and significant effects ($P< 0.05$) were presented with bold letter.
Class A = 1.43 to 1.68kg; Class B = 1.68 to 1.93kg; Class C = 1.93 to 2.41kg; Total = 1.43 to 2.41kg.

Only three (4.69%) flocks had no birds with moderately or very dirty feathers. Five (7.80%) flocks had no birds with footpad dermatitis lesions and twenty-seven (42.19%) flocks had no birds with hock burns lesions. Hock burn was more often absent in class A flocks than in class B flocks. The prevalence of mild hock burns was higher in class B flocks than class A flocks. Significant differences were observed for absence of hock burns (P = 0.04) and mild hock burns (P = 0.03) between class A and B flocks. Breast injuries were present in 1.51% of the total slaughtered birds, ranging from 0.14% to 17.71% per flock. The presence of breast blisters was almost equal in the three weight classes, ranging from 0.86% to 0.97% of the total birds per weight class. Ulcers occurred sporadically in class C showing significant differences (P = 0.04) from other classes.

Variables with some significant Spearman's correlation coefficients (P < 0.01) were presented in Table 2.

Table 2. Significant correlations of Spearman's rho ($P < 0.01$) between variables: clean feathers (DF0), moderately dirty feathers (DF1), very dirty feathers (DF2), absence of footpad dermatitis (FPD0), mild footpad dermatitis (FPD1), severe footpad dermatitis (FPD2), absence of hock burns (HB0), mild hock burns (HB1), severe hock burns (HB2) and breast burns.

Variables		DF0	DF1	DF2	FPD0	FPD1	FPD2	HB0	HB1	HB2	Breast burn
		r									
DF0	*p-value*	1	**-0.699**	**-0.444**	0.257	-0.228	-0.229	0.084	-0.103	-0.015	0.198
DF1		**0.000**	1	-0.198	-0.021	0.270	-0.036	0.123	-0.112	-0.157	-0.322
DF2		**0.000**	0.058	1	**-0.425**	0.095	**0.453**	-0.295	0.306	0.173	0.095
FPD0		0.040	0.866	**0.000**	1	**-0.326**	**-0.936**	**0.591**	**-0.579**	-0.313	-0.313
FPD1		0.070	0.031	0.456	**0.004**	1	0.122	-0.093	0.127	-0.215	-0.166
FPD2		0.069	0.776	**0.000**	**0.000**	0.335	1	**-0.611**	**0.590**	**0.403**	**0.339**
HB0		0.509	0.333	0.018	**0.000**	0.463	**0.000**	1	**-0.991**	**-0.624**	**-0.400**
HB1		0.417	0.379	0.014	**0.000**	0.319	**0.000**	**0.000**	1	**0.564**	**0.352**
HB2		0.905	0.216	0.172	0.012	0.088	**0.001**	**0.000**	**0.000**	1	**0.469**
Breast burn		0.117	0.010	0.455	0.012	0.189	**0.006**	**0.001**	**0.004**	**0.000**	1

DF0 = clean feathers; DF1 = moderately dirty feathers; DF2 = very dirty feathers.
FPD0 and HB0=no lesions; FPD1 and HB1=mild lesions; FPD2 and HB2=severe lesions. Significant correlations ($P < 0.01$) and correspondent r value were presented with bold letter.

Severe footpad dermatitis was positively correlated with the presence of mild hock burns (r = 0.590, P < 0.001), severe hock burns (r = 0.403, P = 0.001) and very dirty feathers (r = 0.453, P < 0.001). Breast burn was positively correlated with the presence of mild hock burns (r = 0.352, P = 0.004), severe hock burns (r = 0.469, P < 0.001), severe footpad dermatitis (r = 0.339, P = 0.006) and negatively correlated with absence of hock burns (r = - 0.400, P = 0.001).

Loadings of each variable to each PC after the varimax normalized rotation and the communalities from the PCs are presented in Table 3.

Table 3. Factor loadings and communalities of variables in the first two components (PC1 and PC2) after varimax normalized rotation.

Variables	**Factor loading**		**[a]CM**
	[b]PC1	**PC2**	
Bartlett's test of sphericity	$P < 0.0001$; df = 21; $\chi^2 = 271.584$		
[c]KMO measure	0.65		
Clean feathers (DF0)	-0.14	0.91	0.85
Moderately dirty feathers (DF1)	-0.17	-0.89	0.83
Absence of footpad dermatitis (FPD0)	-0.80	0.30	0.72
Severe footpad dermatitis (FPD2)	0.84	-0.19	0.75
Absence of hock burns (HB0)	-0.84	-0.19	0.75
Mild hock burns (HB1)	0.83	0.07	0.69
Breast burns (BBurn)	0.64	0.31	0.52
Eigenvalues	3.20	1.88	
Explained variance (%)	45.65	26.92	∑=72.57

[a] CM - communality.
[b] PC - principal component.
[c] KMO - Kaiser-Meyer-Olkin.

Fig. 1 show the projection of the seven selected variables on the two-dimensional space defined by the two PC.

The first and second principal components together (PC1-PC2) (Fig. 1) accounted for 72.57% of the data variance. PC1 showed a relationship between absence of footpad dermatitis (FL = - 0.80) and absence of hock burns (FL = - 0.88) on the left side of the figure and, on the right side, highly mild hock burns (FL = 0.83) and severe footpad dermatitis (FL = 0.84). Mild hock burns and breast burns were also associated, showing both factorial loadings higher than 0.60. The essential parameters for PC2 were clean feathers (FL = 0.91) and moderately dirty feathers (FL = - 0.89) shown in opposite planes.

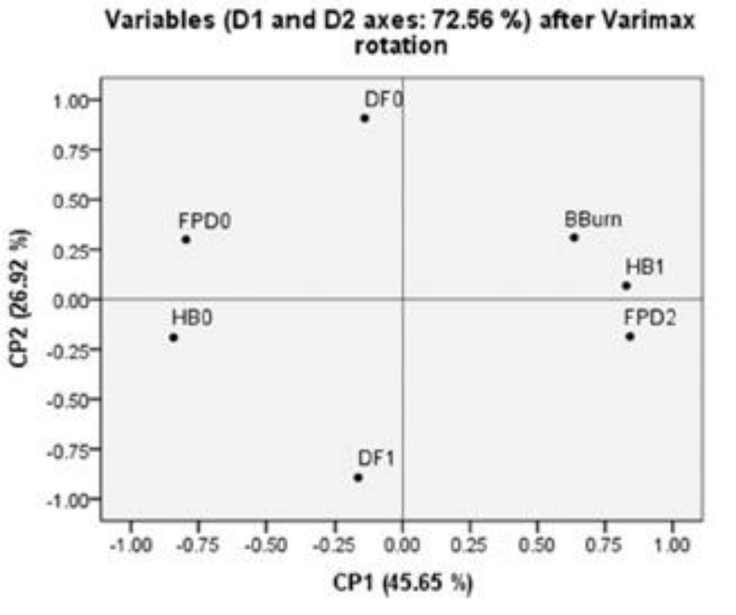

Fig. 1. Loadings for the PC1–PC2 dimensions, after varimax normalized rotation, of the seven variables selected to a principal components analysis: clean feathers (DF0), moderately dirty feathers (DF1), absence of footpad dermatitis (FPD0), severe footpad dermatitis (FPD2), absence of hock burns (HB0), mild hock burns (HB1) and breast burns (BBurn).

2.4. DISCUSSION

Plumage cleanliness is important for thermoregulation and when the feathers are wet or soiled by litter they may lose their protective properties, having negative effects on welfare of birds (Greene et al., 1985; Welfare Quality®, 2009). In our study, the effects of transport duration (≤ 1h; > 1h to ≤ 2h or > 2 h) and of waiting time at the slaughterhouse (≤ 4h; > 4h to ≤ 10h or > 10 h) on dirty feather condition was analysed and no significant effects were observed. Very dirty feathers and severe footpad dermatitis were highly correlated, probably due to a common cause - litter humidity. Taira et al. (2014) clearly demonstrated that reducing litter moisture is crucial for the control of footpad dermatitis. Footpad dermatitis was the most observed lesion, present in 48.92% of feet, in contrast with Haslam et al. (2007) who showed a very low prevalence of moderate or severe foot lesions (11.02%). Other studies showed higher footpad dermatitis percentages such as Allain et al. (2009) who reported a prevalence of more than 90% while Kjaer et al. (2006) reported a similar prevalence (44%) to those obtained in our study. Five flocks (7.8%) had no birds with footpad dermatitis lesions, in accordance with the 6.3% referred by Pagazaurtundua and Warriss (2006) who evaluated the severity of footpad dermatitis lesions in broilers at two slaughterhouses. It should be said that footpad dermatitis scoring systems are not standardized and final scores may differ slightly making difficult to compare results of different studies. Several experiments showed that the origin of contact dermatitis in broilers is multifactorial and its prevalence may be high. Footpad dermatitis is quite frequent, especially on farms that are not properly insulated during the colder months and may reflect a management conflict between heat conservation and ventilation (Meluzzi et al., 2008). However, as it has a significant economic ad welfare impact, both producers and authorities are trying to reduce the prevalence of footpad dermatitis (Shepherd and Fairchild, 2010; Taira et al., 2014). Severe footpad dermatitis were more frequent in class A and class B flocks, while absence of footpad dermatitis was more frequent in class C flocks, although flock classes did not differ significantly for these variables. Farms where higher stocking densities were practiced (class A and B) showed higher prevalence of footpad dermatitis. Several authors agree that footpad dermatitis predominate when high stocking densities are practiced (Dawkins et al., 2004; Hepworth et al., 2010).

A total of 9.67% hock lesions were observed with 7.34% of them mild hock burns and 2.33% severe hock burns. These values are higher than those found by Haslam et al. (2007) for moderate or severe hock lesions (1.29%) but similar (12%) to those shown by Hepworth et al. (2011). The absence of hock burns was more common in class A flocks than in class B flocks, with weight class C having intermediate values. The opposite was found for moderate hock burns, which was most common in class B flocks. Although the prevalence of severe hock burns was numerically highest in class C flocks, flock classes did not differ significantly for this variable. According to Kjaer et al. (2006) and Sørensen et al. (2000), the high prevalence of hook burns in heavier birds may be related to the fact that they spend more time lying on their joints as compared to lighter birds. In addition, significant differences for breast ulcers were observed in heavier birds (weight class C), probably due to their weight, which leads to an increased pressure on their breast.

The prevalence of breast burns was 0.56%, ranging from 0% to 15.47% per flock. This is higher than data reported by other studies, 0.2% by Bruce et al. (1990) and 0.02%, ranging from 0 to 1.56%, by Haslam et al. (2007). Class A flocks were those with the highest percentages of breast burns (1.32%). This result may be related to stocking density reaching its peak before the first “thinning out”. The higher the stocking density, the less the broilers can move and therefore birds spend longer time lying down in contact with the litter. The prevalence of score 2 measures was 28.72% for severe footpad dermatitis; 14.39% for very dirty feathers, followed by 2.33% for severe hock burns; there- fore, considering its severity, footpad dermatitis and dirty feathers were the main welfare problems detected.

The prevalence of birds with breast blisters (0.93%) was lower than that obtained (4.2%) by Allain et al. (2009). Zhao et al. (2009) showed that the occurrence of breast blisters is significantly affected by floor type and density and the differences in clinical scoring are probably due to these rearing conditions, which may diverge between farms and countries. Breast blisters evaluation is easy to perform on the slaughter-line but few references exist on its prevalence (Allain et al., 2009). Another reason for its occurrence is the degree of abdominal defeathering with a greater exposure of the skin to the environment that in turn contributes to the development of breast blisters (Proudfoot et al., 1979).

The prevalence of severe footpad dermatitis and hock burns was correlated, in line with Meluzzi et al. (2008). This correlation is stronger for mild than for severe hock burns. This may indicate that lesions began in the pads and when footpad dermatitis progressed to score 2, mild hock burns arose. This suggestion is in accordance with studies performed by Greene et al. (1985) and Kjaer et al. (2006).

Contact dermatitis is thought to be caused by a combination of moisture, high ammonia content and the presence of other chemicals in the litter (Berg, 2004). The prolonged contact with poor quality litter may explain the positive correlation between the presence of breast burns and severe footpad dermatitis ($r = 0.339$, $P = 0.006$), mild hock burns ($r = 0.352$, $P = 0.004$) and severe hock burns ($r = 0.469$, $P < 0.001$). Allain et al. (2009) reported a similar correlation between breast burns and severe hock burns ($r = 0.45$, $P < 0.01$) and Bruce et al. (1990) demonstrated that flocks with a high prevalence of both lesions showed reduced performance. Hepworth et al. (2011) suggest that management factors that reduce hock burns will also lessen carcass downgrades. The positive associations found for footpad dermatitis, hock burns, and breast burns suggest a common underlying factor, and, based on literature, litter quality seems the most likely cause.

