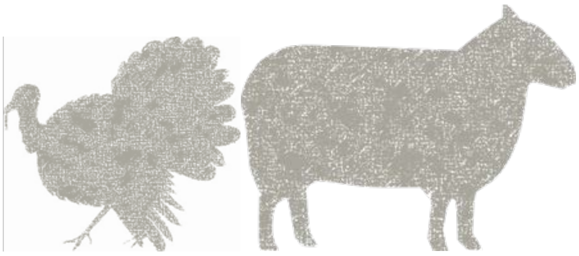
Universidad del País Vasco — Euskal Herriko Unibertsitatea

Development of practical methodology and indicators for on-farm animal welfare assessment



Thesis directed by: Dr. Inma Estevez

PhD candidate: JoannaMarchewka

Doctoral thesis submitted to the:

Department of Zoology and Animal Cellular Biology,
Faculty of Science and Technology,
University of Basque Country.

Vitoria Gasteiz, 2015

To My Mom with love…

Acknowledgment

The present work was carried out at the department of Animal Production at Neiker Tecnalia. Financial support was provided by the Animal Welfare Indicators project (AWIN; FP7-KBBE-2010-4). The PhD was carried also thanks to the technical and economic support from the Ministry for Economic Development and Competitiveness of the Basque Government.

I would like to thank Prof. Inma Estevez who has been my main supervisor for the last three and a half years of work and supported me in all aspects of it. Thank you for offering me this great opportunity and trusting that I would be able to carry successfully this task of completing the PhD degree. Thank you also for your patience, enthusiasm, constant encouragement and ability to make my experience so exciting. Thank you also for always constructive critics which made me a better researcher. I have learned with you about many varied aspects of research world and those experiences have made me convinced about which path to choose for my future. Thank also you for giving me opportunities to participate in so many international congresses and to meet great people during this time period. It all made my PhD studies a great journey.

Thank you to Dr. Roberto Ruiz, the Head of the Animal Production Department at Neiker Tecnalia for his positive attitude and constant support with any arising matter. Since my first day I have felt your patronage over all the undertaken activities. Thank you for making my stay at Neiker facile, thanks to which I was able to focus on the PhD work entirely. Thank you to the whole AWIN team under supervision of Prof. Adroaldo Zanella for great collaborations, time together during the project meetings, all support and advices.

I am grateful to Dr. Maja Makagon's Laboratory at The Animal Science Department at the Purdue University and Dr. Valentina Ferrante at the Department of Veterinary Science and Public Health at University of Milan for providing me the great opportunities of stays. Both Dr. Makagon and Ferrante provided me with great supervision during undertaking my studies at their labs. I have gained new perspectives and experiences while staying with both of you. You both treated me with unexpected hospitality, so that my stays abroad felt nearly like being home. Thank you!

I am grateful to Prof. Javier Loidi from the Department of Plant Biology and Ecology at the University of the Basque Country (UPV/EHU) for formal supervision of my work.

I am also very grateful for a great collaboration and all the support I have received from the industry, while conducting the studies. Many thanks to Grupo AN from Navarra in Spain and Perdue Farms from Indiana, USA and especially to Mark Guinn for making my work not only possible but also so much easier.

Many thanks to all the colleagues and persons working at Neiker Tecnalia. Some of them have become my best friends and a new family. Thank you to Ina for her help during my work and all constructive inputs I received from you. Thank you to Irene, Ane and Xavi, who on daily basis supported me in any aspect of the work and life. Thank you for all the discussions, sharing all the successes and failures, and for never letting me feel alone. Huge thanks to Giuseppe, who was my great support, advisor and friend during my stay and data collection in United States, being part of all the adventures we have gone through during those 3 month. Thank you to Guiomar in helping in my data collections. I am very grateful to Josune for help provided during my stay at Neiker. Thank you to Imanol Etxebarría and Juan Carlos Ochoa de Zuazola for the excellent care of the animals during undertaken experiments. Thank you also to Cristina, Yolanda, Iratxe, Ana, Deiene, Ernesto, Alex and IKT team for all the efforts to help me with the administrative tasks.

Finally, I am deeply thankful to all my family, especially my mom, who has always taken care of me and who helped me to reach this stage. I am grateful to my dad who was always by my side and to Pawel, who made my life so much easier, while helping me to relax in the mean times. All of you are the fuel in times of doubts.

Abstract

Work described in the doctoral thesis entitled “Development of practical methodology and indicators for on-farm animal welfare assessment” was conducted within the frame of Work Package 1 of the AWIN project by Joanna Marchewka. The research project aimed to optimize strategies for welfare assessment including pain in turkeys and sheep. Due to scarce knowledge on turkeys’ welfare and lack of methodology for its evaluation, the first part of work concentrated on the development of a new practical and dynamic protocol for on-farm welfare assessment of turkeys, adjusted to the particularities of large groups in which they are housed. The novel protocol approach based on transect walks, that included the evaluation of the main welfare issues commonly observed in meat poultry, was primarily validated on broilers as a model species. This first approach was used due to the possibility of comparing obtained by transect walks outcomes with results from referenced method based on individual sampling. In order to integrate most relevant behavioral indicators into the developed turkey specific protocol, literature review on this issue was accomplished. The transect-based protocol including welfare and selected behavioral indicators for turkey on-farm welfare assessment was designed and validated for its sensitivity as well as inter observer reliability, providing novel tool for efficient and practical on-farm meat poultry assessment. Second part of the thesis aimed at developing animal based behavioral pain indicators for sheep. Those indicators were not defined, prior to the work conducted within this thesis, for the individual lambs separated from their flock mates. Sheep, as other prey species has developed throughout the evolution process effective mechanisms to avoid showing evident signs of pain. This might in practical framing conditions refer to animals brought individually to a vet's surgery area and

which could reduce visible behavioral indicators of pain. Results of the conducted experiment found that tail docking-related pain is a factor modifying reactions of lambs to isolation, which should be considered as a proof of negative effects on the welfare caused by the common husbandry procedures, as tail docking especially performed without pain relief measures. Outcomes of this work will be integrated into sheep welfare assessment protocols currently being developed within AWIN project, as well as disseminated into everyday on-farm practice.

List of tables

List of figures

Table of contents

Chapter 1: *General introduction*

1.1 Animal welfare

The term "animal welfare" emerged to express the ethical concerns of the society, regarding the risks of decreased quality of life experienced by animals, particularly of those used in agriculture (Sejian, 2011). The main ethical concerns commonly expressed nowadays include: (1) that animals should live natural lives through the development and use of their natural adaptations and capabilities, (2) that animals should feel well by being free from prolonged and intense fear, pain, and other negative states, and by experiencing normal pleasures, and (3) that animals should function well, in the sense of satisfactory health, growth and normal functioning of physiological and behavioral systems (Fraser et al., 1997).

As the welfare of the animals can be assessed correctly only if the meaning of this term is clearly understood, numerous attempts have been made to find the best definition and methods to objectively measure it (Tannenbaum, 1991). The concept of animal welfare that scientists adopt, have a determining influence on the type of animal welfare research they undertake (Duncan & Fraser 1997) and, hence, on the type of information available to society for deciding on animal welfare issues. One thing all scientist seem to agree on is that there is no a single, reliable measure of animal welfare (Mason & Mendl, 1993; Appleby, 1999).

In practice, the definition of animal welfare should reflect a clear concept possible to be scientifically assessed (EFSA, 2006) which can be used by the scientific community and included in laws (Broom, 1991). The definition should also be clear to various stakeholders, such as corporations, consumers, veterinarians, politicians and others

(Hewson, 2003), as they present different attitudes towards animals and preferences for research methodologies (Weber and Zarate, 2005).

The term "animal welfare" is defined by the World Organisation for Animal Health (OIE, World Organisation for Animal Health, 2011; Terrestrial Animal Health Code) as: „how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behavior, and if it is not suffering from unpleasant states such as pain, fear and distress". The EU played a central part in the work leading to the OIE definition, which has now been recognized by more than 170 countries and it is currently used for scientific and legislation purposes.

1.1.1 Approaches to animal welfare

Initially, animal welfare mainly was referred to as the animal's physical health and well-being. On the other hand, some scientists considered welfare also related to the animal's psychological condition and feelings. These two visions became known as the 'biological functioning' school and the 'feelings' school respectively (Duncan, 2004).

According to the biological welfare approach to assure good welfare, the animal must be able to satisfy its biological needs. The term 'need' is understood as a fundamental requirement in the biology of the animal. Needs include obtaining basic resources or responding adequately to particular environmental or bodily stimulus that are essential for the survival or fitness of the individual (Webster, 2005). The range of functional systems controlling basic physiological mechanisms (body temperature, nutritional state, etc.) in conjunction with the behavioral response, will together allow the individual to control its interactions with the environment (Broom, 1981).

Therefore, it can be indicated that when an animal must carry out an action to adjust to its physiological state or environmental situation, it has a need (Broom and Fraser, 2007; Toates and Jensen, 1991). For example, all animals need food, or water, and they would have to perform a series of behaviors to ultimately obtain the required resources. However, even in the presence of the ultimate objective of the activity (feed and water), the welfare of an animal can still be compromised (Broom, 2002).

The Five Freedoms (Brambell Report, 1965, revised by FAWC 1993) is one of the first and more important documents regarding the basic principles to assure the welfare of animals under human management, including those intended for food or which act as working animals. The Five Freedoms were based on the needs that are considered basic to assure the welfare of animals and include:

- *Freedom from thirst, hunger and mal- nutrition* – by ready access to fresh water and diet to maintain full health and vigour.
- *Freedom from discomfort* – by providing a suitable environment including shelter and a comfortable resting area.
- *Freedom from pain, injury and disease* – by prevention or rapid diagnosis and treatment.
- *Freedom to express normal behavior* – by providing sufficient space, proper facilities and company of the animal's own kind.
- *Freedom from fear and distress* – by ensuring conditions which avoid mental suffering.

According to Webster (1994), the attainment of all five freedoms is unrealistic, but may be considered an attempt to achieve the best in a complex situation. In fact, some of the freedoms may conflict in situations where animals are cared for by man. For example, there could be a conflict between the treatment to cure illness/disease and the freedom from fear and distress that may be caused by the handling and/or the procedure. However, generally, the consideration of these five basic principles will assure that animals´ welfare is optimal.

The biological functioning school considers welfare also connected to the absence of a physiological stress response (Duncan, 2005). However, it is essential to consider that not always animals that appear to be distressed show a stress response (Terlouw, 1991), neither that animals showing a stress response, for example when anticipating a reward, will result in reduced welfare (Szechtman, 1974).

Ethical concerns regarding the animals´ quality of life may also arise when the adaptations of an animal do not fully correspond to the challenges posed by its environment (Fraser et al., 1997). A frequently cited definition for animal welfare describes it as the state of an animal regarding its attempts to cope with its environment (Fraser and Broom, 1990). It includes how much an animal has to do to cope and the extent to which it is succeeding in or failing to cope (Broom, 1996). The ability of an animal to adapt to a varying environmental situation is essential for survival. Coping is essentially a reflection of the physical condition of the animal, although its mental state may also contribute to this condition (Fraser and Broom, 1990). Adaptations can involve the animal's anatomy (growing thick fur in winter), physiology (catabolizing glycogen under cold conditions), or behavior (moving to a warm environment). The behavioral repertoire of animals includes many activities that are adaptations to cope with adverse circumstances, while some adaptations involve subjective feelings such as hunger, cold and pain that motivate animals to act in certain ways or that stimulate certain forms of learning (Fraser, et al., 1997).

Many scientists have put particular emphasis in ’80 and ’90 of the twenties century, on the subjective experience of animals in developing scientific conceptions of animal welfare (Duncan, 2005). Some authors presented the idea that welfare is mainly (Dawkins, 1990) or solely (Duncan, 1993) dependent on what the animal feels more than its response. The feelings school considers that welfare relates to what the animal feels, with the absence of negative emotional states creating ‘suffering’. Suffering may include states such as pain, fear, frustration, deprivation and, in some species, boredom (Duncan, 2004).

However, even if we consider that concerns about animal welfare are, in reality, concerns about subjective experience, animal welfare research cannot be limited to the subjective experience of animals because science cannot yet give empirical answers to many ethically relevant questions regarding the subjective experience of animals (Fraser et al., 1997). Therefore, feelings of animals can be derived from their structure and functions and also from their behavior (Dawkins, 1980; Duncan, 1993; Duncan, 2005). In this case welfare should be conceptualized in terms of biological functioning (or possibly natural living) and absence of pain as pain is of pivotal relevance to assure animal welfare.

Pain is a primary indicator that the environment outside the control systems in the brain is having an impact such that, the individual is having difficulty in coping. Because it is large impact on animal health and welfare it remains as large focus area of the research in animal welfare. Pain may also indicate that an animal is likely to fail to cope in the long term (Broom, 2001). Pain has various definitions out of which mostly used are:

- "*An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage*" (IASP, 1979).

- "*An aversive sensory and emotional experience representing an awareness by the animal by the animal of damage or threat to the integrity of its tissues; it changes animal's physiology and behavior to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery*" (Molony and Kent, 1997).

- "*An aversive sensation and a feeling associated with actual or potential tissue damage*" (Broom, 2001).

Pain can result from routine husbandry procedures required to prevent other major health issues, improper management, accidents or reduced health. During production, farm animals are exposed to procedures which can lead to injury, disease and other noxious events and this will have negative consequences for the animal and on production (Fraser and Duncan 1998; Bath 1998). Therefore it is vital for the animal's wellbeing and for economic reasons that we measure and evaluate potentially painful situations in order to reduce suffering and financial losses.

1.2 Relevance of animal welfare for the society, industry and government

1.2.1 Society

Public concern about the welfare of animals has experienced a dramatic increase in the last decade partly because of new knowledge arising from research on animal perception and sentience. There is also growing evidence that consumers view animal welfare to be closely associated with food safety and quality (Harper and Henson, 2000). European consumers require the assurance of meeting animal welfare standards in EU production conditions but also in the countries supplying the European markets with animal products. Community surveys, like the Eurobarometer, indicate that animal welfare concerns among European citizens are found through expectations from governments, industry and stakeholders (Eurobarometer, 2007).

Improvements in the welfare of animals are based primarily on: changes in public attitudes and beliefs on what is consider an acceptable treatment of animals, and changes in farmers attitudes toward animals imposed by governments and regulatory authorities. During the last 30 years, public and political efforts towards improvement in animal welfare have increased dramatically in many countries. Funding from both governmental and non-governmental sources increased to support research in these area and to inform of these efforts. Experts in the field recognize, however, that there is still a gap between the science and the practice of animal welfare, and that policy and actions that aim to protect animals are sometimes implemented in the absence of reliable evidence about their effectiveness (Dawkins, 2006; Lockwood, 2005).

1.2.2 Legislation

Progress regarding farm animal welfare within the European Union has been improved by legislative actions. Animal welfare legislation in Europe was initially elaborated giving priority to the harmonization of the conditions in which animals were maintained through the EU countries. Currently, new animal welfare legislation has more sophisticated objectives such as social interest and economic concerns, but public and animal health issues considered earlier are also taken into account. Such evolution appears to reflect the changes of EU society and the new approach to food safety policies. Animal Welfare legislation in Europe is applied in 27 countries, thus protecting a large number of animals such as laying hens, broilers, calves, pigs, laboratory animals and others and regulates actions such as killing and transport, insuring that animals do not suffer unnecessarily (European Comission, 2010). However, the lack of specific EU legislation and guidance for some categories of animals, as for turkeys and sheep, makes it difficult to ensure these species are sufficiently protected in terms of welfare.

1.2.3 Industry

In the current market industry actions regarding animal welfare are affected mainly by pressure from legislation, followed by the attitude of the consumers. In part, this might be due to the legislative approach undertaken within the EU countries to improve animal welfare, with the consequent reluctance from farmers to deal with imposed changes and paperwork. Although legislative action may sometimes be required to produce major changes in the production systems (e.g. Directive 1999/72, regulation for the welfare of laying hens banning the use of non-enriched cages), it is also essential to place a larger emphasis in farmers education in order to empower farmers to be major actors in the improvement of animal welfare. The lack of knowledge among operators and public officials regarding basic principles of animal welfare and the potential benefits of improved animal welfare practices often leads to resistance to changes for more friendly systems of production. It can be confirmed by the example from North America, where welfare standards established by major fast food chains and supermarkets did produce major unforeseen changes in a very short time.

Although it is well assumed by industry that a better welfare would translate in a better animal performance, there is often a lack of predisposition to proactively address animal welfare. Management practices have major implications over the performance and welfare of animals (i.e. Dawkins et al., 2004 for broiler chickens). The use of pasture, adapted to environment genotypes and individual identification of animals were found to positively affect both productivity and welfare of the dairy cows in Brazil (Costa et al., 2013). This same study found that the absence of health and production records in more than half of the farms prevented farmers from recognizing certain problems. For farmers to identify and address health and welfare concerns it is essential to have easy access to valid and reliable information on the causes of these problems and on preventive measures that can be applied. This goal should be easier to accomplish if the welfare improvement is understood as an additional component to economic returns, product quality and social responsibility.

McInerney (2004) presented a model (Fig. 1) showing the increased productivity and welfare benefits resulting from a better nutrition, health programs and improved housing and management (i.e. from A to B). B in this model represents the point of maximum welfare for animals with important benefits for humans. However, maximum productive output of animal products can be further increased for example with greater intensification, genetic selection and pharmaceutical intervention. This would be achieved up to point E but at a cost to the welfare of animals.

Exploitation of animals beyond this point affects their welfare to such an extent that they are no longer efficient in production terms, and neither humans nor animals derive benefits. The dotted line and point D represent a possible decision on minimum acceptable welfare, for example by legislation, with treatment of animals below this line categorized as cruelty.

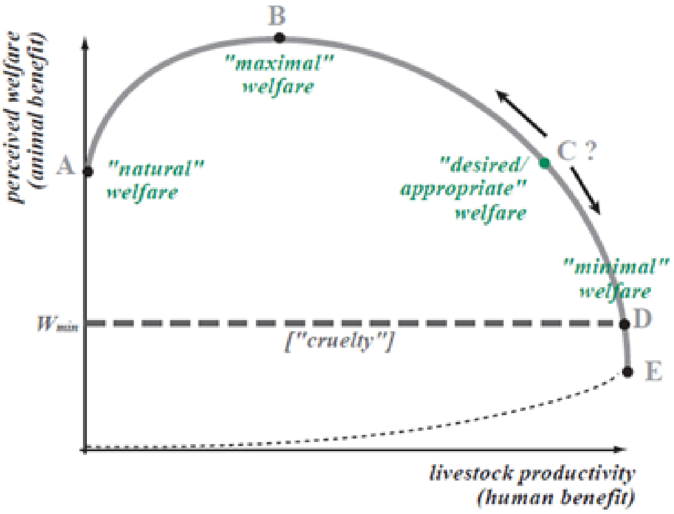


Figure 1. *McInerney's hypothetical relationship between productivity and animal welfare.*

An added difficulty for industry to address animal welfare is that although welfare and performance are directly related when evaluated at the individual animal level, flock 'economic' performance does not necessarily hold a linear relationship with animal welfare (Estevez, 2007). Nevertheless, in this scenario it is essential to consider at which point the economic returns are maximized with minimal welfare impairments. One of the largely discussed examples is the trade of between broiler chickens stocking density and profitability of their production (Estevez, 2007). This study proposed a model in which maximum production with the least possible potential for welfare problems should occur at the point in which yield per unit of space starts to plateau (Fig. 2). The improvement of farm animal welfare should therefore begin with a thorough evaluation of the current situation of the animals, so that potential departures from the expected outcome can be identified and addressed. It is also essential to place a larger emphasis in welfare economics, so that the economic impact of welfare improvements can be considered, and perhaps more solid bridges of understanding can be place between improving animal welfare and farm profitability.

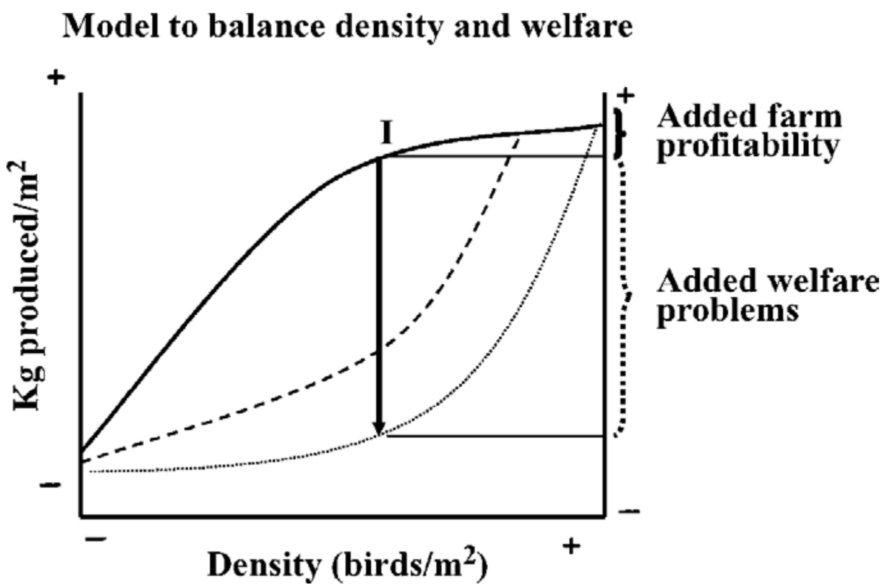


Figure 2. *Model to balance maximum productivity and broiler welfare, based on results of Puron et al. (1995).*

1.3 Welfare indicators

The majority of scientist working in animal welfare recommends taking multiple measurements of parameters that are likely to be relevant to the welfare of the animals (Dawkins, 1980; Fraser, 1995; Mason & Mendl, 1993). The measures or 'indicators' will depend on the base of existing knowledge regarding the species, the question of interest or the context in which it is being assessed. The large discrepancy in the volume of research dedicated to the welfare of different species implies that while for some species there might exist abundant information on most effective parameters, for others we are now learning about basic welfare aspects. Currently, animal welfare indicators can be divided into two major categories: resource-based and animal-based indicators (Mench, 2003; Webster, 2009). Indicators from both groups tend to be evaluative, should be precise and usually quantitative.

Resource-based indicators establish a set of minimum requirements in the physical and social environment of the housed animals and predefine minimum management standards (feeder space, drinkers, space allocation, etc.) that are usually based in current scientific knowledge for the species, or in consolidated practical experience. The animal-based indicators, can be collected on-farm, either by direct inspection of the animal or by indirectly assessing the effects of a response on the environment (e.g. loose faeces on the floor is evidence of diarrhea in the group, although further investigation may be necessary to identify the affected individual). Data can also be collected at a slaughterhouse by the use of disease reporting systems (surveillance), or by consulting production records. For this reason a distinction is sometimes made between direct animal-based measures, taken from the animal, and indirect animal based measures, e.g. taken from records or by remote monitoring of behavior (EFSA, 2012).

In addition to the distinction among direct and indirect animal-based indicators they can be generally divided into four major categories according to the nature of the indicator such as; pathological, physiological, behavioral or productive indicators (Seijan et al., 2007). Curtis (2007) promoted the use of animal-based indicators (e.g. the animal's health because changes in animal-based indicators signal changes in the animal's state of being, and therefore in their welfare. The advantage of animal-based indicators is that they are closely connected to animal welfare state. However they usually are more difficult or might require a larger effort to be assessed as compared to environment-based indicators. For example the indicator of a set amount of space provided per animal is relatively easy to verify, but on the contrary, the possible relationship with animal's welfare is less straightforward to determine (Botreau et al. 2007).

1.4 Welfare assessment

Welfare assessment based on resource-management standards is applied by assigning a score to each environmental or management requirement, that later, using different rules to assemble the information, are compounded into a single score (EFSA, 2012; National Chicken Council, 2014). For example an index system for welfare assessment based on resource measures, was created by Bartussek (1999), proposing a Tier-Gerechtheits-Index (TGI, translated as animal needs index) in reference to a state directive for intensive animal housing legislation in Austria. The concept has been further developed to version TGI 35 L, with an index system for cattle, laying hens and fattening pigs (Bartussek, 1995 a, b, c). Resource-based assessment is generally easier to audit as compared to animal based standards (Mench, 2003), as input factors of the environment are easier to assess than welfare indicators at the animal level. In addition, resource-based standards have the advantage that they should prevent most severe welfare problems from occurring as the conditions are predefined to assure minimum welfare standard.

Resource-based assessment alone can however fail to fully answer questions about the real state of the animals. Although established management and environmental conditions might generally be suitable to meet the animal welfare requirements, these might not necessarily result in an optimum level of welfare, or the results may be uneven within the population due to different reasons. Therefore, lately there has been increasing interest in the development of animal-based methods of animal welfare assessment (Webster, 2009). The RSPCA and the University of Bristol have pioneered the incorporation of animal based welfare assessment techniques into farming systems by assessing the impact of the Freedom Food Scheme in terms of welfare outcomes. The first protocol to be developed, tested, implemented and published was that for dairy cattle (Whay et al., 2003). The protocol was based on direct indices of welfare derived from a combination of direct observations, recordings and farmers' estimates. A similar protocol was later applied to pigs and laying hens (Welfare Quality, 2009). In consequence many recent European farm assurance standards undertook a more animal-centered approach that incorporated the judgment of inspectors regarding the severity of the animal welfare problems encountered.

The Welfare Quality project's (Webster, 2009; Blokhuis et al., 2010) main objective was to deliver reliable, science-based, on-farm welfare assessment protocols for poultry, pigs and cattle as well as a standardized system to convey welfare measures into clear and understandable product information. This large research project led to the development of animal-based, on-farm and slaughter welfare assessment protocols to address the key aspects of animal welfare based on the principles of the Five Freedoms (Fig. 3). These assessment protocols designed for farm animal welfare monitoring can be applied during a single farm visit by an independent inspector and have been tested in many farms across Europe (Blokhuis et al., 2010).

Although there are obvious advantages in increasing the use of animal-based welfare assessment methods, there are also difficulties related to it (Rushen and de Passillé, 2009). Main shortcomings are related to the complexity of the protocols and the intrinsic difficulties to standardize the evaluation criteria by the assessors. In addition, they are time demanding in taking measurements and conducting behavioral observations according to the protocol, resulting in prolonged farm visits disrupting animals and farmers' daily activities.

Further, an issue that is only indirectly assessed by available welfare assessment protocols is the level of pain that animals might be experiencing, which is an issue that to date has not received the necessary attention due to difficulties encountered in determining reliable pain indicators suitable of application at farm level. Recognizing the signs of pain signs as well as its intensity are both essential for an effective pain management and a very important component in welfare assessment. Behavioral and physiological indicators of both short- and long term pain either caused by the animals´ physical or social environment are basic measures of welfare, that however still need to be systematically addressed under commercial conditions (AWIN, 2010).

Identification of pain sings is particularly challenging in some species, since the species have developed throughout the evolution process effective mechanisms to hide signs of pain (Rutherford et al., 2002). Therefore, there is still a large need for valid and practical pain indicators that can be apply during on-farm welfare assessment together with other welfare indicators.

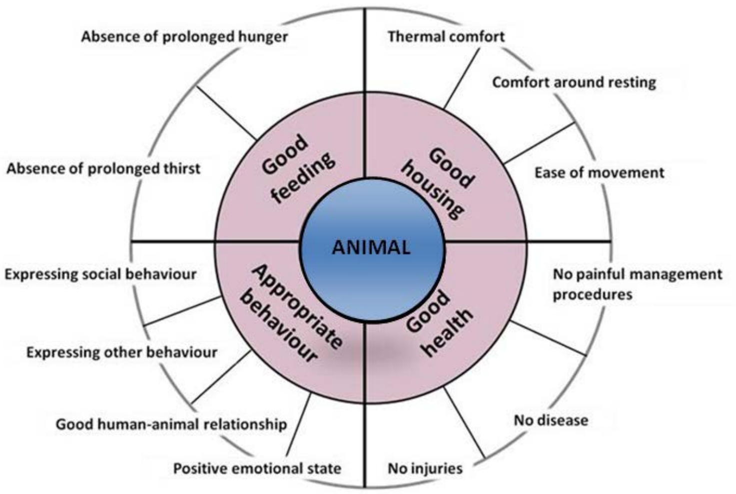


Figure 3. *The four principles and 12 animal-based criteria used as guidelines for good welfare according to the Welfare Quality® (2009) project.*

This combination can be found for instance in welfare assurance schemes as: GLOBAL- GAP Control Points and Compliance Criteria for cattle, sheep, pigs, and poultry (http://www.globalgap. org), Red Tractor Farm Assured Chicken Production Scheme (http://www.assuredchicken.org.uk). Both listed schemes are programs in which participation by farmers is voluntary and adds value to their final products.

In the United States and Canada, most livestock production industries have developed and implemented science-based animal welfare guidelines in response to consumer concerns regarding the humane treatment of animals raised for food. Assurance, that animals are being raised according to these guidelines is provided through voluntary third-party audits rather than legislation (USDA, 2014). For example The National Dairy FARM Program is a nation-wide program that addresses animal well-being in the dairy industry through a third-party verification system. American Humane Association (http://www.americanhumane.org/) or Humane Farm Animal Care (http://certifiedhumane.org/) both are NGO's that provides a welfare certification for most farm animal species according to specific guidelines for humane animal treatment. In addition to this, most producers associations have their own voluntary animal welfare guidelines such as the United Egg Producers or the National Chicken Council, NYSCHAP Cattle Welfare Certification Program. Companies are audit according to these voluntary welfare programs. Specialized professional organizations are responsible for science-based animal welfare training, auditing services or certification programs for animal welfare auditors independent of pork, beef, dairy or poultry production interests (joined Frost, PLLC, FACTA, LLC or PAACO).

