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Neila Ben Sassi

Broiler on-farm welfare assessment: Insights on the applicability of the transect method

Departamento
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Tesis Doctoral

BROILER ON-FARM WELFARE ASSESSMENT: INSIGHTS ON THE APPLICABILITY OF THE TRANSECT METHOD

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Neiker Tecnalia, Instituto Vasco de Investigación y Desarrollo Agrario

Departamento de Producción Animal

Universidad de Zaragoza

Facultad de Veterinaria

Departamento de Producción Animal y Ciencia de los Alimentos

Broiler on-farm welfare assessment: Insights on the applicability of the transect method

Neila Ben Sassi

Under the supervision of: Prof. Inma Estevez

Dr. Xavier Avéros

Broiler on-farm welfare assessment: Insights on the applicability of the transect method

Enfoque sobre la aplicabilidad del método transecto en condiciones comerciales del pollo de engorde

Doctoral thesis presented by

Neila Ben Sassi

Directed by Prof. Inma Estevez (Ikerbasque Research Professor, Neiker-Tecnalia)

Co-directed by Dr. Xavier Avéros (Neiker-Tecnalia)

In order to obtain the doctoral degree within the

Doctorate Program "Animal Production"

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Zaragoza, April 2019

Neiker Tecnalia, Instituto Vasco de Investigación y Desarrollo Agrario
Departamento de Producción Animal

Vitoria-Gasteiz, 27 de Marzo del 2019

Prof. Inma Estevez, Investigadora Principal (Ikerbasque, Basque Foundation for Science y Neiker-Tecnalia) y **Dr. Xavier Averós**, Investigador Colaborador (Neiker-Tecnalia),

Informan:

Que la presente memoria de Tesis titulada **"Broiler on-farm welfare assessment: Insights on the applicability of the transect method"**, que se corresponde con el proyecto de Tesis Doctoral aprobado el 16 de febrero del 2016, y de la que es autora **Neila Ben Sassi,** ha sido realizada bajo su dirección y cumple con las condiciones exigidas para ser presentada y defendida como Tesis Doctoral.

Fdo. Prof. Inma Estevez

Fdo. Dr. Xavier Averós

Neiker Tecnalia, Instituto Vasco de Investigación y Desarrollo Agrario
Departamento de Producción Animal

Vitoria-Gasteiz, 27 de Marzo del 2019

Prof. Inma Estevez, Investigadora Principal (Ikerbasque, Basque Foundation for Science y Neiker-Tecnalia) y **Dr. Xavier Averós**, Investigador Colaborador (Neiker-Tecnalia) certifican:

Que la presente memoria de Tesis titulada **"Broiler on-farm welfare assessment: Insights on the applicability of the transect method"**, de la que es autora **Neila Ben Sassi,** ha sido realizada bajo su dirección.

Que Neila Ben Sassi ha cursado una estancia como investigadora en el Departamento de Ciencias Animales y Acuacultura de la Universidad Noruega de las Ciencias de la Vida del 16 de enero al 15 de mayo del 2017, y del 1 de marzo al 17 de mayo del 2018. Dichas estancias se efectuaron bajo la dirección de la Prof. Ruth C. Newberry.

Que la presente memoria de Tesis ha sido evaluada por dos expertos internacionales, siendo éstos:

- La Dra. Valentina Ferrante (Departamento de Ciencia y Política Ambiental, Universidad de Milano): Doctora por la Universidad degli Studi di Milano, con fecha de expedición del título de doctor en el año 2000.
- La Dra. Joanna Marchewka (Instituto de Genética y Producción Animal, Academia Polaca de Ciencias): Doctora por la Universidad del País Vasco, con fecha de expedición del título de doctor en el año 2015.

Que considerando la memoria de Tesis finalizada, autorizan su presentación en la modalidad de **Mención de Doctorado Internacional** para que sea juzgada por la comisión correspondiente.

Fdo. Prof. Inma Estevez   Fdo. Dr. Xavier Averós

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TABLE OF CONTENT

LIST OF PUBLICATIONS

The present thesis comprises a three Published Scientific Manuscripts together with a fourth manuscript submitted to publication.

BenSassi, N., Across X., Estevez. I. **2016**. Technology and Poultry Welfare. **Animals.** 6: 62-83. doi:10.3390/ani6100062.

BenSassi, N., Averós X., Estevez. I. **2019**. The potential of the transect method for early detection of welfare problems in broiler chickens. **Poultry Science.** . 98: 522–532. doi: https://doi.org/10.3382/ps/pey374.

BenSassi, N., Vas, J., Averós, X., Vasdal, G., Estevez. I, Newberry R. **2019**. On-farm broiler chicken welfare assessment using transect sampling reflects environmental inputs and production outcomes. **Plos One** *(in press)***.**

BenSassi, N., Averós X., Estevez. I. Broiler chickens on-farm welfare assessment: Estimating the soundness of the transect sampling method. **Frontiers in Veterinary Science (***Submitted*).

LIST OF ABREVIATIONS

AWIN	Animal Welfare Indicators
CFD	Computational Fluid Dynamics
C Line	Broiler Cobb genetic line
DOA	Dead on arrival
EU	European Union
FDP	Footpad dermatitis
GIS	Geographic Information System
GS	Gait Score
IRTI	Infrared Thermal Imaging
MCP	Model-based Predictive Controller
OF	Optical Flow
PLF	Precision Livestock Farming
R Line	Broiler Ross genetic line
SHI	Social Hotspot Index
SVM	Support Vector Machine
TCV	Transmission Color Value
UFH	Underfloor Heating
WQ	Welfare Quality
ZOD	Zone Occupation Density

ABSTRACT

Broiler chickens are kept in large flocks of several thousand birds with controlled environmental and management conditions. These conditions tend to deteriorate towards the end of rearing, causing a negative impact on birds´ welfare. To appreciate the magnitude of this impact and rectify it, the use of robust assessment protocols that can be effectively applied under commercial conditions are necessary. The transect method was suggested as a practical assessment tool for the detection of main broiler chicken welfare issues including: leg problems (immobile and lame), illness (sick and terminally ill), wounded (head, back and tail wounds), small, dirty, featherless and dead birds. The assessment is conducted by slowly walking on transects, defined as the path delimited by feeder and drinker lines within a production house. Assessments are conducted by clicking on the evaluation screen of the i-WatchBroiler mobile application as the observer detects welfare issues along the conducted transect. Previous results showed the reliability of the transect method in commercial turkey flocks. Although this new assessment method seems to be effective, research is still needed to further test the transect method under different experimental conditions. The aim of the present Doctoral Thesis was to investigate the practical applicability and soundness of the transect method for on-farm broiler chicken welfare assessment.

In the first Chapter of this Thesis, the opportunities offered by precision livestock farming tools to control, measure and/or improve poultry welfare are reviewed. Technology tools presented in this chapter may indicate welfare, health and management problems. These include sensors for farm environmental monitoring, bird movement or physiological parameters, imaging technologies to detect gait problems and feather pecking, as well as infrared technologies to evaluate birds' thermoregulatory and metabolism changes. Easy data collection and data analysis offered by the use of mobile applications also open new opportunities for easy animal welfare monitoring.

In the second Chapter, the results of investigating the potential of the transect method to detect the effects of bird genetic line, litter quality and transect position (wall vs central), and how these effects change with bird age are presented. A deterioration of most welfare indicators was detected with age. A higher incidence of dirty birds was found with worse litter quality, while higher incidences of immobile, small, sick, dirty and dead were observed next to walls as compared to central transects. An association

between measured on-farm welfare indicators and production outcomes was also established, with incidences of flock illness and mortality being associated with increased dead on arrival at slaughter.

The third Chapter focuses on the effects of increased environmental complexity on the incidence of welfare indicators collected using the transect method and on production outcomes. Environmental complexity referred to the number of provided environmental enrichment types (peat moss, wood shaving bales and boxes). Results showed a decreasing incidence of wounds, welfare problems index (sum of all welfare indictors), as well as of mortality rate, rejections due to wounds, and underweight birds with increased environmental complexity. In the same study, a lower incidence of leg problems and welfare problems index was detected with increased space allowance along with fewer rejections due to wounds and a higher growth rate. Intermittent 16h lighting regimen was associated with lower incidences of illness, wounds and mortality. Similarly to the results of Chapter 2, associations between on-farm welfare indicators and production outcomes were found. Incidence of leg problems, illness, small birds and welfare problems index were positively correlated with rejections due to illness, underweight and total rejections.

The fourth Chapter aimed at evaluating the soundness of the transect method using a capture-recapture approach of a subpopulation of (80 individually marked) birds under commercial conditions. Two observers conducted 4 samplings/house/day, collecting the position of marked birds. The detection and repetition rates per house and transect were estimated, and the effect of flock density, transect number/house (6 vs 8) and sampling time (morning vs afternoon) determined. On average, two-thirds of the marked subpopulation were detected with 23.85% repetition rate when assessing the entire house and 1.66% of repetition per transect. We showed minimum repetition rates if three transects are left between the two assessed. Differences in the distribution index indicate that a random distribution of birds should not be assumed and hence both central and wall transects should be assessed. A representative assessment could be obtained by sampling only two transects according to bootstrapping results of transect collected data.

The results of these studies indicate that the transect method provides logical outcomes when testing the association between incidences of welfare indicators and farm environmental and management conditions. Our results suggest that a sound welfare assessments can be conducted by sampling two transects, one wall and one central, separated by three transects in between. The results obtained in the different studies of this Doctoral Thesis support the validity of the transect method to be used as a practical and sound on-farm welfare assessment method for commercial broiler chicken flocks.