2.5. CONCLUSION

The animal-based measures, collected at the slaughterhouse, allowed for monitoring the welfare of broilers at farm level. Comparing flocks accordingly to average BW, it was shown that absence of hock burns was more common in lighter flocks, mild hock burns and mild footpad dermatitis were more common in medium weight flocks, and severe hock burns and breast ulcer were more frequent in heavier flocks. Considering its severity, footpad dermatitis, dirty feathers and hock burns were the most observed welfare problems in the flocks studied.

It was demonstrated that a meaningful overview of broiler flocks welfare can be obtained by applying simple scoring scales. The welfare evaluation at the slaughterhouse may work, when unsatisfactory welfare reports are obtained, as an early warning sign to adopt procedures in view to improve on-farms birds' welfare.

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CHAPTER IV – WELFARE ASSESSMENT IN LAYING HENS

IV.1. FEAR AND WELFARE INDICATORS ASSESSMENT IN LAYING HENS FROM BARN SYSTEMS

IV.1. FEAR AND WELFARE INDICATORS ASSESSMENT IN LAYING HENS FROM BARN SYSTEMS

Abstract

This study analyses the effects of body weight (BW) and age on fear and welfare indicators and the relationships among welfare indicators. At 50 and 72 weeks of age, 100 laying hens (Novogen brown) were weighed and tested for tonic immobility (TI). After that, a physical examination was carried out to evaluate health conditions, hygiene status, feather damage and claw length. The majority of welfare problems that were detected at 50 weeks had increased by 72 weeks of age. A higher score for keel bone protrusion was observed in lighter (BW ≤ 1.9 kg, $P = 0.014$) and older hens (72 weeks, $P = 0.004$). Heavier hens (BW > 1.9 kg) showed longer TI durations ($P = 0.022$) and older hens required fewer TI inductions ($P = 0.025$) indicating that heavier and older hens were more fearful. Feather damage score on wings was positively correlated with feather damage scores on upper neck ($P < 0.01$) and on back, rump and tail ($P < 0.001$). Hens with higher feather damage scores on the head, back and tail showed a higher probability of having longer TI durations. Hens with skin injuries ($W = 4.100$, $P = 0.043$) were more likely to be induced into TI on the first attempt. Feather damage on back, head and tail and skin injuries increased with age and influenced the increase of fear. Additionally, high cumulative mortality rates (23-26%), keel bone deformations/fractures (57%) and keel bone protrusion (89%) should be considered relevant welfare indicators in laying hens from barn systems.

Keywords: barn system, bone condition, laying hen, fear, feather pecking, welfare indicator

1.1. INTRODUCTION

Laying hens in alternative systems are likely to benefit from such factors as more space and access to perches, nest boxes and littered areas (Ali et al., 2016). The presence of perches may affect the welfare in many ways, reducing fear, improving motor activity and providing preferred resting locations (Lay et al., 2011). Fear is regarded as an undesirable state of suffering and a powerful stressor which seriously affects animal's welfare (Abe et al., 2013), management, performance and profitability (Jones, 1997). Feather pecking and cannibalism have been associated with increased fear and stress in laying hens (El-Lethey et al., 2000, 2001; Freire and Cowling, 2013; Sherwin et al., 2010; Shimmura et al., 2010). The close contact with humans (Campler et al., 2009) and environmental complexity (Rodenburg et al., 2005) can be perceived by hens as potentially dangerous. The degree of fear may be estimated based on the duration of tonic immobility (TI) and on the number of attempts needed to induce TI (Hocking et al., 2001; Schütz et al., 2004). The TI is induced through a brief period of physical restraint during which birds become temporarily paralyzed and unresponsive to external stimuli (Abe et al., 2013).

Feather pecking is another important issue in laying hens, especially for alternative systems where flock management is difficult (Albentosa, et al., 2003; Kjaer et al., 2001). This undesirable behavior is characterized by laying hens pecking at and pulling out the feather of conspecifics and, in severe forms, injuring the skin (Odén et al., 2002; Ramadan and Von Borell, 2008). The presence of feather pecking behavior can be related to poor enrichment during the rearing period (Janczak and Riber, 2015), feeding and foraging behavior (Rodenburg et al., 2013), large flocks (Rodenburg et al., 2005) and high light intensity (Lambton et al., 2010; Mohammed et al., 2010). Mortality is a major health and welfare indicator and among commercial laying hens it is primarily caused by feather pecking and cannibalism (Gunnarsson et al., 1999; Lay et al., 2011). Other causes of mortality include health problems, infections by red mites and smothering in alternative systems (Rodenburg et al., 2008).

The keel bone which is prominent in laying hens is known to be a site of frequent fractures during their productive life (Casey-Trott and Widowski, 2016; Fleming et al., 2004). The increased vertical mobility in alternative systems, where hens must move between vertical levels to access resources, potentially place them at risk of bone fracture due to crash landings or environmental shock (Banerjee et al., 2014). The presence of keel bone deformations or fractures has been associated with chronic pain, reduced productivity and ensuing economic losses (Nasr et al., 2012; 2013).

The dirtiness of feathers and feet, disregarding the possible implications for food safety, can be directly related with management conditions and litter integrity. Usually, alternative systems use hybrid breeds which are more docile when compared with the more traditional hybrids used in cages (Rodenburg et al., 2005). The present study was performed in a traditional floor system equipped with perches at different levels, commonly referred to as a "barn system", housing Novogen Brown hens. This is considered a docile line, but there is no information regarding feather pecking behavior or keel bone fractures or any welfare study for this breed.

This study highlights the relationships between fear and welfare indicators by measuring a variety of different variables to identify behavioral issues, fear, injuries and health problems which can have significant impact on hen welfare.

The aim of the current study was to determine the effects of body weight (BW) (≤ 1.9 kg and >1.9 kg) and bird age (50 and 72 weeks) on fear and welfare indicators and the relationships between fear and welfare indicators in laying hens from two similar barn systems under commercial conditions. A second objective was to test the influence of BW, age and welfare indicators on TI duration and number of TI induction trails.

1.2. MATERIALS AND METHODS

1.2.1. Birds and housing conditions

The study was conducted in two structurally identical commercial farms both defined as barn systems. This system is characterized for having littered floor areas on both sides of one central structure over the manure pit. The manure pit is covered with a slatted floor equipped with rows of long feed troughs, water nipples, nests and perches at different levels. Feeders, drinkers, and nest boxes were on the slatted floor area and on the litter floor. Stocking density was 8.83 hens/m^2 (6 800 hens) in farm 1 and 8.86 hens/m^2 (6 200 hens) in farm 2. Overall, the study included 13 000 Novogen brown shaver pullets obtained at the same time from the same commercial supplier, with the same age and studied in parallel. The birds were beak-trimmed at approximately 10 days, floor reared with access to perches until 17 weeks of age and then transferred to the barn systems until end of lay at 75 weeks of age. Birds were vaccinated against Marek's disease, Newcastle disease, infectious bronchitis, infectious bursal disease and avian encephalomyelitis. From 20 weeks of age, the birds were kept on light/dark regimen of 16:8 h with a light intensity of 35 lux (on average) and the temperature maintained between 16 and 22ºC.

The study took place in February for 50 weeks old hens and in July for hens at 72 weeks.

1.2.2. Tonic immobility (TI) test

One hundred hens at 50 and at 72 weeks of age were randomly caught at the front and back of the pen from different locations (feeders, litter area, nest boxes, slatted floor and perches) and lights were dimmed for ease of handling. Hens were removed individually from the pen and testing took place in the same order as they were caught. Testing was distributed over four consecutive days per farm at 50 and again at 72 weeks of age and processed between 14:30 h and 19:00 h. The TI test was carried out in a separate noise-free room with the light at 35 lux to maintain environmental conditions familiar to the birds. TI was induced using a procedure similar to that described by Jones (1986), placing each bird gently upside down in a cradle and held by the experimenter with one hand by the head and the other hand over the sternum. After 30 s the bird was slowly released and the experimenter looked directly at the bird from a distance of one meter. Birds were allowed to stay in TI for a maximum of 300 s on any occasion, after which they were gently righted. If the bird righted itself within 15 s after TI induction, TI was induced again, up to three attempts per bird. If TI was not attained after three attempts, a score of 0 s was given in accordance to Wang et al. (2013). The number of inductions required to obtain TI and the time until the bird righted itself was recorded for each bird. All catches and TI tests were performed by the same person.

1.2.3. Physical examination of birds

After performing the TI test, each bird was weighed and submitted to a complete physical examination. The average BW across both ages was 1.90 ± 0.15 kg. Hens at 50 weeks of age had an average BW of 1.88 ± 0.13 kg (n = 100), ranging from 1.67 to 2.11 kg. At 72 weeks of age, hens had an average BW of 1.91 ± 0.17 kg (n = 100), ranging from 1.55 to 2.26 kg.

A four-point feather damage-scoring scale (0 = no damage to 3 = very damaged) was applied on 10 body regions: head, upper neck, under neck, back, rump, wings, tail, legs, breast and belly. The total feather score (ranging from 0 to 30 points) was presented as group mean. A four-point scale was also used to record body dirtiness and feet dirtiness (0 = clean to 3 = very dirty) (adapted from Sherwin et al., 2010). Claw length was measured with a digital vernier calliper, to the nearest 0.01 mm, from the root to the claw tip, of centre front and back toe (Shimmura et al., 2010). Skin injuries were recorded using a two-point scale (0 = absence or 1 = presence of injuries or scratches) and presented as percentages. For vent damage, bumble-foot and toe lesions a two-point scale (0 = absence or 1 = presence) was applied (adapted from Sherwin et al., 2010). Keel bone protrusion and keel bone fractures/deformations were determined by palpation using a three-point scale (0 = absence; 1 = slight or 2 = extensive) to record both keel bone protrusion (adapted from Sherwin et al., 2010) and keel bone fractures/deformations (adapted from Casey-Trott and Widowski, 2016; Fleming et al., 2004). Each farmer recorded daily flock mortality on standard record sheets.

1.2.4. Statistical analysis

The effects of age (50 and 72 weeks) and BW (≤ 1.90 kg and > 1.90 kg) on TI duration, number of TI inductions and welfare indicators were estimated by non-parametric Kruskal-Wallis tests and Chi-square tests. BW cut-off was done by mean (1.90 kg) and the sample was split into 94 hens below 1.90 kg and 106 hens above 1.90 Kg. Values are presented as mean (± SD). Statistical significance was set at P level < 0.05.

The potential relationships between keel bone protrusion, keel bone deformations, plumage condition, body dirtiness, feet dirtiness and number of TI inductions were examined using bivariate Spearman rank correlations. Spearman's rank correlation was also carried out to explore possible relationships between feather damage scores in 10 different body parts: head, upper neck, under neck, back, rump, wings, tail, legs, breast and belly. Statistical significance was set at P level < 0.01.

The Generalized Linear Model (GzLM) was used to study the effect of welfare indicators and fixed factors (age and BW) on TI duration and number of TI inductions (McCullagh and Nelder, 1989). A Gaussian error distribution with identity link function was applied for the TI duration and an ordinal logistic GzLM was applied for number of

TI inductions, the ordinal response variable. The number of TI inductions was categorized in accordance with level of fear and thus, category 1 represented a TI induction at the first attempt; category 2 represented a TI induction at the second attempt; category 3 represented a TI induction at the third attempt and in category 4 the TI was not attained after three attempts. The fixed factors were treated as covariates. Regarding categorical variables, the "score 0" was established as the reference category. In ordinal logistic GzLM, the threshold parameters, or the unknown boundary values were also estimated for TI induction. Residual analysis and Akaike Information Criterion (AIC) were measured for goodness of fit. Finally, the significance of the estimated parameters was tested according to a Wald Chi square statistic and non-significant interactions were removed to reduce the number of parameters in the final models. Spearman's correlations, Kruskal-Wallis and Chi-square tests were performed using SPSS 22.0. GzLM was performed using R.

1.3. RESULTS

Animal based-indicators of welfare were collected from 200 laying hens through a complete physical examination looking at dirtiness, health, presence of injuries, keel bone protrusion and deformations, feather damage and claw length. None of the laying hens examined showed evidence of vent damage or presence of bumble-foot and very few hens presented toe lesions. On the contrary, keel bone protrusion, keel deformations, skin injuries, body-feathers dirtiness, feet dirtiness and feather damage were frequently observed. One hundred forty-four hens remained in TI for at least 15 s. The other 56 hens did not show TI response within the three attempts. Twenty four hens (24%) at 50 weeks and 48 hens (48%) at 72 weeks of age remained in TI at the first attempt. The maximum duration of the test (300 s) was recorded on fourteen occasions (7%), ten of which occurred at the first attempt of induction. The effects of age and BW on fear and welfare indicators are presented in Table 1.