1.5 Challenges in on-farm animal welfare assessment

Welfare assessment protocols not only have to be science-base and reliable but also should be practical for on-farm application. In this regard, protocols that are simple to apply and easy to understand by farmers and industry technical staff would have better possibilities of being adopted, and ultimately becoming a relevant tool to support the companies´ decision making process. Up to date animal-based welfare protocols have been developed mostly considering their use by assessors, with limited attempts to make them use-friendly by farmers or stockpersons despite their central role in improving animal welfare on a daily basis (Wiseman Orr et al., 2011). To reach this aim protocols must be easy to understand by farmers as only publishing regulations and controlling their fulfillment are insufficient (Ofner et al., 2003). Protocols that are easy explainable to farmers and designed as a tool for self-evaluation may motivate farmers in their strive for providing better welfare to their animals and to form a basis of dialogue between farmers and other involved parties (Botreau et al., 2007; Ofner et al., 2003).

In addition the protocols should be repeatable, so that end users trust the results and precise to monitor improvements or, on the contrary, decrements in welfare. They also should prioritize the most serious welfare issues to be first addressed and allow farmers to position their farm within others, to make them aware of the conditions which they provide to their animals (Botreau et al., 2007) and what the repercussions might be at the health and economic level. Many designed animal welfare assessment programs follow standards that make a meaningful difference to welfare, however they might be too complex and difficult to meet for a broad spectrum of producers, thereby preventing many from engaging at all (Duncan et al., 2012).

It becomes clear that the farm animal welfare is a complex and a very dynamically developing area of research, attracting much attention of scientists, stakeholders and consumer. Major progress has been made by the last two EU-funded projects: Welfare Quality (2009) and within ongoing project AWIN (2010-2015). Both projects have focused on the development of practical and scientific-base on-farm welfare assessment methods, which were identified as an issue requiring further attention to provide practical solutions. Within AWIN project it was especially important to develop practical welfare assessment solutions for farm species omitted in previous studies and projects such as turkeys. Further, the need to look in more detail into pain issues was recognized. When conducting listed above activities attention has been be paid to the stakeholders’ demands and requirements, to provide the future uptake of the new developments.

1.6 Aims and thesis outline

The overall aim of the series of studies conducted within the PhD thesis entitled: Development of practical methodology and indicators for on-farm animal welfare assessment was to develop and examine the suitability of the welfare indicators and methods for on-farm assessment of selected production animal species.

Special interest was focused on the following general issues:

A. Development of a new practical and dynamic protocol for on-farm welfare assessment of commercial broilers, adjusted to the particularities of large groups in which they are housed and which are very close to one of the focus species within the AWIN project.

B. Application of the newly developed practical and dynamic protocol for on-farm welfare assessment of commercial broilers to turkey flocks, by adjusting it to behavioral differences between species.

C. Development of practical pain indicators for on-farm welfare assessment in sheep.

Hereby the following questions needed to be answered:

1. What are the particularities of production systems and main welfare issues in each of the considered species?
2. Which method should be used to assess most objectively welfare issues for each of the considered species?

3. How to collect information about the selected welfare indicators in the most reliable and practical way?

We hypothesized that transect walks approach will be the best method to evaluate welfare of commercially reared poultry flocks. Further, behavioral indicators will be the most adequate way to measure pain in tail docked lambs in farm conditions.

The first Chapter-Introduction provides an overview regarding animal welfare, its importance within broad society and assessment concepts including pain. The first study described in this thesis regarding issues 1 and 2 investigated the applicability of newly developed practical and dynamic protocol for on-farm welfare assessment of broilers based on transect walks (Chapter 2). Further the behavioral indicators of welfare for turkeys were investigated based on excessive literature review (Chapter 3), in order to select the ones most feasible for on farm welfare evaluation in commercial flocks. The selected, behavioral indicators assessing turkey welfare were added to the protocol described in Chapter 2 and tested in commercial turkey flocks against the individual sampling and load out procedure (Chapter 4).

The experiments described in this thesis regarding issue 3 investigated behavioral pain indicators and vertical activity of lambs which underwent painful procedure of tail docking with or without pain control measures (Chapter 5).

The major findings of the different studies are discussed in the General Discussion (Chapter 6). This chapter also includes potential avenues for improving the collection of information and the potential application of new tools for regarding the future of on-farm animal welfare assessment.

Chapter 2: *Welfare assessment in broiler farms: Transect walks versus individual scoring**

Marchewka, J., T. T. N., Watanabe, V., Ferrante, and I., Estevez. 2013. Welfare assessment in broiler farms: Transect walks versus individual scoring. Poultry Science. 92:2588–2599.

2.1 Introduction

Animal welfare has wide-ranging implications for animal-based companies in the global market as it plays an increasingly important role granting competitiveness and sustainability of commercial animal production. A growing number of countries have adopted specific legislation to assure the welfare of farm species, although often verification of requirements imposed is difficult and expensive (2% of the sector´s value in the EU; EU Commission, 2012). Additionally, other countries such as the U.S. have certified voluntary welfare programs. The need to develop protocols to evaluate animals on-farm with regard to their welfare status was raised by Rousing et al. (2001) and Webster et al. (2008) and some are already available (Welfare Quality®, 2009). These protocols should be characterized by their scientific soundness, possibility to be applied on a commercial farm within a realistic time framework, and ultimately become a relevant tool to support the decision making process. In this regard, protocols that are easy to understand to producers, flock supervisors, and farmers would have better possibilities of being adopted, and this could be achieved by designing welfare assessment protocols that are close to animal care procedures conducted by veterinarians and farmers.

Currently, most scientists agree with the need of designing protocols based on the animal (Main et al., 2007). The use of animal-based welfare indicators is recognized at international level by organizations such as the World Organization for Animal Health (OIE, 2003). The Welfare Quality assessment protocol (Welfare Quality®, 2009), is one of the most recently proposed approaches for on-farm assessment. This protocol has been thoroughly designed, considering all living and welfare requirements of particular species. However, it requires further work with regard to time and labor efficiency as suggested lately by producers (de Jong et al., 2012c). Protocols based on scientifically and practically acceptable methodology becomes especially challenging when the production systems require keeping large numbers of animals in a common housing, as is the case in broilers production.

The welfare of broilers can be challenged by multiple factors such as by their genetic potential for growth, decline of environmental quality, poor management, or excessive density (Dawkins et al., 2004; Estevez, 2007), which may result in contact dermatitis, metabolic, skeletal and muscle disorders or behavioral abnormalities (Dawkins et al., 2004; Estevez, 2007; Meluzzi et al., 2009). Besides the great impact of the welfare status of the animals, all these problems have a major economic relevance for industry. For example in the United States skeletal problems generate annually 200 Million dollars loses to industry (Donoghue, 2012). Therefore, the control of these problems, not only would contribute to a better accountability on animal welfare, but to a higher efficiency of industry.

To assure proper animal care and welfare farmers and flock supervisors conduct routine checks based on walks through the broiler production house in order to screen the health status of the flock. This method allows distinguishing individuals with visible severe welfare issues, providing a quick, estimation of general flock health and welfare status and usually gives bases for future management decisions. It is generally performed in a way to minimize frightening or interrupting the birds, so no direct contact with individuals is included, only visual, which is feasible in for evaluation of welfare indicators such as: lameness, immobility, back dirtiness, sickness, agonizing or dead birds. Although this non-invasive method is well accepted by producers, it does not provide with quantitative data to be able to make reasonable comparisons across the health and welfare status of the birds across farms, or successive flocks of birds within a house.

To date, most scientific assessment methods include bird herding and enclosing, as most of the available studies on broiler welfare evaluation are based on scoring particular welfare deficiencies on the individual level (Welfare Quality®, 2009). For welfare assessment bird samples in diverse numbers are taken usually in random locations of the house, and then scored for the chosen set of welfare indicators (Dawkins et al., 2004; Knowles et al., 2008; Sanotra et al., 2003). This commonly used procedure is time consuming, as it requires catching, enclosing and handling birds, but most importantly, it might be a stress inducing procedure (Jones, 1992), influencing birds´ performance during gait scoring. Furthermore, slower or unfitted individuals might be less likely to escape during catching, as passive coppers (Kolhaas et al., 1999), having the probability of influencing randomness of the procedure.

Walks through performed by animal caretakers, is to a certain extent, a similar strategy for data collection to line transects methodology, which has been successfully used for years in wildlife studies (Buckland et al., 2010). Some aspects of this approach, distance evaluation, were utilized in a non-intrusive method of plumage condition assessment (Bright et al., 2006). However, the methodological differences and results between an approach for welfare evaluation closer to the methods used routinely by animal caretakers and flock supervisors and the classical scientific approach of individual sampling have never been compared. The ideal welfare assessment protocol for on-farm conditions should be a method that provides the dynamism of walk-through inspections but conducted in such a way that provides veracity, inter-observer reliability and that quantitative results obtained can be compared across flocks and farms.

The goal of this study was to compare the welfare assessment results of broiler flocks evaluated according to two different approaches; the transect walks and the individual scoring. The transect walk methodology is based on the idea of walk-through used for broiler care and line transect methodology used in wildlife biology but evaluating the methodology for inter-observer reliability and within- and across-house sensitivity. We compared the results with the individual sampling scoring conducted following the guidelines provided by the Welfare Quality (2009). This is a preliminary study aiming to develop a scientifically sound and practical methodology, combining current scientific findings with the transect approach, for on-farm broiler welfare assessment, with perspective for application in other poultry species.

2.2 Materials and methods

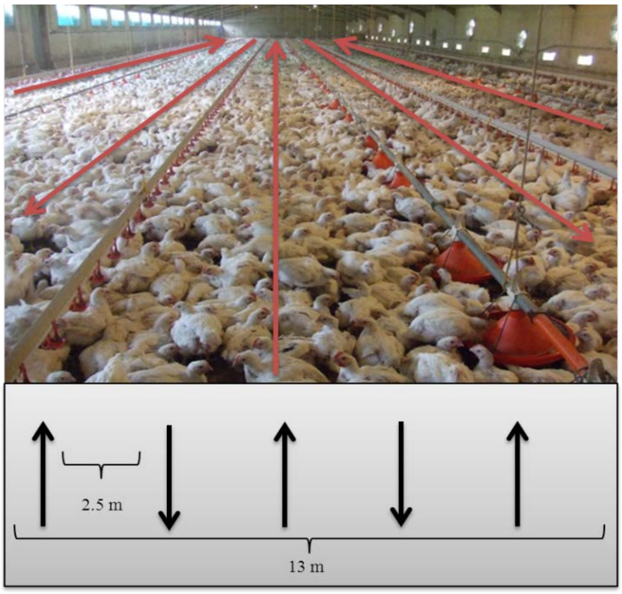
2.2.1 Facilities and animals

The study was conducted from April 30th to May 8th 2012 at six commercial houses (Grupo AN, Navarra, Spain) located in farms in the same geographical region in Northern Spain belonging to the company). Each studied farm had paired houses, with flock sizes/house ranging from 13,220 to 27,540 broilers (COBB 500) reared at a density of 17 birds/m^2. All houses had identical management, other than for the fact that four of the houses used chopped straw as litter substrate, whereas two used wood shavings. All houses were provided with automatic drinkers, feeders and ventilation systems, artificial light and windows allowing natural lighting.

2.2.2 Data collection

We collected data by using two methodologies: the transect walks approach that we developed, and the individual sampling assessment based in the protocols developed by Welfare Quality® (2009).

Transect walks. The transect walk approach is based on the methodology widely and successfully used in wildlife studies for decades (Gates et al., 1968, Buckland et al., 2010). Transects walks for animal welfare assessment in our study consisted of standardized walks divided in randomly set paths covering the full area of the house (Fig. 4a). Broiler houses normally have a rectangular shape, although dimensions may vary across companies and countries. The houses in our study were around 13m wide (variable length) and were divided in five 2.5m wide bands. Transects were numbered from 1 to 5; 1 and 5 being wall and 2, 3 and 4 central transects. Transects width were limited by the location of feeder and drinker lines (for central transects), or the wall and adjacent drinking line (for wall transects), which created invisible barriers to birds´ movements (personal observation). Paired houses at each farm were assessed sequentially by two observers within the same day, when birds were 31-35 days old (birds´ welfare may deteriorate in a day towards the end of rearing). This age range was chosen for assessments instead of the end of production cycle because it is a common procedure in the country to depopulate 25% of the flock at this age. A later evaluation may have provided biased results due to the impact of depopulation catching, which is considered a major stress cause, therefore, providing misleading information about the welfare status of the birds during the production cycle. Observers conducted the data collection independently in each house. Transect walks were performed in random order, in both directions, starting at the entrance wall and the opposite of the entrance wall, alternatively.



a)

b)

***Figure. 4**. a) Design of the transect walks of 2,5 meters within a 13 meter-wide production room. Arrows show the walking path of the observer between lines of feeders and drinkers and b) Data collection during transects -note the short distance to the observer.*

We avoided sequential observations of contiguous transects to minimize the possibility of double counting birds that may have moved momentarily from adjacent scored transects minutes before. The observers walked slowly through the set transect (Fig. 4b) while recording in a spread sheet (Polaris Office) installed in a hand held tablet (ASUS Eeepad TF 101 Transformer). Observations of all occurring incidences of birds within the following categories were recorded: immobile (no attempt to move, even after slight encouragement), limping (visible signs of severe uneven walk), dirty (side and back feathers visibly dirty), sick (bird showing clear signs of impaired health with small and pale comb, red-watery eyes and occasionally unarranged feathering usually found in resting position), agonizing (the bird lies on the floor with closed eyes, breathing with difficulty) and dead.

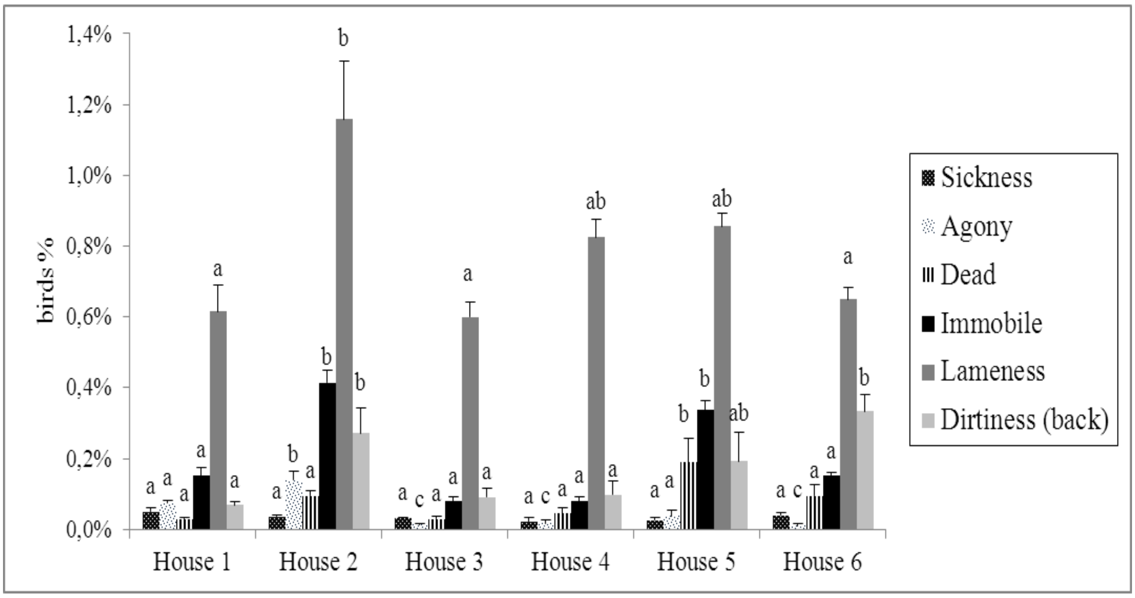


Figure 5. *Mean values (±SEM) of each welfare indicator expressed as percentages for each house obtained by transect walks. Means lacking a common letter (a, b, c) differ ($p \leq 0.05$).*

These are validated welfare indicators, which are considered critical parameters in terms of broiler welfare (EFSA, 2012), can be clearly described, and identified for data collection in broiler flocks, making them ideal for purpose of methodology validation.

Individual sampling. During individual sampling, a separated group of three trained scientist collected birds in six random locations within the house with at least one of them collected in one of the five predefined transects. Each sample consisted of 25 randomly collected birds that were gently pushed to a mobile pen and were kept enclosed during sampling. Each bird was handled gently and individually, weighed on an automatic scale (PCE-WS 30, PCE Instruments) and evaluated, for footpad dermatitis (score 0 to 4), hock burns (0 to 4), breast dirtiness (0 to 2). Afterwards each bird was released away from the scoring area and observed to evaluate gait scoring (scale 0 to 5) when receding. If not showing willingness to move, we used slight encouragement by touching the bird. For each indicator a lower score meant a higher welfare status of the individual. After scoring all birds in the sample, the procedure was repeated in the next location. Although under ideal circumstances we should have had two teams performing dual individual sampling in order to check for inter-observer reliability, this would have required a total of seven people and unfortunately we did not have sufficient man power to do this. In addition, because the individual scoring took half a day per house, the three people team could only do two houses in a day. Therefore, there was not sufficient time to repeat the scoring a second time in each of the paired houses.

2.2.3 Statistical analysis

Transect walks. During transect walks we recorded the number of individuals showing any of the predefined welfare problems. Observed frequencies were transformed into proportions per transect based on the known flock population sized of each particular house, and assuming that birds were randomly distributed through the house.

To test inter-observer reliability and sensitivity of transect evaluation resulting percentages were checked for normality and homogeneity of residual variance. From the whole set of variables immobility, agony and death were non-normally distributed, and were subjected to logarithmic transformation, allowing fulfilling normality requirements. We performed independent mixed-model repeated measures ANOVA for each of the six welfare indicators defined above. The model included transect as repeated measure, house and observer as fixed factors. We included farm as a random statement, as the between houses comparison was the main point of our interest. We included interactions between observer by transect and observer by house, as well as house by transect. LSM differences were adjusted for multiple comparisons by post-hoc Tukey comparison.

We applied bootstrapping techniques to test the precision of the method by taking simulated random samplings combinations from the original data set (Dixon, 1993). Bootstrapping has been used to estimate the accuracy of ecological indices (Dixon, 1993; Stein, 1989) and more recently in a wide range of scientific areas; from genetics (Yang and Rannala, 2012) to economical sciences (Clark and McCracken, 2012). In short, this methodology defines the appropriate model for the observed data, from which it generates n sample data sets using Monte Carlo methods, to finally construct the bootstrap distribution (Efron, 1979; 1987). Expected mean and standard error of the data set for each welfare indicator was calculated by taking random samples of one transect (20% of the information), or combinations of two, three and four transects (40, 60 and 80% of information, respectively). Simulations were run 10,000 times per house and welfare indicator. All variables, except of immobile, were averaged per house due to lack of significant differences across observers. Independent bootstrapping were calculated for the indicator immobile for each observer. We used PROC SURVEYSELECT to perform the bootstrap.

Individual sampling. Data collected in individual samplings were also checked for normality and homogeneity of residual variance. Hock burns, immobility and dirtiness were non-normally distributed, and were subjected to logarithmic transformation. The variables were analyzed by independent mixed-model repeated measures ANOVA. The model included transect as repeated measure and house as fixed factor. However for this analysis the interaction among both factors could not be conducted because of the lack of degrees of freedom. We included farm as a random statement, as for the transect walks analysis. LSM differences were adjusted for multiple comparisons by post-hoc Tukey comparison. All analyses were conducted with SAS 9.3 statistical package (SAS Institute Inc., 2011).

2.3 Results

2.3.1 Transect walks

Sensitivity. Our results showed that transect walks methodology allowed detection of small variations across the studied flocks on the prevalence of the studied welfare indicators. Significant differences across houses were found for the incidence of immobile, limping, with dirty back, agonizing and dead birds (Table 1; Fig. 5). Only incidence of sick birds remained invariable across the studied houses (Table 1 and Fig. 5).

Table 1. *Effect of farm, house (H), observer (Obs), transect (Tr) and the interactions transect with observer and observer with farm for welfare indicators collected by transects.*

Welfare indicator	Analysis of variance components											
	H		Trt		Obs		Tr*Obs		H*Obs		H*Tr	
	$F_{(5,20)}$	p value	$F_{(4, 20)}$	p value	$F_{(1, 20)}$	p value	$F_{(4, 20)}$	p value	$F_{(5,20)}$	p value	$F_{(20,20)}$	p value
Immobile	44.2	<.0001	0.25	0.903	6.3	0.02	1.69	0.191	1.99	0.123	1.24	0.316
Limping	5.32	0.002	0.41	0.799	0.04	0.849	1.48	0.244	2.71	0.0502	0.84	0.645
Dirty	7.17	0.0005	2.24	0.1	0.17	0.683	2.17	0.108	10.64	<.0001	1.45	0.204
Sick	0.7	0.629	0.55	0.699	0.28	0.6	.39	0.81	0.9	0.497	0.49	0.939
Agonizing	17.68	<.0001	1.14	0.365	4.44	0.047	0.42	0.79	2.56	0.06	0.18	0.358
Dead	14.24	<.0001	4.84	0.006	4.35	0.05	0.6	0.666	6.02	0.001	3.85	0.002

*NS - non significant, P-value equal or greater than 0.05.

Inter-observer reliability. Welfare assessment across observers, or the interaction of observer and transect, and observer by house remained consistent for most variables as indicated by the lack of significant differences in the assessment (Table 1 and 2). The effect of observer was only detected for the incidence of immobile and agonizing birds (Table 1), however the inter-observer difference for both variables was not observed for the interaction between house and observer.

On the other hand, house by observer interaction had significant effect on dirty and dead birds. Nonetheless, the significant differences across observers (Table 2) ranged between 0.18%±0.02 and 0.22%±0.03 for the incidence of immobile birds, whereas maximum range of variation across farms and observers for dirty birds was of ± 0.5%.

Table 2. *Mean values (±SEM) of incidence of birds within each welfare indicator category expressed as percentages by observer (Obs) and transect (Tr).*

Welfare indicator	Obs		Tr				
	1	2	1	2	3	4	5
Immobile	0.18 ± 0.02	0.22 ± 0.03	0.20±0.04	0.19±0.05	0.21±0.05	0.21±0.04	0.19±0.04
Limping	0.79 ± 0.06	0.78 ± 0.07	0.76±0.10	0.75±0.07	0.79±0.09	0.74±0.09	0.87±0.15
Dirty	0.18 ± 0.04	0.17 ±0.04	0.21 ±0.08	0.21 ±0.05	0.09 ±0.03	0.14 ±0.04	0.23±0.09
Sick	0.03 ± 0.01	0.04 ± 0.01	0.05±0.01	0.04±0.01	0.03±0.01	0.03±0.01	0.03±0.01
Agonizing	0.04 ± 0.01	0.06 ± 0.01	0.06±0.02	0.03±0.01	0.06±0.02	0.04±0.01	0.05±0.02
Dead	0.09 ± 0.02	0.07 ± 0.01	0.12±0.05	0.08±0.03	0.05±0.01	0.05±0.01	0.10±0.03

Transect effect. No effect of transect location was detected (1 to 5; 1 and 5 being wall transects, and remaining being central transects) for nearly all variables (Table 1 and 2) studied, except for dead which was significant for transect and transect by house effect. Applying bootstrapping techniques showed that the mean for each house was similar to the observed mean value by using as little as 20% of the information for all the variables (representative example in Table 3 and Fig. 6).

Sensitivity. By using individual sampling method we found differences (Table 4) between houses for limping and dirty birds, footpad dermatitis and body weight (Fig. 7 and 8).

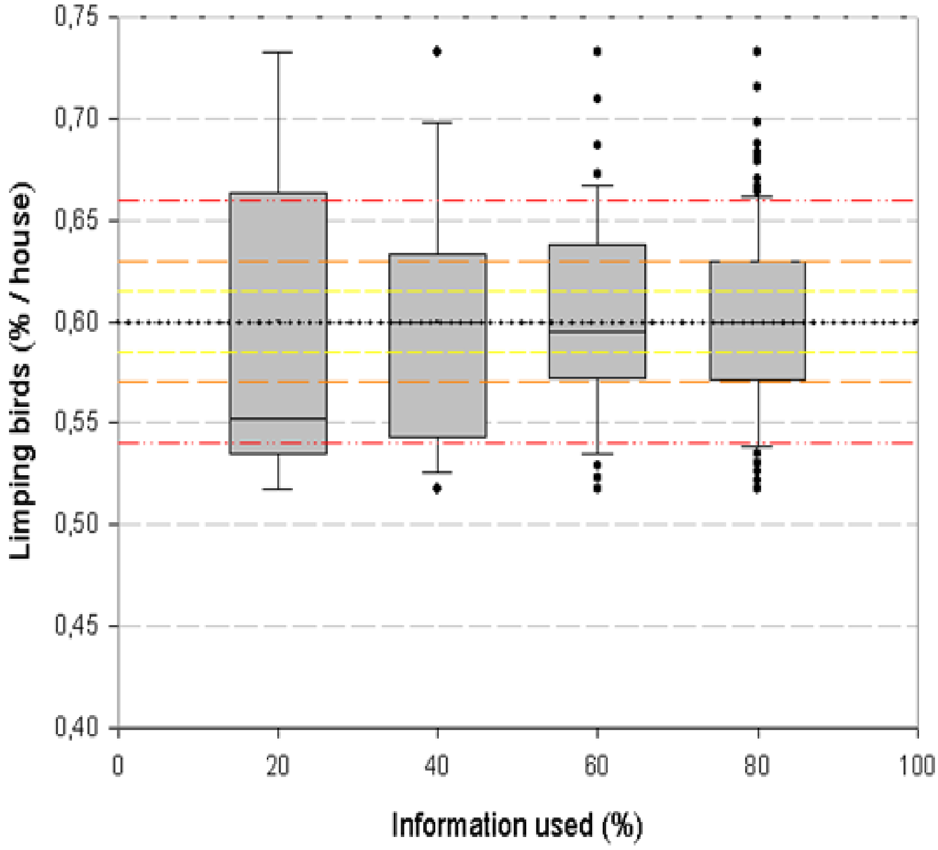


Figure 6. *Mean values (±SD) expressed as percentages obtained from bootstrapping from 20, 40, 60 and 80% of information for limping birds in comparison to mean (dashed line), 2.5% (yellow line), 5% (orange line) and 10% (red line) of error rates of value obtained from 100% of information for house 3 (example).*

2.3.2 Individual sampling

Transect effect. We did not find any significant effect of house, neither transect on any of the variables collected by individual sampling (Table 4).

Table 3. *Mean values (±SEM) for limping and dirty birds presented for 20, 40, 60, 80 and 100% of information used in 10,000 simulations using bootstrapping.*

		% of information used				
Welfare indicator	House	20	40	60	80	100
Limping	1	0.6167±0.1485	0.6171±0.1036	0.6163±0.085	0.6161±0.0735	0.616±0.0828
	2	1.16±0.33	1.1617±0.2326	1.1605±0.1889	1.1586±0.1645	1.1583±0.1834
	3	0.6009±0.0838	0.6005±0.0595	0.5998±0.048	0.6003±0.0419	0.6±0.0469
	4	0.826±0.1045	0.8255±0.0736	0.8249±0.0597	0.8249±0.052	0.825±0.0586
	5	0.8558±0.0755	0.8541±0.054	0.8546±0.0433	0.8547±0.0376	0.8548±0.0395
	6	0.6508±0.0668	0.6507±0.0469	0.6508±0.0387	0.6507±0.0337	0.6505±0.0373
Dirty	1	0.0676±0.0223	0.0677±0.0158	0.0679±0.0129	0.068±0.0112	0.068±0.0124
	2	0.2731±0.1401	0.2736±0.0996	0.2734±0.0816	0.2727±0.0697	0.2723±0.0778
	3	0.0914±0.0476	0.0914±0.0337	0.0916±0.0273	0.0915±0.0237	0.0914±0.0267
	4	0.0983±0.0785	0.0983±0.0557	0.0983±0.0457	0.0982±0.0392	0.0984±0.044
	5	0.1937±0.1611	0.1928±0.1141	0.1922±0.0924	0.1927±0.0807	0.1929±0.09
	6	0.0936±0.3337	0.3344±0.0659	0.3341±0.0531	0.334±0.0462	0.334±0.052

Table 4. *Effect of house (H) and transect (Tr) for welfare indicators collected by individual samplings.*

	Analysis of variance components			
Welfare indicator	H		Tr	
	$F_{(5, 20)}$	p value	$F_{(4, 20)}$	p value
Immobile	0.48	0.7839	0.34	0.8495
Limping	5.88	0.0017	1.43	0.2616
Dirty	8.39	0.0002	0.54	0.7103
Hock burn	3.26	0.0941	0.17	0.8095
Footpad dermatitis	4.00	0.0112	0.95	0.4577
Body weight	6.41	0.0010	0.31	0.8676

*NS - non significant, P-value equal or greater than 0.05.