RESUMEN

Los pollos de engorde se crían en grupos de miles de aves, bajo condiciones ambientales y de manejo controladas. Sin embargo, el entorno puede deteriorarse rápidamente, especialmente hacia el final del periodo de cría, ocasionando problemas de bienestar. La posibilidad de realizar una evaluación efectiva del bienestar del pollo de engorde en condiciones de cría comercial presenta desafíos importantes debido al gran número de animales por lote. El método de los transectos ha sido sugerido como una herramienta práctica para la detección de los principales problemas de bienestar, incluyendo problemas de patas (inmóviles y cojos), enfermedades (enfermos y terminales), heridas (en cabeza, espalda y/o cola), aves pequeñas, sucias desplumadas y muertas. Las evaluaciones se realizan desplazándose a lo largo de los denominados transectos que se definen como el área delimitada entre líneas de comederos y bebederos o por la pared. Las evaluaciones se realizan utilizando la pantalla de evaluación de la aplicación móvil i-WatchBroiler a medida que el observador detecta problemas de bienestar a lo largo del recorrido del transecto. Resultados de estudios anteriores han demostrado la fiabilidad del método de transectos en lotes de pavos comerciales. Aunque este método de evaluación parece ser efectivo, es necesario realizar trabajos adicionales para probar el método de los transecto en diferentes condiciones experimentales. Por lo tanto, el objetivo de la presente Tesis Doctoral es investigar la aplicabilidad práctica y la solidez del método de los transectos para la evaluación del bienestar de pollos de engorde en granjas comerciales.

El primer Capítulo de esta Tesis consiste en una revisión bibliográfica de las herramientas de ganadería de precisión utilizadas para controlar aspectos que afectan directamente al bienestar de las aves de producción, y del pollo de carne en particular. Las herramientas tecnológicas presentadas pueden indicar problemas de bienestar, salud y gestión. Estos incluyen sensores para monitoreo ambiental de la granja, movimiento de aves o parámetros fisiológicos; tecnologías de imágenes para detectar problemas de la marcha y picoteo de las plumas y tecnologías de infrarrojos para evaluar los cambios en la termorregulación y el metabolismo de las aves. Las oportunidades para una fácil recopilación y análisis de datos que ofrece el uso de las aplicaciones también abren nuevas oportunidades para un monitoreo fiable y eficiente del bienestar animal.

En el segundo Capítulo, se presentan los resultados sobre la capacidad del método de los transectos para detectar los efectos de las distintas líneas genéticas del pollo de engorde, la calidad de cama y la posición de los transecto (pared vs centro), y de cómo estos efectos cambian con la edad. Se detectó un claro deterioro de la mayoría de los indicadores de bienestar con la edad de las aves. Una peor calidad de cama resultó en una mayor frecuencia de aves sucias, mientras que en los transectos de pared se observaron frecuencias más elevadas de pollos inmóviles, pequeños, enfermos, sucios y muertos. También se detectó una asociación entre el deterioro de los indicadores de bienestar y los resultados de producción recogidos en matadero. El aumento en la incidencia de enfermos y de la mortalidad en granja se asoció con un aumento de muertos a la llegada al matadero.

El tercer Capítulo se centra en los efectos de incrementar la complejidad ambiental mediante la aplicación de estrategias de enriquecimiento ambiental sobre la incidencia de los indicadores de bienestar recopilados utilizando el método de los transectos y en los resultados de producción. La complejidad ambiental se refiere al número de tipos de enriquecimiento ambiental proporcionados (turba, balas de viruta de madera y cajas). Los resultados mostraron una incidencia decreciente de heridas, índice de problemas de bienestar (suma de todos los indicadores de bienestar), tasa de mortalidad, rechazos por heridas y aves pequeñas con mayor complejidad ambiental. En el mismo estudio, también se detectó una menor incidencia de problemas de patas, un menor índice de problemas de bienestar, menos rechazos debido a heridas, y una mayor tasa de crecimiento con el incremento del espacio disponible. La iluminación intermitente de 16h se relacionó con una menor incidencia de aves enfermas, heridas, y mortalidad. De manera similar a los resultados del Capítulo 2, se encontraron claras asociaciones entre los indicadores de bienestar obtenidos en granja y los resultados productivos. El aumento en la incidencia de problemas de patas, enfermedades, aves pequeñas y el índice de problemas de bienestar se asoció a un aumento en los rechazos por enfermedades, peso bajo y de rechazos totales.

El cuarto Capítulo tuvo como objetivo evaluar la solidez del método de los transectos mediante la técnica de captura-recaptura de una subpoblación (80 individuos marcados individualmente) conocida de pollos de carne en condiciones comerciales. Dos observadores realizaron 4 muestreos/nave/día, recogiendo la posición de las aves detectadas. Se estimaron las tasas de detección y repetición de individuos por nave y transecto, y se determinó el efecto de la densidad, del número de transecto /nave (6 vs 8) y del momento de muestreo (mañana vs tarde). En promedio, se detectaron dos tercios de la subpoblación marcada con un 23.85% de tasa de repetición al evaluar toda la nave y 1.66% de repetición por transecto. Además se ha demostrado que se obtiene una tasa de repetición mínima si los dos transectos evaluados están separados por tres transectos. Las diferencias en el índice de distribución indican que las aves no distribuyen al azar, y por lo tanto, se deben evaluar tanto los transectos centrales como los de pared. Una evaluación representativa se puede obtener muestreando solo dos transectos de la nave de acuerdo con los resultados de bootstrapping de los datos de transectos.

Por tanto se puede concluir que los resultados obtenidos mediante la aplicación del método de los transectos en la evaluación del pollo de engorde serían los esperables al probar la asociación entre la incidencia de los indicadores de bienestar y las condiciones ambientales y de manejo de la granja. Los resultados sugieren que se pueden realizar evaluaciones sólidas de bienestar mediante el muestreo de dos transectos, siendo estos uno central y otro de pared, separados por tres transectos intermedios. Los resultados obtenidos en los diferentes estudios de esta Tesis Doctoral respaldan la validez del método de los transectos que se puede utilizar como un método práctico y sólido de evaluación del bienestar en las granjas comerciales del pollo de engorde.

GENERAL INTRODUCTION

After World War II, Europe faced the challenge of increasing food production in order to satisfy the population needs. Intensive animal production systems that aimed to maximize production outputs while minimizing production costs were since then developed. Chickens were one of the most targeted species for intensive production due to the low labour requirements and rearing costs as compared to other livestock species. With the progress in scientific knowledge, the development of technologies and housing systems with controlled environments, an exponential increment of chicken meat production took place. World chicken meat production increased from 6577 to 66566 million heads between 1961 and 2017 (FAO, 2019). World production was enhanced by genetic selection leading to nowadays most widely used meat chickens line at the global market, the broiler chickens. However, the tremendous increment in production raised important questions related to the farming impact, from the environmental and economic perspective but also at the societal level. The genetic selection for growth rate of broiler chickens and farm management conditions are thought to be the cause of severe health and welfare problems. Increased concerns related to farming practices and its consequences on animal suffering started to emerge from the second half of the twentieth century (Fraser, 2005).

1. Animal welfare

Due to increased societal concerns, livestock production evolved from the necessity of producing in quantity, to the new challenges of the production required in the XXI century. Such challenges include considering the environmental impact of the production systems, the product quality and its impact on human health, and the incorporation of socio-cultural and ethical values (Thornthon, 2010). This last challenge requires the consideration of animal welfare in response to the concerns about animal suffering through the production process. One of the first scientific approaches to animal welfare was developed in the Brambell report (1965; revised by FAWC 1993) which recommended taking in consideration Five Freedoms including: the freedom from thirst, hunger and malnutrition, the freedom from discomfort, the freedom from pain, injury and disease, the freedom to express normal behaviour and the freedom from fear and distress.

A wide range of definitions for animal welfare were developed by scientists and global organizations such as the World Organization for Animal Health which defined animal welfare 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 behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter/killing. Animal welfare refers to the state of the animal. The treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment“ (OIE, 2018; Terrestrial Animal Health Code). The concept of animals coping with its environment involves adapting itself to the challenges the environment may require at an anatomical, physiological, and behavioural level (Broom, 1996). This is important, not only for an ethical point of view, but also for the economic viability of the production system (Broom, 2017). The consideration of animal welfare at the global and farm levels was one of the attributes of the twenty-first century livestock production, in addition to the persisting problems related to the production of food for a growing population, dealing with hunger and poverty in the third-world area and climate change (Pretty, 2008).

1.1. Relevance of considering animal welfare

1.1.1. Animal welfare: An essential component of sustainability

1.1.1.1. Definition of sustainable agriculture

Sustainable development was defined in the Brundtland Report (1987) as “A development that meets the needs of the present without compromising the ability of future generations to meet their own needs”. This includes environmental, economic, ethical and social aspects, and their interactions. Sustainable agriculture involves producing more food for a growing global population, while protecting the environment and reducing or preventing contributions to climate change (Pretty, 2008). With an expected increase of the human population to 9700 million by 2050 (UN, 2015), livestock farming is facing the difficult challenges of reducing environmental footprint and maintaining good profitability, while assuring animal welfare.