During the first scoring at 50 weeks of age, feather damage on breast and under neck was greater than in the other body parts. At 72 weeks, feather damage score was highest on the under neck, tail and head. The feather damage on the head, under neck, back, rump, wings and tail significantly increased with age, as well as the total feather damage score ($Z = -2.7$, 0.84 ± 0.41 vs. 1.09 ± 0.46, $P = 0.007$).

The presence of skin injuries increased from 48.00 ± 0.51% at 50 weeks of age to 78.00 ± 0.42% at 72 weeks (χ^2 = 9.653, *P* = 0.002). A higher score for keel bone protrusion (χ^2 = 11.185, 1.18 ± 0.44 vs. 0.86 ± 0.50, *P* = 0.004) and longer back toe claws (Z = 2.215, 2.03 ± 0.40 vs. 1.88 ± 0.24, *P* = 0.027) were observed in hens at 72 weeks. In contrast, feet dirtiness (χ^2 = 45.019, 2.28 ± 0.57 vs. 1.98 ± 0.55, *P* < 0.001) and breast feather damage (χ^2 = 8.565, 1.58 ± 0.73 vs. 1.10 ± 0.68, *P* = 0.014) improved with age.

Table 1. Mean values ± standard deviation (mean ± SD) and level of significance (P-value) for TI duration (s), number of TI induction trails, plumage damage scores, body dirtiness scores, feet dirtiness scores, keel bone protrusion scores, keel bone deformation/fracture scores and claw length (cm) at two different ages (50 and 72 weeks) and BW (≤ 1.90 kg and > 1.90 kg).

Indicators	**Age**			**BW**		
	50 weeks (n = 100)	**72 weeks (n = 100)**	***P***	**≤ 1.9 kg (n = 94)**	**> 1.9 kg (n = 106)**	***P***
TI duration (s)	59.3±81.6	77.6±86.5	ns	53.4 ±74.6	81.8 ±90.4	0.022
TI inductions trails (No.)	2.60±1.20	2.04±1.21	0.017	2.62 ±1.28	2.06 ±1.13	0.025
Head feather damage (score)	0.64±0.69	1.26±0.90	0.003	0.89 ± 0.87	1.00 ±0.86	ns
Upper-neck feather damage (score)	0.78±0.84	1.14±0.93	ns	0.98 ±1.01	0.94 ±0.79	ns
Under-neck feather damage (score)	1.24±0.98	1.84±0.82	0.009	1.53 ±1.00	1.55 ±0.91	ns
Back feather damage (score)	0.26±0.56	0.68±0.62	0.000	0.43 ±0.58	0.51 ±0.67	ns
Rump feather damage (score)	0.60±0.73	1.10±0.86	0.013	1.00 ± 0.86	0.72 ±0.80	ns
Wings feather damage (score)	0.50±0.61	1.06±0.79	0.001	0.81 ± 0.77	0.76 ±0.76	ns
Tail feather damage (score)	0.96±0.67	1.36±0.66	0.019	1.19 ±0.68	1.13 ±0.71	ns
Legs feather damage (score)	0.92±0.72	0.66±0.66	ns	0.75 ± 0.74	0.83 ±0.67	ns
Breast feather damage (score)	1.58±0.73	1.10±0.68	0.014	1.32 ±0.66	1.36 ±0.81	ns
Belly feather damage (score)	0.92±0.72	0.66±0.59	ns	0.83 ± 0.67	0.76 ±0.68	ns
Total feather damage (mean)	0.84±0.41	1.09±0.46	0.007	0.97 ±0.46	0.96 ±0.44	ns
Body dirtiness (score)	0.28±0.50	1.00±0.40	0.027	0.68 ±0.63	0.60 ±0.53	ns
Feet dirtiness (score)	2.28±0.57	1.98±0.55	0.000	1.98 ± 0.64	2.26 ±0.49	0.021
Claw length centre front (cm)	1.15±0.16	1.18±0.19	ns	1.17 ±0.16	1.16 ±0.19	ns
Claw length back (cm)	1.88±0.24	2.03±0.40	0.027	1.96 ±0.36	1.95 ±0.32	ns
Skin injuries (score)	0.48±0.51	0.78±0.42	0.002	0.60 ±0.50	0.66 ±0.48	ns
Keel bone protrusion (score)	0.86±0.50	1.18±0.44	0.004	1.15 ±0.47	0.91 ±0.49	0.014
Keel bone deformation (score)	0.72±0.73	0.80±0.78	ns	0.70 ±0.75	0.81 ±0.76	ns

Significant effect (*P* < 0.05); not significant (ns).

A BW effect was observed for TI duration (Z = -1.984, 53.38 ± 74.58 vs. 81.83 ± 90.39, *P* = 0.022) indicating that heavier hens are more fearful. Heavier hens had a higher feet dirtiness score (χ^2 = 7.772, 2.26 ± 0.49 vs. 1.98 ± 0.64, *P* = 0.021) and lower keel protrusion score (χ^2 = 6.251, 0.91 ± 0.49 vs. 1.15 ± 0.47, *P* = 0.014). The number of TI induction attempts was higher in younger (Z = -2.377, 2.60 ± 1.20 vs. 2.04 ± 1.21, P = 0.017) and lighter hens (Z = -2.237, 2.62 ± 1.28 vs. 2.06 ± 1.13, P = 0.025) indicating that younger and lighter hens are less fearful.

Keel bone deformations/fractures were shown in 57% of hens (scores 1 and 2), with similar occurrence at 50 weeks (56%) and at 72 weeks (58%). Keel bone protrusion was observed in 89% of hens (scores 1 and 2), 80% at 50 weeks and 98% at 72 weeks, although it was graded as slight in 76% of them (score 1).

At 50 weeks of age the cumulative mortality was 8.40% (farm 1) and 9.15% (farm 2) and at 72 weeks of age, the cumulative mortality was 19.14% (farm 1) and 23.32% (farm 2). At the end of laying period at 75 weeks of age, the cumulative mortality was 23.14 % (farm 1) and 26.32 % (farm 2). Daily mortality was higher mainly at the beginning of lay between 17 and 23 weeks of life and also at the end of laying period between 55 and 75 weeks of life.

Correlations between number of TI inductions and scores of total feather damage, body dirtiness, feet dirtiness, skin injuries, keel bone protrusion and keel bone deformation are presented in Table 2.

Table 2. Spearman correlations coefficient (r) and level of significance (*P-value*) between number of TI inductions, total feather damage score, body dirtiness score, feet dirtiness score, skin injuries score, keel bone protrusion score and keel bone deformation score (n = 200).

Variables	No TI inductions	Feather damage	Body dirtiness	Feet dirtiness	Skin injuries	Keel protrusion	Keel deformation
No TI inductions	1						
Feather damage	-0.123	1					
Body dirtiness	-0.094	0.523***	1				
Feet dirtiness	0.064	0.067	-0.019	1			
Skin injuries	-0.201	0.151	0.232	0.044	1		
Keel protrusion	0.045	0.105	0.296**	-0.380***	-0.092	1	
Keel deformation	0.003	0.005	0.025	-0.031	-0.107	0.210	1

Correlation is significant at **P < 0.01; ***P < 0.001.

A positive correlation was found between total feather damage score and body dirtiness score (r = 0.523, P < 0.001). Body dirtiness was also positively correlated with keel bone protrusion score (r = 0.296, P < 0.01). Feet dirtiness and keel protrusion scores were negatively correlated (r = -0.380, P < 0.001), indicating that as keel protrusion increases, feet dirtiness tends to decrease. Correlations between feather damage scores in 10 different body parts are presented in Table 3.

Table 3. Spearman correlations coefficient (r) and level of significance (*P-value*) between feather damage scores in 10 different body parts: head, upper neck, under neck, back, rump, wings, tail, legs, breast and belly (n = 200).

Variables (scores)	Head	Upper neck	Under neck	Back	Rump	Wings	Tail	Legs	Breast	Belly
Head	1									
Upper neck	0.480***	1								
Under neck	0.485***	0.458***	1							
Back	0.270**	0.433***	0.547***	1						
Rump	0.305**	0.172	0.373***	0.426***	1					
Wings	0.180	0.261**	0.219	0.448***	0.561***	1				
Tail	0.195	0.191	0.276**	0.384***	0.630***	0.624***	1			
Legs	0.050	0.141	0.113	0.114	0.155	0.121	0.185	1		
Breast	0.009	0.089	0.136	0.131	0.162	0.118	0.062	0.342**	1	
Belly	0.080	-0.020	-0.007	0.071	0.123	0.067	0.095	0.517***	0.414***	1

Correlation is significant at **$p < 0.01$; ***$p < 0.001$.

A positive correlation was found between feather damage on the head, upper neck, under neck, back and rump. Feather damage on legs, breast and belly were correlated, although neither of these three body parts correlated to the other seven body parts. A positive correlation was found between feather damage on wings and feather damage on neck, back, rump and tail. The effects of BW and welfare indicators on TI duration and number of TI inductions tested by generalized linear models (GzLM) are presented in table 4.

Table 4. Generalized linear models (GzLM) testing the effects of BW and welfare indicators on TI duration and number of TI inductions. *W-values*, *P-values* and estimate of the coefficient are presented for the explanatory variables.

Dependent variable	(I) TI duration (s) AIC = 1159.786			(II) No of TI inductions AIC = 261.967		
Explanatory variables	*W*-value	*P*-value	Estimate	*W*-value	*P*-value	Estimate
Body weight	3.555	0.059·	89.022	4.619	0.032*	2.692
Head (score 2/3)	2.859	0.091	37.239	-	-	-
Head (score 1)	8.053	0.005*	54.143	-	-	-
Upper neck (score 2)	10.050	0.001***	-73.238	-	-	-
Upper neck (score 1)	6.186	0.013*	-46.280	-	-	-
Back (score 2/3)	10.702	0.001***	109.527	-	-	-
Back (score 1)	0.864	0.353	16.442	-	-	-
Wings (score 2)	3.294	0.070·	-54.756	-	-	-
Wings (score 1)	1.165	0.281	19.291	-	-	-
Tail (score 3)	6.017	0.014*	57.423	-	-	-
Tail (score 2)	0.574	0.449	14.142	-	-	-
Belly (score 2/3)	4.813	0.028*	-51.354	-	-	-
Belly (score 1)	8.463	0.004**	-47.309	-	-	-
Skin injuries (score 1)	-	-	-	4.100	0.043*	-0.766
Threshold TI Induction	-	-	-	5.129	0.024*	-5.452
Threshold TI Induction	-	-	-	3.389	0.066·	-4.400
Threshold TI Induction	-	-	-	2.584	0.108	-3.826

W-value is the Wald statistic test; asterisks indicate significant values: *P < 0.05, **P < 0.01, ***P < 0.001.
Reference categories: Head (score 0); Upper neck (score 0); Back (score 0); Wings (score 0); Tail (score 0/1); Belly (score 0), Skin injuries (score 0).

Significant effects for TI duration and the number of TI inductions are presented in Figure 1 and 2, respectively.

A longer TI duration was obtained in hens with significant feather damage score 1 on head (W = 8.053; P = 0.005), score 2 or 3 on back (W = 10.702; P = 0.001) and score 3 on tail (W = 6.017; P = 0.014) when compared with reference categories (Table 4). On the other hand, shorter TI durations were observed in hens with significant higher feather damage score 2 on upper neck, score 2 on wings and score 1and 2 on belly when compared with score 0.