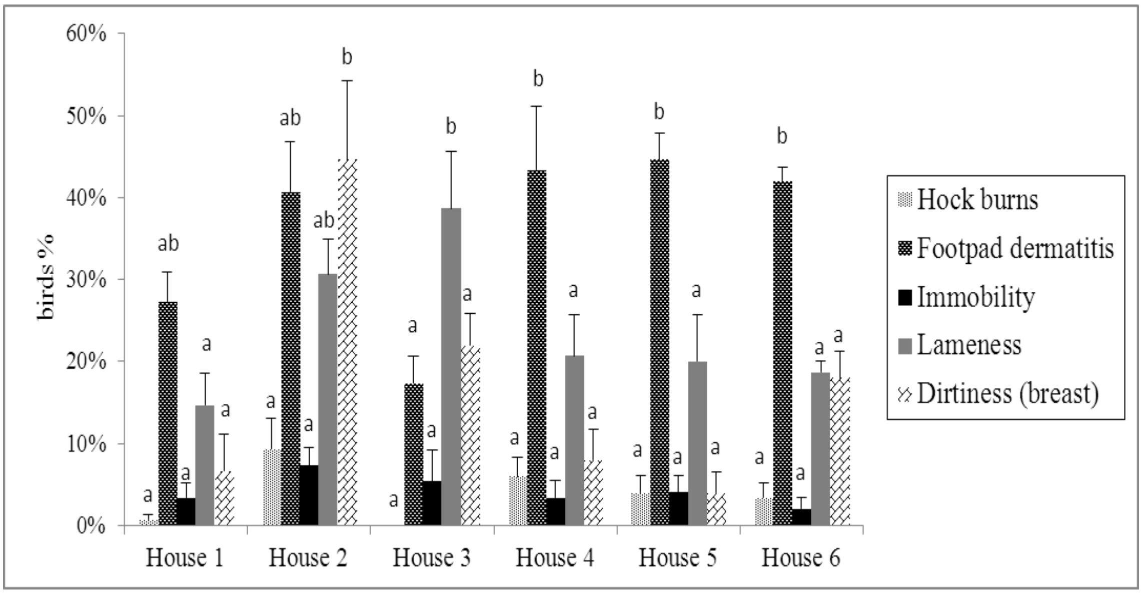


Figure 7. *Mean values (±SEM) of each welfare indicator expressed as percentages for each house obtained by individual samplings. Means lacking a common letter (a, b) differ (p ≤ 0.05).*

2.4 Discussion

2.4.1 Transect Walks

The aim of our study was to explore the soundness of a new approach to welfare assessment for broiler flocks, considering the scientific validity, time and manpower requirements. We also considered the potential acceptability by assessors and producers that might have an interest in self-assessment.

The transect walk approach is based on the routine daily checks conducted by farmers and flock supervisors during inspections, combined with line transect methodology used commonly for evaluating wildlife populations (Buckland et al., 2010). The transect walk approach implies surveying birds throughout the entire production house, registering all individuals falling within each welfare indicator category established in this study within each transect.

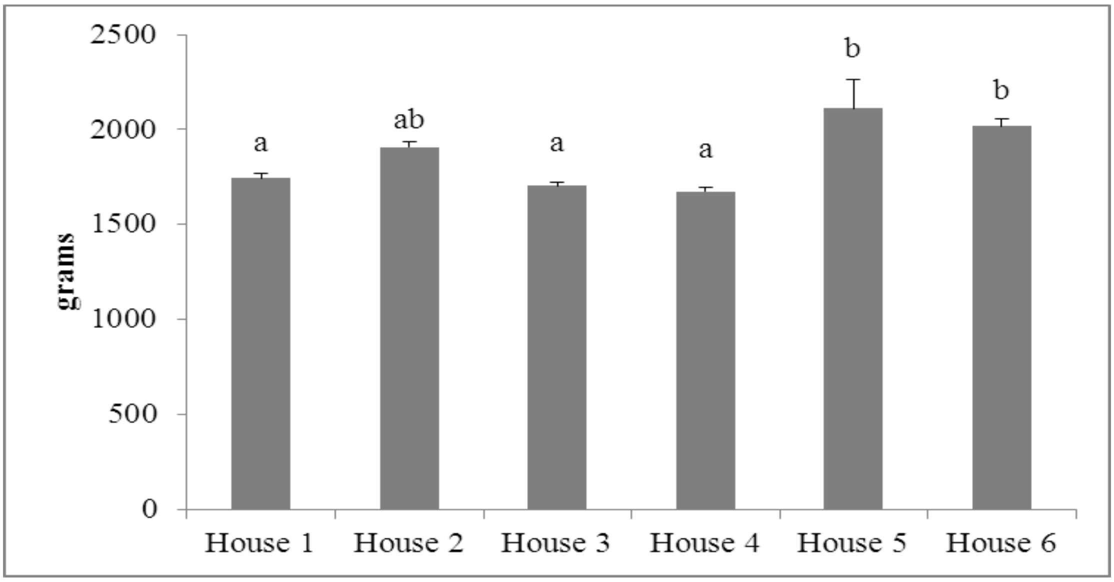


Figure 8. *Mean values (±SEM) of body weight for each house obtained by individual samplings. Means lacking a common letter (a, b) differ ($p \leq 0.05$).*

In this study we homogenized field conditions as much as possible by assigning to the study only houses using birds of identical genetic background (Cobb 500) raised under identical standard management practices within the same geographical region. All houses were sampled when birds were at similar ages (31 to 35 days), and were assessed in less than a month to minimize variations in environmental conditions that may affect the bird´s welfare status (Dawkins et al., 2004).

Despite the homogeneity in housing conditions, our results showed that the transect walk approach was highly sensitive and allowed detection of small, but highly significant, variations in the incidence of the welfare indicators used in this study such as immobility, birds with severe limping, with dirty back, agonizing or dead (Table 1, Figure 2). These indicators are known be critical for the welfare status of broilers (Dawkins et al., 2004; Estevez, 2007), but also have a tremendous economic impact. For example, skeletal problems causing immobility in the UK are responsible for losses estimated in 2 Million pounds per year (Walker, 2012). Other indicators such as back dirtiness (as used in this study) is considered an important welfare indicator connected to litter quality, stocking density (Berg, 1998, Estevez, 2007).

Welfare assessment across observers with the transect walk approach remained consistent for limping, dirty, sick, and dead birds (Table 1 and 2, Figure 3). However, minor, although significant differences were detected for the incidence of immobility across observers and for the interaction of observer with house for dirty and dead birds (Table 1). The significant differences across observers ranged between 0.18 ± 0.02 and 0.22 ± 0.03 for the incidence of immobile birds (Table 2). Considering the scope of the sampling (several thousand birds per flock), and the randomized procedures we used for data collection, it is actually quite remarkable that we only found minor effects across observers, while house assessment results remained consistent with other studies conducted in broilers under commercial (Sanotra et al, 2003; Dawkins et al., 2004; Knowles et al., 2008), and experimental conditions (Kestin et al, 1992). For example, averages of 0.9% birds unable or with impaired walk were found when using a non-invasive method to evaluate walking ability (Dawkins 2004). These results are similar to the values obtained in this study when adding the categories defined as immobile and severe limping. Our results are also comparable to other study (Knowles et al., 2008) in which 0.2% of immobile birds were detected using a method which involved bird handling (Kestin et al, 1992), and using the same methodology (Kestin et al., 1992) an average 0.3% and 2.7% of severely lamed birds were noticed for 28 and 42 day old broilers respectively (Sørensen et al., 2000). On the other hand, the observer by house effects detected for dead birds can be explained by the fact that we were working under commercial conditions and in two of the houses the farmer removed the mortalities in between the data collection of the two observers for two of the houses.

Similarly, an observer by house interaction was detected for dirtiness scoring which could have been caused by natural lightning variation occurring over the time in which the walks were performed. The traditional broilers houses in Spain are provided with windows that are automatically regulated according to changes in environmental conditions. Birds in these houses are normally exposed to a wide range of variation in light intensity during the day. Variations might be more dramatic during early spring when wide range of climatic conditions can occur in the course on one day, when this study was conducted.

Interestingly, and contrary to our initial expectations, we found no effect of transect location (1 to 5; 1 and 5 being wall transects, and remaining being central transects) for any of the welfare indicators, except for the incidence of deaths (Table 1 and 2). This effect could be explained by the farmers´ intervention during data collection period and by the method of the dead birds´ removal by collecting them most likely next to the walls. However overall, the lack of the expected transect location effect obtained in this study suggest that birds varying in welfare status seem to be homogeneously distributed within the house area. Furthermore, these results would at least initially suggest that it would not be necessary to perform all transects in order to obtain a reliable estimation on the welfare status of the broiler flock.

This idea is further supported by the results obtained from applying bootstrapping techniques that allows testing the precision of an estimate by calculating the bias and standard error by taking simulated random samplings combinations from the original data set (Dixon, 1993). The resulting expected mean for each house was similar to the observed mean value by using as little as 20% of the information (Table 3 and Figure 3). These results indicate that, under the conditions of our study, the assessment of an area covering 20% of the house surface will be theorically sufficient to obtain a reliable mean estimation on welfare status of a broiler flock based on the parameters used in this study. If there is interest in getting the closest to real value of the standard error of mean then our results suggest that a minimum 60% of the house area should be evaluated. Given that in this initial study assessing a complete broiler flock by conducting five randomly determined transect walks took close to 4 hours, because location data (measured as distance to the front wall, not used in this manuscript) were also collected, we calculate that if the method is proven for its validity, then farm assessment could be conducted in a time lapse ranging between 30 to 60 minutes and with minimal interference with the daily farm routines.

2.4.2 Individual sampling

Individual sampling is the most commonly used procedure for animal welfare assessment in broilers (Welfare Quality®, 2009; De Jong et al., 2012a) for which a sample between 100-150 birds per flock is recommended due to time and manpower requirements. We were interested in determining how our transect walks approach would compare with this well-known and widely accepted methodology.

The results of the individual sampling (Table 4) showed differences between houses for limping and dirtiness, but not for the incidence of immobile birds. Differences were also detected for the supplementary variables included in the individual sampling such as footpad dermatitis and body weights, but no differences were detected for hock burns. The lack of significant differences across houses for immobility might have been due to the large variation, as indicated by fairly large standard error of mean values for each house, in relation the mean value magnitudes. However, it might be also related to the relative small samples that are considered in individual sampling as compared to the size of the flock (usually several thousand birds) which are justified by the man power requirements of this sampling methodology. In our study it took 3 persons and around 4 hour of work to perform the individual scoring as described in the methodology section above. However, a small sample size would imply that differences regarding the incidence of welfare issues with relatively low incidence (as compared to limping for example) might be more difficult to detect.

An important advantage of the individual sampling methodology than cannot be overlooked is that it allows scoring the incidence of footpad dermatitis and hock burns which are important welfare indicators, in addition to their economic relevance to industry. Our results regarding the values for footpad dermatitis ranging from 18% to 48% for birds with scores 3 and 4 were within the values obtained by a recent study conducted in 386 Dutch flocks, in which 26.1% to 38.4% had mild or severe footpad lesions (De Jong et al., 2012b). With regard to hock burns, an evaluation of more than 2000 birds at the age of 4 weeks showed an incidence of 0,5% (Kjaer et al., 2006), in our study the mean incidence was of 3,6% with farm values ranging between 0% and 8,43%. The much higher upper range of our results might be caused by the older age of birds in our study (more than 30 days old) or due to the fact that the observed flocks were placed in winter, when the incidence of footpad lesion tend to be more important. Regarding body weight, significant and relevant differences were found between houses (Figure 5). However, these particular houses did not appear to be the ones with lower incidence of welfare problems such as lameness, or dirtiness.

Similar to the results obtained by applying the transect walk methodology, we found no effect of transect location for any of the parameters studied, supporting further our assumption regarding the homogeneous dispersion of birds with welfare issues within the house.

2.4.3 Methods comparison

The results of this study show clear major discrepancies between both methods of welfare assessment. The results obtained by individual sampling would indicate a substantially reduced welfare status of broiler flocks when compared to results obtained by applying the transect walk methodology considering the welfare indicators used in this study. The indicators which could be directly compared across transect walks and individual samplings were severe lameness and immobility. Mean incidence of lameness and immobility was 24,18% ±4,68 and 4,22% ±2,3, respectively for individual sampling, while for transect walks mean frequency for lameness was 0,78%±0,07 and a 0,2% ± 0,01.

The discrepancies across the two methods may relate to the observers failing to detect birds within the immobile or limping (severely lame) category during transect walks. This is a likely possibility and further studies should be conducted for improvement of the accuracy and reliability of this new methodological approach for on-farm welfare assessment. However, it should be also considered that when using 25 birds as the sample size in each locations of the house for individual sampling, the effect of scoring just one bird in a given category would already increase the incidence of such category to a 4% incidence for this sample. Therefore, although individual sampling may be ideal for the assessment of large animals in which herd size may be of several hundred (Vasseur et al., 2012), it may be more difficult to apply, or at least bring up some methodological questions when applied to large poultry flocks.

This issue could be easily overcome by increasing sampling size. However, the assessment of 150 birds in our study took between three to four hours for a three experienced people team. The speed of assessment could certainly be improved, but still even if doubling the speed it would take around four hours to sample 300 birds, that would represent a 1% of the population for a 30,000 bird flock.

In this respect, the transect approach is proven to be a more agile methodology in terms of time requirements, but certainly validation of the approach should be first achieved. A transect walk in our experience takes for a trained assessors, between 30 minutes to 1 hour depending on the welfare situation of the flock and house dimensions. According to the evidences supported by the lack of transect effects and bootstrapping methodologies, sampling of only a 20% of the area of the house is required to obtained the mean estimate for the house, which could be achieve by conducting one transect in a maximum of 30 to 45 minutes.

An additional and important concern is also the potential stress effect which the individual sampling may have over the sampled birds. It is known that procedures such as herding, enclosing and handling of the birds causes fear (Newberry and Blair, 1993) and might have large effect on their behavior, including immobility (Duncan and Kite, 1987; Jones, 1992). This reaction known as tonic immobility (TI) is a natural response that provides the bird with an opportunity to escape in an unguarded moment (Thompson and Liebreich, 1987). TI reaction has been correlated with fear and stress indicators as proved by serum corticosterone levels (Lin et al., 2006). During herding into the sampling pen, birds are gently pushed into it and perhaps they are force to walk excessively even when the procedure is carefully performed. This can be painful and tiring for the birds before the evaluation starts (Cordeiro et al., 2009). Additionally, birds which are struggling to walk due to leg disorders and pain in everyday conditions, in these circumstances are likely to show more severe walking difficulties during evaluation.

It is also possible that the herding procedure requiring a number of birds to walk into the portable sampling pens might have also compromised the randomness of the sampling. It is obvious that birds with movement difficulties may have lower chances to escape and, therefore, be easier to catch which may have resulted in samples including a disproportional percentage of birds with high gait scores as compared with the population average. Additionally, in order to be gait scored birds are usually released to the empty area next to the pen, which might induce increased stress and fear reactions. Available literature has shown that broilers react to touching, handling, holding, to the exposure of acute stressors (Jones, 1992; Newberry and Blair, 1993; Marin et al. 2001), or even to the human presence and eye contact can cause behavioral changes in broilers (Zulkifli and Sti Nor Azah, 2004). Therefore, to it seems a likely possibility that the gait scoring evaluation may be affected by the imposed stress of the procedure. On the contrary, because transect walks are conducted slowly without causing mayor disturbances to the birds, results obtained should not be affected by these factors. In addition, because the transect method is based on the sampling of the entire population in the house, or within transect, results are less likely to be affected by the issue of sample size.

Although all these factors interfering with the sampling procedures appear to be realistic possibilities. The question remains on the adequate or ideal validation method. To our knowledge individual sampling methodology has never been scientifically validated, fact that was underlined in a study of footpad dermatitis which is a usual indicator measured with this approach (De Jong et al. 2012a). Clearly the discrepancy in results depending on the applied methodology raise, until further studies are conducted, questions regarding the adequacy of currently available welfare assessment in broiler flocks.

The outcomes of our study revealed large differences between pictures obtained by the two methods analyzed in this study. However, much of the discrepancy can be well explained and justified by the arguments stated above. Certainly the transect methodology still needs to be much testing to assure that lameness and immobility are not overlooked and that the methodology provides a realistic quantitative assessment of the most relevant welfare indicators. Indeed, behavioral assessment will also need to be considered if the methodology is validated in future studies.

2.5 Conclusion

We provided evidence that transect walks have a large potential as prospective approach to on-farm welfare assessment, showing good inter-observer reliability and reduced time and manpower requirements. Because the method is based on daily care farm routine, may be easier to understand and to accept by perspective assessors and producers. However, this work evidenced major discrepancies between welfare indicators estimates according to the method of assessment. Diversity in results may obey to a potential reduced sensitivity to detect welfare issues by the transect approach, which would need to be improved. Nevertheless, individual sampling results might also be affected by the reduced sample size, stress effects and randomization issues. This study provides new insight into constraints and advantages of broiler on-farm welfare evaluation methods, which should be considered in future studies on designing valid and feasible welfare evaluation protocols.

Chapter 3: *Review of the social and environmental factors affecting the behavior and welfare of turkeys (meleagris gallopavo)**

**Marchewka, J., T.T.N., Watanabe, V., Ferrante, and I., Estevez. Review of the social and environmental factors affecting the behavior and welfare of turkeys (Meleagris gallopavo). 2013. Poultry Science. 92:1467-1473.*

3.1 Introduction

Turkey production is considered small when compared to broilers; however this industry has achieved a relevant increase since 1980, escalating from 122 million to 226 million turkeys produced in 2006 within the European Union countries (Food and Agricultural Organization, 2011). Despite their growing relevance, scientific literature regarding the welfare of intensively reared turkeys is scarce when compared to other poultry species. There is a major need for more insight into the factors influencing turkey welfare, not only due to public demands to assure a sustainable production system that foments management practices that take in consideration the welfare of turkeys, but also because this information is needed to reduce losses due to poor bird performance.

Recent studies have shown that 60% of female and 33.8% of 16-week-old male turkeys in commercial facilities showed some degree of footpad lesions (Krautwald-Junghanns et al., 2011). Lupo et al. (2010) indicated that in the French turkey industry the average condemnation rate was 18%, whereas condemnation rate for broilers was lower and ranged 87/10,000 (Lupo et al., 2008). These are only some examples of relevant animal welfare issues that also have important implications for the economic return of turkey production. Knowledge of the main factors affecting the welfare of turkeys and of the means to minimize this impact can not only improve their quality of live, but may be beneficial to industry, by achieving better bird performance, improve carcass quality and reduce mortality and condemnations and.

Unfortunately, there is a lack of scientific studies on the effects of the social and physical environment over the behavior, welfare and performance of commercial turkeys. Most of these studies have been conducted under particular experimental situations (Martrenchar et al., 1999a), therefore, the application of results to commercial practice is difficult. In this paper we review the available scientific literature regarding fundamental factors affecting behavior and welfare of turkeys; this literature is relevant to consider the establishment of science based management practices and to assure animal welfare.

3.2 Review of factors

3.2.1 Density and Group Size

Maintenance of high animal densities per unit of space is a common practice in intensive turkey production systems. Although literature for turkeys is scarce, the abundant references on the effects of density in broilers (for a review see Estevez, 2007) shows the important behavioral, and performance changes that may occur when increasing density, especially when environmental control is not matched up to maintain the demands of the increased number of animals (Dawkins, 2004). This situation may lead to more or less severe performance and welfare problems.

Density and group size are factors which effects are often confounded, together with space availability, as only two parameters can be controlled simultaneously. Therefore individual effects of each contributing factor are difficult to differentiate. Although it is possible to minimize the confusion to a certain extent by using specific experimental designs (i.e. Leone and Estevez, 2008), it is not always a practical approach, especially in applied research, in which the size of the commercial housing is fixed. Keeping in mind those issues, in current review we treat the effects of group size, density as well as the space availability, as have been described in the original study.

The influence of density on the behavior and health of turkey poults was investigated by Martrenchar et al. (1999b), who reduced space allowance from 24 to 15 dm^2 and from 16 to 10 dm^2 for males and females, respectively, until week 12, and from 40 to 25 dm^2 afterwards in case of males. The authors observed gait deterioration at higher density, suggesting stocking density as one of the potential causal factors. They also showed that stocking density had less influence on behaviors such as standing, walking, feeding, drinking, preening and pecking at the environment, or at another bird. However, similar to the findings for other density studies conducted in broilers (Estevez, 1994; Cornetto et al., 2002; Ventura et al., 2012), they found that increased density lead to a significant increment in the frequency of disturbances among resting poults. This behavior is considered as a factor closely linked with carcass quality in poultry.

Turkeys, as animals with a highly competitive social system (Bucholz, 1997) are prone to behaviors leading to the establishment of a social hierarchy. The hierarchy in groups of wild turkeys is based on close kin relationships between relatives, where external males are rejected from the group after moderately aggressive fights, and where the closed units are created for life (Balph et al., 1980; Healy, 1992). The effects of group size, group composition and space availability on the behavior of turkeys have been mainly investigated by Buchwalder and Huber-Eicher (2003, 2004, 2005a). They indicated that insufficient space may lead to increased risk for broken wings due to hitting the pen walls or to other birds during aggressive encounters (Buchwalder and Huber-Eicher, 2004). The incidence of this problem in commercial farms is, so far, unknown, but probably would be more likely to occur in small enclosures rather than in large commercial facilities.

Small groups of familiar toms seem to be able to distinguish non-group members toward whom they display aggressive interactions but the frequency of interactions appear to be modulated by enclosure size (Buchwalder and Huber-Eicher, 2004),. More pecks towards newly introduce unfamiliar tomes were observed in small (2 x 3 m) when compared to large pens (6 x 13 m). Buchwalder and Huber-Eicher (2004) explained these results in terms of a minimum critical distance requirement between opponents, which would be essential to avoid chances of aggressive interactions. Therefore, the newly introduced bird would have been able to keep a larger distance in large pens, resulting in fewer aggressive encounters. These results differed somewhat from other scientific evidences that suggest that aggressive interactions, at least in broilers, occur at a higher frequency in open areas rather than in more crowded regions of the enclosure (Pettit-Riley et al., 2002).

Nevertheless, in another study Buchwalder and Huber-Eicher (2003), found that the response towards non-familiar conspecifics mainly depended on the size of the group in which the foreigner was introduced. The smaller the group (minimum of 6 up to 30 birds), the more intense the aggressive reaction was, with more fights being initiated and more aggressive pecks being delivered. These results do seem to be in accordance with other poultry studies where a reduction in the frequency of aggressive interactions with increased group size has been reported (Estevez et al., 1997, Estevez et al., 2002; Estevez et al., 2003).

Unfamiliarity between several thousand birds of a commercial flock is a common situation in modern turkey rearing systems due to the group becoming too large to allow any form of hierarchical system. In this situation, it is inefficient to even attempt to establish a hierarchy. It has been speculated that the cost in terms of energy necessary for hierarchy formation in large groups of poultry would outweigh the benefits (Estevez et al., 1997). Furthermore, the probability of finding the same individuals over time to get the advantages of dominance will be small (Pagel and Dawkins, 1997). Other social strategies, such as a tolerant social system based on scramble competition, have been proposed to explain the social dynamics in large groups of domestic fowl (Estevez et al., 1997) and they may apply also to turkeys.

Feather pecking is, together with aggressive encounters, an important welfare and management concern in large poultry flocks. They are commonly considered to be linked to large groups, as found for laying hens (Bilcík and Keeling, 2000). No study has looked over the effects of group size over feather pecking in turkeys, but in an experimental study Busayi et al. (2006) compared feather pecking rates of a commercial male line selected for growth and breast yield with a traditional Nebraska Spot turkey coming from small experimental flocks. Higher frequency of pecks and pulls, occurred in males (32%) (Fig. 9), than in females (15%) of commercial line but were not observed in traditional one.

Figure. 9. *The male turkeys at 19 weeks of age.*

However, differences in time budgets across genders were small. Some differences were also observed with regard to maturation, where males showed stronger feather pecks and pulls at 3 weeks of age while females showed the highest frequency at 9 weeks.

3.2.2 Space availability and spatial distribution

Spatial distribution, also referred to as space use patterns, is defined as the localization of birds within the living area in relation to their group mates and resource distribution. Spatial patterns can be very important in terms of bird management as, for example, it has been observed that overcrowding of broilers around the walls of the enclosure cause increase disturbances during the resting period (Cornetto et al., 2002; Ventura et al., 2012), which may increase the risk of scratches and downgrading. Although literature on spatial distribution in turkeys is practically inexistent, one study on nocturnal turkey behavior reported that sleeping areas were mainly located around enclosure walls (Sherwin and Kelland, 1998). Therefore it is expected that turkey's space use would be driven by similar factors as to those in broilers.

In relation to inter-individual distances Buchwalder and Huber-Eicher (2004) observed that the distance between the birds was larger across non-group members than within group members. However, this distance was not the maximum distance that the pen allowed, and 50 cm seemed to be sufficient space between the unfamiliar individual and the other birds of the group. This was interpreted as an attempt to integrate in the group whilst keeping a safe distance to avoid aggressive reactions from encounters Buchwalder and Huber-Eicher (2004). Under commercial conditions, restricted space availability may inhibit birds to fully use the available space. However, detailed studies of space use in broilers demonstrated that space use related more to the size of the enclosure, utilizing a greater amount of space when available, rather than to flock size or density (Leone and Estevez, 2008; Leone et al., 2010). This might be the case for turkeys also.

3.2.3 Aging and Maturation

Changes in time budgets and behavioral repertoire are common in growing animals (Fig. 10). Poultry is no exception. Similar to broilers (Newberry, 1990; Bizeray et al., 2000; Estevez et al., 2003; Pettit-Riley and Estevez, 2001), a general decline in activity with age has been observed in commercial turkeys (Hocking et al., 1999; Martrenchar et al., 1999b; Busayi et al., 2006) together with a general reduction of oral activities such as feeding, foraging, drinking, preening and pecking at the pen walls and fixtures) (Hocking et al., 1999; Busayi et al., 2006). Parallel results were obtained by Sherwing and Kellend (1998) that found a similar decline from 4 to 22 weeks of age in sleeping, environment pecking, wing flapping and running in turkeys maintained in small groups and low density, while the time engaged feeding, standing, sitting, strutting, and preening varied through the study. At 18 weeks birds spent 30 % of their time strutting, which may be interpreted as a threatening behavior but also as courtship towards humans (Bubier et al., 1998). Sherwin and Kelland (1998) indicated that main differences in the behavior of turkeys compared to other poultry species, related to the absence of dust bathing or ground scratching, which are commonly observed in broilers or laying hens. Running and frolicking were observed, but injurious pecking was rarely noticed and feather pecking or cannibalism were not registered at all during development, even though the animals were not beak trimmed, and the light intensities were higher than the ones of commercial facilities.

Similar results were obtained by Hughes and Grigor (1996) studying time budgets of beak trimmed turkey poults up to 12 weeks, kept in small groups of 10-11 birds. Percentage of sitting/sleeping behavior increased over time, while standing/walking behavior primarily declined, and rose at the end of the study. Beak-related behaviors (feeding, drinking, preening, environmental and bird pecking) rose to the peak of 45 % in week two and then declined gradually to around 28% by the end of the study. The general decline in activity with age have been found even when the effects of high stocking density and group size were minimized, and sufficient space was provided to the birds (Sherwin and Kelland, 1998). Reduction in activity also reflected on the distances covered: 27.5 m/30 minutes at 7 weeks to 11.9 at 12 weeks (Buchwalder and Huber-Eicher, 2005b).

Turkeys are known to increase the incidence of feather pecking and cannibalism with age, and this may have practical implications. In a comparative study of traditional and commercial strains of turkeys from 3, to 9 weeks of age the frequency of feather pulls was found to increase with age in both strains, and a higher occurrence of gentle pecks was found in the traditional line, but in no case had effects on mortalities (Busayi et al., 2006). However, damaging pecking in turkeys can occur as early as the first or second week of age (Moinard et al., 2001).

3.2.4 Photoperiod and Lightening

Lighting has profound effects on the physiology and behavior of poultry (Manser, 1996). In modern poultry production, photoperiod and light intensity are strictly controlled in order to promote growth and to avoid excessive feather pecking and cannibalism. Interestingly, even under artificial low light intensity time budgets seem to follow a photoperiod rhythm, with higher proportion of resting, and low standing and walking occurring during midday (Busayi et al., 2006).

Figure 10. *Male turkeys at 2 weeks of age.*

At night, turkeys appear to be mostly inactive, although they may stand two to twelve times during the dark period usually turning around slowly and lying down again (Sherwin and Kelland, 1998).

Although low lighting intensity (1/10 lux) is use to reduce the risk of undesirable behaviors such as feather pecking and cannibalism, it can also inhibit walking, foraging, exploration and social behaviors (Hughes and Grigor 1996; Barber et al., 2004). In general turkeys prefer bright environments as Sherwin and Kelland (1998) demonstrated that turkeys avoided chambers with less than 1 lx light intensity as compared to 5, 10 or 25 lx. But additional studies indicate that turkeys may prefer different light intensities to perform different activities. In this line Barber et al. (2004) demonstrated that in an experimental situation where birds were given continuous access to four rooms with different light treatments (below 1, 6, 20 and 200 lx), at week

2 birds spent most of time in the brightest environment, while at 6 weeks the authors observed partition of behaviors between the two light environments. Resting and perching were only observed in the environment below 1lx, while the rest of behaviors were performed in the two brightest environments. Although environmental enrichment though variation in light intensities may be interesting to improve health and welfare of turkeys this has never been tested under commercial conditions. In any from a management point of view it should be consider that a sudden and temporal increase in light intensity, for bird inspection for example, may lead to fear reaction among birds (Appleby et al., 1992).