According to Chemineau (2016), the three main pillars of agriculture sustainability are:

- The economic pillar: a livestock production system should be economically viable and financially autonomous. This should not only be achieved through increasing the prices of final products, but by controlling the expenses at the farm level.
- The environmental pillar: encompasses the environmental footprints of farming such as greenhouse gas emissions and carbon storage in the soil.
- The social pillar: defined as the ethical quality of the final product related to the farmer working conditions, the use of locally produced food, the rationalized use of antibiotics, and the consideration of animal welfare (i.e. the way the animal was treated during the production and slaughter phases).

The ethical treatment of farmed animals is further mentioned in studies on sustainable farming where it is stated that an animal production system is considered sustainable “if it is acceptable now and if its expected future effects are acceptable, in particular in relation to resource availability, consequences of functioning and morality of actions” (Broom, 2017). This definition emphasizes the multidimensional attributes of current livestock production especially the consideration of animal welfare due to its growing impact on sustainability.

1.1.1.2. Animal welfare and sustainability

Animal welfare was shown to impact the three main pillars of agriculture sustainability. In a case study of cattle farming, the interconnection between farm incomes, carbon footprints and animal welfare was demonstrated (Galioto et al., 2017, Figure 1). While poor animal welfare was shown to result in decreased farm economic efficiency and profitability (Galioto et al., 2017), improving animal welfare results in reduced mortality, improved resistance to disease, reduced use of medication, and improved product quality, therefore leading to financial benefits (Dawkins, 2017). In the study by Galioto et al. (2017), poor animal welfare and increased gas emissions resulted in reduced marginal incomes (Figure 2).

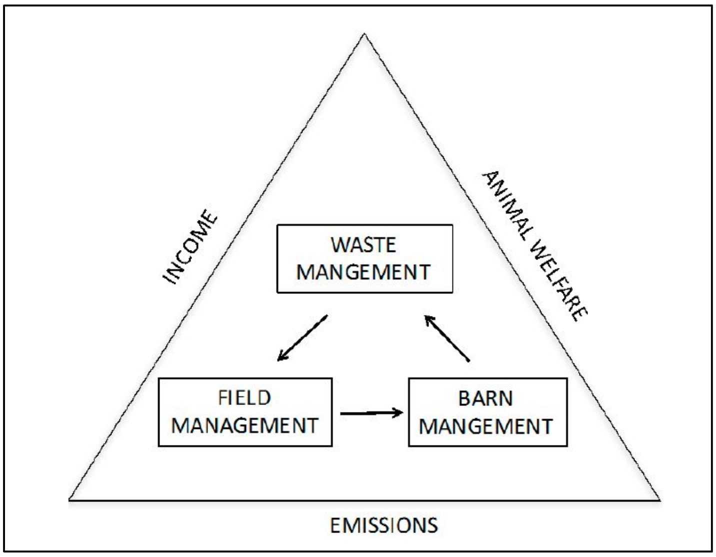

Figure 1: Interconnection between the main production features of livestock farming (adapted from Galioto et al., 2017).

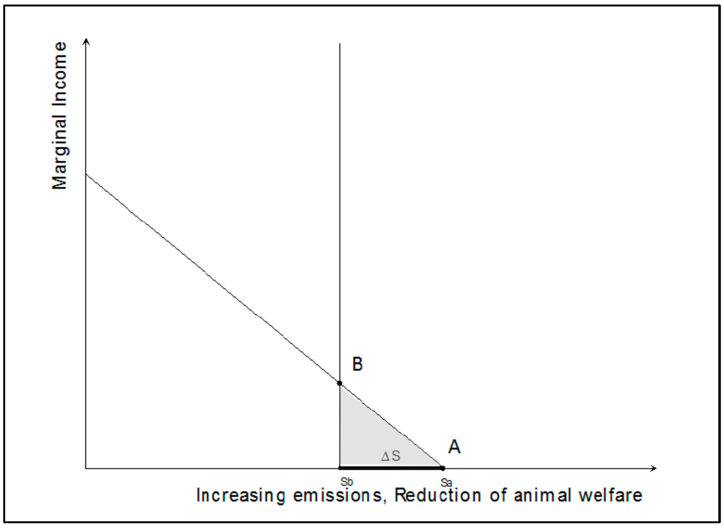

Figure 2: A case study of the marginal income trends with respect to increasing emissions and reduction in animal welfare (adapted from Galioto et al., 2017). The shaded portion corresponds to an improvement in farm profitability noted when moving from point A to B which is in favour of an improved state of animal welfare or a reduction in emissions represented as ΔS)

In broiler chickens, leg problems is one of the main welfare issues limiting birds' mobility and access to feed and water in addition to the pain caused to the animal (Bessei, 2006). Economic losses due to leg problems were estimated to reach 120 $ million per year in the US (Cook, 2000). In Swedish farms, a 10% increase in the prevalence of broiler chickens lameness resulted in a 1% decline in net returns per kilogram produced (Gocsik et al., 2017). These authors evaluated the economic gains derived from reducing lameness through modifications in routine management practices. They studied the impact of longer dark periods, decreased stocking densities, and different types of feeding strategies (meal feeding, restricted feeding, sequential feeding and feeding whole wheat). In addition to an increase in the gross margin and net return to management with the majority of these practices, they demonstrated that even though feed conversion rate may be less efficient with whole wheat feeding, its cost was outweighed by a lower prevalence of lameness, mortalities and feed costs (Gocsik et al., 2017). These results show that improved animal welfare can have wide implications in the system efficiency and in the economic profitability of broiler chickens farms.

From an environmental point of view, standard broiler chicken production practices were shown to have the lowest environmental impact in comparison to other meat production systems (de Vries and de Boer, 2010; Rodić et al., 2011). However, manure and waste management (both at farm and slaughter levels) is still an important environmental issue in broiler chicken production (Rodić et al., 2011). A study estimating the environmental impact of producing 1 kg of broiler carcass weight showed that the chicken rearing was the phase where most impact was observed especially due to food production and processing (Cesari et al., 2017). The high broiler chicken stocking densities used in current production, and the consequent deterioration of environmental quality is one of the main sources of poor welfare (Estevez, 2007) and mortality (Bessei, 2006), but was shown to have a low impact on global warming indicators in comparison with food production (Cesari et al., 2017).

Few studies have estimated the social impact of poor animal welfare. Tallentire et al. (2018) suggested a methodology to incorporate animal welfare indicators within the social life cycle assessment of broiler chicken meat, and therefore, its social impact on sustainability. This method assesses the social and sociological aspects of products, along with their actual and potential positive and negative impacts during its life cycle (UNEP-SETAC, 2009). It generates a Social Hotspot database, with the Social Hotspot Index (SHI), an indicator of the social risks of each product. Tallentire et al., (2018) estimated the quantitative risk of broiler chickens welfare indicator on the variation of SHI. It was shown that SHI was higher in countries where the flock size was very high. Common measures of broiler chicken welfare were reported to be easily incorporated into the estimation of SHI to better understand the impact of animal welfare on the society (Tallentire et al., 2018). Given the economic, environmental and social impact of animal welfare highlighted in these studies, its incorporation as a component of the future global sustainability goals was considered.

Recently, animal welfare was integrated in the “Proposed draft recommendation on sustainable agricultural development for food security and nutrition“, published by The United Nations’ Committee on World Food Security (FAO, 2016). This report stated the importance to “improve animal welfare delivering on the five freedoms and related OIE standards and principles, including through capacity building programs, and supporting voluntary actions in the livestock sector to improve animal welfare“. FAWC (2017) stated that “agriculture cannot be considered sustainable if it is achieved at an unacceptable cost to animal welfare” and that “sustainable agriculture must include a duty of care for the physical and mental needs and natures of farmed animals, and must not have a dependency or prolonged or routine use of pharmaceuticals or on mutilations”. Including animal welfare within the global goals of sustainable agricultural policy formally identifies animal welfare as an independent component of future global agriculture and economy. However, due to the increasing market demands of livestock products, the implications of higher production could imply high pressure on the welfare of intensively farmed animals (Pretty, 2008). To this end, the use of technology and environmental modifications during the rearing period might facilitate the management of increasing animal populations at farm level.

1.2. Improving animal welfare to enhance farm sustainability

The use of technology to control animal environment could be considered one of the major and easiest ways to enhance animal welfare (Galioto et al., 2017). The use of technology, and specifically of precision livestock farming (PLF), was shown to be efficient in maintaining and improving welfare by controlling farm environmental and management conditions (Berckmans, 2014). Enhancing farm sustainability might also be reached by introducing environmental enrichments which was shown to improve animal welfare at the experimental (Cornetto and Estevez, 2001; Ventura et al., 2010) and, in specific cases, the commercial levels (Corkery et al., 2013). In the bibliographic review by Estevez and Newberry (2017), the contribution of environmental enrichment to sustainable poultry productions was discussed by showing its use and applicability in different poultry species. Riber et al., (2017) reported efficient effects of elevated resting places and cover panels in broiler breeders. They also presented a literature review of all types of environmental enrichment used in broiler chickens (Riber et al., 2018).