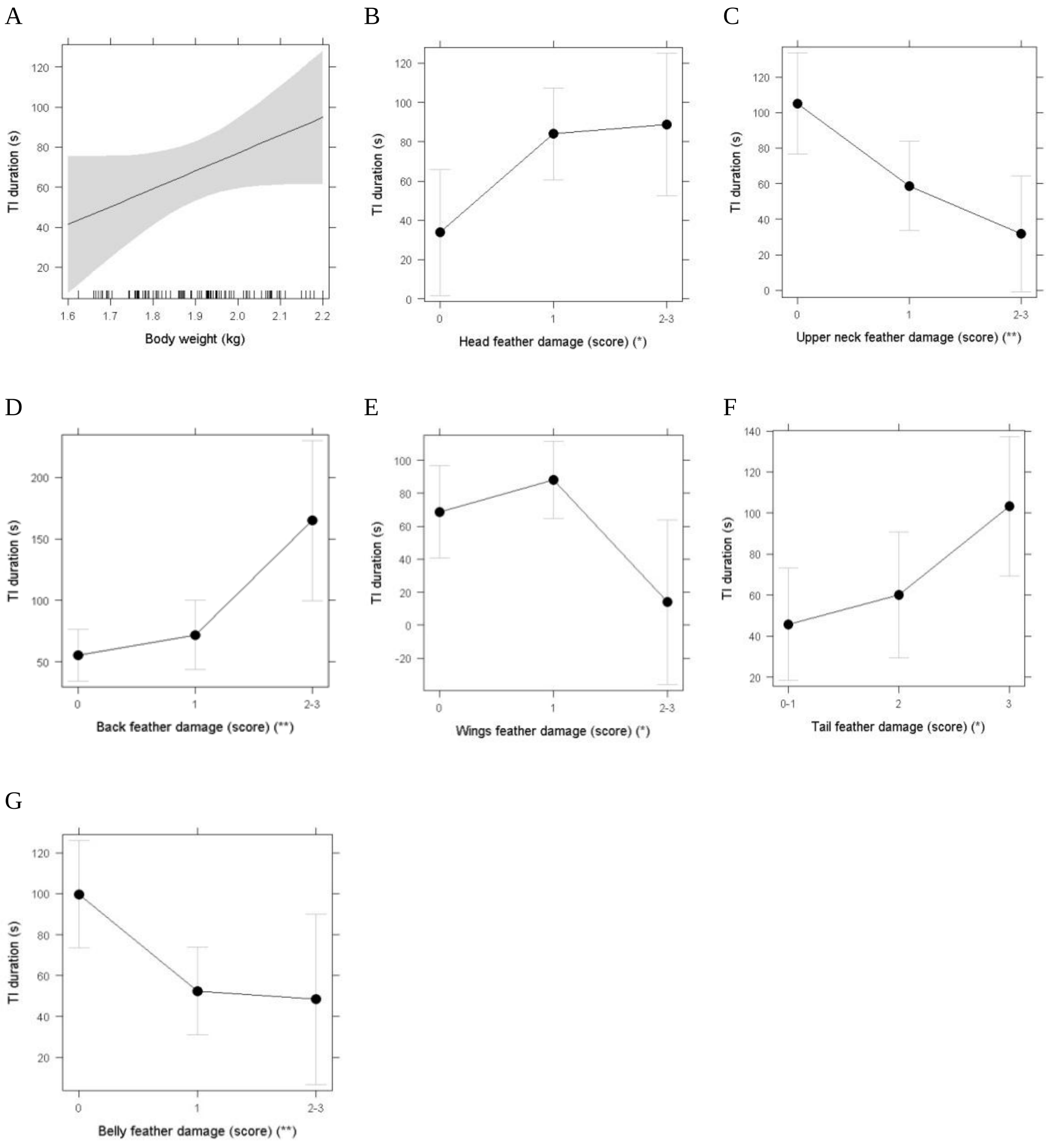

Figure 1. Effects of BW (A), head feather damage score (B), upper neck feather damage score (C), back feather damage score (D), wings feather damage score (E), tail feather damage score (F) and belly feather damage score (G) on TI duration (seconds).

Hens with skin injuries (W = 4.100, P = 0.043) were more likely to be induced into TI on the first attempt. The increase in BW (W = 4.619, P = 0.032) increased the probability of hens being induced at first attempt.

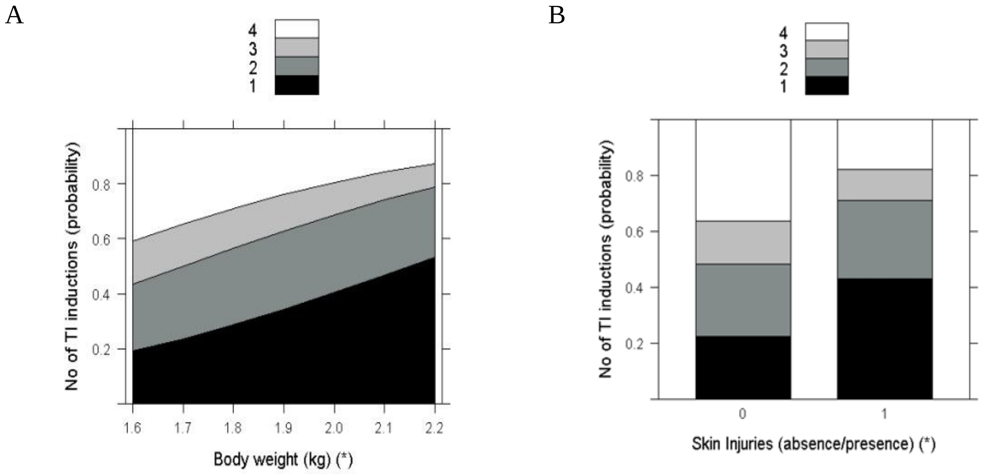

Figure 2. Effects of BW (A) and skin injuries (B) on the number of TI inductions (probability).

Ordinal scale: 1 = TI induction at the first attempt; 2 = TI induction at the second attempt; 3 = TI induction at the third attempt; 4 = TI was not attained after three attempts.

1.4. DISCUSSION

Differences in the fear response and welfare indicators were observed for hens with 50 and 72 weeks of age and between BW groups (≤ 1.90 kg and > 1.90 kg). Heavier hens presented longer TI durations and needed fewer attempts to attain it and were considered more fearful. Older hens required fewer TI induction attempts than those at 50 weeks of age and were therefore regarded as being more fearful. According to Campler et al. (2009) captivity may be a source of fearful experiences and the environmental and social factors will inevitably affect fear-related responses. The laying hens may have experienced TI anti-predator response in aversive situations, like aggressive pecking, attacks, threat and chasing from other birds and the long-term effects of stress situations may justify the older birds being more fearful. The increase of fear with age was previously observed in some other studies conducted in laying hens at various stages of production until 68-70 weeks of age (Anderson et al., 2004; Hansen et al., 1993; Uitdehaag et al., 2008). On the opposite, Hocking et al. (2001) and Albentosa et al. (2003) reported in their experiments that fear decreased with age; however the fear assessment was conducted in young hens with 0 to 31 weeks and 4 to 12 weeks of age, respectively. Additionally, Campo and Carnicer (1994) suggested that a stronger TI reaction is expected in older birds as a result of the increase of corticosterone levels, although more recent investigation is needed.

Hens with skin injuries remained in TI more often at the first attempt of induction. Therefore, the presence of injuries may be a welfare indicator related with fear. This finding is in line to Wahlström et al. (2001) and De Haas et al. (2013) experiments showing that fearful hens are probably subjected to a higher frequency of aggressive pecking behavior which resulted in injuries. Back feather damage had a significant influence on TI duration, being a longer TI duration expected in hens with back feather damage scores 2 or 3. Tail feather damage score 3 significantly increased with TI duration and, consequently, a higher scores of tail feather damage have a higher probability to increase the fear response.

A positive correlation was found between feather damage on wings and feather damage on back, rump and tail and according to Bilcík and Keeling (1999) feather damage is usually caused by severe feather pecking. In line with correlations of the present study, Ramadan and Von Borell (2008) showed that wings, rump, tail and back are the main targets for feather pecking. On the other hand, the positive correlations found for feather damage on legs, belly and breast can be important to identify factors, other than pecking behavior which can influence these variables similarly. The feather lost in these three parts can be related to walking and laying activity and with the brood patch on the underside of birds, the latter suggested by Bilcík and Keeling (1999). Abrasion due to different parts of the environment may also lead to wear, feather damage and feather loss (Guinebretière et al., 2013; Heerkens et al., 2015).

Despite, total feather damage score is often used as a convenient measure to assess feather pecking in flocks of laying hens (Gunnarsson et al., 1999; Rodenburg et al., 2005), the correlations obtained in present study suggested that total feather damage score should not comprise the legs, belly and breast parts. Moreover, by performing the GzLM was demonstrated that hens with higher feather damage on belly have a higher probability of having shorter TI durations.

Light intensity inside the housing systems was on average 35 lux, which is considered high and, for that reason, a potential contributing cause of feather pecking (Nicol et al., 2013; Odén et al., 2002). Contrary to almost all other welfare measures, feet cleanliness improved with age, from February to July and this could be attributed to temperature and litter condition, since feet cleanliness represents a positive indicator of management and environmental conditions.

Older and lighter hens presented a significantly higher keel bone protrusion. In line with the present study, Sherwin et al. (2010) showed that hens from barn systems were the lightest and had the greatest prevalence of severe keel protrusion. It is, therefore, an important indicator to consider in this housing system. Sherwin et al. (2010) also suggested that BW and keel protrusion are indicators of possible emaciation.

The prevalence of keel deformations/fractures was 56%. A high prevalence of keel bone deformations was also reported by Freire et al. (2003) and Rodenburg et al. (2008) in aviary systems (55% and 97%); and Käppeli et al. (2011) in aviary and floor pen systems (73%), being indicative of a very prevalent welfare problem in alternative systems.

Mortality rate found in both farms at the end of lay was high and in accordance with Lay et al. (2011) mortality can reach unacceptable high levels in alternative systems resulting from high risks of feather pecking and cannibalism. Both farms in our study had a large litter area which allowed hens to scratch, thus preventing excessive claw growth (Vits et al., 2005). Claw length was in average 19.5 mm for the centre front toe and 11.6 mm for the back toe, similar to those found in other studies carried out in large litter systems (Shimmura et al., 2010). The large amount of litter and more bird movement in alternative systems promoted foraging and scratching (Shimmura et al., 2010; Lay et al., 2011; Rodenburg et al., 2012). Therefore, welfare problems due to claw length over-growth, such as claw breaking and, in more severe cases, toe injuries and wounds, seem to be minimized in barn systems.

1.5. CONCLUSION

Fear, feather pecking, feather damage, keel bone deformations and flock mortality remain common problems in barn systems. A meaningful overview of the welfare of laying hens in barn systems can be obtained by applying simple scoring scales, such as those proposed in this study. The statistical models performed for TI duration and number of TI inductions showed that the increase in BW, presence of skin injuries, high back, head and tail feather scores had impact in the increase of fear response.

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IV.2. INFLUENCE OF DIFFERENT HOUSING SYSTEMS ON PREVALENCE OF KEEL BONE LESIONS IN LAYING HENS

IV.2. INFLUENCE OF DIFFERENT HOUSING SYSTEMS ON PREVALENCE OF KEEL BONE LESIONS IN LAYING HENS

Abstract

The present study aimed to investigate the effect of three housing systems (furnished cages - FC, Barns - B and free range - FR) on the prevalence and severity of keel bone protrusion and deformations. These health and welfare indicators were measure at the slaughterhouse, using a 4-point scale (0=absence, 1=slight, 2=moderate and 3=severe). Keel bone deformation was also categorized in relation to the presence of compression over the ventral surface, deviation from a 2D straight plane and deviation from the transverse (C-shaped) or median sagittal (S-shaped) plane. The housing system had a significant effect on prevalence of keel bone deformation ($\chi2 = 45.465$, df = 6, $P < 0.001$). In FR systems 60.40 % of hens presented keel bone deformation, followed by 54.20 % in FC and 53.50 % in B; however higher scores for keel bone deformations were more frequent in B systems. Although, keel bone protrusion was observed in all laying hens systems, the majority of hens only presented a slight degree (score 1) of protrusion. A positive correlation was obtained for keel bone protrusion and emaciation. The results could be used to initiate detailed investigations into problematic issues that occur during the laying period to improve the health and welfare conditions on farms.

Key words: Animal welfare, Housing system, Laying hen, Keel bone deformation, Slaughterhouse

2.1. INTRODUCTION

The keel bone is an extension of the ventral surface of the sternum, progressing along the midline of the sagittal plane. The keel spans from the cranial *Carina apex* to the caudal tip, with the keel spine tapering off as it approaches to the caudal portion of the keel (Casey-Trott et al., 2015). Due to its exposed anatomical location the keel bone is usually the first point of contact when collisions occur (Scholz et al., 2008), and therefore appears prone to damage (Donaldson et al., 2012). In avian species, the keel serves as an anchor for wing muscles attach, hereby providing adequate leverage for flight (Claessens, 2009).

In domestic fowl, the flight is not sustained over great distances, but instead fowl focus flight is associated to their anti-predator survival techniques on short bursts of direct-lifting flight, being the ratio between the body weight and pectoral muscle mass essential for flight (Duncker, 2000). The keel bone also plays an essential role in expanding and contracting the thoracic cavity during the respiratory process. Therefore, it is important to the successful of daily function of birds in both, flight and respiratory efficiency (Claessens, 2009). Furthermore, due to their genetic selection for high egg production, laying hens are at risk of rapid depletion of body reserves. Thus, the modern breast conformation of laying hens with a prominent keel bone may be a predisposition factor for its damage (Fleming et al., 2004; Sherwin et al., 2010). Gregory and Robins (1998) demonstrated that scoring the body condition of hens, according to the keel protuberance and breast muscles size, were well correlated with fat and muscle development. Visual assessment of the body condition directly in the slaughter line is straightforward and it is regularly used during meat inspection for condemnation of emaciated carcasses (Graft et al., 2017).