Regarding the type of lighting some studies have shown that the use of fluorescent, as compared to incandescent lighting, reduced the incidence of injuries in tails and wings, while incidence of tail and wing injuries was positively correlated with the intensity (5, 10, 36 or 70 lx) of fluorescent lights (Moinard et al., 2001). Potential benefits from use of fluorescent lights is less bright, reason why turkeys may perceived it as lower light intensity (Lewis et al., 2000), or it may relate to the composition and proportion of red light that they contain (10% fluorescent as compared to 70% to incandescent; Moinard et al., 2001). Other types of lighting types are known to have powerful effect over the behavior of turkeys. Studies by Gill and Leighton (1984) found birds maintained in low intensity blue light were more docile and less active. Sexual behavior in these pens was at a minimum, and social interactions, were rare. In contrast, birds exposed to high intensity intermittent white light were hyperactive and showed extreme flightiness during handling.

Another aspect that should be considered in turkey management is that Turkeys are known for having potential for vision in the UV-A spectral range, and it is possible that plumage may contain visual information detectable only under in UV-A wave bands

(Hart et al., 1999). In fact results from Hart et al. (1999) and Moinard and Sherwin (1999) suggest that turkeys preferred UV-A-enriched environment to one illuminated by fluorescent light alone. In modern housing the use of fluorescent or incandescent lamps that emit low levels of UV-A spectrum, may limit the natural communication conveyed by the plumage of turkeys. In fact, Hart et al. (1999) suggested that provision of supplementary UV light may reduce the incidence of visually mediated, aberrant behaviors.

Besides light intensity and type, the lighting program has been proven to have a significant effect on the behavior of turkeys and may be used to improved bird management. For example Classen et al. (1994) demonstrated that turkey male poults of a heavy strain reared to 188 days of age in a 6L: 18D at 7 days increasing to 20L: 4D by 63 days, or starting with 6L:18D increasing to 10L: 14D from 84 to 112 days, showed a superior walking ability and sat less often as compared to birds maintained at constant 24L:0D. Lewis et al. (1998) investigated the influence of 4 different photoperiods (8, 12, 16 or 23h) with light intensities of 1 or 10 lx on the behavior of male turkeys. Light intensity did not influence feeding behavior, but injurious pecking took place at a higher frequency for the 12h photoperiod 10 lx combinations. On the other hand, Sherwin et al. (1999) carried out an experiment comparing 12L/24h with 8(2L)/24h finding that even though some patterns of intermittent lightning were effective in reducing the frequency of injurious pecking behavior, they compromised other welfare indicators, such as the musculo-skeletal function and the occurrence of blindness (Sherwin et al., 1999).

3.2.5 Feeding

The number of studies dedicated to the effects of diet composition, the form in which is presented, and its availability may influence behavioral patterns and welfare in turkeys is very limited. Six to twelve week old turkey poults feed with pellets spent less time feeding, when compared to younger age birds fed with crumbs (Hughes and Grigor, 1996). Contrarily, Hale and Schein (1962) found that 12 week old pellet-fed birds spent more time feeding, less time drinking and preening and resting, and had higher engagement in other behaviors when compared to mesh-fed ones. Main differences between these results may relate to genetic factors due to 30 year difference between them, the age of the birds and to how the feed was presented.

Nutritional enrichment in the form of whole wheat provided in separate feeders, replacing 10% of wheat from their regular diet, has been used with the objective of increasing time dedicated to feeding, and decrease time availability for injurious pecking (Mirabito et al., 2003). A positive effect of the intervention was detected during the first 2 weeks. However, from 9 weeks onwards increased feeding frequency was only detected during the evening and in general, the provision of whole meal had little effect on the feeding behavior, and no effects on the turkeys' pecking behavior.

Feed restriction is a commonly used management practice in the breeder turkey industry to control male body weight for optimal semen production, to manage risk of heat stress, or musculo-skeletal lesions. However, food deprivation can have a negative impact in the welfare of turkeys which may manifest through changes in their behavior patterns. Hocking et al. (1999) compared the behavior of *ad libitum* and feed restricted commercial large white turkey male line from 8 to 28 weeks. *Ad libitum* fed birds mainly showed standing, walking and preening behavior (44 to 77% of the time budget), while feed restricted birds showed high frequencies of oral activities such as pecking on pen walls and furnishings (20 to 59% of the time budget depending on week). It was emphasized by the authors that first signs of the increased oral activity and reduction of sitting was observed already two weeks after restriction began.

3.2.6 Transport

Catching and transport of live turkeys, as for other poultry, may be one of the most stressful events in the bird's lifetime if not done properly. Pre-transportation procedures such as inadequate catching and crating have major negative impact on birds' welfare, varying from mild stress to the death before arriving to the slaughterhouse. Therefore the way in which these procedures are conducted can have a dramatic impact in carcass quality and economic profit. Most of the available studies in turkeys describes the direct effects of the procedures on animal welfare in the form of deaths on arrival (DOA) (Wichman et al., 2010). A large scale study conducted by Petracci et al. (2006) in Italy showed an average DOA of 0.38 up to 0.52 during summer. Causing factors are suspected to be similar to broilers: thermal stress, acceleration, vibration, motion, impacts, fasting, withdrawal of water, social disruption and noise, incorrect transport of sick or injured animals and the human factor (Mitchell and Kettlewell, 1998; Prescott et al., 2000; Petracci et al., 2006).

For turkeys, there seem to be some benefits of automatic as compared to manual crating in terms reduction of body damage and heart rate (Prescott et al., 2000). Even though the birds were herded into the module using an automatic loading system, the manual handling proved to be more stressful than the automatic conveyance. The human participation during the manual crating procedure was the most influencing factor on turkeys' stress indicators.

Recently Wichman et al. (2010) described the effect of crate height (45, 50 or 90 cm) during 6h confinement on the behavior of turkeys. While turkeys could not stand in the lowest crates, they stood 35 and 43% of the total time in 50 and 90 cm height crates, respectively. More stepping, turning and preening were performed in 50 and 90 cm, whereas in the 40 cm crates more rising attempts were observed. The conclusion of this study was that 40 cm crates decreased the possibility of birds to move and to change postures. However a potential danger that should be considered is that bigger crates can lead to further carcass damages due to scratches made by the nails among crated birds.

3.3 Discussion

Based on the literature published in last two decades it is clear that behavior and welfare of commercial turkeys is affected by the conditions in which they are reared (for example: Buchwalder and Huber-Eicher, 2003; Busayi et al., 2006). Clear evidences of negative consequences of modern rearing conditions can be observed at the farm level (Martrenchar, 1999a). Many studies concentrated on problems related to the use of too large density and group sizes (Martrenchar et al., 1999b; Buchwalder and Huber-Eicher, 2003; 2004; 2005a; Busayi et al., 2006) and on the other hand on the lack of space for performing natural behviours (Sherwin and Kelland, 1998; Buchwalder and Huber-Eicher 2004). The results of rearing turkeys in those conditions were measured mainly by occurrence of such behaviors as agresivity, disturbance in social, resting and comfort behaviors or negative changes in general activity and locomotion. Other investigated environmental factors, having large effect on turkeys behavior, were lightning type (Moinard et al., 2001), light intensity (Hughes and Grigor, 1996; Barber et al., 2004; Sherwin and Kelland, 1998) and lightning program (Classen et al., 1994; Lewis et al., 1998; Sherwin et al., 1999). Turkeys showed preferences for variability of light intensities to perform particular behaviors, as well as for fluorescence (Lewis et al., 2000; Moinard et al., 2001), UV-A enriched environments (Hart et al., 1999; Moinardet al., 1999) and not constant photoperiod (Classen et al., 1994; Lewis et al., 1998; Sherwin et al., 1999). Lack of fulfilling physiological requirements with regards to the lightning caused decreased levels of welfare expressed by: injurious pecking, feather pecking and cannibalism but also deprived locomotion and activity budgets. Aging and maturation were found to have large influence on the turkeys' behavior (Hocking et al., 1999; Martrenchar et al., 1999b; Busayi et al., 2006; Sherwing and Kellend, 1998; Hughes and Grigor, 1996). Most apparently birds at 4 weeks of age showed first signs of decreased locomotion abilities and less active behaviors (Sherwing and Kellend, 1998), while the injurious pecking levels increased already after week 3 (Busayi et al., 2006). Nutritional factors, like diet changes (Hughes and Grigor, 1996; Hale and Schein, 1962), addition of fodder enrichments (Mirabito et al., 2003) or food restriction (Hocking et al., 1999) influenced activity, general behaviors, or oral activity. Catching, crating (Prescott et al., 2000) and transport (Wichman et al., 2010), as influencing factors for turkeys welfare were shown to be substantial, causing death of birds if not processed in a correct manner by using sufficient catching methods, cage sizes or environmental conditions.

As shown above, if minimum physiological requirements of farmed turkeys are not fulfilled, birds show large behavioral adjustment to the conditions they are facing. Therefore, their behavior can be regarded as a rapidly changing indicator of welfare. Out of wide scope of behavioral adjustments shown by turkeys, presented studies have proved that on one hand increased aggression, feather pecking or cannibalism and on the other decreased activity, locomotion and disturbed time budgets schemes, are the components which are mostly influenced.

Recent and past literature about turkeys provides evidences that there is a need for better insight into their behavior in wide perspective. In broilers, researchers saw the need of developing statistical models for that kind of complex analysis, showing behavioral changes depending on the influencing factors levels variations and combinations (Estevez et al., 2007). In turkeys it is however still necessary to get better understanding of effects related to some factors, like for example spatial distribution or common management procedures causing pain, on behavior, which have not been investigated yet for this specie. Additionally, importance of avoiding sudden and temporal changes in environmental conditions should be scientifically proved and underlined (Appleby et al., 1992).

On the other hand behaviors related to social interactions or communication between individuals should be understood better in order to allow insight into functioning of the groups in which turkeys are kept (Estevez et al., 2007). Some comparisons with regard to those aspects can be performed to broilers but behavioral reactions of turkeys might not always be corresponding (Sherwin and Kelland, 1998).

The main task set for scientists currently would be to suggest optimal level ranges which ensure birds' well-being, manifested by acceptable behavioral profiles, and to disseminate them into practical conditions. That kind of science based management procedures should be implemented primarily to solve the most severe welfare problems met in turkey production: increased aggression, feather pecking and cannibalism, leg disorders or injuries among birds and secondly they could also give a base for development and setting thresholds of animal based indicators used for assuring turkeys on-farm welfare.

3.4 Conclusion

Over the years scientists have been not only trying to find the underlying causes for the welfare deprivation, which were reviewed in current study, but also to suggest possible solutions to improve the current on-farm situation. This aims not only at increasing welfare status of the birds but also could be very beneficial for the farms economy. To our opinion this can be done by defining and applying complex sets of rearing and environmental conditions to be applied at the farm. For example applying optimal density and related group size to particular age group could improve the gait scores, disturbances between birds would decrease, while final carcass qualities increase. Additionally, it would be beneficial to take under account balance between turkey needs of integration and safe distance by use of environmental enrichments combined with adjusted light regimes or even by providing different light levels across the house to provide possibility of behavior variations within the room. That kind of suggestions of complex solutions might seem costly and labor consuming, however the tremendous impact of pulled, optimal environmental conditions on poultry performance improvement was already stressed (Dawkins et al., 2004), so it is clear that by optimizing the conditions of rearing, production levels could be increased, while flocks health-related costs and rejection rates decreased, aligning the economical inputs or even bringing profits to the farmers.

Chapter 4: *The transect method: a novel approach to on-farm welfare assessment of commercial turkeys**

**Marchewka, J., I., Estevez, G., Vezzoli, V., Ferrante, and M. M., Makagon. 2015. The transect method: a novel approach to on-farm welfare assessment of commercial turkeys. Poultry Science. 94: 7-16.*

4.1 Introduction

The lack of effective and efficient protocols for the evaluation of commercial turkeys impedes the ability of turkey producers to evaluate the effects of management practices on bird productivity and welfare, or provide stakeholders with science-based assurances as to the welfare status of flocks. The ability for producers to monitor bird welfare can have important impacts on their economic revenue. Welfare related issues, including leg and mobility problems, aggression towards other turkeys, have been cited as major causes of economic loss for this industry (Krautwald-Junghams et al., 2011). The development and validation of universal, reliable, quantitative and easy to apply methodologies for on-farm turkey welfare assessment is a critical step towards monitoring the incidences of, understanding the causes of, and formulating remedies for these types of concerns.

Available science-based welfare assessment protocols for other meat poultry (e.g. Welfare Quality Assessment Protocol for Poultry; Welfare Quality, 2009) often require the corralling and handling of birds. These types of methods are not practical for use on turkey farms as the large body sizes, heavy weights, and active and flighty nature of turkeys make their handling difficult and potentially dangerous for the birds and handlers. Methods that do not require the handling of birds provide a more feasible option. For example, Dawkins et al. (2004) proposed a method for the assessment of broiler gait that relies on the visual inspection of a subsample of birds as they take 10 steps. However, a limitation of this and similar approaches is that they are time consuming and allow only for the assessment of a relatively small proportion of the flock, particularly given the relatively large sizes of turkey flocks. This could lead to skewed flock level estimates of incidences of welfare issues.

Currently available information about the state of commercial turkey flocks has been obtained mainly from animal centered protocols focused on the condition of the birds at the slaughter plant (DOAs, condemnations, bruising, etc), both pre- and post-mortem (e.g. St-Hilaire et al., 2003; Grandin, 2011). This information does not necessarily represent the on-farm welfare condition of the birds as it is confounded by the effects of loading, transportation and lairage. Additionally, it does not provide information about many of the welfare categories that can be assessed on-farm.

The TW approach, an assessment method that has recently been deemed practical for evaluating the welfare of broilers (Marchewka et al., 2013a), may resolve many of the practical and sampling challenges associated with the assessment of large turkey flocks. The TW method is based on line transect methodology, a technique routinely used in ecological studies to estimate animal biodiversity and abundance (Burnham et al. 1980, Butler et al., 2006). In short, an assessor walks the house along predetermined paths counting the incidences of birds' representative of predefined welfare indicator categories. The method requires no animal handling and allows for the visual assessment of the entire flock. An additional strength of the approach is that it bears similarity to the daily poultry flock checks conducted by farmers, and is therefore easy to adopt.

The overall goal of this study was to examine the suitability of the TW method for the on-farm assessment of turkey welfare. We compared the results and inter-rater reliabilities of turkey welfare assessments made using the TW method to those made using two other approaches: an individual scoring approach modified from Dawkins and colleagues (2004), and the individual scoring of turkeys during L. The assessments focused on large toms at the end of the production cycle, as we hypothesized that welfare-related issues would be most evident within this production group, and because assessments made at the end of the production cycle would be most likely to be consistent with the evaluation made during L. We additionally compared the results of the three assessments with the company's perception of how the flocks on the farms would be faring based on past production data.

4.2 Materials and methods

The study was conducted between September 23rd and November 18th, 2013 on 10 commercial turkey tom farms located in the Midwestern United States. One flock per farm was included in the study. The farms were selected based on the focal flock's age at the time of the study, and the company's opinion as to whether the farm fared well (five farms) or needed improvement (five farms) with regard to bird performance. The research team was blind as to each farm's classification until data collection was completed.

4.2.1 Facilities and animals

All farms belonged to a single turkey company, and were managed using standardized protocols. Hybrid turkey flocks were raised in grow-out houses from six wks of age until the end of the production cycle, which took place at approximately 19 to 20 wks of age when the birds reached an average BW of 20 kg. The stocking density at the beginning of the production cycle was between 3.5 and 3.6 birds/m^2. The turkeys were originated from five breeder flocks. They were raised on wood shaving alone or mixed with rice hulls. All houses had mesh windows on the sides of the buildings, were equipped with automatic drinkers and feeders, and with either manually or automatically controlled ventilation systems. Natural light, which entered the house through the windows, was supplemented with artificial lighting for a total of 23 h of light per day. Flock management information is summarized in Table 6.

With the exception of one house, which measured 15.3 x 152.4 m, all of the study houses had identical dimensions (12.5 x 152.4 m). House measurements were confirmed using laser range finder (BOSCH GLM 825, Stuttgart, Germany). For the purposes of data collection, each house was divided longitudinally into four transects. Transects were approximately 3m wide, and their widths were limited by the feeder and drinker lines, or the wall and adjacent drinking line (Fig. 11). The presence of these physical barriers hindered the birds' movement between adjacent transects as they walked away from approaching humans.

4.2.2 Data collection

Each of the 10 flocks was evaluated at 19/20 wks of age by two observers using the TW and S methods within the same day. A final flock evaluation, L, took place during the load out process, when birds were moved out of the house and loaded onto transportation trucks, which occurred within 48 h of the initial evaluation period. All three evaluation methods included the same welfare indicator categories, which are presented in Table 5.

The duration of each evaluation, and cumulative mortality data were obtained from flock records at 19/20 wks of age are shown in Table 6.

Two observers assessed each flock simultaneously, but independently in order to allow for a subsequent evaluation of inter-observer reliability. Both observers had previous experience conducting poultry welfare assessments, but had limited experience working with turkey flocks. While one of the observers had previous experience evaluating broilers using TW, the other was new to this methodology.

Table 5. *Description of the birds' behavior and appearance in each of the welfare indicator categories. Birds meeting any of the descriptors within a category were counted as belonging to that category. Individual turkeys could be classified as belonging to more than one category.*

Welfare indicator	Description
Immobile	Bird not moving when approached, or after being gently touched; birds are only able to move by propping themselves up on their wings.
Lame	Bird walks with obvious difficulty; one or both legs are not placed firmly on the ground; bird is moving away from the observer but stopping after 2-3 paces to rest; bird has legs shaking syndrome.
Head wounds	Bird has visible marks on the head, snood, beak or neck related to fresh or older wounds.
Back wounds	Bird has visible fresh or older, including bleeding, wounds on the back and/or wings.
Vent wounds	Bird has visible wounds around tail, or on its sides, including fresh, older or bleeding wounds.
Aggression towards mate	Bird chases or pecks, hits, flies into or leaps onto another bird.
Human interaction	Bird perceptibly hits human with the wings, or runs into, jumps onto or pecks the human.
Mounting	Bird mounts another bird.
Dirty	Very clear and dark staining of the back, wing and or tail feathers of the bird, not including light discoloration of feathers from dust, covering at least 50% of the body area.
Featherless	Missing feather on the majority of the back area, including the wings.
Small	Easily distinguishable females or individuals that are approximately ½ the size of an average bird in the flock.
Sick	Bird showing clear signs of impaired health, including with a small and pale comb, red-watery eyes, and disarranged feathers. These birds are usually found in a resting position. Also birds with pendulous crops; birds with a pendulous crop hanging in front of the breast, with missing or deformed body parts (excluding birds with leg deformations accounted for as lamed), or with pale/yellowish body color.
Terminal	Bird with large wounds or lying on the ground with head rested on the ground or back, usually with half closed eyes; Bird must be breathing visibly.
Dead	Dead

Prior to the onset of data collection the observers were provided with on-farm training during which they walked through a house with a producer and discussed examples of turkeys that they deemed to be representative of each animal welfare indicator category.

Transect walks (TW). The TW approach for on farm welfare evaluation of turkey flocks was based on methodology previously described for broilers (Marchewka et al., 2013a). The two observers walked the length of each transect recording all observed incidences of birds falling into any of the predefined welfare indicator categories. The order in which transects were walked were selected randomly, except that sequential observations of contiguous transects were avoided in order to account for double counting of birds. Observers moved slowly in order to minimize disruption to the flock during scoring (Fig. 11).

Individual scoring (S). The individual scoring method was adapted from Dawkins et al. (2004). One hundred and four randomly chosen turkeys, 26 birds per transect from two random locations along it, were evaluated. Each bird was followed visually as it took up to 10 steps and then scored using the predefined welfare indicator criteria (Table 5).

In order to ensure that both of the observers were evaluating the same bird, a laser pointer was used to identify the focal birds. With his or her eyes closed, one observer fixed the pointer on a spot. If the pointer indicated a bird, that turkey was assessed by both observers. The procedure was repeated if no bird was present in the indicated spot.

Table 6. *Total number of birds placed, management (M) details, cumulative mortality calculated up to 19/20 wk of age, and duration of each of the data collection procedure (L, TW and S) is listed for each focal house.*

Farm	Total nr of birds placed/house	M	Drinker	Litter	Antibiotic use allowed	Light	Cumulative mortality- 19/20 wk (%)	Load time (h)	Transect time (min)	Individual time (min)
1	6545	Optimal	bell	rice hull	no	incandescent	24.0	3.5	28.3	30
2	6660	sub-optimal	bell	rice hull + wood shavings	yes	incandescent	11.4	5	35.1	27
3	6485	Optimal	nipple	rice hull + wood shavings	yes	incandescent	13.3	4.5	35.8	32
4	6460	Optimal	bell	rice hull + wood shavings	no	incandescent	20.2	3.5	36.6	29.5
5	6660	sub-optimal	bell	rice hull	yes	incandescent	13.0	5	46.6	40
6	6560	sub-optimal	nipple	rice hull + wood shavings	no	compact fluorescent	18.8	3.5	26.9	32
7	6560	Optimal	bell	rice hull + wood shavings	no	compact fluorescent	11.4	3	25.8	34
8	8462	Optimal	nipple	rice hull + wood shavings	yes	incandescent	11.4	7.5	43.0	35
9	6502	sub-optimal	bell	rice hull + wood shavings	yes	compact fluorescent	15.9	4	29.4	31
10	6660	sub-optimal	bell	rice hull + wood shavings	yes	incandescent	10.9	4.5	32.6	45
Mean							15.03	4.40	34.01	33.55
SE							1.44	0.41	2.17	1.69

Load out evaluation (L). During L, consecutive batches of 40-50 birds were herded into a corridor made out of wooden panels that funneled the turkeys towards the loading belt. The corridor was divided by a middle panel separating the birds into two groups of approximately 20-25.

Figure 11. *Observer during a TW data collection. The transects are limited by the drinkers (left) and feeder line (right).*

Turkeys were individually evaluated as they walked towards the loading belt and past the observers who stood at the sides of the corridors. The data collected in this fashion was considered to be the "gold standard", as it provided the observers with the opportunity to assess each bird from a flock from close distance. The same indicator categories were considered as for the other two methods (Table 5), with the exception of interactions with humans as the observers were separated from the birds by a wooden barrier. Once the majority of birds were moved out of the house, the observers walked the house, recording mortalities and evaluating any birds that may have been left behind.

Slaughterplant data. Data collected routinely at the slaughterplant was acquired for each focal flock. The following indicators were used in current study: livability; condemned: DOA, whole, parts; age at slaughter; weight gain per day and average weight gain.

4.2.3 Statistical analysis

Incidence of welfare indicators were calculated for each flock, therefore the analyses were conducted as house as the experimental unit (10 houses total). Prior to statistical analysis the recorded frequency data on the numbers of individuals counted within each welfare indicator category were transformed into proportions per transect based on the known flock population in each house at the time of assessment. It was assumed that turkeys distributed randomly throughout the house.

In order to meet normality and homogeneity of residual variance assumptions all variables were arc sin square root transformed. An independent mixed-model repeated measures ANOVA was performed for each of the 14 welfare indicator categories using the PROC MIXED procedure in SAS 9.3 (SAS Institute Inc., 2011). The model included method of assessment, observer and producer assigned management level (faring well or sub-optimal) as fixed factors, and the interactions between observer by method and management, as well as management by method. Farm nested within the management category was included as a random statement, as well as its interactions with method, observer and the three way interaction with method and observer. LSM differences were adjusted for multiple comparisons using the post-hoc Tukey test. As turkeys were not able to interact with assessors during loading, the model for this indicator variable was run only for the other two methods.

Spearman correlations calculated using the PROC CORR script in SAS 9.3 (SAS Institute Inc., 2011) software were used to test the relationships between all variables collected during evaluations made using TW, S, L, data collected at the slaughterplant and cumulative mortality levels reported to 19/20th wks of turkey age.

4.3 Results

Effects of fixed factors and interactions included in the analysis of variance on all evaluated indicators are summarized in the Table 7.

Welfare assessment remained consistent across observers for all of the indicators (Table 8). The interaction between observer and method did not affect the incidences of the welfare indicators with the exception of immobility as evaluated by TW. Nonetheless, the significant differences across observers, which ranged between 0.74% ± 0.2% vs. 0. 75% ± 0.2% for the incidence of immobile birds, were very small in numerical terms.

The effects of assessment method on welfare indicator outcomes are presented in Table 9. As compared to the TW and L methods, the S method yielded higher estimates of incidence of lame turkeys and turkeys with head wounds, and lower estimates of the incidence of featherless, terminal and dead birds, and birds engaging in mounting behaviors.

The percentages of sick birds evaluated using TW or S differed from the percentage calculated during L assessments. All three methods differed in the detected percentages of immobile and dead birds.

Table 7. *Effect of observer (Obs), method (Me), management (Mg) and their interactions for all scored welfare indicators.*

		Analysis of variance components					
Welfare indicator		Obs	Me	Ma	Obs*Me	Ma*Obs	Ma*Me
Immobile	F	4.33	30.64	1.3	4.33	1.33	0.65
	p-value	0.071	<0.0001	0.288	0.0292	0.2823	0.5361
Lame	F	0.06	32.06	0.53	0.07	0.02	0.15
	p-value	0.8095	<0.0001	0.488	0.9369	0.8822	0.8653
Aggression towards mate	F	2.5	2.5	2.5	2.5	2.5	2.5
	p-value	0.1526	0.1136	0.1526	0.1102	0.1526	0.1136
Mounting	F	1	5.35	0.54	1	1	1.73
	p-value	0.3466	0.0167	0.4843	0.3874	0.3466	0.2086
Human interaction	F	2.87	34.39	0.04	1.26	0	0.15
	p-value	0.1289	0.0004	0.8436	0.2905	0.9998	0.7094
Head wounds	F	1.72	21.17	0.32	0.67	0.15	0.79
	p-value	0.2267	<0.0001	0.5871	0.5223	0.7119	0.4715
Back wounds	F	1.4	1.18	0	1.97	0.38	1.51
	p-value	0.2703	0.3335	0.9613	0.1686	0.5552	0.2507
Vent wounds	F	0.34	0.63	0.01	0.35	0.7	1.13
	p-value	0.5759	0.544	0.9432	0.7083	0.4271	0.3479
Small	F	0.36	0.24	0.68	0.74	0.02	0.09
	p-value	0.5657	0.7895	0.432	0.4906	0.8852	0.9144
Featherless	F	0.04	6.65	0.07	0.04	0.78	0.85
	p-value	0.8406	0.0079	0.7949	0.9568	0.4039	0.4443
Dirty	F	0.7	0.42	0.5	1.16	1.16	1.72
	p-value	0.4268	0.6646	0.5006	0.335	0.3122	0.2097
Sick	F	1.32	13.07	4.23	1.49	1.31	3.33
	p-value	0.2832	0.0004	0.0738	0.2509	0.2857	0.0619
Terminal	F	0.09	11.11	1.45	0.09	1.25	0.65
	p-value	0.7714	0.0009	0.2635	0.9141	0.2957	0.537
Dead	F	1	65.36	1.26	1	1	1.93
	p-value	0.3466	<0.0001	0.2942	0.3874	0.3466	0.1772

The results of the Spearman correlations analysis between welfare indicators as collected by TW and L (excluding interaction with humans, which was not observed during L), cumulative mortality levels reported at 19/20 wks and the parameters collected at the slaughter plant are presented in Table 10.

Table 8. *Mean values (±SEM) of incidence of birds within each welfare indicator category expressed as percentages for each observer (Obs).*

Welfare indicator	Obs 1 (%)	Obs 2 (%)
Immobile	0.741±0.161	0.752±0.162
Lame	6.428±1.314	6.371±1.244
Aggression towards mate	0±0	0.001±0.001
Mounting	0.009±0.003	0.011±0.004
Human interaction	0.253±0.072	0.154 0.053
Head wounds	3.416±1.001	3.491±1.027
Back wounds	0.350±0.077	0.291±0.056
Vent wounds	0.139 0.052	0.140±0.052
Small	0.921±0.016	0.899±0.158
Featherless	0.024±0.009	0.022±0.007
Dirty	0.069±0.032	0.1±0.044
Sick	0.445±0.0105	0.397±0.097
Terminal	0.032±0.009	0.032±0.008
Dead	0.168±0.041	0.168±0.041

We did not find any differences between management classifications in the percentages of birds within particular welfare indicator categories. Effects of management by method, or management by observer interactions were also not significant.

Table 9. *Mean values (±SEM) of incidence of birds within each welfare indicator category expressed as percentages for each method (Me).*

Welfare indicator	Transect	Individual sampling	Load out
Immobile	0.60±0.11[a*]	0.19±0.09[b]	1.45±0.24[c]
Lamed	2.36±0.34[b]	12.74±1.92[a]	4.10±0.67[b]
Aggression toward mate	0.002±0	0±0	0±0
Mating	0.02±0.01[a]	0±0[b]	0.01±0[a]
Human interaction	0.31±0.05[a]	0.10±0.07[b]	N/A
Head wounds	1.16±0.15[b]	7.50±1.81[a]	1.70±0.31[b]
Back wounds	0.22±0.02	0.38±0.13	0.35±0.05
Vent wounds	0.05±0.01	0.29±0.10	0.08±0.01
Small	0.59±0.06	1.35±0.31	0.79±0.06
Featherless	0.04±0.01[a]	0.00±0.00[b]	0.03±0.01[a]
Dirty	0.07±0.02	0.14±0.08	0.04±0.01
Sick	0.05±0.01[b]	0.50±0.16[b]	0.71±0.09[a]
Terminal	0.03±0.00[a]	0±0[b]	0.06±0.01[a]
Dead	0.14±0.02[b]	0±0[c]	0.37±0.06[a]

*small letter subscripts indicate *p*-value smaller then 0.05

4.4 Discussion

Considering the large size and flighty nature of commercial turkeys, and particularly that of turkey toms, evaluation of their welfare status using strategies developed for other poultry species, which typically require the corralling or handling of birds, can be challenging. In addition to being inefficient with respect to time requirements, these methods are likely to disrupt the flock, and are potentially dangerous for both the turkeys and the evaluators. The current study compared the TW approach with two other welfare assessment methods that do not require the handling of birds: individual sampling of a random sample of birds and flock evaluation as birds were loaded out of the house for transportation. None of these methods had previously been evaluated for use on commercial turkey farms. The S and TW approaches were selected for this study as they have successfully been used for the evaluation of broilers (Dawkins et al., 2004; Marchewka et al., 2013a).