1.2.1. Precision livestock farming and mobile tools

Technology tools have been used in livestock farming since the last century, mainly to register management data. The first massive application of technology was available in dairy farms with the individual electronic milk meter, followed by commercialized oestrus detection devices, rumination tags and an online, real-time milk analyser (Halachmi and Guarino, 2016). In broiler chickens, the first use of technology at the farm level occurred early this century (Corkery et al., 2013). Application of technologies for farm management is nowadays referred to as PLF. PLF is defined as the management of livestock production using the principles and technologies of process engineering (Berckmans, 2014). Data from diverse sources are collected through sensors, sound and imaging devices, and analysed to create an automatic management system (Berckmans, 2014). In dairy cattle for example, image processing was used to automatically detect early stages of lameness that would permit the application of mitigation strategies reducing animal suffering and associated economic losses (Van Nuffel et al., 2015). Sound sensors in pig farms permit the detection of respiratory infections through the registration of cough frequencies, which would likely reduce the need for antibiotics (Vranken and

Berckmans, 2017). Detecting the emergence of poultry diseases using technology was presented in a literature review (Astil et al., 2018), showing the potential of these methods to prevent disease dissemination. In addition to detect animal health and welfare challenges, the use of technology can prevent important losses due to damaged farm equipment (e.g. broken ventilation systems or a damaged feeder). Sensors are the most commonly used technological devices at the farm level as they are usually relatively cheap and non-invasive to the animal. In broiler chickens, sensors are used to measure temperature, humidity and gas concentrations (carbon dioxide, ammonia) which directly impact chickens health and welfare.

Despite the many possibilities that PLF technologies brings to the livestock industry including the improvement of animal welfare, production efficiency and labour, its implementation has been quite limited so far. The high price of some devices may have been an issue, but perhaps the technological complexity and data interpretation have been a more limiting challenge limiting the uptake of the technological developments at the industrial level. For instance, while sensors are useful for real-time data collection and are quite inexpensive, the access to data is conditional to connecting the device to a computer, downloading the file, and applying further processing (e.g. calculation of mean and variation) in order to interpret the stored data. In many other cases, it is unclear how the economic investment in technology devices will benefit the farmers in the daily work.

However, other set of technologies such as mobile tools have opened a huge set of possibilities in a simple, effective and inexpensive way. The development of mobile tools was initially focused on social human interactions, but the range of such tools has increased tremendously in the past years with a profound effect on the lives of millions of people. Mobile tools have spread to other sectors including wildlife studies (Madder et al., 2012) and are becoming more popular for the livestock industry (e.g. animal trading and management of pastoral area) (Butt, 2015; Debsu et al., 2016). Mobile applications are some of the most used technology tools as it provides farmers with an interactive platform for data access and use (Steinberger et al., 2009). They are generally easy and freely downloaded in any smartphone. Mobile applications provide a platform for simple data gathering, visualization, interpretation and sharing (Lantzos et al., 2013). In fact, most technology devices are nowadays sold with correspondent mobile applications that, through a touch screen, provide the farmer with data outputs and graphic illustration. Mobile applications are revolutionizing the livestock production of the twenty-first century as they provide an easy and affordable way of data management for busy farmers (Lantzos et al., 2013).

1.2.2. Environmental enrichment programs

Environmental enrichment refers to the additions and modifications to the production system that facilitate the biological adaptation of animals to their environment and improve their welfare (Newberry, 1995). One of the main purposes of environmental enrichment programs is to increase the animal's ability to handle behavioural and physiological challenges (Newberry, 1995) which are directly related to animal welfare. Van de Weerd and Day (2009) identified four criteria for a successful environmental enrichment program. It should 1) increase species-specific behaviour, 2) maintain or improve animal health, 3) improve the economics of the production system, and 4) be practical to employ (Van de Weerd and Day, 2009). Types of environmental enrichment include: social, occupational, physical and nutritional enrichments (Mkwanazi et al., 2019). In poultry species, Estevez and Newberry (2017) classified environmental enrichment in: enrichments providing structural complexity (including cover panels, perches, and barriers), visual enrichment (through varying lighting sources and colours), foraging enrichment (through the provision of straw bales or worm running inducers), and enrichments to promote comfort behaviour (including dust substrates and water for bathing behaviours).

In dairy cows, most common environmental enrichment consists in increasing social contact through pair housing of calves and providing opportunities of exploration and play. Some of these enrichment strategies were demonstrated to have economic benefits (Mandel et al., 2016). In broiler chickens, the most common environmental enrichment tools mentioned in recent bibliography reviews include perching structures, devices to increase cover (cover panels, vegetation, bales of hay), foraging and bathing materials, lighting and provision of outdoor areas (Estevez and Newberry, 2017; Riber et al., 2018). Even though very few studies reported the economic implications of introducing environmental enrichment in commercial farms, Leone and Estevez, (2008) demonstrated an increment of 4.5

more chicks produced per hen in broiler breeders in one production cycle (which was equivalent to 400$ investment per flock and an increase returns of 6000$).

PLF and environmental enrichment might contribute to enhance farm sustainability through the improvement of animal welfare by either controlling the farm environment or improving animal ability to cope with its environment. However, the effectiveness of these tools cannot be appreciated without measuring its impact in terms of animal welfare, which demonstrates the necessity of robust welfare assessment protocols at farm level.

2. Animal welfare assessment

2.1. Relevance of assessing animal welfare

As stated above, animal welfare impacts the three pillars of farm sustainability (i.e. economic, environmental and social) (Chemineau, 2016). This shows the need to establish animal welfare assessment that, ideally, can be frequently carried out throughout the production cycle. Routine procedures including farmers' daily flock checks along with visits of veterinary services have always been fundamental practices to identify emerging issues that could impair welfare and cause economic losses. Even though routine inspections are not science-based assessment protocols, they provide farmers and veterinarians with qualitative indications on the flock health and welfare through the production cycle. Research on animal behaviour and welfare has contributed to identify management aspects as risk factors for broiler chickens including the effect of high stocking densities, air quality, temperature and lighting regimen (Estevez, 2007; SCAHAW, 2000). The scientific evidences resulted in better management practices and in establishing regulations by the European commission (EU, 2007) and through the European Food Safety Authority reports to standardize practices starting from the incubation, along through the rearing and slaughter phases (Berg et al., 2012). Once rules and guidelines are established, it is required that inspections take place to ensure the application of the regulations (Main et al., 2014) and provide consumers with additional information regarding animal welfare conditions.

Besides, the inclusion of animal welfare within the global goals of sustainable agricultural policy (FAO, 2016) is expected to imply new investment programs, expertise, technologies, education and training (Buller et al., 2018). Such activities will promote the development and implementation of welfare assessment methods, along with inspections and certification schemes to guarantee compliance with the market demands. The existing approaches to assess animal welfare and their application are described in the flowing sections.

2.2. Approaches to welfare assessment

2.2.1. Resource-based measures

Resource-based measures mainly relate to the environment and management conditions. It includes measures related to the quality of the stockpersons (stockman competence and handling skills), the environment (housing conditions, type of floor, cleanliness, water and food facilities, type of bedding, thermal comfort, ventilation system, stocking density) and other management factors (such as appropriate health plans; Butterworth, 2009). Resource provision is supposed to prevent the animal from severe welfare issues as they assure minimum welfare standards. However, resource-based welfare assessment do not inform of the real state of animals, which raised the need to develop animal-based measures.

2.2.2. Animal-based measures

Animal-based measures are parameters directly related to the animals and are usually categorized as: physiological, clinical, behavioural and performance measures (Sejian, 2007). Animal welfare assessment protocols are based on a series of welfare indicators derived from direct or indirect observations. Such indicators are also referred to as outcome-based measures, because data are collected directly, by observing the animal, or indirectly, by assessing the animal response to its environment (e.g. blood or faeces analyses). Slaughter outcomes are considered animal-based measures as they report on carcass issues (e.g. injuries, burns, diseases, mortality at arrival) related to the welfare condition of the living animals, both at the farm and during their transport to the slaughterhouse (EFSA, 2012). Both resource-based and animal-based approaches provided the baseline for welfare assessment protocols in different species including broiler chickens. They also made possible the development of certification and labelling schemes as a result of the market demands for higher animal welfare standards.

2.2.3. Application of animal welfare assessment: Certification schemes

To date, a unique compulsory labelling exists, promoted by the European commission and based on the legislation for laying hens (EC, 1999) to define the production system (e.g. eggs provided by hens reared in cages, barns, free-range or organic). Besides legislation, voluntary or self-imposed labelling schemes are adopted by producers and retailers in the European Union (EU) and other parts of the world (Passantino et al., 2008). Certification companies can base their assessment on resource-based indicators (e.g American Humane) or animal-based indicators (e.g the Royal Society to Prevent Cruelty to Animals (RSPCA), AENOR Comform).

According to Main et al. (2014), a regular monitoring of resource and animal-based measures through certification schemes results in preventive and corrective actions that maximize levels of welfare. This approach implies a continuous improvement by permanent assessment of the conditions and reviewing their implications on welfare at the farm and retailer levels (Webster, 2009). Certification schemes aim to provide consumers with assurance on certain welfare levels, and/or promote welfare improvement within their scheme. The standardization of welfare assessment measures appears to be an important component for consumer information systems (Main et al., 2014).

2.3. Existing welfare assessment methods in broiler chickens

2.3.1. The Welfare Quality protocols

The Welfare Quality® (WQ) protocols were the first animal-based protocols developed for the assessment of cattle, pigs and poultry (Welfare Quality®, 2009) and are recognised worldwide. The WQ protocols are based on the principles of the Five Freedoms stated in the Brambell report (1965; revised by FAWC, 1993). They include the assessment of resource and animal-based indicators such as: housing factors, feeding and management, animal health and behaviour, presence of injuries or diseases, along with human-animal interactions (Botreau et al., 2007a, b). All protocols are based on twelve criteria, organized in four main principles: good feeding, good housing, good health and appropriate behaviour (Welfare Quality®, 2009).