The European Union (EU) Council Directive 1999/74/EC established that laying hens may only be housed in either furnished cages or alternative systems, from 1st January 2012. Within the adoption of this regulation, the provision of perches in all types of housing systems became mandatory (European Union, 1999).

The presence of perches has been associated with higher incidence of damages in the keel bone (Scholz et al., 2008; Sandilands et al., 2009; Hester et al., 2013; Ali et al., 2016). High-impact injuries, unequal wing-loading during wing-flapping, perch use and compression fractures due to osteoporosis are the main causes of keel bone damage which can take different forms, including fractures, deformations, or indentations along the ventral edge of the bone (Pickel et al., 2011; Hester et al., 2013; Harlander-Matauschek et al., 2015). Soft perches increase the spread of pressure on the keel bone during perching, reducing keel bone deviations (Stratmann et al., 2015). However, in some countries raised slatted floors are currently used as perches and, in these cases, the pressure of the keel bone during perching is reduced (Donaldson et al., 2012). Recent research confirms that laying hens' keel bone is particularly susceptible to fractures (Casey-Trott and Widowski, 2016). The role of collisions as a cause of fractures was investigated by Toscano et al. (2012) using an ex vivo protocol to model bone fracture in laying hens and these that greater collision energies resulted in an increased likelihood of fractures and of greater fracture severity. Harlander-Matauschek et al. (2015) also emphasizing that more research should be addressed to the relationship between keel bone deformations and keel bone fractures. Impact injuries leading to fractures of keel bone can cause acute and/or chronic pain, which in turn may depress behaviour of laying hens and reduce their productivity, ensuing economic losses (Fleming et al., 2004; Nasr et al., 2012; 2013).

Previous studies have reported differences on prevalence of keel deformations which seems to depend on housing systems. In general, a range of 25 to 36% was previously reported for commercial layer cages flocks (Sherwin et al., 2010; Wilkins et al., 2011; Petrik et al., 2015) and approximately 48 to 90% in non-cage flocks in alternative housing systems, such as barn and free-range (Sherwin et al., 2010; Käppeli et al., 2011; Wilkins et al., 2011; Stratmann et al., 2015; Regmi et al., 2016).

Some risk factors closely linked to keel bone deformations, such as different housing designs (Wilkins et al., 2011; Stratmann et al., 2015), perch materials and designs (Pickel et al., 2011; Scholz et al., 2014; Stratmann et al., 2015), nutrition (Riber et al., 2018), reduced breast muscle mass of modern layers (Fleming et al., 2004) or genetic factors (Whitehead, 2004; Stratmann et al., 2016) have been investigated. Sandilands et al. (2009) suggest that the risk of keel bone damage can be reduced by preventively assessing each new housing system. Osteoporosis is also prevalent in hens from cages due to lack of exercise (Lay et al., 2011). However, hen's genetic improvements may influence health and bone strength (Whitehead, 2004; Fleming, 2006). To the best of our knowledge, this is the first study measuring, keel bone deformations and protrusion through a visual assessment at the slaughterhouse, using a 4-point scoring system. Previous investigations have shown that the prevalence of keel bone deformations within flocks increases throughout the laying period until the end-of-lay. In this context, it is important to collect these health and welfare information in slaughterhouses of laying hens.

The present study aimed to investigate the effect of three housing system (furnished cages - FC, barns - B and free range - FR) on the prevalence, severity and morphology of keel bone lesions.

2.2. MATERIAL AND METHODS

This study was conducted in a Portuguese poultry slaughterhouse. Sixteen batches of end-of-lay hens were assessed with regard to the condition and integrity of the keel bone. From a total of 41,435 slaughtered hens, 18,920 were from furnished cages (FC); 12,125 from barns (B) and 10,390 from free range (FR). On average, FC hens had 92 weeks of age (83 to 102 weeks), B hens had 78 weeks (75 to 84 weeks) and FR hens had 86 weeks (83 to 87 weeks), correspondingly to a body weight of 1.91 kg, 1.88 kg and 1.89 kg.

Breeds used in this study were HN brown, Lyline brown, ISA brown, Lohmann brown and Novogen brown which were reared identically in conventional aviary systems until the 17 week of age. Hereafter, birds were transferred to 16 adult eggs production systems namely, six FC (two ISA and four Lohmann), five B systems (three Novogen and two Lohmann) and five FR systems (three Lohmann and two HN).

The B system consisted of a traditional floor system with litter, slats over a manure pit and equipped with perches at different levels. Perches were composed of a circular metal pipe with a diameter of 5 cm. FR systems were characterized by having multi-levels with perches, nest boxes and feeders on each level. Birds were provided with continuous daytime access to land mainly covered with vegetation and access to outside via popholes. Perches were of the same material as used in barns.

Data were collected by inspection on slaughter line immediately after defeathering. From each flock, one hundred hens were randomly assessed for keel bone protrusion, deformations and morphology of damage. Detailed descriptions were used to standardize the lesions as presented in the assessment protocol (Table 1). Deformations were conducted using a 4-point scoring scheme, adapted from Scholz et al. (2008), indicating the severity of keel bone damage. For keel bone protrusion a 4-point scoring was used in adaption of Gregory and Robins (1998).

Table 1. Summary of assessment protocol conducted directly at the slaughter line.

Health and welfare variables	Measures for scoring
Keel bone protrusion	Score 0 = no protrusion (well-developed relatively round breast muscle with limited protuberance at the keel bone) Score 1 = keel less prominent (relatively well-developed breast muscle) Score 2 = keel prominent (moderately development of breast muscle) Score 3 = prominent ridge on the keel (scarce overall breast muscle)
Keel bone deformations	Score 0 = no deformation Score 1 = slight deformation Score 2 = moderate deformation Score 3 = severe deformation
Morphology of deformation	Compression over the ventral surface of the keel Deviation (moderate) from a theoretical 2D straight plane or a transverse (C-shaped) Deviation (severe) from a transverse (C-shaped) or a median sagittal (S-shaped) plane
Ascites, septicaemia and emaciation	Score 0 = absence Score 1 = presence

2.2.1. Statistical analysis

Pearson's chi-squared test (χ^2) was used to test the differences between the observed and expected frequencies of keel bone deformations and keel bone protrusion with regards to the housing system (furnished cages - FC, barns - B and free range - FR). Data followed a normal distribution and the *P-value* was set at 0.05.

Spearman's correlation coefficients ($P < 0.01$) were calculated to determine the relationship between keel bone deformations, keel bone protrusion, emaciation, septicaemia and ascites. Data analysis was carried out using XLStat (release 2011, Addinsoft).

2.3. RESULTS

Table 2 shows the frequencies of keel bone deformations (4-point) according to the type of housing system (FC, B and FR).

Table 2. Pearson's chi-square value (χ^2) and frequencies of keel bone deformation according to the 4-point scale and housing system.

	Keel bone deformation (%)			
Production system	**Score 0**	**Score 1**	**Score 2**	**Score 3**
Furnished cages (FC)	45.8^a	45.6^a	7.8^a	0.8^a
Barns (B)	46.5^a	35.5^a	13.0^b	5.0^b
Free range (FR)	39.6^b	49.1^a	8.7^a	2.6^a
χ^2 = 45.465, df = 6, p < 0.000				

In each row, systems with different superscript letters indicates statistical differences (p< 0.05).

The frequencies of keel bone deformations differed between the housing systems (χ^2 = 45.465, df = 6, P < 0.001). The prevalence of keel bone deformation was significantly higher in FR (60.4 %), followed by FC (54.2 %) and a lower prevalence was observed in B (53.5 %) system. However, the majority of the keel bone deformations were of slight degree (score 1). Moderate (score 2) and severe (score 3) deformations were more frequent in B flocks (18.0 %), followed by FR (10.3 %) and FC (8.6 %).

The frequencies of different morphologies (compression, moderate deviation and severe deviation) in relation to the type of housing system (FC, B and FR) are presented in Table 3.

Table 3. Pearson's chi-square value (χ^2) and frequencies of different keel bone shapes (compression, minor deviation and severe deviation) according to the type of housing system (FR, B and FC).

	Morphology of the damage (%)		
Production system	**Compression**	**Moderate deviation**	**Severe deviation**
Furnished cages (FC)	48.3^b	24.9^b	26.8^c
Barns (B)	32.4^a	28.5^b	39.2^b
Free range (FR)	28.7^a	47.3^a	24.1^a
χ^2 = 77.212, df = 4, P < 0.000			

In each row, systems with different superscript letters indicates statistical differences (p< 0.05).

The frequencies of keel bone morphology differed between the housing systems (χ^2 = 77.212; P < 0.001). In hens from B and FR systems, the compressive morphology was less observed, differing significantly from FC hens (48.3 %). On the opposite, hens from B showed most frequently a severe deviation of the keel bone (39.2 %).

Table 4 shows the frequencies of keel bone protrusion (4-point) according to the type of housing system (FC, B and FR).

Table 4. Pearson's chi-square value (χ^2), the number of degrees of freedom (df) and frequencies of keel bone protrusion (4-point) according to the type of housing system (FR, B and FC).

	Keel bone protrusion (%)			
Production system	**Score 0**	**Score 1**	**Score 2**	**Score 3**
Furnished cages (FC)	18.0^{c}	$68.2^{a,b}$	13.2^{a}	0.6^{a}
Barns (B)	12.0^{a}	73.5^{a}	13.0^{a}	1.5^{a}
Free range (FR)	6.4^{b}	65.4^{b}	26.4^{b}	1.8^{a}
$\chi2 = 68.77$, df =6, $P < 0.001$				

In each row, systems with different superscript letters indicates statistical differences (p< 0.05).

Keel bone protrusion was more frequent in hens from B with 93.6 %, followed by 88.0 % in FR hens and 82.0 % in FC hens. However, the majority of hens presented only a slight degree of keel protrusion, ranging from 68.2-73.5 % per housing system. Higher scores for keel bone protrusion were observed in hens from B with 26.4 % (score 2) and 1.8 % (score 3).

Keel bone deformations was positively correlated with keel protrusion ($r = 0.590$; $P < 0.001$). Keel protrusion was also positively correlated with emaciation ($r = 0.359$; $P < 0.001$) and with septicaemia ($r = 0.251$; $P < 0.001$).

2.4. DISCUSSION

This study demonstrates that keel bone deformations remain a prevalent welfare problem in all housing systems. A prevalence of 60.4 % was observed in FR, 54.2 % in FC and a lower prevalence of 53.5 % was observed in B systems.

Nicol et al. (2006) in a study with 36 barn flocks obtained a prevalence of 60% for keel bone deformations and fractures. Similar or even higher prevalence were found by Freire et al. (2003), Rodenburg et al. (2008), Käppeli et al. (2011) in alternative (non-cage) systems. In accordance to Blatchford et al. (2016) the prevalence of keel bone deformations within flocks would increase throughout the laying period, reaching the higher incidence (40–78%) in end-of-lay hens from alternative husbandry systems (Wilkins et al., 2004; Petrik et al., 2015; Blatchford et al., 2016). However, the most surprising result obtained in the present study was the prevalence of keel bone deformations in FC. It was higher than those obtained in the most recent research conducted in laying hens from cages systems (Sherwin et al., 2010; Wilkins et al., 2011; Petrik et al., 2015). According to Käppeli et al. (2011) current levels of keel bone deformations in cages may raise some concerns about the imminent introduction of higher activity husbandry systems. In agreement with Fleming et al. (2006), more active housing systems does improve bone strength, but does not necessarily result in lower fracture incidences due to the higher probability of traumatic accidents. In addition, Käppeli et al. (2011) considered the rules imposed by EU legislation, which banned the use of conventional battery cages, an unexpected challenge for laying hen industry, leading to an increased prevalence of keel bone damage. Early, Vits et al. (2005) has also enquired about the intensive use of perches in furnished cages that seemingly increases the occurrence of keel bone deformations. However, various studies indicate that keel bone deformations are more likely to arise in laying hens with weaker bones. In this context, the finding from this study can be related to the current layer hen genotypes which probably are not sufficiently robust to withstand production demands. Osteoporosis occurs with the decrease of the amount of structural mineralized bone tissues leading to bone fragility and higher susceptibility to fracture (Whitehead, 2004). In agreement to Riber et al. (2018) the large amounts of calcium required for eggshell production, starting at the onset of lay, it is possible that for high-producing layers—the cartilaginous keel bone receives less than adequate calcium for proper ossification during the early laying period which continues until approximately 40 weeks of age. The genetic selection is referred by Fleming et al. (2004) as a mean for improving the skeletal characteristics of hens.