To the best of our knowledge, this is the first time the results of a welfare evaluation conducted during the load out process are presented for poultry. Although this approach is time consuming, and therefore not practical for everyday use, it provided us with the opportunity to sample all of the birds in the flock. In addition to comparing the welfare indicator data collected using each of the three methods, we examined practical aspects of the methodologies including the inter-observer reliability of each method, and its time requirements.

Because a validated set of welfare indicators is not available for turkeys, we evaluated the birds based on welfare indicators developed for other species of meat poultry (Welfare Quality, 2009) and based on the review of the available literature for turkeys (Marchewka et al., 2013b). Several indicators were added to account for differences we expected to see between turkeys and broilers based on their temperament and size, older age at slaughter and ability to reach sexual maturity, and their relatively higher activity levels (Huff et al., 2003). These factors were suspected to result in higher severity of wounds, especially on the head and back, higher frequency of interactions with flock mates, and higher incidence of sexual behaviors noticed previously in turkey flocks (Marchewka et al., 2013b).

The welfare indicator categories were developed based on preliminary observation of turkey toms, as only male flocks were included in this study. It is possible that additional or different indicators may be appropriate to use in female flocks (Martrenchar et al., 2001, Huff et al., 2007).

We found no differences between observers, as well as for observer and method interaction, except for the proportion of immobile birds as evaluated using the TW method. Differences obtained for immobile birds across observers were similar to the result obtained earlier for broilers by using TW (Marchewka et al., 2013a). In both studies although differences across observers for immobility were significant, the actual difference in values were minimal. In this study, the numerical differences between observers were only 0.01%, which is too small to be considered as a major constraint of the TW approach. Therefore the results highlight that the detection of important and well defined welfare indicators in turkeys can be reliably achieved by multiple observers with minimal training.

On the other hand differences in the data collected using the three assessment methods were found for all but five of the indicator categories. Overall, the identified differences separated the S method from the TW and L assessments. The S method yielded different results for eight of the 13 welfare indicators evaluated during L, including the proportion of turkeys that were lame, featherless, terminal, showing head wound, and exhibiting mounting behaviors, sick, immobile or dead.

Table 10. *Spearman correlations between indicators collected by TW, S, during L at the slaughterplant and mortality levels at 19/20 wks. Due to large size the entire correlation table was divided into 3 tables. Significance of the correlations is indicated as follows: * for $p<0.05$; ** for $p<0.01$; *** for $p<0.001$.*

		Load out													
	Welfare indicator	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Load out	Immobile (2)	-0.35													
	Lame (3)	-0.37	0.96***												
	Human int. (4)	0.29	-0.29	-0.41											
	Mounting (5)	0.41	-0.03	-0.08	0.43										
	Head wounds (6)	0.03	0.09	0.1	-0.52	0.03									
	Back wounds (7)	-0.16	0.48	0.49	-0.41	0.15	0.08								
	Vent wounds(8)	0.35	-0.28	-0.21	-0.17	0.07	-0.37	0.05							
	Small (9)	-0.62	0.37	0.5	-0.41	0.06	0.03	0.59	0.02						
	Featherless (10)	0.19	-0.39	-0.55	0.31	0.5	-0.14	-0.1	0.3	-0.24					
	Dirty (11)	-0.07	0.34	0.5	-0.41	-0.05	0.2	0.3	-0.23	0.46	-0.54				
	Sick (12)	-0.38	0.71**	0.71**	-0.41	-0.03	0.65*	0.38	-0.69**	0.39	-0.47	0.51			
	Terminal (13)	-0.21	0.55	0.63	-0.18	0.12	-0.13	0.61	0.08	0.77**	-0.35	0.52	0.36		
	Dead (14)	-0.33	0.5	0.58	-0.06	0.2	-0.02	0.58	-0.2	0.81**	-0.36	0.51	0.5	0.93***	
Transect walks	Immobile (15)	-0.22	0.89**	0.85**	-0.17	-0.03	0.07	0.42	-0.18	0.24	-0.46	0.04	0.56	0.44	0.39
	Lame (16)	-0.31	0.89***	0.85**	-0.29	-0.02	0.1	0.53	-0.09	0.36	-0.36	0.02	0.56	0.48	0.43
	Aggression t. mate (17)	0.05	0.04	-0.03	0.58	0.04	-0.49	0.16	-0.35	-0.03	-0.05	0.16	-0.03	0.32	0.39
	Human int.(18)	0.47	-0.56	-0.66*	0.17	-0.01	0.1	-0.18	-0.12	-0.69**	0.38	-0.19	-0.31	-0.72**	-0.67*
	Mounting (19)	0.42	-0.44	-0.58	0.46	0.74**	-0.05	0	0.19	-0.26	0.9***	-0.42	-0.4	-0.28	-0.23
	Head wounds (20)	0.24	0.2	0.24	-0.41	0.23	0.89***	0.18	-0.22	0.03	-0.27	0.18	0.6	0.01	0.08
	Back wounds (21)	-0.12	0.62	0.53	0.41	0.36	-0.26	0.16	-0.2	0.05	-0.1	-0.25	0.24	0.23	0.27
	Vent wounds (22)	0.21	-0.22	-0.18	0.06	0.27	-0.32	0.02	0.85**	0.16	0.33	-0.44	-0.58	0.2	0.02
	Small (23)	-0.38	0.13	0.26	-0.17	0.23	-0.01	0.48	0.2	0.83	-0.19	0.09	0.14	0.62	0.67*
	Featherless (24)	0.21	-0.25	-0.46	0.53	0.5	-0.17	-0.09	0.09	-0.44	0.83**	-0.79**	-0.39	-0.48	-0.42
	Dirty (25)	-0.27	0.43	0.48	0.29	0.12	-0.21	-0.01	-0.57	0.33	-0.43	0.62	0.44	0.45	0.58
	Sick (26)	-0.47	0.56	0.6	-0.52	-0.24	0.68*	0.21	-0.56	0.3	-0.56	0.25	0.85**	0.08	0.21
	Terminal (27)	0.03	0.18	0.07	0.17	-0.52	-0.3	-0.18	-0.35	-0.52	-0.19	0.06	0.01	-0.23	-0.27
	Dead (28)	-0.38	0.42	0.47	-0.52	0.01	0.61	0.53	-0.35	0.68*	-0.25	0.51	0.77**	0.62	0.71*
Sampling	Immobile (29)	0.09	0.44	0.44	-0.17	0.37	0.09	0.52	0	0.09	-0.09	0.09	0.26	0	0
	Lame (30)	-0.3	0.83**	0.81**	-0.29	-0.34	0.1	0.24	-0.25	0.08	-0.59	0.06	0.55	0.21	0.16
	Human int. (31)	0.52	-0.17	-0.17	-0.17	0	0.44	-0.17	-0.17	-0.61	-0.09	0.18	0.09	-0.62	-0.61
	Head wounds(32)	0.24	-0.11	-0.07	-0.23	0.37	0.76*	0.1	-0.09	0.01	-0.05	-0.07	0.32	-0.19	-0.07
	Back wounds (33)	0.03	0.42	0.49	-0.26	-0.35	-0.15	-0.04	0.38	-0.04	-0.51	-0.09	-0.03	0.15	-0.06
	Vent wounds (34)	-0.04	0.42	0.34	0.51	0.2	-0.65*	-0.19	0.11	-0.11	0.04	-0.27	-0.19	0.12	0.04
	Small (35)	-0.35	-0.21	-0.06	0.18	0.02	-0.34	-0.14	0.12	0.37	-0.26	-0.09	-0.24	0.04	0.13
	Dirty (36)	0.39	-0.06	0.11	-0.17	-0.13	-0.22	-0.16	0.42	-0.16	-0.46	0.36	-0.28	-0.06	-0.23
	Sick (37)	0.07	0.15	0.14	0.19	0.82**	0.35	0.19	-0.33	0.2	0.19	0.15	0.39	0.03	0.23

		Slaughter information							
Welfare indicator		Livability	Condemned	DOA	Whole	Parts	Av GW	Age	Gain/day
	Mortality	-0.95***	-0.58	-0.08	-0.25	-0.72*	0.66*	0.69*	064*
Load out	Immobile	0.49	0.82**	0.58	0.89***	0.82**	-0.49	-0.38	-0.37
	Lame	0.49	0.78**	0.41	0.85**	0.85**	-0.59	-0.50	-0.43
	Human int.	-0.29	-0.29	0.41	-0.17	-0.52	0.41	0.23	0.41
	Mounting	-0.46	-0.20	0.21	0.07	-0.37	0.27	0.05	0.25
	Head wounds	0.08	0.13	0.00	0.15	0.21	-0.02	0.33	-0.16
	Back wounds	0.16	0.26	-0.05	0.37	0.27	-0.56	-0.43	-0.36
	Vent wounds	-0.48	-0.36	-0.43	-0.25	-0.38	0.04	-0.09	0.01
	Small	0.58	0.26	-0.22	0.24	0.50	-0.76*	- 0.91***	-0.58
	Featherless	-0.30	-0.38	-0.01	-0.41	-0.52	0.51	0.22	0.34
	Dirty	0.13	0.02	-0.38	0.07	0.37	-0.12	-0.38	0.18
	Sick	0.55	0.61	0.36	0.62	0.73	-0.41	-0.18	-0.33
	Terminal	0.31	0.14	0.01	0.35	0.35	-0.57	-0.71*	-0.28
	Dead	0.43	0.18	0.09	0.35	0.37	-0.60	-0.7*	-0.32
Transect walks	Immobile	0.36	0.84**	0.71**	0.98***	0.68*	-0.60	-0.23	-0.56
	Lame	0.42	0.84**	0.63*	0.95***	0.71**	-0.67*	-0.32	-0.65*
	Aggression t. mate	0.04	-0.24	0.13	-0.16	-0.21	0.13	-0.08	0.42
	Human int.	-0.52	-0.48	-0.26	-0.56	-0.59	0.70	0.73*	0.62
	Mounting	-0.52	-0.51	0.00	-0.40	-0.69*	0.55	0.33	0.44
	Head wounds	-0.13	0.20	0.15	0.38	0.16	-0.13	0.32	-0.25
	Back wounds	0.16	0.66*	0.86**	0.81**	0.35	-0.33	-0.17	-0.35
	Vent wounds	-0.31	-0.23	-0.11	-0.06	-0.34	-0.16	-0.19	-0.26
	Small	0.30	0.15	-0.12	0.24	0.20	-0.78**	-0.71*	-0.71*
	Featherless	-0.30	-0.09	0.40	-0.08	-0.46	0.37	0.40	0.15
	Dirty	0.35	0.24	0.23	0.26	0.40	-0.11	-0.44	0.15
	Sick	0.56	0.75*	0.33	0.66*	0.77**	-0.55	-0.11	-0.62
	Terminal	0.09	0.10	0.21	-0.02	0.10	0.32	0.31	0.43
	Dead	0.52	0.19	-0.06	0.24	0.47	-0.47	-0.39	-0.35
Sampling	Immobile	-0.17	0.52	0.26	0.61	0.26	-0.26	-0.04	-0.26
	Lame	0.43	0.89***	0.63	0.9***	0.77**	-0.54	-0.12	-0.54
	Human int.	-0.52	-0.09	-0.13	-0.09	-0.17	0.52	0.7*	0.44
	Head wounds	-0.25	0.05	0.04	0.20	-0.08	-0.11	0.33	-0.30
	Back wounds	0.01	0.49	0.26	0.58	0.38	-0.39	-0.07	-0.44
	Vent wounds	0.04	0.42	0.65*	0.49	0.19	-0.04	-0.19	-0.04
	Small	0.16	0.12	-0.13	0.03	0.06	-0.48	-0.49	-0.49
	Dirty	-0.47	-0.06	-0.34	0.02	-0.04	0.08	0.03	0.16
	Sick	-0.12	0.16	0.27	0.30	0.03	0.02	-0.04	-0.02
Slaughter information	Livability								
	Condemned	0.6							
	DOA	0.2	0.6						
	Whole	0.4	0.89***	0.73**					
	Parts	0.79**	0.88***	0.3	0.72*				
	Av GW	-0.65*	-0.6	-0.1	-0.6	-0.64*			
	Age	-0.6	-0.3	0.2	-0.2	-0.5	0.7*		
	Gain/day	-0.6	-0.65*	-0.2	-0.6	-0.6	0.92***	0.5	

	Welfare indicator	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
		Transect walks														Sampling							
Transect walks	Lame (16)	0.98***																					
	Aggression towards mate (17)	-0.07	-0.15																				
	Human int. (18)	-0.59	-0.62	0.16																			
	Mounting (19)	-0.46	-0.40	0.09	0.46																		
	Head wounds (20)	0.31	0.31	-0.51	-0.05	-0.07																	
	Back wounds (21)	0.78**	0.72	0.13	-0.45	-0.03	0.03																
	Vent wounds (22)	0.00	0.09	-0.34	-0.40	0.26	-0.11	0.11															
	Small (23)	0.25	0.35	-0.16	-0.68*	-0.11	0.16	0.21	0.49														
	Featherless (24)	-0.13	-0.09	0.02	0.40	0.82**	-0.15	0.31	0.25	-0.20													
	Dirty (25)	0.22	0.12	0.53	-0.36	-0.32	-0.18	0.33	-0.48	0.07	-0.42												
	Sick (26)	0.59	0.59	-0.40	-0.32	-0.55	0.67*	0.24	-0.43	0.21	-0.36	0.16											
	Terminal (27)	0.05	-0.05	0.54	0.41	-0.28	-0.43	-0.01	-0.60	-0.77**	-0.07	0.23	-0.12										
	Dead (28)	0.24	0.33	-0.02	-0.39	-0.20	0.49	-0.14	-0.22	0.44	-0.44	0.21	0.55	-0.31									
Sampling	Immobile (29)	0.52	0.52	-0.25	0.00	0.05	0.35	0.52	-0.04	0.17	0.18	0.00	0.35	-0.17	-0.09								
	Lame (30)	0.93***	0.88**	-0.15	-0.45	-0.66*	0.26	0.62	-0.18	0.03	-0.25	0.18	0.67*	0.27	0.13	0.44							
	Human int. (31)	-0.17	-0.26	-0.25	0.7*	0.05	0.44	-0.26	-0.48	-0.61	0.00	-0.17	0.17	0.26	-0.26	0.38	0.00						
	Head wounds(32)	0.10	0.12	-0.59	0.05	0.16	0.90	0.02	0.09	0.30	0.09	-0.34	0.51	-0.65*	0.27	0.39	0.02	0.39					
	Back wounds (33)	0.64*	0.60	-0.39	-0.51	-0.62	0.12	0.39	0.38	0.12	-0.31	-0.13	0.25	0.03	-0.23	0.25	0.72*	-0.05	0.00				
	Vent wounds (34)	0.49	0.42	0.16	-0.42	-0.04	-0.42	0.8**	0.27	-0.04	0.27	0.34	-0.19	0.19	-0.49	0.22	0.42	-0.33	-0.42	0.47			
	Small (35)	-0.01	-0.03	-0.17	-0.41	-0.23	-0.18	0.22	0.32	0.63	-0.13	0.18	0.05	-0.46	-0.24	0.13	-0.03	-0.31	0.11	0.21	0.24		
	Dirty (36)	0.02	-0.08	-0.25	-0.02	-0.39	0.01	-0.08	0.13	-0.08	-0.46	0.07	-0.10	0.04	-0.45	0.31	0.13	0.43	0.00	0.56	0.16	0.31	
	Sick (37)	0.12	0.12	-0.13	-0.05	0.44	0.48	0.39	-0.12	0.28	0.29	0.30	0.28	-0.52	0.22	0.56	-0.09	0.19	0.59	-0.36	0.04	0.16	-0.13

In contrast, data obtained using the TW differed from L data in only three categories: sick, immobile and dead. The numerical differences between TW and L were however relatively small, below 1% for all 3 differing indicators. The lack of agreement between TW and L versus S method may be explained by potential constraints of the sample of birds sampled using the latter method. A sample size of approximately 100 individuals is typically suggested in available welfare assessment protocols for poultry (WQ, 2009; De Jong et al., 2012). Therefore we sampled 104 turkeys using the S method. However, sampling only 104 turkeys from a flock of approximately 4,500 may have led to high estimates for some of the indicators, as identification of a welfare indicator in just one bird is equivalent to a 0.96% increase in the incidence of that indicator at the flock level, and to low estimates of other indicators, as only 2.3% of the entire flock is evaluated. The sampling scheme may have further accounted for the underestimation of the incidence of immobile and dead individuals. Individuals in these categories were typically laying on the ground, obstructed from view by standing turkeys, and were therefore unlikely to be randomly selected for sampling using the laser pointer method employed in this study. These limitations in the sample obtained using the S versus TW method, in combination with the relatively large sizes of turkeys versus broilers, may explain why the differences found between TW and S for turkeys were smaller than ones reported for broilers (Marchewka et al., 2013a).

The proportions of sick, immobile and dead turkeys were highest when evaluated during L than using either of the other methods. This likely reflects the increased visibility of these birds during L, as sick, lame and immobile individuals were likely to be left behind after the load out process was completed, and could easily be counted by the observers. These birds would have likely been sitting on the ground, obstructed from view by surrounding turkeys during S and T assessments. Additional factors that may have contributed to the increased number of observed immobile and dead birds include bird fatigue and flock disruption that occurs during the load out process, as well as differences in the timing of the two evaluations as L was carried out up to 48h later than TW (Buchwalder & Huber-Eicher, 2003; Marchewka et al., 2013b). The difference in the incidence of sick birds could have been attributed to the different views from which the birds were scored. During TW birds are typically evaluated from behind as they moved away from the observers, while during L the turkeys were scored as they moved towards or stood next to the observers. As the most frequent reason for assigning a turkey to the sick category was the pendulous crop (personal observation; earlier: Rigdon et al., 1960), it is possible that this condition was underestimated when the turkeys were observed from behind.

Additional support for the accuracy of the TW method can be obtained from the correlation analysis. Overall, a high number of strong correlations were detected with regards to the incidence of indicators scored by TW and L. The number of birds having problems in terms of immobility, lameness and head and vent wounds assessed using the two methods had particularly high ($r>0.85$) degrees of agreement. Conversely, the incidence of lameness was the only category that evaluated using S that correlated with evaluations made using TW and L. The lack of correlation found between numbers of immobility turkeys using S further supports that this method may not be suitable for detecting this welfare issue.

Data collected using the on-farm assessments correlated with important production parameters collected at the slaughter facility. Bird condemnations (whole or part) correlated positively with mobility assessments made using all three on-farm assessment methods. Mobility assessments made using TW additionally correlated with birds found DOA. Back wounds evaluated using TW, but not S or L, were highly and positively correlated with the proportion of birds DOA, and proportion of birds condemned whole. This could partially be related with the angle from which the birds were evaluated. TW and S were conducted from behind the birds, while the birds were evaluated front and side during L. The differences between S and TW could be explained by differences in sample size, as described above. The angle of assessment is a less obvious explanation for why the proportion of condemned (total, whole and parts) birds was correlated with the frequency of sick birds as evaluated using the TW method only. The majority of birds deemed “sick” had a pendulous crop, which one would assume would have been easier to observe from the front of the birds, as was the case during L.

Several expected correlations were noted. For example, flocks affected by lameness may be expected to be less active, which is likely the reason behind the negative correlation between the recorded amount of interactions with humans and proportion of birds affected by lameness. Similarly, as expected, high frequencies of mounting observed during TW were correlated with the proportion of featherless birds in the flock assessed using L ($r=0.9$) but also TW ($r=0.74$).

From the perspective of the practicality of on-farm application, not surprisingly, the L data collection method proved to be the most time consuming. The time requirements of this method were limited by the pace of the loading crews, the number of birds in the flock at the time of L, as well as their condition. The time necessary for conducting TW (34.0 ± 2.2 min) was similar to that needed for the S (33.5 ± 1.7 min), highlighting the efficiency of the TW method which allowed for the evaluation of the entire flock versus 104 randomly selected individuals.

No differences in welfare measures were detected between houses assigned by the company to the two management categories. This study focused on flocks raised in one geographical area, during one season within the same year, and on farms belonging to the same company and therefore using very similar husbandry protocols, and house lay outs. This overall similarity may be one reason why we were not able to detect differences in the management levels assigned by the company using any of the turkey welfare assessment methods. This result may also be due to differences in the criteria used for identifying "well managed" farms (Botreau et al., 2007; Duncan et al., 2012). Such issues as mobility problems, dirtiness or aggression towards humans cannot be directly detected after finishing the production cycle and obtained from the performance reports. In the case of this study, where turkey carcasses were used for parts as a final product, it can be especially difficult to deduce from slaughterplant data the welfare of the birds on farm (St-Hilaire et al., 2003). The company's impression of the farms was additionally based on long term observations of their performance. We have obtained data from one flock only, getting a snap shot of the current on-farm situation. It is likely that data collected from multiple flocks from each farm would have better aligned with the producer's perception, underlining the importance of developing methodology that allows for this type of systematic data to be collected in a simple manner.

Overall, evidence collected during this study indicates that the transect-based on-farm welfare assessment method for meat poultry could be a feasible method for assessing the welfare of turkeys, including large toms. The method can be a time-efficient and practical tool for turkey companies and farmers to evaluate the welfare status of the flocks. The results of such evaluations can be linked to historical and management data as well as economically important outputs. Information collected over several flocks could be used to control and predict arising welfare issues giving producers the ability to develop and implement preventative strategies. In light of the increasingly high demand among consumers for guaranteed standards in animal welfare (Barbut, 2010), the TW could also serve as an animal-bases assessment during internal or external welfare evaluations or audits.

4.5 Conclusion

The data supports that the TW method is a reliable tool for on-farm assessment of turkey welfare. This method is practical, efficient, and easy to implement under field conditions as it resembles the techniques typically used by farmers to check on their flocks, and requires minimal training to produce reliable data when used by different observers. Importantly, for 10 of the 13 welfare indicator categories sampled during L, when all birds in the flock were individually assessed, the TW method yielded similar results. The differences that were identified, the percentage of immobile, dead and sick turkeys, may equally well have been due to the increased visibility of affected birds during the L procedure, or the impact of the L procedure on these welfare indicators.

The non-handling S method, which was used to evaluate a random sample of 104 birds per flock, either over- or under- estimated the prevalence of many of the welfare indicators (8 out of 13) as competed to the results obtained during L. The utility of the S method seems to be constrained by the sample size and strategy. Although it is possible that the utility of this method could be improved by increasing the size of the sample, given that the time required evaluating the 104 turkeys using the S methods is similar to that required for the entire house to be assessed using the TW method, increasing the sample size may not be practical.

Chapter 5: *Behavior of tail docked lambs as pain indicator**

**Marchewka, J., I., Beltrán de Heredia, R., Ruiz, X., Averos and I., Estévez. 2015. Behavior and vertical activity of tail docked lambs as pain indicators. Livestock Science; Paper submitted.*

5.1 Introduction

Some of the documented behavioral changes occurring within the first hours after the application of a potentially painful procedure such as tail docking in lambs (Ovis aries) include agitation, bleating, lateral and ventral recumbency, lip curling, kneeling, knee walking, writhing and other abnormal postures (i. e. Lomax et al., 2010; Molony et al., 2002; Mellor and Murray, 1989). Most of these studies observed the treated individuals in, or soon after returning to their social environment.

Expression of pain-modulating mechanisms is highly variable, where some stimuli can cause inhibition while others can cause facilitation of nociception and pain (Fields & Basbaum 1999). Pain (Hild et al. 2010), as other stress responses in sheep can be buffered by the social environment such as presence of group mates (Nicol, 1995, Gonzalez et al., 2013). On the contrary, isolation of animals living in groups under natural or optimal welfare conditions deprives them companionship providing protection from dangers. This results in intensified stress responses of separated individuals, necessary to solitary overcome environmental threats (Kikusui, et al., 2006).

Sheep, as a prey species, have evolved to hide any signs of pain that can make them more vulnerable to predation. Predator avoidance is such a priority, that it might, at least temporarily, displace awareness of pain (Rutherford et al., 2002). The phenomenon of pain suppression upon exposure to stressful stimuli is commonly known as stress-induced analgesia (SIA) (Butler and Finn, 2009), and occurs as an adaptive mechanism where situations are perceived to be intensely stressful and dangerous by the animal. Once the organism is no longer in danger, elevated nociception, expressed upon extinction of the aversive response, could be beneficial, as otherwise carrying a normal activity may aggravate the injury. SIA and following it elevated nociception could occur in isolated lambs experiencing pain, while not in ones that only experience stress of separation from flock mates. On the other hand hyperalgesia, an exaggerated response to noxious stimuli (Nolan, 2000), takes place through the release of catecholamine and causes sensitization of nociceptors. It can be observed in sheep after the application of a psychological stressor (Chapman & Turner 1986). Submitting lambs to isolation stressor could provoke elevated and more obvious pain reactions then within a social group. However, no information about the direction of those reactions is currently available.

Anesthesia and analgesia have been proven to decrease pain behaviors and postures in farm animals, as reactions to pain caused by tail docking (reviewed by Sutherland and Thucker, 2011). Local anesthesia is defined as any technique to induce the absence of sensation in part of the body in response to a painful stimulation. Drugs that produce anesthesia may or may not provide analgesia (Fish et al., 2008; ILAR, 2009). Analgesia causes the absence of pain in response to stimulation that would normally be painful. An analgesic drug can provide analgesia by acting at the level of the central nervous system or at the site of inflammation to diminish or block pain signals (Fish et al., 2008; ILAR, 2009). Therefore, those two measures give a possibility to grade the applied pain levels by being applied separately or together. The effects of applying various pain levels, modulated by anesthesia and analgesia, on lamb reactions under isolation are also currently unknown.

Based on model suggested by Henry, (1992) the level of control over stressful stimulus differs between animals according to experienced pain levels. Isolation may allow investigating pain levels based on, practical and feasible in on-farm conditions, behavioral indicators. Locomotion and fear behavior responses collected in the arena tests were found replicable across time and thus true measures of behavior in an individual sheep (Dodd et al., 2012). Vertical movements, related to climbing on the pen wall, need more attention and understanding, as they may serve as potential valuable behavioral indicators. They have been reported previously for rats, as an important behavioral indicator during open field test (Canini et al., 2009). Being able to identify reliable behavioral pain indicators under field conditions could have important implications for the design of future pain exploring studies, as well as for the everyday on farm practices (Morris, 1994). This is also of a high importance due to current attention of governments and consumers to improvement of farm animal welfare by fulfilling set standards of rearing animals free from fear and pain (FAWC, 2009).

The aim of current study was to identify behavioral signs that could potentially be used as pain indicators under field conditions in sheep, by analyzing the responses to tail docking in lambs in which the procedure was conducted with or without pain control measures. We stated the hypothesis that lambs which underwent tail docking will differ in activity and behavioral response from sham-tail docked lambs. We expected to find differences between treatments groups during first part of the study, when the pain levels were expected the highest, at least for tail docked group. We anticipated it especially that we deprived the lambs of social buffering effect of the group during test, exposing them in this way to potentially higher stress levels.

5.2 Materials and methods

5.2.1 Animals and facilities

The present study was carried out at the experimental dairy sheep farm of Neiker-Tecnalia (Arkaute, Spain) and complied with the requirements of the European

Directive 86/609/ECC regarding the protection of animals used for experimental and other scientific purposes. Twenty four Latxa female lambs (*Ovis aries*), born between 23rd and 30th of January 2012, were used. Lambs were individually recognized with numbered plastic ear tags. Three days prior to the start of the experiment, the lambs were removed from a single flock and randomly assigned to four pens. The holding pens were located in a barn with solid walls and windows allowing natural lighting. The pens were constructed with grey PVC panels, and were provided straw bedding, a feeder and a drinker each. Fresh straw was added periodically as required to maintain the good bedding conditions. Feeding was based on *ad libitum* access to hay and concentrate. Prior to the start of the experiment lambs were weighted (body weight ranged between 12.20 and 17.90 kg). The treatments were applied when the animals were 47±2 days old.

5.2.2 Experimental treatments

All 24 lambs were randomly divided in groups of six, but balancing groups according to body weight. Each group was allocated to 1 of 4 pens, to which 1 of the following treatments was assigned: tail docking (TD) with rubber ring without pain control, TD with anesthesia (TDA), TD with anesthesia and analgesia (TDAA) and control (C) equally handled but without TD or pain relieve application. Treatments were applied on day 0 under veterinarian supervision.