After assessment, a score is assigned to each measure that it is later included to form part of a compounded, single score through a three-step aggregation process: measures are aggregated into criteria, criteria into principles and principles into an overall assessment (Botreau et al., 2007a, b). Following this process each farm is assigned an overall welfare score that could be categorized as excellent, enhanced, acceptable or not classified. Even though the WQ are the most science based referred protocols, some aspects could be improved. For example, critiques rose in relation to the calculation of the final score which was described by some authors to be subjective, given that few measures were reported to be less important in comparison with iceberg indicators (de Vries et al., 2013; Czycholl et al., 2017). The farm score is calculated in a way that could compensate poor conditions in one measure with better scores from another one, which might provide a biased perspective of the real welfare condition (Buijs et al., 2017). The feasibility and application of the WQ protocol at commercial level is another challenge (de Jong et al., 2016; Buijs et al., 2016) as 4 to 7 hours are required for implementation which might limit the number of sampled farms (Blokhuis et al., 2013).

2.3.2. Animal welfare indicators project: The transect method

The Animal Welfare Indicators (AWIN) was the second largest European project focusing on animal welfare. The main goals of AWIN were to develop, integrate and disseminate animal-based measures of welfare with an emphasis on pain recognition and assessment. Welfare indicators for different species such as sheep, goats, horses, donkeys and turkeys were identified, and assessment protocols for on-farm evaluation developed.

The development of a protocol for turkeys presented challenges due to the size and flighty nature of this poultry species, reasons why the WQ approach could not be applied. Trying to address this challenge, the transect method was developed as a potential alternative. It was first tested in broilers (Marchewka et al., 2013) before adapting it to turkeys (Marchewka et al., 2015). This welfare assessment protocol consists on slow walks along transects (paths delimited by feeder and drinker lines) (Figure 3) during which the observer collects the number of birds showing one of the pre-defined welfare indicators (Marchewka et al., 2013; 2015). This protocol is conducted using interactive apps (i-WatchBroiler, 2018; i-WatchTurkey, 2017), as welfare indicators are observed. General data characterizing the farm, the housing conditions and the current flock are first introduced in the app. Once that is completed, welfare assessment data can be introduced on the evaluation screen of the apps as the assessor walks on the predefined transects. The apps later provide a calculation of the mean incidence for each welfare indicator with a graphic illustration of the current flock mean in comparison with the mean calculated from all the flocks previously assessed. This provides the farmer with the opportunity to visualize quantitative data regarding the welfare state. These apps also give the possibility to download the data for further processing.

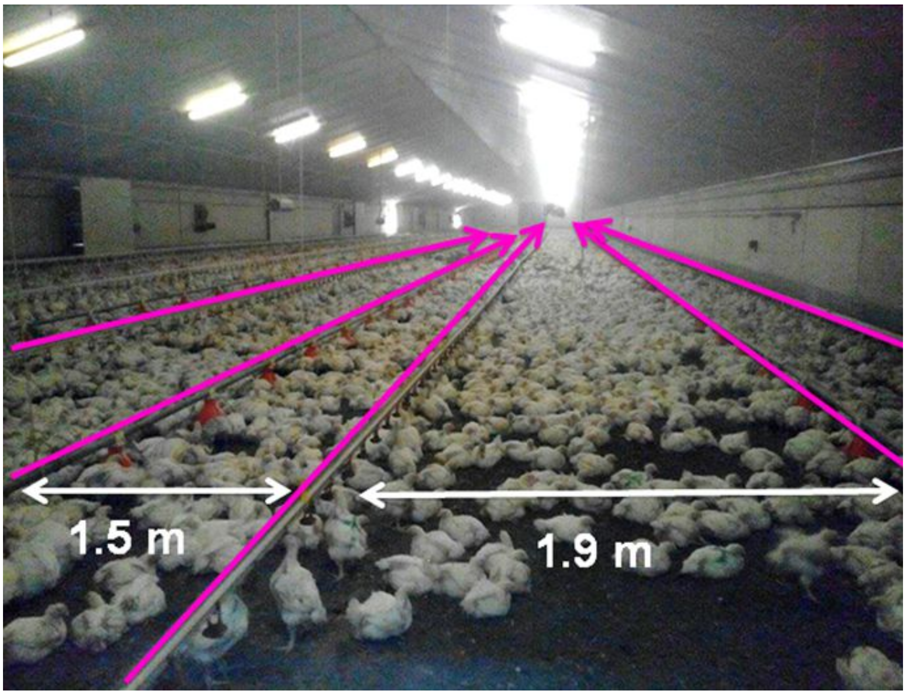

Figure 3: Transect delimitation by feeder and drinker lines and average sizes

The transect method was validated in commercial turkey farms against the gold standard of assessing the entire flock at the end of the rearing period (Marchewka et al., 2015). For broiler chickens, the selected welfare indicators included leg problems, presence of wounds, presence of diseases, dirtiness, small, and featherless birds. The transect results were compared with the individual sampling method of the WQ protocol in broiler chickens (Marchewka et al., 2013). The results highlighted important differences between methods that were explained on the basis of differences in sample size and in the way bird mobility assessment is conducted. As assessing chickens one by one might not be feasible due to the flock size of broiler chickens, further studies were necessary to determine the method usefulness as a practical tool for on-farm welfare assessment.

3. Challenges of effective welfare assessments using the transect method

The transect method is based on the assumption that birds are homogeneously distributed in the house. However, assessment is conducted along transects that are located in different areas of the house where birds welfare might differ. These attributes come with concerns related to the transect method usefulness given that little is known about its capacity to detect birds with impaired welfare, or the possibilities of counting them more than once during a house assessment. These concerns, along with the inter-observer reliability, are relevant in order to find out about the method practical applicability, soundness and usefulness in commercial broiler chicken farms.

3.1. On-farm practical applicability

The study by comparing the transect method with the individual sampling of the WQ protocol showed discrepancies between both methods in the estimation of common welfare indicators. Limitations related to each method might be the reason for some of the reported discrepancies (Marchewka et al., 2013). For the specific case of the transect method, little is known about its capacity to detect the impact of farm conditions on the animals, an aspect of paramount relevance for its implementation at the commercial scale. In addition, the relationship between on-farm welfare impairment and the consequent production outcomes at slaughter (Jacobs et al., 2017a, b) should be further investigated with data collected using the transect method.

Animal welfare impairment might be caused by aspects related to the animals themselves, or can be associated with environmental farm conditions. Examples of bird related aspects include age (Sørensen et al., 2000; Cordeiro et al., 2012) and genetic makeup (Jang et al., 2013; Dinev et al., 2012). Farm environmental conditions may include stocking density and litter quality (Estevez, 2007), lighting regimen and ventilation program (SCAHAW, 2006) or the use of environmental enrichment (Newberry, 1995). Skeletal disorders are among the most common causes of culling and mortality in broiler chickens as they start developing between 3 and 5 weeks of age causing lameness and immobility (Bradshaw et al., 2002). Genetic selection for rapid growth and muscle development affects bone quality (Bennett, 2008; Shim et al., 2012; González-Cerón et al., 2015), increasing the risk for lameness, twisted legs, and tibial dyschondroplasia (Oviedo-Rondon et al., 2006). The gait score (GS) evaluated using Kestin el al. (1992) five-point scale was positively correlated with bird age when using optical flow techniques (Dawkins et al., 2012, Figure 4). Rapid growth has also been linked to cardiovascular diseases, ascites and bacterial diseases (Bessei, 2006; Northcutt et al., 2003).

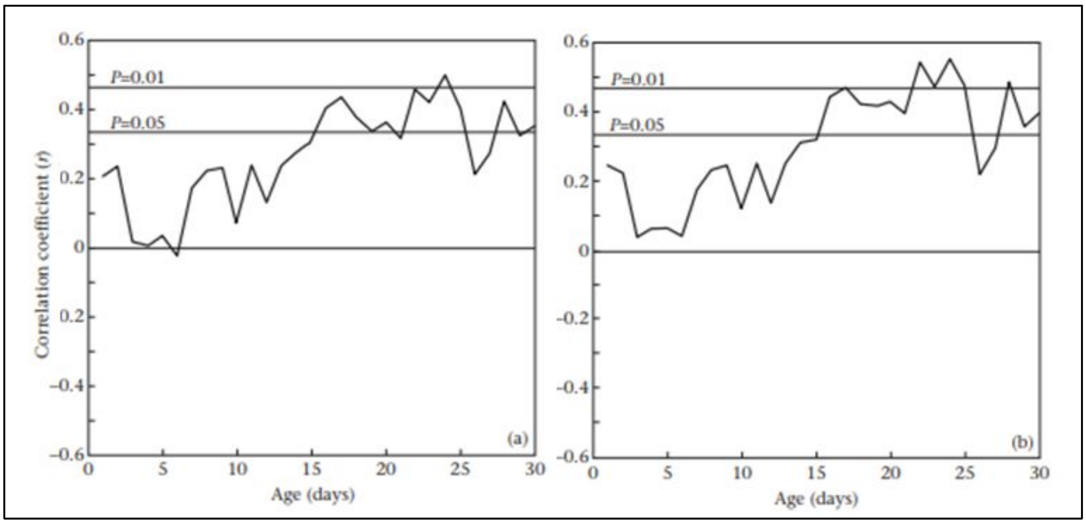

Figure 4: Effect of bird age on correlation coefficients (r) between gait score and optical flow measures (Adapted from Dawkins et al., 2012). (a) Correlation between gait score and skew of flow; (b) Correlation between GS and kurtosis of flow. In each case, the thick line indicates the value of r^2 and the thin lines indicate the thresholds for different levels of significance.