In relation to severity of lesions, moderate and severe keel bone deformations were more frequent in hens from B (18.0 %), followed by FR (10.3 %) and, finally, FC (8.6 %). Several reports concluded that almost all moderate and severe keel bone deformations resulted from traumatic bone fractures and callus formation which are associated with chronic pain (Fleming et al., 2004; Scholz et al., 2008; Nasr et al., 2013; Petrik et al., 2015). These reports showed a high prevalence of moderate and severe keel bone deformations in alternative systems, suggesting that painful fractures are more probable in B and FR systems. These fractures may often be intensified due to the action of the breast musculature which causes additional movement and discomfort. Furthermore, a fractured keel is unlikely to be detected in a commercial laying hen house as easily as a long bone fracture and the animal may experience prolonged unnecessary suffering as a result of this (Fleming et al., 2004). A strong correlation between keel bone deformation and protrusion ($r = 0.590$; $P < 0.001$) was found, suggesting that a higher protuberance of the keel bone can be a predisposition factor for the occurrence of keel deformations/fractures. In this respect, a high prevalence of keel bone protrusion was identified in all housing systems. Nevertheless, 68.2 to 73.50 % of laying hens per housing systems presented only a slight degree of keel bone protrusion (score 1). A higher protuberance of the keel bone can be also a consequence of the reduced mobility and therefore the access to resources (feed, water, and nest boxe) (Ribet et al., 2018). These negative consequences are likely to differ among housing systems, too. For example, caged hens living in a highly restricted area and therefore the vital resources are more accessible.

Hens from B presented a significantly higher frequency of moderate protrusion with 26.4 % compared with hens from FR (13.2%) and FC (13.0 %). In addition, a positive correlation was found between keel bone protrusion and emaciation ($r = 0.359$; $P < 0.001$), showing that keel protrusion is an indicator of possible emaciation. These findings are in agreement with Sherwin et al. (2010) showing that B hens were the lightest at *post mortem* and had the greatest prevalence of severe keel protrusion. The type of housing system had a large effect on emaciation prevalence, but all housing systems produced hens that had protruding keel bones (Sherwin et al., 2010). Recently, Grafl et al. (2017) in an experiment assessing health and welfare at the slaughterhouse showed that hens with better body condition are correlated with significantly higher body weight.

The housing system has a significant effect on the morphology of the keel bone damage ($\chi2 = 77.212$, $P < 0.001$). Hens from FC presented a significantly higher compressive keel deformation (48.3 %) than B hens with 32.4 % and FR hens with 28.7 %. This could be due to osteoporosis which occurs normally in cage systems and it is also characterized by causing more often compressive lesion of the keel bone. On contrary, B hens showed more frequently severe deviations including D-deviation or S-deviation of the keel bone (score 2 or 3) (39.2 %). Significantly more deformations occur in the pens equipped with metal perches than in those equipped with plastic perches (Käppeli et al., 2011). In the case of a collision, harder materials such as metal cause more injuries compared with plastic perches. The high frequencies of keel deformations observed in the present study may be related to the fact that all perches were of metal. On the other hand, the severe deviations which occur more frequently in B may be a result of high impact trauma from high distances. In B system, the resources were on the floor and the perches were at different levels. In FR systems, each level had nest boxes and feeders without the need of hens to move between different levels to access resources; however the perches were at different levels. For this reason, multilevel perches potentially place laying hens at risk of bone breakage, due to crash landings or impacts with the environment due to movements between different levels to access resources (Scholz et al., 2008; Banerjee et al., 2014).

Selection of specific bone traits associated with bone strength as well as the related differences in body morphology (i.e., lower index of wing loading) have potential to reduce keel bone damage in commercial settings. Also, the housing environment (i.e., aviary design) may have additive effects (Stratmann et al., 2016). Käppeli et al. (2011) recommend bone strength to be considered in genetic selection of modern laying hybrids in order to reduce the prevalence of broken keel bones. Bishop et al. (2000) showed that it is possible to select laying hens with stronger bones without compromising laying performance. Further research should be conducted to improve recommendations for aviaries design, perch type and the array of perches within the system (Käppeli et al., 2011).

Good keel quality should be a prerequisite for all housing systems since the keel appears particularly vulnerable to fracture. In future design of aviaries, efforts should be taken to include conditions that help prevent accidents and keel bone deformations. It is also important to study the effects of keel bone damage on the affective states of laying hens especially on highly motivated natural behaviour such as perching.

2.5. CONCLUSION

A meaningful overview of keel bone integrity for laying hens can be obtained by using simple scoring scales at the slaughterhouse, highlighting the importance of using simple welfare indicators to be collected at the slaughterhouse. The type of housing system had a great effect on the prevalence of keel bone deformations/fractures. The high prevalence of keel bone protrusion in all housing systems indicates that breast muscle mass decreases in modern laying hens, with a more uncovered keel bone. Preventive measures must be taken on farms to avoid the occurrence of fractures which cause unnecessary suffering on animals for an extended period of time.

It is crucial to identify several areas of action to implement minimum standards for the protection and welfare for laying hens which can highlight specific problems that must be checked at the slaughterhouse as those proposed in the preset study.

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IV. 3. CAUSES OF CARCASS CONDEMNATION IN END-OF-LAY HENS ACCORDING TO FOUR DIFFERENT HOUSING SYSTEMS

IV. 3. CAUSES OF CARCASS CONDEMNATION IN END-OF-LAY HENS ACCORDING TO FOUR DIFFERENT HOUSING SYSTEMS

Abstract

Causes of condemnation in end-of-lay hens were studied at slaughterhouse and effects of age, body weight (BW) and housing system were investigated. A total of 1,156 (0.183%) birds were found dead on arrival (DoA) and 20,754 carcasses out of 629,331 (3.298%) were condemned during *post mortem* inspection. The main reasons for condemnation were peritonitis, septicaemia, salpingitis, emaciation and tumours. The means percentages of condemnation by septicaemia and emaciation differed according to age and BW groups and were more common in batches of younger (≤ 87 wks) and lighter (≤ 1.88 kg) birds. Moreover, peritonitis and ascites differ significantly between age groups, occurring more frequently in batches of older hens. The presence of tumours of the reproductive system was more frequently observed in older and heavier hens. This result raises the possibility of tumours being correlated with the higher number of prior ovulatory events. The total condemnation rate was lower in hens from organic systems, followed by free-range, and was significantly different from barns and cage systems. Carcasses with ascites and peritonitis were found more commonly in hens from cages and barns and both differed significantly from organic systems. Salpingitis was statically more prevalent in barns, presenting differences from organic and free-range systems. Monitoring condemnation causes of end-of-lay hens at slaughter provided a better understanding of health and welfare issues in different housing systems and allowed to identify potential welfare problems, which can be used to improve management and welfare on-farms.

Key words: Condemnation cause, Tumour, Emaciation, Laying hen, Slaughterhouse, Housing system

3.1. INTRODUCTION

Meat inspection is one of the most widely implemented and longest running surveillance systems. It was primarily introduced to identify meat unfit for human consumption, and progressively, recognized as a relevant source of data for monitoring animal health and welfare conditions (Stärk et al., 2014; Huneau-Salaün et al., 2015). A good example of this was the adoption of the Directive 2007/43/EC which provided minimum standards to ensure the protection of broilers during intensive production (European Union, 2007). Under this Directive, the official veterinarian should monitor and follow-up parameters at the slaughterhouse which are important to identify welfare problems in poultry farms (Saraiva et al., 2016).

Conditions or diseases relatively common in birds can be recognized during slaughterhouse inspection as some pathological changes will affect the carcass appearance at the time of slaughter (Collins and Huey, 2015; Grafl et al., 2017). The *post mortem* examination is important to identify some conditions as ascites which is difficult to detect on farms or at *ante mortem* inspection (EFSA, 2012). Cachexia and emaciation are difficult to differentiate at the slaughter-line, though the latter is associated with presence of disease (Bremner and Johnston, 1996; Haslam et al., 2008). Septicaemia has been referred as an important cause of rejection in poultry, principally due to bacterial infection by *Escherichia coli*. Other common organisms include *Staphylococcus* spp., *Pasteurella* spp. and *Salmonella* spp. (Bremner and Johnston, 1996; Collins and Huey, 2015). Infections with *E. rhusiopathiae* and *P. multocida* have also been reported in hens housed in litter-based systems, but not from in cage systems (Fossum et al., 2009). Ascites in laying hens is often associated with diseases of the abdominal cavity particularly, peritonitis and tumours (Tiwari et al., 2013) and is considered a sign of poor welfare in poultry farms (Butterworth and Niebuhr, 2009). Ovarian carcinoma and other reproductive system tumours can be highly prevalent in laying hens with metastasis affecting mainly the peritoneum and the visceral organs (Barua et al., 2009; Saraiva et al., 2013). Salpingitis is also a recurrent pathology in laying hens and is characterized by distension and inflammation of oviduct with accumulation of caseous exudate of fibrin, granulocytes, yolk and shell material (Landman et al., 2013). Concurrent peritonitis can arise through the compromised oviduct wall, leading to the spread of *E. coli* into the abdominal cavity and the accumulation of caseous exudate (Grist, 2006; Landman et al., 2013). Abscesses are less common in poultry than in other animals, but they can be found in laying hens with bumble-foot or following injurious due to feather pecking or cannibalism (Bremner and Johnston, 1996; Sherwin et al., 2010). Bruising can occur during crating, transport, uncrating and shacking of poultry (Nijam et al., 2005) and the number of animals dead on arrival (DoA) is considered a major welfare and health indicator of flocks transported to the slaughterhouse (Weeks et al., 2012).

It is important to determine the reasons and rates of condemnation in order to manage meat quality and safety, as well as to improve animal health and welfare (Salines et al., 2017). Moreover, pathologies of reproductive system are usually associated with decrease in egg production which in turn can be an indicator of environmental stress (Zanella et al., 2000).

The aim of this study was to determine the prevalence of DoA birds and of carcass condemnation causes in end-of-lay hens flocks and investigate the effects of age, BW and housing system.

3.2. MATERIAL AND METHODS

3.2.1. Population

The study was performed along three consecutive years and includes the assessment at the slaughterhouse of 224 batches (shipments) from 54 farms of laying hens. The production system in the source farms were: cages, 34 (62.96%); barns, 10 (18.52%); free-range, 5 (9.26%); organic free-range, 5 (9.26%). The hybrids used were Lohmann Brown, ISA Brown, Lohmann Selected Leghorn, Hy-Line Brown and Novogen Brown. All hens were vaccinated against Marek's disease, avian encephalomyelitis and infectious bronchitis. Batch size (number of birds per shipment) was on average 2,815 ± 970, ranging from 275 to 8,360 hens. Hens were, on average, 87 wks age old, ranging from 68 to 131 wks per batch. The average BW per batch was 1.88 kg, ranging from 1.55 to 2.18 kg.

3.2.2. Inspection at the slaughterhouse

The number of birds found DoA were recorded at the slaughterhouse. *Post mortem* inspection was performed at the slaughter-line on 629,331 carcasses. The number and percentages of condemnation causes were record per batch of transport. The most relevant condemnation reasons were identified by the same official veterinarian and consisted of: abscesses/cellulitis, ascites, extensive bruising, emaciation, salpingitis, septicaemia, peritonitis and tumours. In all these cases carcasses and offal were condemned and considered unfit for human consumption. Other causes of condemnation, unrelated to bird health condition and welfare, included mechanical trauma, faecal contamination and poor bleeding. These were not included in this study and represented only 0.05% (290 birds) of carcasses condemned.

3.2.3. Statistical analysis

The effect ($P < 0.05$) of age, BW and housing production system was studied using non-parametric tests (Mann-Whitney-Wilcoxon and Kruskal-Wallis tests) for percentage of DoA per batch of transport, as well for abscesses/cellulitis, ascites, emaciation, extensive bruising, peritonitis, salpingitis, septicaemia, tumours and total condemnation per slaughtered hens in which batch of transport. The cut-off for the analysis of age and BW effect was made by mean values. These two groups consisted respectively, of batches with ages between ≥ 68 and ≤ 87 wks (n = 135) and between > 87 and ≤ 131 wks (n = 89) and in batches of hens with average BW between ≥ 1.55 and ≤ 1.88 kg (n = 121) and between > 1.88 and ≤ 2.18 kg (n = 103). As regards to the housing system, the effect of four different production systems were investigated namely, organic (n = 10), free-range (n = 14), barn (n = 66) and cage (n = 134) systems. Data analysis was carried out using XLStat (release 2011, Addinsoft).