The TD treatment was performed with an elastrator to stretch the elastic band around the tail. For the TDA and TDAA treatments the compounds, dose and administration site are presented in Table 11. Bupivacaine hydrochloride, known to have rapid and long lasting (up to more than 5 h) anesthetics effects (Babst and Gilling, 1978) was used as local anesthetics. This compound was combined with epinephrine to improve the action of epidural administered local anesthetics having its peak 5 min after administration (Ratajczak-Enselme et al., 2007). In the TDAA treatment, Flunixin meglumine was used in combination with the above described anesthetic agents, as it is a potent non-steroidal anti-inflammatory drug, whose analgesic efficacy has been confirmed in both laboratory animals and clinically in domestic species. This anesthetic agent peaks 12 to 16 h post administration, and the effects last for 24-36 h (Ciofalo et al., 1975, 1977; Welsh and Nolan, 1995).

5.2.3 Experimental procedures

The experimental procedures were carried out for 9 days (-2, -1, 0, 1, 2, 3, 4, 5 and 6). Blood samples for cortisol analyses were collected from 3 randomly chosen lambs in alternative days starting on day -2 while in their home pens. Behavioral testing of all individuals took place in uneven days, starting on day -1 to avoid interference of the blood sampling procedure.

The days of behavioral testing, each group was chosen in random order and directed to a nearby waiting pen (measuring 1 x 2 m). Immediately after arrival, 1 of the lambs was taken at random from the waiting area and placed in an adjacent open field testing arena (measuring 2 x 3 m). Remaining individuals waited in the holding pen. The testing arena was built with opaque, 1.5 m high walls and was provided with straw litter. No water or feed was provided in the testing arena. After testing (described below) the lamb was redirected to the holding pen and a new individual was placed in the testing arena. The testing procedure was repeated until all lambs from each particular treatment were tested. The testing order of all groups was random and varied for each day of behavioral observations. The testing procedure was the same for all day of data collection, except for day 0, for which prior to testing each lamb underwent the treatment procedure. The procedure took no more than 2 min per individual.

Behavioral observations were done by the same person on all the testing days and consisted of 2.5 min direct sampling observations, where frequency of all occurring behaviors was noted. Data were collected using the software Chickitizer (Sanchez and Estevez, 1998), which allows collecting the behavior and the location of the individual of interest in XY coordinates.

Spatial data will be processed and prepared in a separated manuscript. The sequences were also video recorded (Panasonic HDC-HS80, Osaka, Japan), initially as a precaution.

The direct observation ethogram used for sampling included: run, stand, walk, explore, rest, and urinate (Dwyer, 2004). Due to unexpected behaviors occurring during tests (i.e. exploratory (EC) and abrupt (AC) climbs on the walls, defined as a climb on the pen walls, requiring at least both front legs to be off the ground), and to the fact that both types of climbs occurred very fast and frequently, the video recordings were used to assess their frequency during the 2.5 min observations.

Therefore, we distinguished these 2 types of climbs due to observed differences in duration and their type, which are confirmed in the literature (Dodd et al., 2012). EC were defined as climbs that lasted longer than 2 s and AC as climbs that lasted up to 2 s. In EC, lambs were upright on their back legs, with the front legs placed on the pen walls and their head over the fence. AC consisted of a vigorous run ending with a jump on the pen wall in which the individual attempted to hook with the legs to the top of the wall.

Table 11. *Protocol of the treatments applied to 4 groups of lambs.*

Treatment	Code	Nr of pens (animals)	Protocol	
Control	C	1 (6)	Catching the animal	-
Anesthesia	TDA	1 (6)	Catching the animal and placing the rubber ring on the tail Bupivacaine hydrochloride (0.50%) and 1:200,000 epinephrine bitartrate. 1.5 ml per animal via subcutaneous, precisely in the region proximal to the placement of the rubber ring, applied 0.5 ml and 0.5 ml ridges on each side	Treatment applied 2 min before placing the rubber ring on the tail
Anesthesia + analgesia	TDAA	1 (6)	Catching the animal and placing the rubber ring on the tail Anesthesia protocol was the same as for TDA group Additional application of 2.5 ml meglubine flunexin (intramuscular)	Treatment applied 2 min before placing the rubber ring on the tail
Only tail docking	TD	1 (6)	Catching the animal and placing the rubber ring on the tail	-

During video observations we also looked for behavioral changes observed within the first hour after tail docking in, or soon after returning to their social environment: agitation, bleating, lateral and ventral recumbency, lip curling, kneeling, knee walking, writhing and other abnormal postures (i.e. Lomax et al., 2010; Molony et al., 2002; Mellor and Murray, 1989). However, none of them was observed. Blood collection took place on even days between 10 and 12 am. Samples of 10 ml of blood were collected from 3 random lambs per group. Two persons conducted the procedure: one held the animal while an experienced veterinarian collected the blood sample from the jugular vein, using a 0.9 x 25 mm needle, into non-coated BD Vacutainer® (UK) tubes.

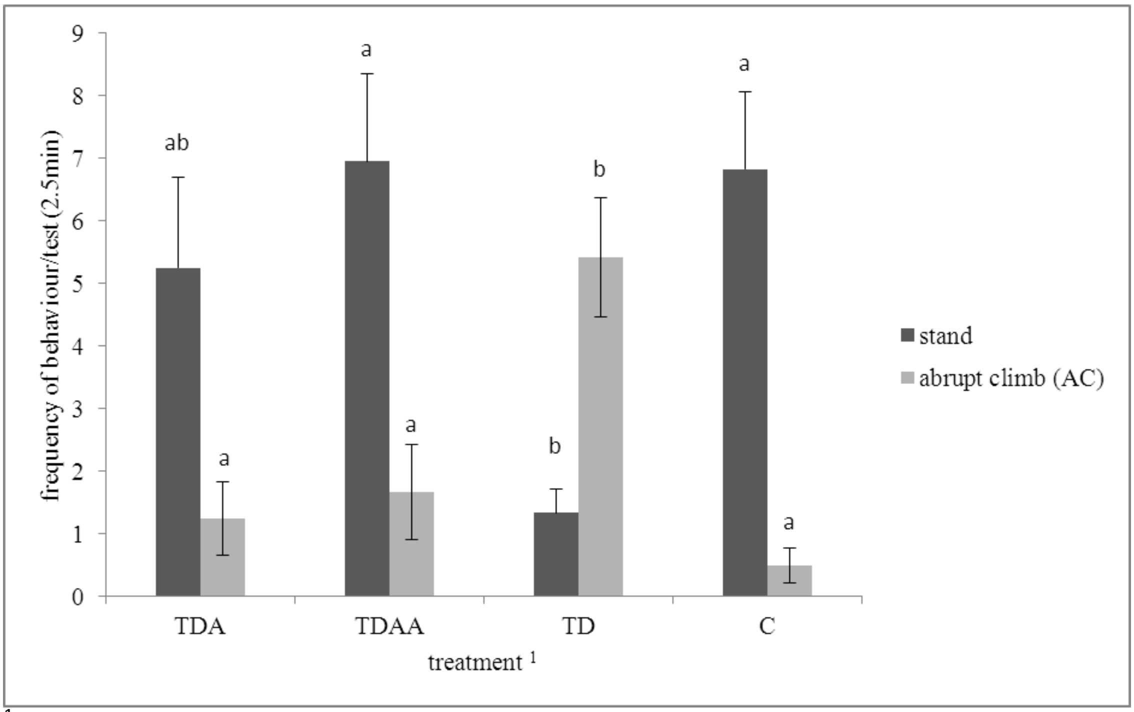
Samples were placed on ice immediately after collection, and later transported to the laboratory where they were allowed to clot at room temperature. Blood samples were then centrifuged for 15 min, at 350 *g* and 4°C. The serum fraction was extracted and stored at -20°C until processing. Cortisol was evaluated by radioimmunoanalysis using Coat-a-Count cortisol kits (PITKCO-9, 2010-10-21 - Siemens Healthcare Diagnostics LTD. Sir William Siemens Sq. Frimley, Camberley, UK GU16 8QD). A 0.5 mcg/dl – 50 mcg/dl calibration interval for serum cortisol was used, and the detection limits were 0.2 mcg/dl. Inter- and intra-assay variation coefficients were 6%. Samples were analyzed using 1470 Wizardtm, Wallac analyzer (Perkin Elmer Life Sciences, Massachusetts, USA).

5.2.4 Statistical analysis

From the focal observations the frequencies of the behaviors run, stand, walk, explore, rest and urinate, as well as frequencies of EC and AC were calculated per lamb for each testing period. The frequencies of a particular behavior for each individual were not standardized according to the total frequency of behaviors per test since the duration of tests was equal for all lambs. Variables were square root transformed prior to statistical analysis to meet the ANOVA requirements. A repeated measures model was built, including treatment and day as fixed factors, and lamb, nested within treatment, as a random factor. Values obtained on day -1 were included as covariates into the models. The PROC MIXED procedure was used with spatial power matrix accounting for uneven time distances between days, including day as repeated measure. The least square means for all significant effects ($P<0.05$) was computed using LSMEANS option. The same model was used to evaluate cortisol levels (ng/ml) from 3 lambs per treatment. All the analysis was performed in SAS (SAS Inst. Inc., Cary, NC).

5.3 Results

The results of the statistical analysis are presented in Table 2. We found a clear effect of treatment on the frequency of standing (Fig. 12), where C and TDAA lambs were standing more than TD lambs. In addition, TD lambs performed significantly higher AC as compared to all other treatments (Fig. 12). No other significant effects of treatment were detected. Resting and urination frequencies were close to zero and therefore disregarded.



[1] treatment: TDA: tail docking with anesthesia; TDAA: tail docking with anesthesia and analgesia; TD: tail docking; C: control. Tail docking in all treatments done with rubber ring

Figure 12. *Effect of day on the mean frequency ±SEM of standing and AC per 2.5 min test. Within each variable, different letters indicate significant LSM differences across treatments (P < 0.05).*

On the other hand, EC frequency was affected by the treatment by day interaction (Table 12, Fig. 13). Significant differences across treatments were only evident on day 5, with C lambs showing significantly lower frequencies of EC when compared to TD, TDA or TDAA.

Table 12. *Results of the ANOVA model for the effect of treatment (T), day (D) and interaction on the behavior and cortisol levels of lamb.*

Behavior	Analysis of variance components					
	T		D		T x D	
	F value	p	F value	p	F value	p
Explore	$F_{(3,19)}$=0.02	0.997	$F_{(3,60)}$=5.14	0.003	$F_{(9,60)}$=1.13	0.356
Run	$F_{(3,19)}$=0.73	0.554	$F_{(3,60)}$=9.21	<.0001	$F_{(9,60)}$=0.64	0.759
Stand	$F_{(3,19)}$=5.98	0.005	$F_{(3,60)}$=3.36	0.024	$F_{(9,60)}$=0.48	0.880
Walk	$F_{(3,19)}$=1.14	0.359	$F_{(3,60)}$=13.87	<.0001	$F_{(9,60)}$=1.32	0.2483
EC	$F_{(3,19)}$=0.94	0.440	$F_{(3,60)}$=9.66	<.0001	$F_{(9,60)}$=2.10	0.043
AC	$F_{(3,19)}$=3.37	0.040	$F_{(3,60)}$=0.63	0.599	$F_{(9,60)}$=0.62	0.775
Cortisol (ng/ml)	$F_{(3,31)}$=0.81	0.500	$F_{(3,31)}$=4.78	0.008	$F_{(9,319)}$=1.99	0.076

Interestingly, there were no differences in the frequency of EC across days for the C group, whereas a clear increment in this frequency was observed for all other groups, and especially for TD (Fig. 13). In addition, this interaction showed a trend for cortisol levels (Table 12). Most importantly, cortisol levels tended to differ between groups TD and C on days 0 and 6, being higher for TD lambs (3.93±0.73 and 2.15±0.37 for days 0 and 6, respectively) than for C (1.03±0.19 and 0.69±0.22 for days 0 and 6, respectively).

In addition, a clear day effect was detected for all indicators (exploring, running, standing, walking, EC), except for AC (Table 12). Independently of the treatment, lambs walked, run and stood more, and explored less on day 0 as compared to any other days (Table 13). We observed less EC on the walls on the first 2 days (0 and 1) than on days 3 and 5. Cortisol levels followed a similar patter with significantly higher values detected on day 0 as compared to all other days.

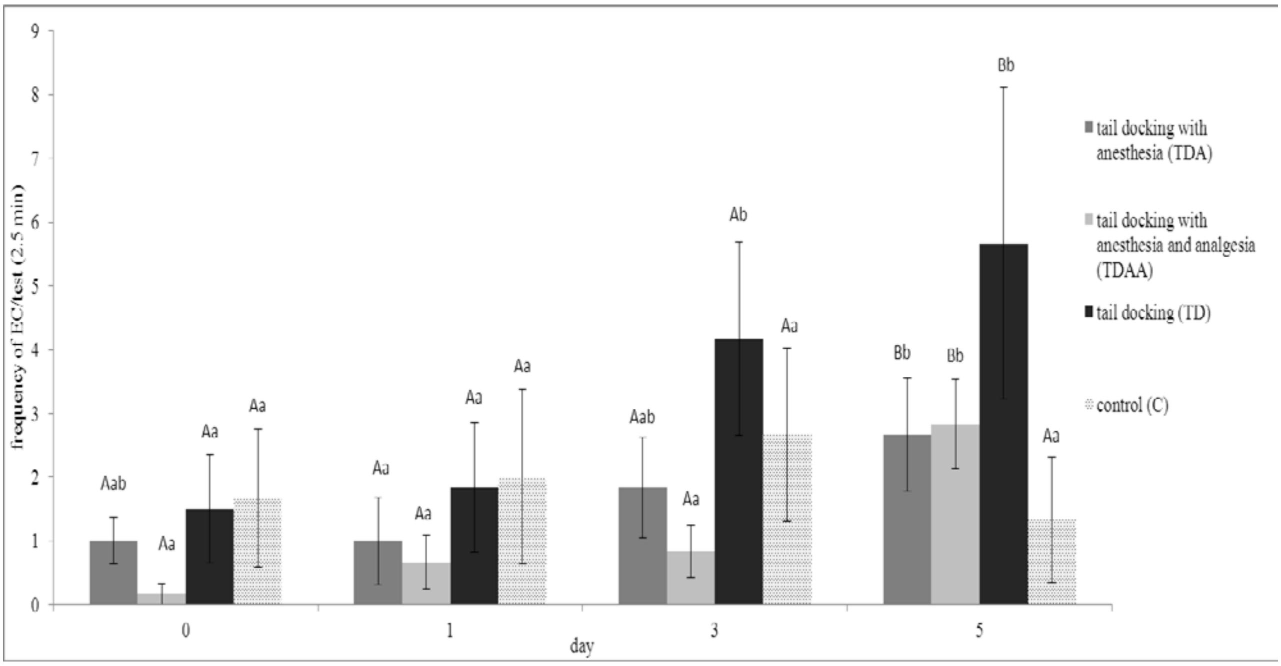


Figure 13. *Effect of treatment by day interaction on the mean frequency ±SEM of EC per 2.5 min test. Major letters indicate significant LSM differences across treatments (P < 0.05) on a particular day. Within each variable, small letters indicate significant LSM differences across days (P < 0.05) for each treatment.*

Table 13. *Changes in the studied indicators (mean values (±SEM)), along the experimental days.*

Behavior	0	1	3	5
Explore	3.67±0.81^{a}	8.71±1.23^{b}	7.38 ±1.14^{bc}	5.75±1.01^{c}
Run	11.88±2.12^{a}	4.46±1.09^{b}	4.54±1.02^{b}	3.71±1.16^{b}
Stand	7.17±1.24^{a}	4.33±1.37^{b}	3.96±1.10^{b}	4.92±1.32^{b}
walk	13.42±1.78^{a}	4.13±0.80^{b}	7.17±1.08^{c}	7.83±0.93^{c}
EC	1.08±0.36^{a}	1.38±0.45^{a}	2.38±0.58^{b}	3.13±0.75^{b}
AC	2.88±0.89	2.46±0.83	1.88±0.83	1.63±0.58
	0	2	4	6
Cortisol (ng/ml)	3.01±0.53^{a}	1.62±0.40^{b}	1.50±0.32^{b}	1.36±0.23^{b}

$^{a–c}$ Means within a row with different superscripts differ (P<0.05)

5.4 Discussion

This study aimed at identifying behavioral signs that could potentially be used as pain indicators in sheep under field conditions. The capacity to identify reliable behavioral pain indicators on-farm could have important practical implications for pain management in farm practices (Morris, 1994). This is also important due to current attention of governmental agencies and civil society organizations to the improvement of farm animals´ welfare by fulfilling set standards of rearing animals free from fear and pain (FAWC, 2009).

Our study was conducted on lambs tested under isolation, prior and after undergoing tail docking with or without pain control measures. We separated lambs from their flock mates in order to overcome the conditioning effects of social buffering.

We expected to find clear behavioral and activity differences across treatments, especially soon post-procedure when the differences in pain levels were expected to be highest. We found a clear treatment effect on standing behavior and AC frequencies. TD lambs stood least often, whereas AC frequency was the highest for this treatment. Although no treatment effects were detected for walking or running, differences in AC and standing observed in TD lambs suggest higher distress levels in these lambs over the study period. This was perhaps the result of the combined effects of pain caused by the application of the treatment and of isolation.

The low standing behavior frequency that may be indicative of restlessness, as well as high AC frequency related to escape attempts, may be part of a natural fight or flight reaction. These results would to some extent agree with previous studies in which high locomotor activity was considered as a sign of distress in the context of social isolation

(Boissy et al., 2005), whereas increased incidence of active behaviors were considered as an attempt to escape or remove the perceived pain (Graham et al., 1997).

In young sheep, the strong buffering effect of the social group, when exposed to a fear eliciting stimulus, was demonstrated by a lower incidence of fast movements and escape attempts, as opposed to the behavior of isolated ones characterized by higher values of fast movements and escape attempts (González et al., 2013). TD lambs in our study showed similar reactions in regard to the AC response. However, high frequencies of running and walking, and a low frequency of exploration were generally observed on the day of treatment application in all groups regardless of the treatment. González et al. (2013) suggested that the stress reactions due to social isolation were substantially stronger than those elicited by the presence of a fear inducing stimulus. Similarly, initially high activity levels observed in this study would suggest that lambs were reacting more to the distress caused by isolation rather than to the potential pain caused by the treatments. However, as the tests were subsequently repeated, a clear reduction in the behavioral activity was observed during the experiment, suggesting that lambs somehow may have become habituated to the isolation testing conditions, and an extinction mechanism of the response may have occurred (Erhard et al., 2006).

Despite the strong interfering effects that isolation may have had over the lambs behavioral response, the much lower frequency in standing and clearly higher AC response observed in TD lambs as compared to all other treatments suggest that the reaction of TD lambs was different. AC consisted of a vigorous run, ending with a jump from a distance onto the pen wall, in which the individual attempted to hook with the legs to the top of the wall. This behavior was considered as a clear attempt to escape. Therefore, it is possible that TD lambs experienced a SIA mechanism, resulting from isolation and occurring only in tail docked lambs without pain control measures. SIA cascade would have allowed the lambs to remain active and mobile, in order to search for possibilities to escape from a danger (or pain) providing them with potential higher survival chances.

Interestingly, none of the studied behavioral indicators, except for EC, was affected by the treatment by day interaction. From the application of the treatment to day 3, when pain severity should be expected to be highest, there were no differences among TD, TDA and TDAA as compared to C lambs. Differences were detected only on day 5, in which C lambs showed the lowest EC frequency as compared with all TD treatments. For those groups, the frequency of EC steadily increased from day 0 to 5 (Fig. 2). As stated previously, it is possible that in our study, isolated lambs might have experienced a SIA-like syndrome, as social isolation is considered very distressing for sheep (Boissy et al., 2005). It may be possible that the combination of social isolation stress and pain originating from the procedure was sufficient to induce some form of natural analgesia during the first three days of testing, although not sufficient to totally overcome the reactions detected in TD lambs.

In tail docked, group housed lambs, previous studies showed behavioral or postural abnormalities within the first 3 h post treatment application (Archer et al., 2004; Graham et al., 1997; Grant, 2004; Kent et al., 1998; McCracken et al., 2010; Molony et al., 2002). The increase in the incidence of active behaviors and time spent in abnormal postures were described as reliable indicators of the severity of acute pain resulting from rubber ring castration and tail docking (Molony et al., 2002; McCracken et al., 2010), while the reduction observed after 3 h post-procedure was interpreted as a reduction in acute pain (Graham et al., 1997). There is, however, evidence of long lasting neuroma formation in tail docked lambs (Fisher and Gregory, 2007; French and Morgan, 1992), which is often associated with pain due to abnormal nerve discharges

(Breward and Gentle, 1985). Pathological changes including reddening and swelling of the skin immediately proximal to the ring around the tail docking location have been observed 4 wks post treatment application (Lomax et al., 2010). Long-term post amputation pain due to tail docking has also been proved in dogs (Gross and Carr, 1990). In the current study, however, the lack of differences between treatments, except for EC frequency, prior to day 5 do not allow us to make assumptions about the incidence and severity of pain that tail docked lambs may have experienced.

It is difficult to explain the progressive increase in EC frequency over time to yield significant differences for TD treatments 5 days after treatment application. An increase in vertical activity, similar to EC, has been observed in rats injected with metyrapone, a chemical agent used to provoke stress-like biological syndrome causing SIA (Canini et al., 2009). Metyrapone induced a rapid and abrupt decrease in the open field exploration while provoking a higher frequency of low speed movements (Canini et al., 2009). A SIA effect on rat behavior was observed immediately post the stressor application (Canini et al., 2009) up to 21 days (Pinto-Ribeiro et al., 2004). When the acute pain transmutes into chronic, the effects of SIA might be reversed and therefore reactions to pain may increase (Rivat et al., 2007). A similar process might have occurred in the TD lambs in our study, as probably the ring presence could still be perceived but pain was no longer acute due to the appearance of ischemic necrosis (Lomax et al., 2010). We suspect that in our study SIA effect might decrease prior to day 5 causing exaggerated reactions of tail docked lambs afterwards. We were not able to clearly determine whether EC were attempts to establish social contact with flock mates, or they were related to exploration of the environment. However, distinguishing vertical from horizontal movements of isolated individuals has been previously reported to provide important information regarding their state (Canini et al., 2009). Therefore, further attention should be paid to EC frequency as it was the only indicator which we found to be affected by the treatments over time.

We did not find a significant interaction of treatment by the day on cortisol levels, but there was a trend. We found higher cortisol levels for TD lambs as compared to C lambs, on days 0 and 6. Previous research has shown that plasma cortisol concentration was significantly higher in the lame than in healthy sheep, remaining up for up to three months after the apparent resolution of the clinical lesion (Ley et al., 1994). Therefore it is possible to suggest that after deactivation of SIA mechanism pain perception may have increased with the consequent increment in cortisol levels.

Pain perception is known to be heavily influenced by the context (Ford and Finn, 2008). SIA has been seldom reported in relation to potentially painful husbandry procedures. Mulesing was reported as potential trigger for the release of β-endorphin, a potent analgesic lasting for up to 60 min (Shutt et al., 1987). Physical (continuous pinching of the skin for 60 min) and isolation stress applied to sheep caused excretion of serum α-endorphin, endogenous opioid peptides providing benefits in terms of analgesia and well-being (Hashizume, et al., 1994). However most of the studies focused on the molecular and neural mechanisms of SIA, paying less attention to its effects on the behavior, which will be most practical to assess pain triggering situations under on-farm conditions.

Under isolation conditions, we did not observe any of the pain indicators commonly reported for group housed, tail-docked lambs such as rolling, foot stamping, kicking and easing quarters (Molony and Kent 1997; Kent et al., 1998). The only exception was restlessness, on any of the days including day 0. This would suggest that lambs simultaneously subjected to stress and pain conditions may have diverse coping mechanisms, depending on the social environment in which they are.

The results we obtained are relevant to ongoing discussions about welfare and pain assessment, and provide new perspectives on the behavioral indicators requiring further exploration. If the SIA mechanism is affecting the tail docked lambs behavior when isolated, it is possible that similar reaction can take place with regard to other pain sources combined with various stressing factors. If SIA mechanism is activated in lambs to hide their real condition, evaluation of their pain becomes even more challenging than previously assumed (Kavaliers and Colwell, 1991), especially taking into account that the human (observer) may be perceived as a potential predator, what might be sufficient to induce SIA (Caine, 1992). The trigger of the SIA mechanism has been described as indicator of reduced welfare in itself (Fraser and Broom, 1997), due to persisting pain background as well as costly maintenance of SIA state.

In practice, when evaluating the condition of animals under suspected pain, usually separated from the flock for veterinary examination, the eventual onset of SIA should be predicted. On the other hand it needs further exploration, if farm animals would be more used to, and not fearful towards stockpersons, then would it be possible to avoid the SIA in order to perceive the real health status of the animal? The delayed and elevated nociception occurring post SIA should also be accounted for when scoring pain in animals, since what is considered as an extreme pain response can be a delayed effect of earlier acute pain. Finally, the reason for the increase in EC frequency on day 5 is unclear. For the moment we question whether the observed reactions are related to a potential transition from acute to chronic or no pain, or they should rather be explained by habituation to the testing procedure.

5.5 Conclusions

The hypothesis about differences in behavior and activity between tail docked with or without pain control measures and control lambs was partially confirmed in the present study, since there were differences in AC and standing between TD and C. Pain reactions were possibly covered by the onset of SIA mechanism resulting from the combination of pain and fear from isolation, especially during the initial stage of the study. Differences in the frequency of EC between treatments, being highest for TD, were only found on day 5. This might suggest the involvement of the SIA mechanism up to 5 days post treatment application. Testing lambs under isolation has a marked effect on their pain responses and prevents from detecting pain indicators from their behavior. Those findings might have important implications for the design of further pain exploring studies, as well as for everyday on-farm practices.

Chapter 6: *General discussion*

6.1 General discussion

The aim of the thesis entitled "*Development of practical methodology and indicators for on-farm animal welfare assessment*" was to develop a practical and scientifically based on-farm welfare assessment protocol for turkeys and to determine potential pain indicators in lambs. While reaching these goals, the commonly accepted definition of animal welfare proposed by the OIE (World Organisation for Animal Health, OIE, 2008) "*animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behavior, and if it is not suffering from unpleasant states such as pain, fear and distress*" was followed.

The development of the on-farm welfare assessment protocol for turkeys was divided into three phases. Phase one included testing the welfare assessment prototype in commercial broiler flocks, in order to determine if the concept had potential to be further developed. The choice of testing it in broilers obeyed to a number of reasons. Most welfare indicators for meat poultry have been focused on this species, which significantly facilitated testing the new assessment method. Additionally, testing the prototype on broilers allowed us to avoid potential constraints of working with large size turkeys, which are difficult to handle constraining the individual sampling procedure, required in order to compare methodologies. Broiler farms were further much more accessible in Northern Spain as compared to the turkey flocks. This factor was crucial to test the potential of the method in a timely manner.

Phase two required the adjustment of the initial prototype to the particularities of turkey behavior and welfare issues. Therefore, a thorough literature review was conducted on the behavior and welfare of turkeys. After adjusting the proposed approach to turkey specific characteristics, we tested the prototype in ten commercial turkey flocks located in Indiana, U.S.A. This work was possible through the collaboration with Dr. Makagon, at the University of Perdue. The study was conducted during two ages towards the end of the production period (16 and 19 wks.). Given the potential issues derived from bird catching observed in the initial broiler study, for turkeys, we choose to compare the outcomes of three assessment methods, neither of which required bird catching: the transect method, individual sampling (modified from Dawkins, 2004), which were compare with individual assessment of all birds collected at load out and was considered the 'golden standard'.

The second part of the thesis aimed at developing pain indicators for sheep at the animal level. Sheep, as other prey species, developed throughout the evolution process, effective mechanisms to avoid showing evident signs of pain, causing pain indicators not evident. Search for potential behavioral pain indicators in lambs was experimentally conducted in a novel way, as the individuals were tested under isolation and over a period of six days after treatment application.

The results of the above studies were discussed in detail in each corresponding chapter. Further general considerations on the welfare assessment methods (transect walk method) and indicators (pain indicators) are addressed below. Additionally, the potential relevance of the results obtained from the perspective of the society and stakeholders, and the future challenges which were identified after performing current research are also discussed.

6.2 The transect walk method for on-farm welfare assessment

The transect walk method developed through the studies showed great potential to be a practical and reliable tool for on-farm meat poultry welfare assessment, such as for broiler and turkeys, and possibly other species that are maintained in large flock sizes. The transect method was proved to be consistent across observers and sensitive to detect small differences across flocks for both species. The opportunity to compare welfare assessment results with meat quality parameters in the turkey study (condemnations, downgrades) proved the existence of a direct relationship between both outputs, providing further supporting evidences on the reliability of this new method. Furthermore, from a practical stand point, the transect walk was found feasible, easy to apply under field conditions and not demanding in terms of personnel and time requirements as compared to other assessment methods. Initial experience indicates a good acceptability by producers as they can relate to the method easily and perceive potential benefits from their application in everyday practices.

6.2.1 Advantages of the transect method:

- Non-intrusiveness

Ideally, welfare assessment should be non-intrusive and cause minimal disturbance to the animals (Webster, 2005). This is partly for reasons of practicality and cost, but mainly because it is essential not to cause stress to the animals or disturb their behavior, both which occasionally can have costly consequences (e.g. piling up resulting in mortality if birds get too scared). The transect method does not require any bird handling, and disturbances are reduced to the minimum as the method only requires that the assessor slowly walk through the flock in a standardized manner. The indicators chosen for the transect protocol can all be collected by observing the individuals from a close distance which is unlikely to cause any fear or panic reaction.