In addition to genetic aspects, the deterioration of litter quality has a major impact on welfare indicators such as the incidences of hock and footpad burns (de Jong et al., 2014). Litter quality is strongly related to stocking density as humidity and temperature become difficult to control (Estevez, 2007), and may result in the deterioration of plumage conditions (de Jong et al., 2015; Li et al., 2017, Figure 5).

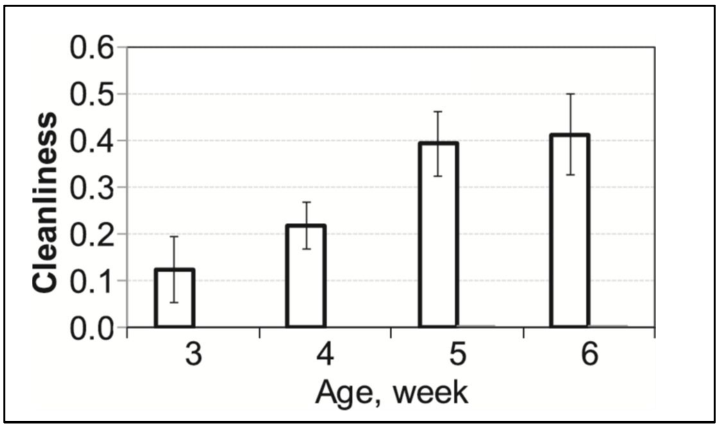

Figure 5: Mean±SE of plumage cleanliness scores according to bird age in broiler houses (rice hull litter) (Adapted from Li et al., 2017).

On the other hand, environmental enrichment was demonstrated to have a positive impact on poultry health stimulating their behavioural repertoire, in addition to being useful to reduce fear and aggressive interactions (Estevez and Newberry, 2017). Some of the most common forms of environmental enrichment are perches and platforms, which can strengthen leg muscles and joints by stimulating movement (Bailie et al., 2014; Presby et al., 2014). Other forms of environmental enrichment in poultry include the provision of materials to stimulate ground scratching and dustbathing behaviours (Olsson and Keeling, 2005; Guinebretiére et al., 2014) which also promotes physical activity involving the movement of legs and wings. Dust bathing materials can contribute to improve leg health as a negative association has been reported between lameness and dustbathing frequency (Vestergaard and Sanotra, 1999).

Increased space allowance was shown to stimulate walking in broiler chickens (Leone et al., 2010) in addition to an improved walking ability (Knowles et al.,

2008) and a reduced incidence of contact dermatitis and mortality (Hall, 2001). Lighting regimen has also a direct impact on birds' activity level and biorhythms. Thus, increasing darkness periods was associated with decreased mortality due to metabolic and skeletal diseases (Schwean-Lardner et al., 2013).

Broiler chickens showed a strong preference for house walls to rest (Newberry and Hall, 1990; Buijs et al., 2010), especially when their welfare is impaired (Aydin et al., 2016) which could result in an uneven distribution of birds with welfare issues. Given that transects can be central (delimited by feeder and drinker lines) or wall (delimited by a wall and drinker/feeder line), comparing the consistency in the prevalence of welfare problems between both types of transects would give practical information for a sound estimation of the flock welfare issues. As bird age and genetic line, litter quality, environmental enrichment and space allowance are factors known to impact on broiler welfare, investigating the capacity of the transect method to detect variation in the prevalence of welfare indicators when varying these factors might provide insights on its usefulness to be implemented in commercial broiler chickens farms.

Production outcomes collected at the slaughter house, such as death on arrival (DOA), is a commonly used welfare indicator which is closely associated with flock health (Jacobs et al., 2017b). Other slaughter parameters informing on the welfare status of broilers chickens at the farm level include feather condition, footpad dermatitis and hock burns (Saraiva et al., 2016; de Jong et al., 2015). In addition, strong correlations were found for turkeys between on-farm welfare indicators and production outcomes such as downgrades or condemnations (Marchewcka et al., 2015). Given the known relationship between on-farm welfare indicators and slaughter parameters, verifying the association among on farm assessment data using the transects and slaughter outcomes will help to support the validity of the method.

3.2. Data soundness

3.2.1. Inter-observer reliability

Inter-observer reliability describes the extent to which two observers obtain similar results when measuring the same sample simultaneously (Martin and

Bateson, 2004). Given that the transect method assesses welfare based not only on injuries and dirtiness on the animal body but also on the bird posture, movement and behaviour (e.g. to determine sickness and/or leg problems), achieving sufficient consistency between observers may represent a real challenge for the practical implementation of the transect method as a valid welfare assessment tool. Indeed, assessments are normally conducted on a high number of simultaneously moving birds, which might complicate data collection and perhaps affect the inter-observer reliability. Testing the inter-observer reliability would thus, provide an appreciation of the consistency that could be obtained in reporting welfare issues between observers.

3.2.2. Strength of assessments of freely moving birds

Investigating the influence of farm environmental and management conditions during transect assessment might provide insights on its practical applicability, but does not inform about the soundness of the method in terms of its capacity to detect, overlook and/or double count impaired birds within and between transects. Most welfare assessment methods are based on evaluating one bird at a time while they are in an enclosure (e.g. the individual scoring of the WQ protocol). When conducting transects, a high number of freely moving birds are simultaneously assessed and among them, the identified impaired population. Flock density might vary according to rearing conditions or thinning, which may affect the observation of impaired birds, increasing the likelihood of double counting the same individuals. Assessment results might also vary according to birds activity level during the day (Hocking et al., 1996), enclosure dimensions (Leone et al., 2010; Mallapur et al., 2009), and/or bird distribution within the house (Cornetto and Estevez, 2001). Such variations might result in imprecise findings if assessment is not carefully conducted. This suggests the necessity of investigating the robustness of the method in detecting a known bird population in order to evaluate its capacity to report true and consistent results.

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MAIN OBJECTIVES

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The general objective of this Doctoral Thesis is to investigate the usefulness, practical applicability and soundness of the transect method as a welfare assessment method in commercial broiler chickens flocks.

To this end, the specific objectives of this Doctoral Thesis are:

1. To provide an overview of existing precision livestock farming methods used for controlling house environment and poultry welfare assessment (Chapter 1).

2. To determine the potential of the transect method to detect differences in welfare status on commercial broiler chickens according to the predicted effects of bird age and genetic line, litter quality or house area (Chapter 2).

3. To investigate the potential of the transect method to detect the positive impact of implementing environmental enrichment strategies, on broiler chickens welfare indicators (Chapter 3).

4. To determine the predictive capacity of the transect method regarding the associations between flock welfare indicators and its production outcomes (Chapters 2 and 3).

5. To determine the soundness of the transect method through capture-recapture techniques in order to determine the detection and repetition rates of a known subpopulation of birds (Chapter 4).

I

CHAPTER 1: Technology and poultry welfare

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Abstract

Consideration of animal welfare is essential to address the consumers' demands and for the long term sustainability of commercial poultry. However, assessing welfare in large poultry flocks, to be able to detect potential welfare risks and to control or minimize its impact is difficult. Current developments in technology and mathematical modelling open new possibilities for real-time automatic monitoring of animal welfare and health. New technological innovations potentially adaptable to commercial poultry are appearing, although their practical implementation is still being defined. In this paper, we review the latest technological developments with potential to be applied to poultry welfare, especially for broiler chickens and laying hens. Some of the examples that are presented and discussed include the following: sensors for farm environmental monitoring, movement, or physiological parameters; imaging technologies such as optical flow to detect gait problems and feather pecking; infrared technologies to evaluate birds' thermoregulatory features and metabolism changes, that may be indicative of welfare, health and management problems. All these technologies have the potential to be implemented at the commercial level to improve birds' welfare and to optimize flock management, therefore, improving the efficiency of the system in terms of use of resources and, thus, long term sustainability.

1. Introduction

Public concern regarding the conditions in which producing animals are maintained has led to the need for developing methods to verify minimum animal welfare standards. As defined by the World Organization for Animal Health (OIE, 2011), "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 behaviour, and if it is not suffering from unpleasant states such as pain, fear and distress". However, to prove and verify animal welfare requirements in practice is not simple. In intensive poultry production a large number of factors, such as stocking density, environmental deterioration, unsuitable social environments, thermal stress, or difficulties in accessing essential resources can be major sources of stress that can lead to welfare deterioration and reduced performance (Muiruri and Harrison, 1991; Appleby et al., 1993; Baxter, 1994; Belnave and Muheereza, 1997; Meluzzi and Sirri, 2009; Tactacan et al., 2009). Many of these factors can be controlled through well-established management practices to provide birds with an optimal environment. However, the sharp control of the temperature and relative humidity required to minimize the occurrence of welfare problems in poultry (Appleby et al., 2004; Dawkins et al., 2004) might not be easy to achieve under high density or if the available farm equipment is inadequate. In addition, unforeseen situations or potential interactions among factors may be difficult to predict and control, thus potentially impacting on welfare. Welfare assessment serves to verify that the conditions to satisfy welfare standards during production are indeed met.