3.3. RESULTS

The number of animals transported in 224 batches for slaughter were 630,487 and from these, 1,156 (0.183%) were found DoA. The *post mortem* examination included the inspection of 629,331 carcasses and offal at the slaughter-line and 20,754 (3.298%) carcasses were condemned by different causes.

The number and percentages of condemnation reasons, as well as mean, standard error, range, minimum and maximum values of DoA, condemnation causes and total condemnation, expressed as an average percentage per batch, are summarized in Table 1.

Table 1. Number and percentage of carcasses condemnation, as well as mean, standard error and range of variables expressed as a mean percentage.

Variables	**No. condemned (%)**	**Mean (%)**	**SE of Mean (%)**	**Range (%)**	**Minimum (%)**	**Maximum (%)**
Dead on arrival	1,156 (0.183)	0.174	0.014	1.667	0.000	1.667
Abscesses/Cellulitis	65 (0.010)	0.010	0.003	0.551	0.000	0.551
Ascites	1,579 (0.251)	0.250	0.013	1.248	0.000	1.248
Extensive bruising	77 (0.012)	0.011	0.011	0.273	0.000	0.273
Emaciation	3,690 (0.586)	0.584	0.031	3.314	0.056	3.370
Peritonitis	4,964 (0.789)	0.794	0.028	2.386	0.000	2.386
Salpingitis	3,837 (0.610)	0.615	0.025	3.304	0.000	3.304
Septicaemia	4,346 (0.691)	0.661	0.053	9.068	0.000	9.068
Tumours	2,196 (0.349)	0.349	0.021	2.108	0.000	2.108
Total condemnation	20,754 (3.298)	3.273	0.132	19.285	0.612	19.897

The mean percentage of total condemnation of carcasses and offal rejected during *post mortem* inspection was 3.273% and differed considerably between batches, ranging from 0.612% to 19.897%. From the total condemned (20,754) birds, 4,964 (23.92%) were condemned by peritonitis, 4,346 (20.94%) by septicaemia and 3,837 (18.49%) by salpingitis. Abscesses/cellulitis and extensive bruising were considered minor causes of condemnation, comprising together only 0.68% of rejections. Septicaemia represented, at least in one batch, a maximum value of condemnation of 9.068%. The effect of age groups (≥ 68 to ≤ 87 wks, n = 135 and > 87 to ≤ 131 wks, n = 89) on percentages of DoA birds, of condemnation causes and total condemnation is presented in Table 2.

Table 2. Level of significance of percentages (Mean ± standard deviation) for DoA, abscesses/cellulitis, ascites, emaciation, bruising, peritonitis, salpingitis, septicaemia, tumours and total condemnation according to age groups (≥ 68 to ≤ 87 wks, n = 135 and > 87 to ≤ 131 wks, n = 89).

Variables	**Age ≥ 68 to ≤ 87 wks (n = 135)**	**Age > 87 to ≤ 131 wks (n = 89)**	***P*-value**
Dead on arrival (%)	0.193 ± 0.232	0.144 ± 0.143	ns
Abscesses/Cellulitis	0.011 ± 0.037	0.009 ± 0.060	ns
Ascites (%)	0.233 ± 0.196	0.274 ± 0.181	0.021
Emaciation (%)	0.612 ± 0.513	0.513 ± 0.362	0.002
Extensive bruising	0.012 ± 0.037	0.011 ± 0.041	ns
Peritonitis (%)	0.802 ± 0.404	0.865 ± 0.429	0.030
Salpingitis (%)	0.634 ± 0.391	0.622 ± 0.344	ns
Septicaemia (%)	0.664 ± 0.970	0.554 ± 0.364	0.002
Tumours (%)	0.332 ± 0.275	0.383 ± 0.372	0.046
Total condemnation	3.307 ± 2.250	3.220 ± 1.493	ns

Significant difference ($P < 0.05$); no significant difference (ns).

Septicaemia (P = 0.002) and emaciation (P = 0.002) were more common in younger hens (≥ 68 to ≤ 87 wks). In contrast, ascites (P = 0.021), peritonitis (P = 0.030) and tumours (P = 0.046) were more frequent in older hens (> 87 to ≤ 131 wks). Rates of total condemnation and of salpingitis were similar among age groups. The effect of two BW groups (≥ 1.55 to ≤ 1.88 kg, n = 121 and > 1.88 to ≤ 2.18 kg, n = 103) on percentages of DoA birds, of condemnation causes and of total condemnation, is presented in Table 3.

Table 3. Level of significance of percentages (mean ± standard deviation) for DoA, abscesses/cellulitis, ascites, emaciation, bruising, peritonitis, salpingitis, septicaemia, tumours and total condemnation according to BW groups (≥ 1.55 to ≤ 1.88 kg, n = 121 and > 1.88 to ≤ 2.18 kg, n = 103).

Variables	**BW ≥ 1.55 to ≤ 1.88 kg (n = 121)**	**BW > 1.88 to ≤ 2.18 kg (n = 103)**	***p*-value**
Dead on arrival (%)	0.213 ± 0.235	0.127 ± 0.143	<0.001
Abscesses/Cellulitis (%)	0.009 ± 0.035	0.013 ± 0.059	ns
Ascites (%)	0.254 ± 0.194	0.249 ± 0.188	ns
Emaciation (%)	0.649 ± 0.537	0.489 ± 0.330	<0.001
Extensive bruising (%)	0.011 ± 0.039	0.011 ± 0.039	ns
Salpingitis (%)	0.677 ± 0.393	0.580 ± 0.345	0.009
Septicaemia (%)	0.726 ± 1.016	0.482 ± 0.307	<0.001
Peritonitis (%)	0.816 ± 0.408	0.790 ± 0.422	ns
Tumours (%)	0.324 ± 0.217	0.394 ± 0.400	0.046
Total condemnation (%)	3.499 ± 2.294	3.007 ±1.502	ns

Significant difference (*P* < 0.05); no significant difference (ns).

Emaciation (P < 0.001), salpingitis (P = 0.009), septicaemia (P < 0.001), were more frequent in batches with BW below the mean value (= 1.88 kg). In contrast, tumours (P = 0.046) were more frequently observed in heavier hens. The percentage of birds with abscesses/cellulitis, bruising, peritonitis did not differ between BW groups. The effect of different housing systems on percentages of DoA birds, of condemnation causes and total condemnation is presented in Table 4

Table 4. Level of significance of percentages (Mean ± standard deviation) for DoA, abscesses/cellulitis, ascites, bruising, emaciation, peritonitis, salpingitis, septicaemia, tumours and total condemnation according to the housing system (organic, n = 10; free-range, n = 14; barn, n = 66 and cage, n = 134).

Variables	Organic system (n = 10)	Free-range (n = 14)	Barn system (n = 66)	Cage system (n = 134)	*p*-value
Dead on arrival (%)	0.109 ± 0.096	0.147 ± 0.131	0.207 ± 0.264	0.177 ± 0.177	ns
Abscesses/Cellulitis	0.000 ± 0.000	0.004 ± 0.013	0.004 ± 0.021	0.013 ± 0.059	ns
Ascites (%)	0.110 ± 0.075^{a}	0.163 ± 0.169^{ab}	0.238 ± 0.155^{bc}	0.276 ± 0.208^{c}	0.001
Extensive bruising (%)	0.000 ± 0.000	0.000 ± 0.000	0.005 ± 0.020	0.016 ± 0.047	ns
Emaciation (%)	0.462 ± 0.223	0.545 ± 0.461	0.640 ± 0.532	0.570 ± 0.436	ns
Peritonitis (%)	0.498 ± 0.140^{a}	0.614 ± 0.249^{ab}	0.818 ± 0.343^{bc}	0.824 ± 0.459^{c}	0.011
Salpingitis (%)	0.360 ± 0.173^{a}	0.492 ± 0.272^{ab}	0.663 ± 0.299^{c}	0.628 ± 0.412^{bc}	0.001
Septicaemia (%)	0.348 ± 0.130	0.535 ± 0.515	0.693 ± 0.614	0.682 ± 0.909	ns
Tumours (%)	0.193 ± 0.139	0.306 ± 0.237	0.300 ± 0.242	0.388 ± 0.361	ns
Total condemnation	1.935 ± 0.564^{a}	2.657 ± 1.328^{ab}	3.366 ± 1.570^{bc}	3.391 ± 2.234^{c}	0.011

In each row, means with different superscript letters differs significantly ($P<0.05$); no significant difference (ns).

Total condemnation rate was lower in hens from organic systems (1.935%), followed by hens from free-range (2.657%) and was similar in hens from barns (3.366%) and cages (3.391%). However, only hens maintained in organic production systems differed significantly from barn and cage hens. Peritonitis (P = 0.011) differed significantly between hens from organic and barn systems (P = 0.022) and between organic and cage systems (P = 0.038), with cage systems presenting the highest prevalence (0.824%). Salpingitis followed the same tendency, with significant differences between organic and barn systems (P = 0.002) and between organic and cage systems (P = 0.011), with barn systems presenting the highest prevalence (0.663%). Ascites (P = 0.001) was more frequently identified in hens from cages (0.276%), presenting significant differences with hens from organic systems (P = 0.008) and free-range (P = 0.029). Organic and barn systems also differed statistically (P = 0.037).

3.4. DISCUSSION

In the present study, end-of-lay hens from 54 different farms were transported in 224 batches to slaughter and condemnation causes were identified at the slaughter-line. The average mortality was 0.174%, ranging from 0 to 1.667% per batch. Few studies have been conducted on transport mortality in laying hens, however Weeks et al. (2012) in a survey of 13.3 million hens transported to slaughter in Great Britain observed an average mortality of 0.27% (median 0.15%). Another study (Petracci et al., 2006), showed an higher DoA mean value in Italian laying hens slaughterhouses (1.22%), with a very wide variation interval from 0 to 6.60%. Accordingly to the authors the pre-slaughter mortality in laying hens was critical due to injuries produced during catching, cage removal and crating, since osteoporosis in laying hens increases by the end of laying period leading to a higher occurrence of factures. Additionally, Newberry et al. (1999) monitored DoA rates of hens transported for slaughter in Canada and the United States and observed that mortality ranged from 0.7% to 2.3% depending on the duration of the journey. These authors explained high mortality rates by claiming that relatively few poultry processing companies are willing to accept hens because of their low meat value compared with broiler chickens and turkeys. For this reason, hens in Canada and the United States tend to be transported longer distances to slaughter than other types of poultry. In the present study, no significant age group effect or housing system effect was observed on DoA rates. However, a BW effect was found for DoA rate ($P < 0.001$), with lighter hens presenting a higher average mortality rate (0.213 ± 0.235% vs. 0.127 ± 0.143%). This is consistent with Weeks et al. (2012) showing that highly significant risk factors ($P < 0.001$) related to the conditions of birds on farm, namely lower BW will increase the risk of mortality on transport. From all carcasses totally condemned, the most relevant carcass condemnation reasons were peritonitis (23.92%), septicaemia (20.94%), salpingitis (18.49%), emaciation (17.78%), tumours (10.58%) and ascites (7.61%). Carcasses with extensive bruises, which were caused by traumatic lesions during pre-slaughter procedures, were subjected to total condemnation at the slaughter-line but represented only 0.37% of condemnations. Besides few reports performed on prevalence of condemnation causes in laying hens, Fossum et al. (2009) compared the mortality causes in laying hens in different housing systems and observed that colibacillosis was the most predominant disease in all housing systems. The most frequent pathological findings associated with colibacillosis consisted in acute or subacute fibrinous salpingitis, oophoritis and peritonitis. According to Wahlstrom et al. (2001) the main causes of hens mortality on farms included cannibalistic wounds, salpingitis and coccidiosis. Kajlich et al. (2016) assessed, during *post mortem* examination, the prevalence and severity of lesions in non-cage hens from commercial farms and verified that septicaemia lesions were observed in 23.1% of condemnations. Fulton et al. (2017) in an attempt to provide early detection of health problems in 16 egg-producing flocks, necropsied a representative sample to determine the cause of hen death, and the top 15 causes of normal mortality included egg yolk peritonitis, salpingitis, septicaemia, internal layer and prolapsed vent.