- Inter-observer reliability

Inter-observer reliability provides a score of how much homogeneity, or consensus, there is in the ratings given by observers. It is useful in refining the tools, for example by determining if a particular scale is appropriate for measuring a particular variable. High levels of inter-observer reliability are one of main criteria which provide evidence of the validity of the method (Hewetson et al., 2006; Meagher, 2009). If various assessors do not agree, either the scale is defective or the assessors need to be re-trained. We have successfully tested the inter-observer reliability for nearly all indicators included in the transect protocol. The concordance in the results obtained suggest that the method was powerful enough for two persons to assess the welfare status of the flocks in the same way, while reducing any possible discrepancies between them to a minimum. The excellent inter-observer reliability is a strong argument in favor of the adequacy of the method. In addition, if the method would be used for auditing, assurance or official programs, high inter-observer reliability is desire to guarantee the objectivity and fairness of the evaluation process.

- Population level approach

Up to date nearly all available on-farm animal based welfare assessment protocols in meat poultry were centered in the evaluation of a sample of individuals collected in representation of the flock. The evaluation of the entire population in animal production species is nearly impossible mainly due to workload constraints (Scott et al. 2009). Whenever measures are taken from a sample of animals, it is essential to assure that the sample is unbiased and representative (e.g. in terms of sex, age, body size, etc.) of the population. Nevertheless, evaluation of the sample is always an inference of the real situation including an error. In order to minimize problems it is important to consider how the representative sample is chosen (random strongly advised) and the sample size requirements to provide sufficient accuracy (De Jong et al., 2012).

As described for both current studies on broilers and turkeys, the selection of unbiased and representative sample is a challenge, even for experienced scientist. Randomness of the sample in broiler assessment appeared to have been compromised by the walking ability of the birds. Even though the procedure was performed with the intention of catching the birds at random, it seemed likely that birds with poor walking ability might have had higher chances of being caught. In addition to this, in the case of turkeys, catching and handling a sample of birds is considered dangerous for both humans and birds because of their size and power, especially at the end of rearing when most welfare assessment protocols are applied. Because of this reason it was essential that the protocol would try to avoid as much as possible any herding or catching of turkeys. An innovative approach needed to be undertaken to overcome these difficulties.

The transect method was developed considering the need to collect information about welfare status of species housed in large numbers which was recognized earlier (EFSA, 2012). However, the overall scoring is the result of the evaluation of the individuals, as the transect method implies the assessment of all individuals within the transects conducted. In this method, the occurrence of each welfare indicator is presented as a percentage of incidences in the flock population, giving a good straight forward overview of the general welfare status of the evaluated flock.

6.2.2 Challenges of the transect method

The result of this work demonstrates that the newly developed transect method has the potential to be reliable and practical for meat poultry commercial assessment. Nonetheless, additional studies are required in order to provide stronger evidences of its reliability under a range of environmental conditions and species, and also to conveniently address potential weaknesses of the method.

- Indicators persistence

The choice of indicators to be included in the protocol was an important task. In order to remain practical and feasible in on-farm conditions the number of indicators to be included in the protocol was reduced to those known to have the largest impact in meat poultry. We focused on the most important and clearly defined welfare deficiencies, which can be clearly understood and identified by persons with a minimal training.

Next to requirement for each indicator to be meaningful with respect to animal welfare, they also needed to be clearly visible on the birds, assuring the feasibility of the method (FAWC, 2009; Knierim and Winckler, 2009). Both broilers and turkeys of modern strains are white (although there are some exceptions), which makes possible noticing darker patches on their body. Those patches can be caused by: dirtiness, blood and wounds or for example missing feather patches. Further, individuals with movement irregularities or immobile in between a large number of evenly walking birds are relatively easy to detect. However, the detection of other important welfare indicators used to evaluate condition of meat poultry such as footpad dermatitis, hock burns or breast blisters is not possible without handling the individuals. Therefore, the transect protocol may need to be complemented by further evaluation of birds at the slaughter plant. It is also possible that some relationships between collected on farm indicators and the ones scored at the slaughter exist, which would further improve welfare assessment by transect method.

- Robustness

To be appropriate and effective in assessing welfare of animals at the farm level the protocols should fulfill criteria of validity and robustness. This includes considerations such as how often and how long, at what time of the day, at what stage of production, etc., they should be applied (EFSA, 2012). The protocols performance might be situation dependent: sometimes measures that are valid during one stage of the production cycle might not be applicable at other times, or the situation during summer might be different from that during winter. Systems using natural ventilation can be significantly influenced by the actual climatic condition. Therefore, the time point of the farm visit might be a critical aspect of a reliable welfare assessment.

In order to assure that the transect method is robust, selected indicators should not be dependent on the external conditions. For instance, panting might not be considered a robust indicator as it scoring will depend greatly on the temperature and humidity inside the production house and may change quickly. On the other hand, indicators included into transect protocol such as lameness or injuries levels, sickness, dirtiness, etc. are of course related to the external conditions and the ongoing interactions in the flock. However they will remain visible and stable for a longer period of time after those conditions return to optimal. Thus, they can be considered as robust. Dirtiness is the only factor, from the ones selected, that may slightly vary in appreciation according to light intensity, and therefore will not be considered as robust as other indicators.

Therefore it is important to check if the visibility is high enough to provide reliable data collection.

An additional important remark is that the transect protocol has been designed for use in commercial on farm conditions where large numbers of birds are housed. This protocol is not appropriate for use in small enclosures under experimental conditions. In smaller enclosures the presence of the observer might be more disturbing and therefore affect the veracity of the assessment.

- Early assessment

The aim of any welfare assessment protocol is to quantify the 'state' of the animals so that preventive measures or corrective actions can be implemented in favor of the animals. A less often considered aspect is that these improvements can also revert in increased benefits by improving farm productive efficiency. Thus far, assessing protocols were too complex to be suitable for application by farmers. However, the simplicity of the transect protocol regarding the parameters considered, the way data are gather, and the availability of additional tools to facilitate the assessment, creates real possibilities for easy assessment by the farmer. It has been also suggested that a good welfare assessment protocol should be integrative (Webster, 2005). It means that the measures used to assess animal welfare should, as far as possible, be designed to integrate the long-term consequences of husbandry practices.

These aspects open the possibilities for the transect protocol to go beyond the role of assessment tool, by potentially helping to predict the condition of the birds based on information collected at earlier ages. Early health and welfare assessment could open the possibility of implementing corrective actions to prevent disease outbreaks or control the issues arising early in the flock. For instance, skin lesions and feather loss can be used as integrative measures of social problems in poultry. Some of the results in turkeys already evidenced a high correlation of scorings across age and slaughter plant outcomes. Nonetheless, still further work is needed in order to establish the potential strength of the relationship between early welfare assessment and health or meat quality outcomes both for broiler chickens and turkeys.

- Benchmarking

The transect protocol could have a large potential to be used for benchmarking purposes. Benchmarking can be used to track changes in successive flocks within the same farm over time as a part of good farm management. It gives a chance to determine the evolution of welfare issues of a particular flock over time, making possible not only the evaluation of the effectiveness of corrective actions (Colditz et al., 2014), but also its quantification. The capability of easy quantitative assessment would allow comparisons of results between farms with similar housing systems or management practices, facilitating the identification of optimal housing or management established according to specific animal-based outputs. Using the appropriate set of animal-based measures is especially useful to confirm improvements in welfare following an implemented production system change. Collecting data regularly on welfare assessment using the transect protocol on the flock, house, farm and company level opens new opportunities for management improvements by selecting husbandry practices with best outcomes. Benchmarking could also be used by breeding companies and legislators to track changes over time as a consequence of changes in selection criteria or following particular interventions or initiatives (EFSA, 2012).

6.2.3 Further applications of the transect method

The transect method has the potential to be applied for various purposes in farming practice and monitoring in other species. It may be used to screen the farms by managers or farm advisors, in order to take correct management decisions, or by veterinary practitioners involved in flock health management. Auditing or accreditation organizations may use the method for easy health and welfare assessment according to their accreditation schemes, being a useful tool to determine if farm conditions and management satisfies the necessary criteria to be part of a quality assurance or labeling scheme. The transect method could be used to guarantee that a farm satisfies animal welfare requirements set according to legislation, or to evaluate effects of changes in animal welfare legislation in practice, normally conducted by the competent/responsible authority.

The transect method has been tested initially for meat poultry flocks. Nonetheless the method is susceptible of application to other species. Primarily, this approach could be fairly easily applied to other intensively indoor-reared meat poultry such as ducks or quails. Some issues need, however, further exploration in case of poultry reared in less intensive or free range systems.

Previously, an attempt to conduct bird evaluation in organic laying farms based on observing number of individuals within transect was applied (Bestman and Wagenaar, 2003). In this study hens were scored, both inside and outside the house. This was only possible because the scoring was conducted over a predefined known number of birds in the flock in which the assessment was based. However, in free range systems the birds move freely between the house and the outdoor area, making it difficult to estimate the total bird number per transect, which is required for an accurate calculation of the incidence of the different welfare issues. Another obstacle may come from the large variety of the outdoor areas, which may be of irregular shape, while the lack of visual obvious borderlines may hinder designation of the transects and their regularity. This last issue may be resolved by land-marking the transects previously. However, it might be more efficient to simply apply the transects indoors, prior to the opening of the outdoor areas in the morning.

The same obstacle may limit potential use of the transect walks method in other free ranging farm species. For example sheep are generally managed in groups, and similarly to poultry, shepherds, routinely perform brief observations of the flocks identifying individuals with health issues. This approach, similarly to poultry, involves minimal disturbance to the herd, is feasible under on-farm conditions, and requires few resources apart from the observational skills and knowledge of the protocol by the assessor. Additionally, in case of sheep herds the total number of animals is always known. Therefore it should be possible to develop variants of the method for application under such conditions.

6.3 Pain indicators in lambs

The capacity to identify reliable behavioral pain indicators in producing animals has important practical implications for pain management in farm practices (Morris, 1994) as pain can be a major welfare concern in most production systems (EFSA, 2014). During their life farm animals, including sheep, might experience painful procedures that are considered necessary to increase productivity or for health reasons. However, the effects of potentially painful procedures on the welfare of farm animals are difficult to evaluate, without knowing how to assess pain. This remains very a difficult task for sheep due to the lack of known clear and practical pain indicators.

A common procedure used in animal farming which is considered painful is tail docking (Sutherland and Tucker, 2011). Tail docking is commonly performed in sheep flocks to reduce fly strike risks and to improve fertility (French et al., 1994; Webb-Ware et al., 2000). Tail docking, if necessary, is recommended to be conducted in young animals up to two months of age, according to FAWC (2008). At this age the procedure is considered to be less painful (Molony et al., 1993), while wounds are supposed to heal easier. With the objective of identifying practical behavioral pain indicators an experiment was conducted using the model of tail docking in lambs. The responses of one month old lambs tail docked by using rubber rings, with or without the application of pain control measures were studied.

6.3.1 Challenges of the study

Large difficulties in detecting pain signs in lambs were suspected at the onset of the study due to the evolutionary pressures to hide them to diminish predation risk (Butler and Finn, 2009). Gregariousness is one of the most important characteristic of sheep driving their behavior. Presence of group members, through social buffering, has been shown to reduce fear reactions to novel objects (Guesgen et al., 2014; Gonzalez et al., 2013) and to maintain a state of well-being and a normal physiological range that may even alleviate pain levels (Kikusui et al., 2006). Therefore, in order to overcome the difficulties in identifying subtle behavioral pain indicators and the potential buffering effects of the group members, the study was conducted under conditions of isolation from their flock mates.

For this study purpose, the experimental lambs were introduced to their home pens seven days prior to the habituation period and remained in stable groups during the length of the study. To minimize the stress caused by the novel testing environment all lambs were exposed to an habituation period of the testing arena that was similar to the length and conditions of the test (2,5 minutes). The habituation took place during two consecutive days before the onset of the study, and it was performed in the presence of the observer standing outside the testing arena. Other studies reported similar habituation periods repeated over one until few days (see Forkman et al., 2007).

Even though the habituation period prior to testing was performed according to previously accepted methods (Forkman et al., 2007), all lambs showed decreased activity levels over the following testing days. This may indicate that the habituation was not completed before the testing started. The distressing effect of isolation itself was hard to distinguish from effect of the treatments. Regarding the results obtained we may further speculate that tail docked lambs without pain control showed the lowest ability or required longer time to habituate to the testing conditions as compared to lambs in control groups or to which pain mitigation treatments were applied. This is supported by the finding that exploratory climbs in lambs with no pain control remained on a similar level across the days of observations, while for other lambs they increased. It may be possible that isolation will always remain a strong testing condition to which lambs will have difficulties to habituate to.

During the testing procedure the experimental lambs were deprived of any physical or visual contact with conspecifics, while remaining under human observation that might have been perceived as a threat or as a potential ´predator´. This situation may have also been a cause of stress which might have added to cover the pain perception by the experimental lambs. It is uncertain how the testing conditions might have modified the behavioral signs of pain, or which mechanisms were launched due to experienced stress, as behavioral responses may be modulated by endogenous analgesic mechanisms that are triggered in response to pain (Nijs et al., 2012). SIA mechanism (Butler and Finn, 2009) may be one of the explanations of the lack of clear differences in the behavior of lambs in the experimental treatments as compared with the control group.

It has been speculated that showing an abnormality (including abnormal behavior in response to pain) may increase predation risk (Dwyer, 2004). Therefore, as result of the evolutionary pressures prey animals are less likely to show any signs of pain (Underwood, 2002) even when severe, which may have been the situation in this study. However, it should not be assumed that because there were not obvious signs of pain the animals did not experienced pain. Perhaps pain mechanisms should be further investigated under experimental conditions prior to interpreting lambs behavior in field conditions.

On the view of the results obtained, it could be recommended for future experiments to adjust the stress associated with the experimental conditions by buffering the response with the presence of another familiar conspecific (Porter et al., 1995) when tested. Another possibility could be could be to ´simulate´ the presence of another individual by introducing mirror panels during isolation period in order to reduce the magnitude of the responses to this psychologically distressing situation (Parrott et al., 1988). These alternative approaches may allow reducing the distress of isolation covering the signs of pain, but still controlling for potential social buffering effects.

It is also possible that the results of the current study may have been altered by other factors. For instance, the diversity in behavioral expressions may vary within a species (Niemelä et al., 2012). When the a treatment elicits different behavioral responses this might be the result of the diverse sensations experienced by the animals, for example due to differences in tissues that are damaged or damaged in different ways (Kent, 2000). Lambs housed in a group assigned to the same treatment and tested under identical conditions in this study have shown fairly variable reactions that could be explained as result of variation in pain sensitivity (Molony and Wood, 1992). This effect could be to some extent reduced by using larger sample sizes to help overcame statistically inter individual variations. However, this implies the use of more experimental animals undergoing a painful procedure. Additionally, the use of a suite of behaviors potentially indicative of pain may be better to determine the existence, duration and intensity of the noxious experience, while the use of a single behavior which may be misleading (Dinniss et al., 1999). It is also possible that pain perception is to such an extent an individual trait that it should not be evaluated on the group but individual level.

6.3.2 Further considerations about pain detection on-farm

This study has shown that the large effect of isolation makes almost impossible to determine visible effects of pain caused by tail docking. From the practical stand point these results suggest that procedures that are likely to cause pain should be undertaken in the presence of other individuals, so that at least the strong stress caused by isolation can be reduced. Therefore further work is necessary to find the most suitable pain indicators in sheep by considering their social needs and evolutionary mechanisms. This need was acknowledged by a newly published study which confirmed that the social context influence the behavioral expression of pain by lambs (Guesgen et al., 2014). Recent study demonstrates that the occurrence of social buffering on lamb pain behavior depends on social aspects such as the relationship between the actor and observer and on previous experience.

In addition, the current study shows that behavioral signs of pain in lambs are subtle and context dependent. The ability to detect elusive behavioral abnormalities by farmers or veterinarians in order to control pain should be part of good stockmanship. To standardize and facilitate this skill, and educate stockpersons, clear animal based pain indicators are still needed.

6.4 Conclusions

The overall aim of the studies conducted within the PhD thesis entitled: “Development of practical methodology and indicators for on-farm animal welfare assessment” was to develop and examine the suitability of the welfare indicators and methods for on-farm assessment of selected production animal species. The results of the studies conducted have shown that the transect method fulfills the requirements of a modern welfare assessment protocol suitable for easy application to large commercial meat poultry flocks. The new approach of the transect method has good inter-observer reliability and sensitivity, and has the advantage of being non-intrusive for the animals. In addition, the excellent correlation found between the on farm indicators used in the study and slaughter plant parameters provides additional benefits to the users. Thus, not only the use of transects can help to improve the conditions of the animals during rearing, but can be a way for producers to improve economic returns. While further studies are still needed to fully validate the method under different scenarios and ages of application, the transect method appears to be a promising tool to be used for early assessment. Early verification of potential health and welfare risks in the flocks would allow implementing corrective actions to redirect the situation, or to minimize potential impacts. The method has potential to be use in other meat poultry species easily and potentially in other production systems or species with adjustments.

To test the suitability of welfare indicators for pain assessment we investigated the behavioral response to tail docking in lambs with or without pain control measures in an isolation test. We applied the isolation during the testing procedure in order to increase the chances of detecting any pain reactions by avoiding the potential covering effect caused by social buffering in such gregarious species. However, the lambs´ behavior appeared to be largely affected by the stress caused by isolation, more so than by the procedure in itself. Even though the usual habituation to testing conditions was applied, the combined effect of novel environment and isolation was stronger than predicted. We suspected that some survival mechanisms which covered the direct pain reactions of the lambs were evoked during tests, causing difficulties to clearly identify pain indicators. This study showed the importance of considering the social environment when studying pain indicators which should be considered when designing future studies.

The main aims of current work were accomplished successfully, as it provided new knowledge and an innovative tool to help improve on-farm animal welfare assessment. In addition, the welfare assessment protocol was easy to understand to stakeholders. Additionally to the main outcome, this work has achieved the first science-based, welfare assessment in male turkey flocks at the end of the production cycle, never done previously in commercial on-farm setting.

The pain assessment was found to be a very difficult task, considering the large effect of the experimental situation on the lamb behavior. The pain still remains important but not sufficiently explored issue within animal welfare areas of knowledge requiring further investigations. In conclusion some of the outcomes of current studies can be directly applied to improve on-farm practices, as in case of the transect method, or can help in further work on development of welfare indicators to assess sheep pain.

References

References

Appleby, M. 1999. What should we do about animal welfare? Oxford: Blackwell Science Limited.

Appleby, M. C., S. F. Smith, and B. O. Hughes. 1992. Individual perching behavior of laying hens and its effects in cages. Br. Poult. Sci. 33:227-238.

Babst, C. R., and B. N. Gilling. 1978. Bupivacaine. Anes Prog 25: 87–91.

Balph, D. F., G. S. Innis, and M. H. Balph.1980.Kin Selection in Rio Grande Turkeys: A Critical Assessment. The Auk 97:854-860.

Barber, C. L., N. B. Prescott, C. M. Wathes, C. Le Sueur, and G. C. Perry. 2004. Preferences of growing ducklings and turkey poults for illuminance. Anim. Welfare. 13:211-224.

Barbut, S. 2010. Past and future of poultry meat harvesting technologies. World's Poultry Sci J. 66:399-410.

Bartussek, H. 1995a. Animal needs index for cattle, TGI 35 L, March 1995. Federal Research Centre for Alpine Agriculture, Gumpenstein, Austria.

Bartussek, H. 1995b. Animal needs index for laying hens, TGI 35 L, November 1995. Federal Research Centre for Alpine Agriculture, Gumpenstein, Austria.

Bartussek, H. 1995c. Animal needs index for fattening pigs, December 1995. Federal Research Centre for Alpine Agriculture, Gumpenstein, Austria.

Bartussek, H. 1999. A review of the animal needs index (ANI) for the assessment of animals' well-being in the housing systems for Austrian proprietary products and legislation. Livest. Prod. Sci. 61: 179-192.

Bath, G. F. 1998. Management of pain in production animals. App. Anim. Behav. Sci. 59: 147-156.

Berg, C. C. 1998. Foot-pad dermatitis in broilers and turkeys. Diss. (sammanfattning/summary) Skara: Sveriges lantbruksuniv., Acta Universitatis agriculturae Sueciae. Veterinaria, 1401-6257; 1998:36 ISBN 91-576-5442-5 [Doctoral thesis]

Bestman, M. W. P., and Wagenaar, J. P. 2003. Farm Level Factors Associated with Feather Pecking Damage in Organic Laying Hens. Livest. Prod. Sci. 80: 133–140.

Bilčík, B. and L. J. Keeling. 2000. Relationship between feather pecking and ground pecking in laying hens and the effect of group size. Appl. Anim. Behav. Sci. 68:55-66.

Bizeray, D., C. Leterrier, P. Constantin, M. Picard, and J. M. Faure. 2000. Early locomotorbehavior in genetic stocks of chickens with different growth rates. Appl. Anim. Behav. Sci. 68:231-242.

Blokhuis, H. J., I. Veissier, M. Miele, and B. Jones. 2010. The Welfare Quality® project and beyond: safeguarding farm animal well-being. Acta Agriculturae Scandinavica Section A, Animal Science, 60: 129–140.

Boissy, A., A. D. Fisher, J. Bouix, G. N. Hinch, and P. Le Neindre. 2005. Genetics of fear in ruminant livestock. Livest. Prod. Sci. 93: 23-32.

Botreau, R., M. Bonde, A. Butterworth, P. Perny, M. B. M. Bracke, J. Capdeville, and I. Veissier. 2007. Aggregation of measures to produce an overall assessment of animal welfare. Part 1: A review of existing methods. Animal 1:1179-1187.

Breward, J., and M. J. Gentle. 1985. Neuroma formation and abnormal afferent nerve discharges after partial beak amputation (beak trimming) in poultry. Experientia. 41: 1132-1134.

Bright, A., T. A. Jones, and M. S. Dawkins. 2006. A non-intrusive method of assessing plumage condition in commercial flocks of laying hens. Anim. Welfare. 15:113-118.

Broom, D.M., 1981. Biology of Behavior. Cambridge (UK), Cambridge University Press, p: 320.

Broom, D.M. 1991. Animal welfare: concepts and measurement. J.Anim. Sci. 69:4167-4175.

Broom, D.M. 1996. Animal welfare defined in terms of attempts to cope with the environment. Acta Agric. Scand. Sec. A. Anim. Sci. Suppl. 27: 22-28.

Broom, D.M. 2001. The use of the concept of Animal Welfare in European conventions, regulations and directives. In: Food Chain 2001, pp. 148-151. SLU Services, Uppsala.

Broom, D. M. 2002. Does present legislation help animal welfare? Landbauforschung Völkenrode. 227: 63-69.

Broom D. M. and A. F. Fraser. 2007. Domestic Animal Behavior and Welfare. Wallingford (UK), CAB Int., 437 p.

Bubier, N. E., C. G. M. Paxton, P. Bowers, and D. C. Deeming. 1998. Courtship behavior of ostriches (struthiocamelus) towards humans under farming conditions in Britain. Br. Poult. Sci. 39:477-481

Buchholz, R. 1997. Male dominance and variation in fleshy head ornamentation in wild turkeys. J. Avian Biol. 28:223-230.

Buchwalder, T., and B. Huber-Eicher. 2003. A brief report on aggressive interactions within and between groups of domestic turkeys (meleagrisgallopavo). Appl. Anim. Behav. Sci. 84: 75-80.

Buchwalder, T., and B. Huber-Eicher. 2004. Effect of increased floor space on aggressive behavior in male turkeys (meleagrisgallopavo). Appl. Anim. Behav. Sci. 89:207-214.

Buchwalder, T., and B. Huber-Eicher. 2005a. Effect of group size on aggressive reactions to an introduced conspecific in groups of domestic turkeys (meleagrisgallopavo). Appl. Anim. Behav. Sci. 93:251-258

Buchwalder, T., and B. Huber-Eicher. 2005b. Effect of the analgesic butorphanol on activity behavior in turkeys (meleagrisgallopavo). Res. Vet. Sci. 79:239-244.

Buckland, S. T., A. J. Plumptre, L. Thomas, and E. A. Rexstad. 2010. Line transect sampling of primates: Can animal-to-observer distance methods work? Int. J. of Primatol. 31:485-499.

Burnham, K. P., D. R. Anderson, and J. L. Laake. 1980. Estimation of density from line transect sampling of biological populations. Wildl Monogr 72:3-202.

Busayi, R. M., C. E. Channing, and P. M. Hocking. 2006. Comparisons of damaging feather pecking and time budgets in male and female turkeys of a traditional breed and a genetically selected male line. Appl. Anim. Behav. Sci. 96:281-292.

Butler, M. J., W. B. Ballard, M. C. Wallace, and S. J. Demoaso. 2006. Road-based surveys for estimating wild turkey density in the Texas rolling plains. J Wildlife Manage. 71:1646-1653.

Caine, N. G. 1992. Humans as predators: observational studies and the risk of pseudohabituation. In: H. Davis and H. Balfour, editors, The Inevitable Bond: Examining Scientist-Animal Interactions. Cambridge University Press: Cambridge, UK. p. 357-364

Canini, F., S. Brahimi, J. B. Drouet, V. Michel, A. Alonso, A. Buguet, and R. Cespuglio. 2009. Metyrapone decreases locomotion acutely. Neurosci. Lett. 4571: 41-44.

Chapman, C. R., and J. A. Turner. 1986. Psychological control of acute pain in medical settings. J. Pain Symptom Manag. 1: 9–20.

Ciofalo, V. B., M. B. Latranyi, J. B. Patel, and R. I. Taber. 1977. Flunixin meglumine: A non narcotic analgesic. J. Pharmacol Exp Ther. 200: 501-507.

Clark, T. E., and M. W. McCracken. 2012. In-sample tests of predictive ability: A new approach. J. Econometrics 170:1-14.

Classen, H. L., C. Riddell, F. E. Robinson, P. J. Shand, and A. R. McCurdy. 1994. Effect of lighting treatment on the productivity, health, behavior and sexual maturity of heavy male turkeys. Br. Poult. Sci. 35:215-225.

Colditz, I.G. D.M. Ferguson, T. Collins, L. Matthews, and P. H. Hemsworth. 2014. A Prototype Tool to Enable Farmers to Measure and Improve the Welfare

Performance of the Farm Animal Enterprise: The Unified Field Index. Animals. 4: 446-462.

Communication from The Commission to The European Parliament, The Council and The European Economic and Social Committee on the European Union Strategy for the Protection and Welfare of Animals: 2012-2015. 2012. Brussels.

Cordeiro, A. F. S., I. A. Nääs, and D. D. Salgado. 2009. Field evaluation of broiler gait score using different sampling methods. Braz. J. Poultry Sci. 11:149-154.

Cornetto, T., I. Estevez, and L. W. Douglass. 2002. Using artificial cover to reduce aggression and disturbances in domestic fowl. Appl. Anim. Behav. Sci.75:325-336.

Costa, J. H. C., M. J. Hötzel, C. Longo, and L. F. Balcão. 2013. A survey of management practices that influence production and welfare of dairy cattle on family farms in southern Brazil. J. Dairy Sci. 96:307-317.

Curtis, S. 2007. Commentary: Performance indicates animal state of being: A cinderella axiom? Prof. Anim. Sci. 23: 573-583.

Dawkins, M.S. 1980. Animal Suffering, the Science of Animal Welfare. Chapman and Hall, London, UK.

Dawkins, M. 1990. From an animal‘s point of view: motivation, fitness and animal welfare. Behav. Brain Sci. 13:1-61.

Dawkins, M.S., C.A. Donnelly, T.A. Jones. 2004. Chicken welfare is influenced more by housing conditions than by stocking density. Nature 427: 342–344.

Dawkins, MS. 2006. A user’s guide to animal welfare science. Trends in Ecology & Evolution. 21: 77-82.

De Jong I. C., J. Van Harn, H. Gunnink, A. Lourens, and J. W. Van Riel. 2012. Measuring foot-pad lesions in commercial broiler houses. Some aspects of methodology. Anim Welfare 21:325-330.

De Jong I. C., T. Perez Moya, H. Gunnink, V. A. Hindle, and C. G. van Reenen. 2012. Simplifying the Welfare Quality® assessment protocol for broilers In: Minding Animals 2012 Conference, Utrecht, The Netherlands, 2012-07-03/ 2012-07-06.

Dinniss, A. S., K. J. Stafford, D. J. Mellor, R. A. Bruce, and R. N. Ward. 1999. The behavior pattern of lambs after castration using a rubber ring and/or castrating clamp with or without local anaesthetic. New Zeal. Vet. J.l 47: 198-203.

Dixon, P. M. 1993. The bootstrap and the jackknife: describing the precision of ecological indices. In: Design and analysis of eco- logical experiments. Chapman & Hall, London.

Dodd, C. L., W. S. Pitchford, J. E. Hocking Edwards, and S. J. Hazel. 2012. Measures of behavioral reactivity and their relationships with production traits in sheep: A review. Appl. Anim. Behav. Sci. 140: 1-15.

Donoghue, A. USDA-ARS Poultry Production and Product Safety Research Unit (PPPSRU) Fayetteville, Arkansas: (accessed on the 11th of February 2013); http://www.ars.usda.gov/main/site_main.htm?modecode=62-26-00-00 (2012).

Duncan, I.J.H. 1993. Welfare is to do with what animals feel. J. Agr. Env. Ethics, 6, Suppl. 2: 8-14.