First attempts to assess animal welfare were resource-based, because assessing minimum resource requirements is generally easier than to evaluate the impact of the production conditions on animals (Mench, 2003). Resource-based assessment intends to warrant the provision of the necessary environmental conditions for an optimal animal welfare. However, to verify that such conditions did not compromise the welfare of animals it was essential to develop methods based on the impact over the animals themselves. Animal-based assessment methods that were later developed, such as the Welfare Quality® (WQ) poultry assessment protocol provides a thorough assessment of the impact of the actual rearing conditions on poultry welfare (Welfare Quality®, 2009). At a commercial level, however, the implementation of the full protocol is difficult and time consuming, thus, a simplified and effective protocol was developed for broilers (de Jong et al., 2015). More recently, a different welfare assessment approach based on the transect method was proposed for commercial broilers and turkeys (Marchewka et al., 2013; 2015). Initial results suggest that this method is simple and practical for on-farm application and seems to have a good-inter-observer reliability for the detection of major welfare issues in meat poultry.

Despite the important efforts to simplify the available welfare assessment protocols for poultry, their implementation within the European Union (EU) legal framework could impose increased biosecurity challenges and production costs, which may hinder economic profit for farmers (Berkmans, 2014). Available technological innovations currently tested for welfare assessment could greatly help to encompass a better environmental control and improve welfare while minimizing costs (Mollo et al., 2009; Corkery et al., 2013; Berkmans, 2014). In addition, the reduction in health and welfare problems would lead to a more efficient and sustainable production in the long term.

Precision livestock farming (PLF), defined as the management of livestock production using the principles and technologies of process engineering (Wathes et al., 2008), is based on automatic data acquisition, access, and processing (Mollo et al., 2009). Data from diverse sources are collected through smart sensors and compiled to a central database, where they will be later analysed to create an automatic management system based on real-time monitoring to control animal performance, health, and welfare (Berkmans, 2014). According to Mollo et al. (2009), PLF must be able to automatically manage commercial poultry farm equipment (including feeders, fans, heating systems, and sprinklers) based on the collected information. Different studies on broiler chickens and laying hens have shown the importance of technology and PLF to study birds' behaviour and welfare (Moura and Naas, 2006; Robins and Philips, 2011; Corkery et al., 2013; Berkmans, 2014). Although most technologies are still in the experimental phase, some are already available and can be introduced on commercial poultry farms (Kashiha et al., 2013; Marchewka et al., 2013; 2015) with good results.

As welfare depends on both management practices and the use of adequate equipment, different technological advances are emerging to improve both. For example, keel bone breakage risk in laying hens is higher in barns and aviaries as compared to cages (Siegford et al., 2016). However, new devices can be installed to detect poor management practices or to identify any behavioural or health issue occurring and, therefore, contribute to improved farm design and equipment use.

This paper reviews available technologies that have the potential to be implemented for a better control of the environment to improve poultry welfare, or to be applied for an automatic welfare assessment. The practical applications and the potential impact of such technologies are discussed.

2. Sensors

In the last few years' tremendous advances were achieved in sensing technology in terms of diversity, accuracy, and affordability. Sensors, especially wireless sensors, have a wide range of applications in civil and environmental engineering emergency management and agriculture (Mayer, 2005; Ruiz-Garcia et al., 2009). Their application to farming is more recent with first applications aiming to reduce management costs and improve animal health (Ruiz-Garcia et al., 2009). As sensing technology has become progressively more affordable, and in many cases less complex, research interest into potential applications to assess, control, and improve animal welfare is expanding and it is expected to increase with time. Table 1 summarizes the most relevant sensors tested for application in poultry with potential to benefit welfare.

2.1. Environmental Sensors

Environmental conditions, in particular inadequate temperature, relative humidity, and the length of exposure have a major impact on broiler welfare, mortality, and performance (Dawkins et al., 2004; Jones et al., 2005). Exposure to elevated levels of noxious gases like carbon dioxide and ammonia is also known to reduce growth, feed conversion, and immunological response (Wang et al., 2010). Even a two weeks' exposure to high carbon dioxide concentrations in one day old chicks is sufficient to increase the incidence of late mortalities and alter heart characteristics (Olanrewaju et al., 2008). Thus, any efforts to better monitor and control environmental conditions will have a direct impact on bird welfare.

Table 1. Main sensor technologies and potential applications to improve poultry welfare.

Sensor Type		**Applications**
Air quality		Indoor climatic conditions' assessment Broilers' final weight prediction
Sound	Broiler incubation	Monitoring hatching windows for better productivity
	Broilers	Feed intake measurements Growth prediction Thermal comfort estimation within farms
	Laying Hens	Stress detection induced by environmental temperature variation and fear Determination of feather pecking conditions
Locomotion		Assessing locomotion deficiency in broilers Use of Geographic Information Systems (GIS) to evaluate space use and different behaviours in laying hens Study of hens' jumps between perches and its impact on bone breakage occurrence Study of hens' use of pop holes and its effect on keel fracture incidence
Health status		Detection of avian influenza by the measure of broilers' temperature variations Detection of avian influenza by the measure of broilers' activity

Although real-time multi-sensor monitoring and control of the environmental conditions (besides temperature) is not commonly applied in commercial poultry farms, current advances in sensing technology, with higher capabilities at affordable prices, will permit the development of systems for a precise control of the production environment. Some examples of current developments include multi-sensing systems to monitor environmental temperature, differential indoor atmospheric pressure, and air velocity in broiler flocks (Bustamante et al., 2008) to automatically assess the adequacy of the ventilation system design and functioning, which is highly relevant to provide a comfortable environment to poultry. Using sensors to simultaneously collect temperature, relative humidity, carbon dioxide, and ammonia concentrations, Jackman et al. (2015) developed a good prediction model to calculate final mean bird weight in broiler flocks. The model showed excellent, house specific prediction ability ($r^2 = 0.89$) between the predicted and observed bird weight based on the conditions of the rearing environment. The development of continuous real-time environmental monitoring combined with advanced modelling tools could be used to provide a warning system to potential deviations from targeted weight gains which may also be a good indication of health or welfare risks; thus, having real potential to assure the optimal and sustained environmental conditions.

2.2. Acoustic Sensors

Bioacoustics studies the characteristics and the biological significance of sounds emitted by living organisms (Tefera, 2012). Birds in particular, rely on acoustic communication for their social interactions and for alarm signalling (Corkery et al., 2013). Some forms of acoustic signalling can also be considered as reliable stress indicators (SCAHAW, 2001) and, thus, is an interesting approach when looking for reliable welfare assessment indicators. Acoustic studies can range in complexity from the simple establishment of differences on the frequency of emitted vocalizations to intricate analyses on sound physical properties. The later used to be laborious and complex (Marx et al., 2001), but current available bioacoustics software, like Raven software (Cornell Lab of Ornithology, Ithaca, NY, USA), has made this type of analysis somewhat easier, thus, becoming a practical tool for behavioural and welfare studies.

Using relatively simple acoustic parameters such as vocalization frequency, Koene et al. (1999) and Zimmerman et al. (2000) were able to detect episodes of food deprivation in broilers and in laying hens, while Bright (2008) showed higher rates of squawks and total vocalizations in laying hen flocks with feather pecking problems. Based on complex sound analyses and algorithmic procedures, Aydin et al. (2014) were able to distinguish sound signals corresponding to pecking (characterized by a sudden increase in amplitude follow by a sudden decrease) from all other signals in the range of 1000 Hz to 5000 kHz (using a 6th order Butterworth filter). Based on these analyses, together with the recording of the feed uptake (recorded with the traditional feed weighing system), they developed a model to predict feed intake in broilers, which was highly correlated with pecking sounds. A later study, used peak frequency vocalizations emitted by broiler flocks (analysed using Adobe® Audition™ CS6) to predict growth, as they found that these vocalizations were inversely proportional to bird age and weight (Fontana et al., 2015). Based on these finding, the authors suggested that the automatic analysis of peak frequency vocalizations would allow the development of prediction tools and, therefore, would permit health and welfare assessment at the farm level, with potential to be used as an early warning system.

Sound analyses have been proven to be powerful to determine the adequacy of the thermal environment. Thus, Moura et al. (2008) estimated thermal comfort and chick performance based on the analyses of amplitude vocalizations and the noise frequency spectrum (using Cool Edit® and Audacity®) of broiler chicks placed under varying environmental temperatures, while collecting their behavioural response in parallel. They showed that when temperature decreased, the amplitude and frequency of the vocalizations increased as birds grouped together to reduce heat loss, while during thermal comfort the amplitude and frequency of vocalizations stabilized. Pereira et al. (2014) identified thermal stress conditions based on broiler vocalizations and verified the existence of different vocalization patterns in heat stressed birds. In this case, the study was based on four vocalization acoustic parameters: energy, bandwidth, and first and second formant (using Praat® and Matlab® software). Thermal stress together with fear induced by routine management practices were the main sources of stress considered by Lee et al. (2015), who tested their effects on vocalization patterns of laying hens. They developed an automatic online-monitoring prototype that used bird sounds to notify producers of a stressful situation. The system was developed with support vector machine techniques that were able to classify the sound emitted by laying hens (using Praat 5.3.52 and Weka 3.6 (http://www.cs.waikato.ac.nz/ml)) into categories such as physical stress, thermal stress, and mental stress due to fear. Results were validated with real sound records, showing 96.2% accuracy in detecting stress episodes.

Sound analysis has also been used during incubation as a tool to reduce the hatching window (time interval between the first and the last hatching egg), as it is considered a key factor directly related to broiler welfare and performance (Van de Ven et al., 2011). If hatching occurs early, it increases the probability of dehydration and early mortality, but late hatching also increases the risk of poor hatchability, pipped eggs, live-embryo unhatched eggs, and reduced chick quality (The Poultry Site, 2016).