In relation to condemnation causes, ascites was more frequent in batches of older hens (P=0.021). In broilers, the continuous selection for either growth rate or feed conversion ratio, increases the pressure on metabolic processes and on the oxygen demand, increasing the occurrence of ascites (Gupta, 2011). However, in hens the presence of ascites has been associated with ovarian and oviduct carcinoma and is one of the features of advanced stages of ovarian cancer in chickens (Urick et al. 2008). Based on this assumption, Tiwari et al. (2013) collected ascites-derived cells from hens, which were maintained in short-term culture for determination of vascular endothelial growth factor (VEGF) expression. The mentioned study was the first to characterize malignant tumour cells derived from ascites of hens that had developed ovarian tumour. Barua et al. (2009) reported that laying hens develop epithelial ovarian tumours and exhibit a high rate of ovarian cancer (25 to 40%) in advanced ages. These findings further support the concept that ovulation with repeated cycles of rupture and repair of the ovarian epithelium may increase the number of proliferative events and genetic errors (Carver et al., 2011). The high ovulatory rate, almost daily, raises the possibility that tumours are correlated with the number of prior ovulatory events, justifying the presence of a higher prevalence of ascites in older and heavier hens.

Peritonitis was more frequently observed in older hens (P = 0.030). The continuous ovulation in laying hens is likely to contribute to a higher peritonitis incidence in older hens, due to abdominal posture when various ovum are released into the abdominal cavity, being surrounded by fibrinous material (Urick et al., 2008; Saraiva et al., 2013). Landman and Eck (2015) evaluated the pathogenesis, prevention and treatment of peritonitis, and the economic and welfare impact. The economic losses consisted mainly in the decrease egg production (Landman et al., 2013) with drops in egg production of about 1% to 2% (Zanella et al., 2000).

The percentage of carcasses condemned because of septicaemia was higher in younger (P = 0.002) and lighter hens (P < 0.001). This result can be related to downgrade conditions as a consequence of presence of infectious agents or management procedures that also lead to a low growth rate. In poultry farms, the infectious agents tend to be common to the whole flock and the identification of the microorganism causing disease is highly recommended (Ansari-Lari et al., 2007; Collins and Huey, 2015). The most common bacterial infection diagnosed in laying hens has been *E. coli*, which has been reported in many countries as a frequent cause of disease in commercial laying hens, as well as in hens in experimental trials (Vandekerchove et al., 2004; Jordan et al., 2005; Fossum et al., 2009).

Emaciation in more frequent in lighter birds ($P < 0.001$) which is consensual among studies. According to Sherwin et al. (2010) all housing systems produce a large amount of emaciated hens, but the type of housing system has a large effect on emaciation prevalence. Hens kept in cages were considerably heavier than those kept on litter (Wezyk et al., 2006; Sherwin et al., 2010). This can be explained by the fact that hens exposed to litter and soil are subjected to greater opportunity for disease and parasites spreading (Lay et al., 2001).

The total condemnation rate was lower in hens from organic systems, followed by free-range, and was significantly different from barns and cage systems. Carcasses with ascites and peritonitis were found more commonly in hens from cages and barns and both differed significantly from organic systems. Percentages of condemnation by peritonitis and by salpingitis in organic systems differed from both, barns and cages systems. Peritonitis was the most prevalent cause of condemnation in all housing systems, however with a higher percentage in hens from barns and cages. Salpingitis was statically more prevalent in barns, presenting differences from organic and free-range systems.

Emaciation was the second most frequent cause of condemnation in hens from organic (0.462%) and free-range systems (0.545%), however presented a lower prevalence of carcasses condemnation when compared to birds from barns (0.640%) and cages (0.570%). On contrary, septicaemia was the second most frequent cause of condemnation in barns and cage hens followed by salpingitis. Emaciation in hens from organic free-range systems can probably be justified by difficulties hens face in locating resources (Janczak and Riber, 2015). Feeding patterns can have an important effect on the intestinal microflora composition of hens, which may impact the host nutritional status and intestinal health (Wang et al., 2016; Cui et al., 2017).

Moreover, helminths have been more common in alternative systems compared with cages where they were rarely identified (Permin et al., 1999). Additionally, Kaufmann et al. (2011) observed in 18 organic free-range farms that almost all hens (99.6%) harboured at least one helminth species. A major challenge facing organic animal production systems is the management and treatment of health-related issues because in organic flocks the use of antibiotics and anthelmintics is restricted (Rodenburg et al., 2012; Sutherland et al., 2013).

Carcasses with ascites were found more commonly in hens from cages and barns, differing both from organic systems. Free-range and cage systems also differed for ascites. Probably birds in a more confined environment as cage and barn systems have a higher probability to induce ascites by reducing oxygen availability. However, the higher prevalence of ascites observed in cage hens can also be related to the fact that hens in cages are normally slaughtered at older ages compared to hens from alternative non-cage system.

3.5. CONCLUSIONS

The present study determined the main causes of carcass condemnation in end-of-lay hens and evaluated the influence of age, BW and housing systems on these. Ascites, peritonitis and tumoural lesions increased significantly with age, while emaciation and septicaemia were observed more frequently in younger hens. Regarding BW, it was shown that DoA birds, emaciation, salpingitis, and septicaemia were more frequent in lighter hens, which can be related to the presence of infectious agents or poor management procedures that may lead to a low growth rate. The type of housing systems influenced the percentage of ascites, peritonitis, salpingitis and total condemnation rates, with hens from barns and cages showing statistical differences from organic systems.

General health status of flocks under different ages, BW and housing systems may vary leading to carcasses of different quality and safety. Monitoring condemnation causes of end-of-lay hens at slaughter can help to support farm managers and veterinarians to initiate management check-ups, define the age of slaughter and improve health and welfare in their housing systems.

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CHAPTER V – DISCUSSION

DISCUSSION

Consideration of animal welfare is essential to address the consumers' demands and for the long term sustainability of commercial poultry (Ben et al., 2016). The results from these studies indicate that an industrialized agri-food systems is held primarily responsible for the perceived problems in the poultry value chain (Sonntag et al., 2018).

Poultry production is faced with different types of stresses that are responsible for issues of animal welfare as well as economic losses. Moreover, the immunity decreases when animals are stressed (Lara et al., 2017). For example, the pre-slaughter stress can result in variations in the glycogen storage and metabolic changes of muscle, responsible for quality poultry meat (Santonicola et al., 2017).

Recent attention has been given to developing welfare assessment tools for research purposes and for use directly on poultry farms. Historically, most of these tools have relied on resource-based measures and on management-based measures, but it was unclear how well they correlate with outcomes indicative of positive animal welfare (Blatchford, 2017). The Welfare Quality Assessment protocol for poultry (WQA) offers researchers a tool that has been validated, tested for repeatability, and standardized across farms (Welfare Quality®, 2009). However, many measures are still in need of validation. Assessing welfare in large poultry flocks can be done on farms, during transport and at different stages of slaughter process, being able to detect potential welfare risks and therefore to control or minimize its impact. New technological innovations potentially adaptable to commercial poultry are appearing, especially for broiler chickens and laying hens, although their practical implementation is still being defined. Technologies such as optical flow to detect gait problems on farms and feather pecking behaviour; infrared technologies to evaluate birds' thermoregulatory features and metabolism changes (Ben et al., 2016). All these technologies have the potential to be implemented at the commercial level to improve birds' welfare and to optimize flock management, therefore, improving the efficiency of the system in terms of use of resources and, thus, long term sustainability (Ben et al., 2016). Meanwhile the European Union, meat inspection aims to protect public health by ensuring that minimal hazardous material enters in the food chain. It also contributes to the detection and monitoring of animal diseases and welfare problems but its utility for animal surveillance has been assessed partially for some diseases only (Huneau-Salaun et al., 2015; Salin et al, 2017). Using the example of poultry production, during meat inspection at the slaughterhouse an taking in account the sensitivity of official veterinarians appeared to be very high to detect most of the conditions studied because is performed at batch level and applied to a high number of birds per batch (Huneau-Salaun et al., 2015; Saraiva et al., 2016). However, selective breeding programs are rapidly advancing, enhanced by both genomics and new quantitative genetic theory that offer potential solutions by improving adaptation of the bird to existing and proposed production environments (Muir et al., 2014). The outcomes of adaptation could lead to improvement of animal welfare by increasing fitness of the animal for the given environments, which might lead to increased contentment and decreased distress of birds in those systems (Beaumont et al., 2010). Moreover, evaluation of some welfare and conditions of laying hens is also essential to understand the influence of genetics, housing design, and management factors on their welfare (Blatchford, 2017).

Loading density, trailer microclimate, transport duration, animal size and condition, management factors including bedding, ventilation, handling, facilities, and vehicle design are main effects of welfare for broilers and laying hens (Mitchell & Kettlewell, 2004; Schwartzkopf-Genswein, 2012). These factor can have impacts on welfare (stress, health, injury, fatigue, dehydration, core body temperature, mortality and morbidity), as well as meat quality (shrink, bruising, pH, color defects and water losses) to varying degrees (Schwartzkopf-Genswein, 2012). It is clear that the effect of road transport is a multi-factorial problem where a combination of stressors rather than a single factor is responsible for the animal's well-being and meat quality post transport (Mitchell & Kettlewell, 2004). Achieving optimal animal well-being during pre-slaughter, carcass and meat quality will entirely depend on the quality of the animal transport process (Grashorn, 2010).

The poultry species are capable of feeling several states of suffering including fear, frustration and pain (Duncan, 2002). A start has been made to elucidate these states and the conditions that cause them, but much remains to be done. Duncan (2002) showed evidence suggests that the poultry species may also be capable of experiencing pleasure and concluded that, although poultry welfare is all to do with the subjective feelings, it is possible to be objective and scientific about these feelings. Differences in risk perceptions of public health and food safety hazards in various poultry husbandry systems by various stakeholder groups, may affect the acceptability of those husbandry systems (Van et al., 2018).

CHAPTER VI – CONCLUSIONS

CONCLUSIONS

The conclusion of the present work highlighted that handling at harvesting, transport, lairage and slaughter of poultry can adversely affect birds' welfare. By assessing several welfare indicators in batches of broilers transported from farms to slaughterhouse important risk factors affecting broiler welfare were identified. Dead on arrival rate, presence of bruises and dehydration were the most important identified indicators. Long transport distances and lairage durations, catching birds after midnight, inadequate catching and crating procedures can be factors with negative impact on ensuring high standards of welfare in broiler chickens.

The animal-based measures, collected at the slaughterhouse of broilers, allowed the monitoring of broilers welfare at farm level. It was shown that body weight influenced the occurrence of some lesions. As example, the absence of hock burns was more frequent in lighter flocks, while severe hock burns and breast ulcer were more frequent in heavier flocks. Considering its severity, footpad dermatitis, dirty feathers and hock burns were the most observed welfare problems in the flocks studied.

A meaningful overview of the welfare of laying hens in barn systems can be obtained by applying simple scoring scales, such as those proposed in this study. Fear, feather pecking, feather damage, keel bone deformations and flock mortality remain common problems in alternative production systems. The statistical models performed to test fear in laying hens, namely duration of tonic immobility and number of tonic immobility inductions showed that the increase in body weight, presence of skin injuries, high back, head and tail feather scores had impact in the increase of fear response.

The main causes of carcass condemnation in end-of-lay hens were influenced by age, body weight and housing systems. Ascites and peritonitis lesions increased with age, while emaciation and septicaemia were observed more frequently in younger hens. Dead on arrival, emaciation, and septicaemia were more frequent in lighter hens, which can be related to the presence of infectious agents or poor management procedures that may lead to a low growth rate. The type of housing systems influenced the percentage of ascites, peritonitis, salpingitis and total condemnation rates.

Monitoring condemnation causes of end-of-lay hens at slaughter can help to support farm managers and veterinarians to initiate management check-ups, define the age of slaughter and improve health and welfare in their housing systems.

This study is of great interest and practical applicability. It was demonstrated that a meaningful overview of broiler flocks welfare can be obtained by applying simple scoring scales. The welfare evaluation at the slaughterhouse may work, when unsatisfactory welfare reports are obtained, as an early warning sign to adopt procedures in view to improve on-farms birds' welfare.

On the other hand, the limited number of studies evaluating the welfare of laying hens under commercial conditions, highlight the importance of this work with the possibility of obtaining sustained data on a comprehensive scientific basis that allow classifying and comparing the laying hens welfare in different production systems and stages of the food chain.

A high prevalence of keel bone protrusion was observed in all housing systems showing the significance and applicability of these data. Keel bone integrity for laying hens can be obtained by using simple scoring scales at the slaughterhouse, highlighting the importance of using simple welfare indicators to be collected at the slaughterhouse. The type of housing system had a great effect on the results obtained for keel bone deformations. Preventive measures must be taken on farms to avoid the occurrence of fractures which cause unnecessary suffering on animals for an extended period of time.

It is crucial to identify several areas of action to implement minimum standards for the protection and welfare for laying hens which can highlight specific problems that must be checked at the slaughterhouse as those proposed in the preset study.

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