Duncan, I.J.H. 2004. A concept of welfare based on feelings. In The well-being of farm animals: challenges and solutions (G.J. Benson & B.E. Rollin, eds). Blackwell, Ames. pp: 85-101.

Duncan, I.J.H. 2005. Science-based assessment of animal welfare: farm animals. Rev. Sci. Tech. OIE. 24:483-492.

Duncan, I. J. H., and V. G. Kite. 1987. Report for 1986-1987. AFRC Institute of Animal Physiology and Genetics Research. Edinburgh Research Station, Edinburgh. Pages 30-36.

Duncan, I. and D. Fraser. 1997. Understanding animal welfare. In: Appleby, M.C. and Hughes, B.O. (eds) Animal Welfare, pp. 19-31. CAB International. Wallingford, UK.

Duncan, I. J. H., M. Park, and A. E. Malleau. 2012. Global animal partnership's 5-step™ animal welfare rating standards: A welfare-labelling scheme that allows for continuous improvement. Anim Welfare. 21(Suppl. 1): 113-116.

Dwyer, C. M. 2004. How has the risk predation shaped the behavioral responses of sheep to fear and distress? Anim. Welfare 13: 269-281.

Efron, B. 1979. Bootstrap methods: another look at the Jackknife. Ann. Stat. 7:1–26.

Efron, B. 1987. Better bootstrap confidence intervals. Am. Stat. Assoc. J. 82:171–200.

Erhard, H. W., D. A. Elston, and G. C. Davidson. 2006. Habituation and extinction in an approach-avoidance test: an example with sheep. Appl. Anim. Behav. Sci. 99: 132–144.

Estévez, I. 1994. Efecto del tamaño de grupo y de las condiciones de manejo en el comportamiento y uso del espacio del gallo doméstico (Gallusgallus). [Effect of group size and rearing conditions on the behavior and use of space in broiler chickens]. Ph.D. Diss. Univ. Córdoba, Spain.

Estevez, I. 2007. Density allowances for broilers: Where to set the limits? Poultry Sci. 86:1265-1272.

Estevez, I., R. C. Newberry, and L. A. De Reyna. 1997. Broiler chickens: A tolerant social system? Etologia 5:19-29.

Estevez, I., R. C. Newberry, and L. J. Keeling. 2002. Dynamics of aggression in the domestic fowl. Appl. Anim. Behav. Sci. 76:307-325.

Estevez, I., L. J. Keeling, and R. C. Newberry. 2003. Decreasing aggression with increasing group size in young domestic fowl. Appl. Anim. Behav. Sci. 84:213-218.

Eurobarometer, 2007. Attitudes of EU citizens towards animal welfare. Special Eurobarometer 270, European Commission. Home page address: http://ec.europa.eu/ food/animal/welfare/ index_en.htm

European Food Safety Authority. 2006. Scientific Colloquium "Principles of Risk Assessment of Food Producing Animals: Current and future approaches". Available from http://www.efsa.europa.eu/en/supporting/pub/111e.html

European Food Safety Authority (EFSA) (2012). – Scientific Opinion on the use of animal-based measures to assess welfare of broilers. EFSA Panel on Animal Health and Welfare. Question no.: EFSA-Q-2011-00808. EFSA J., 10 (7), 2774. doi:10.2903/j.efsa.2012.2774.

European Food Safety Authority (EFSA) AHAW Panel (EFSA Panel on Animal Health and Welfare). 2014. Scientific Opinion on the welfare risks related to the farming of sheep for wool, meat and milk production. EFSA Journal 2014;12(12):3933, 128 pp. doi:10.2903/j.efsa.2014.3933

European Union (EU) Directive 2010/63/EU

Farm Animal Welfare Council. 1993. Second Report on Priorities for Research and Development in Farm Animal welfare. MAFF Publ., Tolworth, London, UK.

Farm Animal Welfare Council. 2009. Report on the Welfare of Farmed Animals at Slaughter or Killing – Part Two: White Meat Animals "http://www.fawc.org.uk/pdf/report-090528.pd

Fields, H. L., and A. I. Basbaum. 1999. Central nervous system mechanisms of pain modulation. In: P. D. Wall and R. Melzack, editors. Textbook of Pain. Churchill Livingstone, New York. p. 243–257.

Fisher, M. W. and N. G. Gregory. 2007. Reconciling the differences between the length at which lambs' tails are commonly docked and animal welfare recommendations. Proceedings of the New Zealand Society of Animal Production. 67: 32–38.

Food and Agricultural Organisation database: http://faostat.fao.org. (Retrived on: 27.09.2012).

Ford, G. K., and D. P. Finn. 2008. Clinical correlates of stress-induced analgesia: Evidence from pharmacological studies. Pain 140: 3-7.

Forkman, B., A. Boissy, M. Meunier-Salaün, E. Canali, and R. B. Jones. 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiol. and Behav. 92: 340-374.

Fraser D. 1995. Science, values and animal welfare: exploring the 'inextricable connection'. Anim. Welfare. 4:103-117.

Fraser, A.F., and D. M. Broom. 1990. Farm animal behavior and welfare, 3rd Ed. Baillière Tindall, London.

Fraser, A. F., and D. M. Broom. 1997. Farm Animal Behavior and Welfare, 3rd Edition. CAB International: Wallingford, UK.

Fraser, D., D. M. Weary, E. A. Pajor, and B. N. Milligan. 1997. A scientific conception of animal welfare that reflects ethical concerns. Anim. welfare. 6:187-205.

Fraser, D., and I.J.H. Duncan. 1998. 'Pleasures', 'pains' and animal welfare: toward a natural history of affect. Anim. Welf. 7: 383-396.

French, N. P. R. Wall, and K. L. Morgan. 1994. Lamb tail docking: a controlled field study of the effects of tail amputation on health and productivity. Vet. Rec. 134: 463-467.

Gates, C. E., W. H. Marshall, and D. P. Olson. 1968. Line transect method of estimating grouse population densities. Biometrics 24:135-145.

Gill, D. J., and A. T. Leighton Jr. 1984. Effects of light environment and population density on growth performance of male turkeys. Poultry Sci. 63:1314-1321.

González, M., X. Averós, I. Beltrán de Heredia, R. Ruiz, J. Arranz, and I. Estevez. 2013. The effect of social buffering on fear responses in sheep (ovis aries). Appl. Anim. Behav. Sci. 149: 13-20.

Graham, M. J., J. E. Kent, and V. Molony. 1997. Effects of four analgesic treatments on the behavioral and cortisol responses of 3-week-old lambs to tail docking. Vet J. 153: 87-97.

Grant, C. 2004. Behavioral responses of lambs to common painful husbandry procedures. Appl. Anim. Behav. Sci. 87: 255-273.

Gross, T. L., and S. H. Carr. 1990. Amputation neuroma of docked tails in dogs. Vet. Pathol. 27: 1-62.

Guesgen M.J., N.J. Beausoleil, E.O. Minot, M. Stewart, and K.J. Stafford, Social context and other factors influence the behavioral expression of pain by lambs. 2014. Appl. Anim. Behav. Sci. 159: 41-49.

Hale, E. B., and M. W. Schein.1962. The behavior of turkeys. In: Hafez ESE editors. The Behavior of Domestic Animals. London: Bailliere/Tindall/Cox.

Harper, G.C., and S.J. Henson. 2000. Consumer values and farm animal welfare – the Comparative Report. The University of Reading. United Kingdom. EU FAIR CT98-3678.

Hart, N. S., J. C. Partridge, and I. C. Cuthill. 1999. Visual pigments, cone oil droplets, ocular media and predicted spectral sensitivity in the domestic turkey (meleagrisgallopavo). Vision Res. 39:3321-3328.

Hashizume, T., S. A. Haglof, and P. V. Malven. 1994. Intracerebral methionine-enkephalin, serum cortisol, and serum beta-endorphin during acute exposure of sheep to physical or isolation stress. J. Anim. Sci. 72: 700-708.

Healy, W. M. 1992. Behavior (pp 46–65) in The Wild Turkey: biology and management (J. G.Dickson, Ed.). Stackpole Books, Harrisburg, Pennsylvania.

Hewson, C. J. 2003. Can we assess welfare? Canadian Vet. J. 44: 749–753.

Hewetson, M., T. M. Christley, I. D. Hunt, and L. C. Voute. 2006. Investigations of the 340 Reliability of the Observational Gait Analysis for the Assessment of Lameness in 341 Horses. Vet. Rec. 158: 852-858.

Hocking, P. M., M. H. Maxwell, and M. A. Mitchell. 1999. Welfare of food restricted male and female turkeys. Br. Poult. Sci. 40:19-29.

Huff, G. R., W. E. Huff, J. M. Balog, and N. C. Rath. 2003. The effects of behavior and environmental enrichment on disease resistance of turkeys. Brain Behav Immun. 17:339-349.

Huff, G. R., W. E. Huff, N. Rath, A. Donoghue, N. Anthony, and K. Nestor. 2007. Differential effects of sex and genetics on behavior and stress response of turkeys. Poultry Sci. 86:1294-1303.

Hughes, B. O., and P. N. Grigor. 1996. Behavioral time-budgets and beak related behavior in floor-housed turkeys. Anim. Welfare 5:189-198.

International Association for the Study of Pain (IASP), Pain terms: a list with definitions and notes on usage. 1979. Pain. 6:249–52.

Jones, R. B. 1992. The nature of handling immediately prior to test affects tonic immobility fear reactions in laying hens and broilers. Appl. Anim. Behav. Sci. 34: 247-254.

Kavaliers, M., and D. D. Colwell. 1991. Sex differences in opioid and non-opioid mediated predator-induced analgesia in mice. Brain Res. 568: 173-177.

Kent, J. E., V. Molony, and M. J. Graham. 1998. Comparison of methods for the reduction of acute pain produced by rubber ring castration or tail docking of week-old lambs. Vet. J. 155: 39-51.

Kent, J. E., R. E. Jackson, V. Molony and B. D. Hosie. 2000. Effects of acute pain reduction methods on the chronic inflammatory lesions and behavior of lambs castrated and tail docked with rubber ring at less than two days of age. Vet. J. 160: 33–41.

Kestin, S. C., T. G. Knowles, A. F. Tinch, and N. G. Gregory. 1992. The prevalence of leg weakness in broiler chickens and its relationship with genotype. Vet. Rec. 131:190–194.

Kikusui, T., J. T. Winslow, and Y. Mori. 2006. Social buffering: Relief from stress and anxiety. Philosophical Transactions of the Royal Society B: Biological Sciences 361: 2215-2228.

Kjaer, J. B., G. Su, B. L. Nielsen, and P. Sørensen. 2006. Foot-pad dermatitis and hock burn in broiler chickens and degree of inheritance. Poult. Sci. 85:1342–1348.

Knierim, U., and C. Winckler. 2009. On-farm welfare assessment in cattle: validity, reliability and feasibility issues and future perspectives with special regard to the Welfare Quality. Anim. Welfare, 18: 451-458.

Knowles, T. G., S. C. Kestin, S. M. Haslam, S. N. Brown, L. E. Green, A. Butterworth, S. J. Pope, D. Pfeiffer, and C. J. Nicol. 2008. Leg disorders in broiler chickens: prevalence, risk factors and prevention. PloS One 3, e1545.

Krautwald-Junghanns, M. -E, R. Ellerich, H. Mitterer-Istyagin, M. Ludewig, K. Fehlhaber, E. Schuster, J. Berk, S. Petermann, and T. Bartels. 2011. Examinations on the prevalence of footpad lesions and breast skin lesions in british united turkeys big 6 fattening turkeys in Germany. Part I: Prevalence of footpad lesions. Poultry Sci. 90: 555-560.

Leone, E. H. and I, Estévez. 2008. Use of space in the domestic fowl: Separating the effects of enclosure size, group size, and density. Anim. Behav, 76:1673-1682.

Lewis, P. D., G. C. Perry, and C. M. Sherwin.1998.Effect of intermittent light regimens on the performance of intact male turkeys. Animal Sci. 67:627-636.

Lewis, P. D., G. C. Perry, C. M. Sherwin, and C. Moinard. 2000. Effect of ultraviolet radiation on the performance of intact male turkeys. Poultry Sci. 79:850-855.

Ley S. J., A. E. Waterman, A. Livingston, and T. J. Parkinson. 1994. Effect of chronic pain associated with lameness on plasma cortisol concentrations in sheep: a field study. Res. Vet. Sci. 57: 332–335.

Lin, H., S. J. Sui, H. C. Jiao, J. Buyse, and E. Decuypere. 2006. Impaired development of broiler chickens by stress mimicked by corticosterone exposure. Comp. Biochem. Phys. A. 143:400-405.

Lockwood, R. 2005. Tracking the "State of the Animals": Challenges and opportunities in assessing change. In: The State of the Animals III, 2005, eds. DJ Salem & AN Rowan, pp. 1-14. Washington, DC: Humane Society Press.

Lomax, S., H. Dickson, M. Sheil, and P. A. Windsor. 2010. Topical anaesthesia alleviates short-term pain of castration and tail docking in lambs. Aust. Vet. J. 88: 67-74.

Lupo, C., S. Le Bouquin, V. Allain, L. Balaine, V. Michel, I. Petetin, P. Colin, and C. Chauvin. 2010. Risk and indicators of condemnation of male turkey broilers in western France, february-july 2006. Prev Vet Med 94:240-250.

Main, D. C. J., H. R. Whay, C. Leeb, and A. J. F. Webster. 2007. Formal animal-based welfare assessment in UK certification schemes. Anim. Welfare 16:233-236.

Manser, C. E. 1996. Effects of lighting on the welfare of domestic poultry: A review. Anim. Welfare 5:341-360.

Marchewka, J., T. T. N. Watanabe, V. Ferrante, and I. Estevez. 2013a. Welfare assessment in broiler farms: Transect walks versus individual scoring. Poult Sci. 92:2588-2599.

Marchewka, J., T. T. N. Watanabe, V. Ferrante, and I. Estevez. 2013b. Review of the social and environmental factors affecting the behavior and welfare of turkeys (meleagris gallopavo). Poult Sci. 92:1467-1473.

Marin, R. H., P. Freytes, D. Guzman, and R. Bryan Jones. 2001. Effects of an acute stressor on fear and on the social reinstatement responses of domestic chicks to cagemates and strangers. Appl Anim Behav Sci. 71:57-66.

Martrenchar, A., D. Huonnic, J. P. Cotte, E. Boilletot, and J. P. Morisse. 1999b. Influence of stocking density on behavioral, health and productivity traits of turkeys in large flocks. Br. Poult. Sci.. 40:323-331.

Martrenchar, A. 1999a. Animal welfare and intensive production of turkey broilers. World's Poultry Sci. J. 55: 149-152.

Mason, G., and M. Mendl. 1993. Why is there no simple way of measuring animal welfare? Anim. Welfare. 2: 301-319.

McCracken, L., N. Waran, S. Mitchinson, and C. B. Johnson. 2010. Effect of age at castration on behavioral response to subsequent tail docking in lambs. Vet. Anaest. Analg. 37: 375-381.

McInerney, J. 2004. Animal Welfare, Economics and Policy. Defra, London, UK. (w-p: https://statistics.defra.gov.uk/esg/reports/animalwelfare.pdf).

Meagher, R. K. 2009. Observer ratings: Validity and value as a tool for animal welfare research. Appl. Anim. Behav. Sci. 119: 1–14.

Mellor, D. J. and L. Murray. 1989. Effects of tail docking and castration on behavior and plasma cortisol concentrations in young lambs. Res. Vet. Sci. 46: 387-391.

Meluzzi, A., F. Sirri, C. Castellini, A. Roncarati, P. Melotti, and A. Franchini. 2009. Influence of genotype and feeding on chemical composition of organic chicken meat. Italian J. Anim. Sci. 8:766-768.

Mench, J.A. 2003. Assessing animal welfare at the farm and group level: A United States Perspective. Anim. Welfare. 12: 493-503.

Mirabito, L., L. Andre, and I. Bouvarel. 2003. Effect of providing 'whole wheat' in the diet on pecking behavior in turkeys. Br. Poult. Sci. 44:776-778.

Mitchell, M. A. and P. J. Kettlewell. 1998. Physiological stress and welfare of broiler chickens in transit: Solutions not problems! Poultry Sci. 77:1803-1814.

Moinard, C., P. D. Lewis, G. C. Perry, and C. M. Sherwin. 2001. The effects of light intensity and light source on injuries due to pecking of male domestic turkeys (meleagrisgallopavo). Anim. Welfare 10: 131-139.

Moinard,C. and C. M. Sherwin. 1999. Turkeys prefer fluorescent light with supplementary ultraviolet radiation. Appl. Anim. Behav. Sci. 64:261-267.

Molony, V., and G. N. Wood. 1992. Acute pain from castration and tail docking of lambs. In: C. E. Short and A. van Poznak (Ed.) Animal Pain. pp 385−395. Churchill Livingstone, New York.

Molony, V., J. E. Kent, S. M. Fleetwood-Walker, F. Munro, and R. M. C. Parker. 1993. Effects of xylazine and L659874 on behavior of lambs after tail docking. Proc. 7th IASP World Cong. On Pain, Paris, p 80.

Molony, V. and J. E. Kent. 1997. Assessment of acute pain in farm animals using behavioral and physiological measurements. J. Anim. Sci. 75: 266-272.

Molony, V. and Kent, J.E. 1997. Assessment of acute pain in farm animals using behavioral and physiological measurements. Journal of Animal Science 75: 266-272. Written paper of presentation given by V. Molony at 87th Annual meeting of the American Society of Animal Science, Florida, USA, July 1995.

Molony, V., J. E. Kent, and I. J. McKendrick. 2002. Validation of a method for assessment of an acute pain in lambs. Appl. Anim. Behav. Sci. 76: 215-238.

Morris, D. G., T. R. Kuchel, and S. Maddocks. 1994. Stress responses in lambs to different tail docking methods. Proc. Aust. Soc. Anim. Prod. 20: 202–205.

Newberry, R. C. and J. W. Hall. 1990. Use of pen space by broiler chickens: Effects of age and pen size. Appl. Anim. Behav. Sci. 25: 125-136.

Newberry, R. C., and R. Blair. 1993. Behavioral responses of broiler chickens to handling: effects of dietary tryptophan and two lighting regimes. Poult. Sci. 72:1237-1244

Nicol, C. J. 1995. The Social Transmission of Information and Behavior. Appl. Anim. Behav. Sci. 44: 79–98.

Niemelä, P. T., N. DiRienzo, A. V. and Hedrick. 2012. Predator-induced changes in the boldness of naive field crickets, Gryllus integer, depends on behavioral type. Anim. Behav. 84: 129-135.

Nijs, J., E. Kosek, J. Van Oosterwijck, and M. Meeus. 2012. Dysfunctional endogenous analgesia during exercise in patients with chronic pain: to exercise or not to exercise? Pain Physician 15: 205-2013.

Nolan, A. M. 2000. Patterns and management of pain in animals. In: Morton, D. (Ed.), Pain: its Nature and Management in Man and Animals, International Congress and Symposium Series. Lord Soulsby of Swaffham Prior, vol. 246. Royal Society of Medicine Press, p. 93–100.

Office International des Épizooties (OIE). 2008. 2nd OIE Global Conference on Animal Welfare. Cairo, Egypt (pp. 20-22). October. Retrieved June 12, 2009, from www.oie.int/eng/A_AW2008/ANG_Final%20Recommendations.pdf

Ofner, E., T. Amon, M. Lins, and B. Amon. 2003. Correlation between the results of animal welfare assessments by the TGI 35 L Austrian Animal Needs Index and health and behavioral parameters of cattle. Anim. Welfare. 12: 517-578.

OIE (Office International des Epizooties), 2011. Terrestrial Animal Health Code. Available from http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.7.1.htm

Pagel, M. and M. S. Dawkins. 1997. Peck orders and group size in laying hens: 'futures contracts' for non-aggression. Behav. Process. 40: 13-25.

Parrott R. F., K. A. Houpt, and B. H. Misson. 1988. Modification of the responses of sheep to isolation stress by the use of mirror panels. Appl. Anim. Behav. Sci. 19: 331-338.

Petracci, M., M. Bianchi, C. Cavani, P. Gaspari, and A. Lavazza. 2006. Preslaughter mortality in broiler chickens, turkeys and spent hens under commercial slaughtering. Poult. Sci. 85:1660- 1664.

Pettit-Riley, R. and I. Estevez. 2001. Effects of density on perching behavior of broiler chickens. Appl. Anim. Behav. Sci. 71: 127-140.

Pinto-Ribeiro, F., A. Almeida, J. M. Pêgo, J. Cerqueira, and N. Sousa. 2004. Chronic unpredictable stress inhibits nociception in male rats. Neurosci. Lett. 359: 73-76.

Porter, R., R. Nowak R, and P. Orgeur. 1995. Influence of a conspecific agemate on distress bleating by lambs. Appl. Anim. Behav. Sci. 45: 239-244.

Prescott, N. B., P. S. Berry, S. Haslam, and D. B. Tinker. 2000. Catching and crating turkeys: Effects on carcass damage, heart rate, and other welfare parameters. J.Appl. Poultry Res. 9:424-432.

Puron, D., R. Santamaria, J. C. Segura, and J. L. Alamilla. 1995. Broiler performance at different stocking densities. J. Appl. Poult. Res. 4: 55–60.

Ratajczak-Enselme, M., J. P. Estebe, F. X. Rose, E. Wodey, J. M. Malinovsky, F. Chevanne, G. Dollo, C. Eccofey, and P. Le Corre. 2007. Effect of epinephrine on epidural, intrathecal, and plasma pharmacokinetics of ropivacaine and bupivacaine in sheep. Brit. J.Anaesth. 99: 881-890.

Rigdon, R. H., T. M. Ferguson, and J. R. Couch. 1960. Pendulous crops in turkeys-an anatomic and pathologic study. Am J Vet Res. 21:979-986.

Rivat, C., E. Laboureyras, J. -P Laulin, C. Le Roy, P. Richebé, and G. Simonnet. 2007. Non-nociceptive environmental stress induces hyperalgesia, not analgesia, in pain and opioid-experienced rats. Neuropsychopharmacology 32: 2217-2228.

Rousing, M. T., M. Bonde, and J. T. Sorensen. 2001. Aggregating welfare indicators into an operational welfare assessment system: A bottom-up approach. Acta Agr Scand A. 51:53-57.

Rushen, J. and A. M. de Passillé. 2009. The scientific basis of animal welfare indicators. In: Smulders FJM and Algers B (eds) The Assessment and Management of Risks for the Welfare of Production Animals. Food Safety Assurance and Veterinary Public Health, Volume 5. Wageningen Academic Press: Wageningen, The Netherlands

Rutherford, K. M. D. 2002. Assessing pain in animals. Anim. Welfare 11: 31-53.

Sanchez, C., and Estevez, I., 1998. The Chickitizer v.4. University of Maryland,College Park, MD, USA.

Sanotra, G. S., C. Berg, and J. D. A. Lund. 2003. A comparison between leg problems in Danish and Swedish broiler production. Anim. Welfare 12:677-683.

SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513.

Scott, K., Binnendijk, G. P., S. A. Edwards, J. H. Guy, M. C. Kiezebrink, and H. M. Vermeer. 2009. Preliminary evaluation of a prototype welfare monitoring system for sows and piglets (Welfare Quality® project). Anim. Welfare. 18: 441-449.

Sejian, V. 2007. Measurment of animal welfare. In: The short course manual on animal welfare and behavior Srivastava R. S., S. Bag, B. C. Das, and V. P. Varshney, (Eds.) IVRI., Izatnagar, pp:57-60.

Sejian, V., J. Lakritz, T. Ezeji and, R. Lal. 2011. Assessment methods and indicators of animal welfare. Asian J. Anim. Vet. Adv. 6: 301 - 315.

Sherwin, C. M. and A. Kelland. 1998. Time-budgets, comfort behaviors and injurious pecking of turkeys housed in pairs. Br. Poult. Sci. 39:325-332.

Shutt, D. A., L. R. Fell, R. Connell, A. K. Bell, C. A. Wallace, and A. I. Smith. 1987. Stress-induced changes in plasma concentrations of immunoreactive beta-endorphin and cortisol in response to routine surgical procedures in lambs. Aust. J. Biol. Sci. 40: 97-103.

Sørensen, P., G. Su, and S. C. Kestin. 2000. Effects of Age and Stocking Density on Leg Weakness in Broiler Chickens. Poultry Sci. 79:864-870.

Stein, M. L. 1989. Asymptotic distributions of minimum norm quadratic estimators of the covariance function of a Gaussian random field. Ann. Stat. 17:980 -1000.

St-Hilaire, S., S. Arellano, and C. S. Ribble. 2003. Association between cellulitis (enlarged sternal bursa) and focal ulcerative dermatitis in Ontario turkeys at the time of processing. Avian Dis. 47:531–536.

Sutherland, M. A., and C. Tucker. 2011. The long and short of it: a review of tail docking in farm animals. Appl. Anim. Behav. Sci. 135: 179-191.

Szechtman, H., P.G. Larnbrou, A.R. Caggiula and E.S. Redgate. 1974. Plasma eortieosterone levels during sexual behavior in male rats. Horrn. Behav. 5: 191.

Tannenbaum, J. 1991. Ethics and animal welfare: The inextricable connection. JAVMA, 198 (8): 1360-1376.

Terlouw, E.M.C., A.B. Lawrence, J. Ladewig, , A.M.B. de Passillé, J. Rushen, and W. Schouten. 1991. A relationship between stereotypies and cortisol in sows. Behav. Process. 25:133-153.

Thompson, R. K. R., and M. Liebreich. 1987. Adult chicken alarm calls enhance tonic immobility in chicks. Behav. Process. 14:49-61.

Toates F., and P. Jensen 1991. Ethological and psychological models of motivation - towards a synthesis. In: International conference on simulation of adaptive behavior (Ed. by Meyer, J. A. and Wilson, S. W.): MIT Press: Cambridge.

Underwood, W. J. 2002. Pain and distress in agricultural animals. Journal of the Am. Vet. Med. Assoc. 221: 208–211.

Vasseur, E., J. Rushen, D. B. Haley, and A. M. de Passillé. 2012. Sampling cows to assess lying time for on-farm animal welfare assessment. J Dairy Sci. 95:4968-77.

Ventura, B. A., F. Siewerdt, and I. Estevez. 2012. Access to barrier perches improves behavior repertoire in broilers. PLoS ONE 7, Article number: e29826.

Walker, A. 2012. Leg health in broilers. (accessed on the 11th of February 2013); http://www.agrowebcee.net/fileadmin/user_upload/faw/doc/reports2/REVIEW_ON_WELFARE_OF_BROILERS_01.pdf

Webb-Ware, J. K., A. Vizard, and G. R. Lean. 2000. Effects of tail amputation and treatment with and albendazole controlled-release capsule on the health and productivity of prime lambs. Aust. Vet. J. 78: 838-842.

Weber, R. E. F., and A. V. Zarate. 2005. Welfare in farm animal husbandry – current definitions and concepts as basis for practical oriented research with focus on fattening pig husbandry. Arch. Tierzucht. 48: 475-489.

Webster, J. 1994. Animal Welfare - A cool eye towards eden. Blackwell Science, Oxford, UK.

Webster, J. 2005. Animal Welfare: Limping towards Eden. Blackwell Publishing Limited.

Webster, A. J. F. 2009. The virtuous bicycle: a delivery vehicle for improved farm animal welfare. Anim. Welfare. 18: 141-147.

Webster, B., B. D. Fairchild, T. S. Cummings, and P. Stayer. 2008. Validation of a Three-Point Gait-Scoring System for Field Assessment of Walking Ability of Commercial Broilers. J. Appl. Poultry Res. 17:529-539.

Welfare Quality®. 2009. Welfare Quality® assessment protocol for poultry (broilers, laying hens). Welfare Quality® Consortium, Lelystad, The Netherlands.

Welfare Quality®. 2009. Welfare Quality® assessment protocol for poultry (broilers, laying hens). Welfare Quality® Consortium, Lelystad, The Netherlands.

Welsh, E. M., and A. M. Nolan. 1995. Effect of flunixin meglumine on the thresholds to mechanical stimulation in healthy and lame sheep. Res. Vet. Sci. 58: 61-66.

Whay, H. R., D. C. J. Main, L. E. Green, and A. J. F. Webster. 2003. Animal-based measures for the assessment of welfare state of dairy cattle, pigs and laying hens: consensus of expert opinion. Anim. Welfare. 12:205-217.

Wichman, A., M. Norring, M. Pastell, B. Algers, R. Pösö, A. Valros, H. Saloniemi, and L. Hänninen. 2010. Effect of crate height during short-term confinement on the welfare and behavior of turkeys. Appl. Anim. Behav. Sci. 126:134-139.

Wiseman-Orr, M. L., E. M. Scott, and A. M. Nolan. 2011. Development and testing of a novel instrument to measure health-related quality of life (HRQL) of farmed pigs and promote welfare enhancement (Part 2). Anim. Welfare. 20: 549–558.

World Organisation for Animal Health (OIE), Terrestrial Animal Health Code, 12th Ed. OIE, Paris (2003).

Yang, Z., and B. Rannala. 2012. Molecular phylogenetics: Principles and practice. Nat. Rev. Genet. 13:303-314.

Zulkifli, I., and A. Siti Nor Azah. 2004. Fear and stress reactions, and the performance of commercial broiler chickens subjected to regular pleasant and unpleasant contacts with human being. Appl Anim Behav Sci. 88:77-87.