Differences in hatching times may also affect feeding behaviour in broilers (Nielsen et al., 2010) and pronounced fearfulness in early male hatches (Løtvedt, 2014). Given the long term consequences of the hatching window on broiler welfare and performance, it is essential to monitor the final phase of the incubation process to minimize the risk of early and late hatches. Exadaktylos et al., (2011) conducted a sound analysis within the 2000–4000 Hz region (using a 10th order Butterworth filter) to identify the moment at which embryos reached or passed the internal pipping stage, according to the peak frequency of the sounds, to then apply an adjusted temperature profile and narrow the hatching window. The developed algorithm based on sound analyses detected 93%–98% of the chicks reaching the internal pipping state. Although this was an experimental study, it would not be surprising to see further acoustic analysis applications during incubation to benefit bird health and welfare.

In summary, these studies show a full range of potential applications of sound analysis to optimize the conditions of the rearing environment and to detect behavioural problems such as feather pecking, hunger episodes, or thermal stress. Because sound technology has been around for a number of years, and some parameters are simple to assess, it has real potential for practical commercial implementation to improve health and welfare in poultry.

2.3. Movement Sensors

Freedom of movement is an intrinsic component of animal welfare (Brambell Report, 1965), thus, to assure optimal welfare animals should be able to move freely. However, rearing conditions may hinder movement in poultry due to high density, housing space availability, and design or health condition, among other factors (Newberry and Hall., 1990; Cornetto and Estevez, 2001; Leone and Estevez, 2008; Naas et al., 2009). Thus, movement (or lack thereof) is a direct indicator of the welfare status in poultry. Movement sensors have been used to study different aspects of movement in broilers and in laying hens. Piezoelectric crystal sensors were used to determine locomotion deficiencies, one of the major indicators of broiler welfare (EFSA, 2012), by examining the peak vertical force on both feet during walking sequences (Naas et al., 2010). With this equipment, the authors were able to detect an asymmetry of the peak force in each foot that led to uneven walking in male broilers, which is a first approach towards real time broiler gait assessment.

A combination of Geographic Information System (GIS) with wireless sensor attached to the birds' body (Mica2 Dot radio mobile) was used by Daigle et al. (2014) to study in detail the relationship between movement and behaviour in laying hens. A series of nodes installed through the hens' environment (Mica2 Dot stationary radio nodes) acted as beacons for the detection of the sensors, and a base station collected the data from the stationary nodes. In addition, video recordings allowed the association of the observations with the output of the sensors producing spatial explicit data which permitted analysis of the spatiotemporal variation in individual hens under experimental conditions. The data were used to map the spatial configuration of hens' home range distribution to finally investigate their association with welfare indicators such as health status, expression of natural behaviours, and their emotional state. The results obtained by Daigle et al. (2014) showed large inter-individual variability in time spent performing specific behaviours, home range size, and home range overlap with conspecifics. According to the authors, this variability could explain individual hen's condition, and could be indicative of illness, injury, or changes in social dynamics. On the basis of these results, Daigle et al. (2014) suggested that a better understanding of confined animals' behaviour and space use, by using GIS technology, may help to advance in-housing design and management practices to improve the welfare of laying hens. However, the implementation of such a technology on the commercial scale would not be cost effective as the authors were actually interested in studying the behaviour of individual non-caged hens.

In alternative housing systems, movement of laying hens across perches and other housing equipment may increase the risk of bone breakage which is a source of poor welfare (Nasr et al., 2012). Banerjee et al. (2014) used a 3-axis ADL335 accelerometer (Analog Devices, Norwood, MA, USA) placed in laying hens and similar communication equipment to the one used by Daigle et al. (2014) to calculate landing forces, height of the jumps, and the initiation and landing times when jumping from perches at variable heights to a lower landing surface in an experimental set up. The accelerometers continuously collected the acceleration data in three axes as birds jumped. A pressure mapping system and a video camera were installed as a validation method to measure the landing forces and to record the jumps. Banerjee et al. (2014) reported an average landing force of 15.85 and 20.8 KJ when jumping from heights of 41 to 61 cm, respectively. This technological approach capable of assessing the landing forces on laying hens differing in age, size, and plumage integrity may enable the improvement of housing and perching design (Banerjee et al., 2014), which is important to resolve some persistent issues on aviary systems that are relevant for the welfare of laying hens.

Richards et al. (2012) studied the effect of pop hole use on the percentage of keel fractures in free range laying hens tagged with radio frequency identification (RFID) transponders. Flocks of different ages were monitored during two laying periods and keel fracture incidence was evaluated through regular palpations. It was shown that the average percentage of keel fractures increased with hens' age but higher keel scores were registered when pop holes were less utilized. The authors concluded that fractured hens had a lower ability to use the pop holes to access the outdoor range which reduced the welfare advantages provided by free range housing systems. Siegford et al. (2016) reviewed and discussed different technologies used for assessing hens' activity and location and their impact on welfare condition depending on the farm's housing system.

2.4. Sensors for Health Status Detection

Wireless systems equipped with body temperature sensors and accelerometers have been used under experimental conditions to detect chickens infected with highly pathogenic avian influenza six hours before death (Okada et al., 2009). The same team later developed more sensitive equipment based on wireless 3-axis accelerometers and a radial lead thermistor that sent the data on activity and temperature to wireless sensor nodes to detect signs of avian influenza. The authors proved the ability of the method to detect abnormal states caused by the disease twice as early as with body temperature sensors alone with a reported detection ratio of 100% (Aydin et al., 2010). Even though this sensing equipment can prevent economic losses and welfare issues due to disease spread, it would be unpractical and too expensive to fit all individuals with surveillance equipment in a typically large poultry flock. However, sensors could be fit to a subpopulation of sentinel birds, which may be as effective for prevention or as an early detection strategy at least in high risk-areas. In addition, as variation in temperature and reduced activity are common general symptoms for many diseases, this basic equipment could be used as a warning for other health risks as well.

3. Image Technology

3.1. Image Analysis

Skeletal disorders and contact dermatitis are major broiler welfare issues (EFSA, 2012) that are still a matter of concern from the welfare and the economic stand point. Good farm management and a good use of novel technology may be of great relevance in the upcoming years to minimize such problems. For instance, in a study conducted in broilers (Aydin et al., 2010), the "Eyenamic Software" (Leroy et al., 2006) was used to calculate birds' activity level by processing calibrated recorded video images. Then they calculated differences in pixel intensity values in comparison to the previous image to calculate an activity index. This system was used to assess the relationship between automatic gaits with gait score obtained by human experts, to later develop an automatic activity index tool capable of detecting leg problems. In this study, video images were processed with the Eyenamic software to calculate an activity index of six birds, which were given a gait score by experts according to Kestin et al. (1992). The method was sensitive to detect severely affected birds with gait scores 4 and 5, but not for moderately affected birds. The authors indicated that if further validation can be obtained, this automatic activity monitoring tool has the potential be used to detect high gait scores (4 and 5) in commercial farms.

An image analysis prototype was evaluated for its adequacy as a tool for automated footpad dermatitis scoring as compared to the traditional human scoring at the slaughter plant (Vanderhasselt et al., 2013). Experimental birds were assessed and selected for each of the five categories of footpad dermatitis according to the Welfare Quality® protocol for poultry (Welfare Quality, 2009) in semi-commercial conditions. Those birds were transported to the slaughter plant five days later where their feet were first assessed with the automated image-analysis assessment prototype system (Meyn Food Processing technology B.V.) and then assessed by the same expert that performed the first evaluation at the farm. Agreement between both assessment methods was initially poor because the automated system does not consider the depth of the lesion. However, the results improved considerably when considering only those birds for which the automatic system produced a dermatitis scored for both footpads yielding stronger correlations with the expert scoring ($r = 0.68$ and $r = 0.74$ for farm and slaughter plant, respectively) (Vanderhasselt et al., 2013). Even though the system still requires adjustments, the automatic image analysis offers a great potential for automatic footpad dermatitis assessment that could be implemented in a relatively short time period at slaughter plants.

3.2. Optical Flow

A particular type of image analysis is the optical flow analysis (OF) that has been used in many applications including traffic flows (Bellomo et al., 2009), movement of glaciers (Giles et al., 2009), cell and sperm motility (Cheng et al., 2009), and, lately, in the analysis of movement in confined broilers (Dawkins et al., 2012; 2013). One of the main advantages of OF is that it allows the automatic and continuous assessment of moving images containing hundreds of individuals (Sonka et al., 1999) and, thus, could be a practical approach for the assessment of movement related welfare issues in commercial poultry.

OF detects the brightness change rate in pixels of a moving image and has specific statistical properties such as the mean flow rate, variance, skew, and kurtosis that can be used to detect its association with variations in gait score, pododermatitis, hock burn, and mortalities in broiler flocks (Dawkins et al., 2012). In a study conducted in 24 commercial flocks, Dawkins et al. (2012) detected a negative correlation between mean flow and flock mortality in 30-day-old broilers, while both the skew and kurtosis were positively correlated with the incidence of mortalities, culls, hock burns, and mean gait score. However, in a later study conducted with the aim of finding a more direct relationship between OF, behaviour, and welfare, Dawkins et al. (2013) were only able to find significant positive correlations for skew and kurtosis with the number of birds walking for at least 10 s, concluding that OF is probably more sensitive to flock uniformity or lack of it.

A combination of OF and Bayesian regression was used by Roberts et al. (2012) to predict health and welfare on a continuous basis. Mean, variance, skewness, and kurtosis were estimated daily using an OF algorithm. Gait score was assessed on day 28 in 60 birds/flock. In addition, daily mortality, culls, weights, growth rate, water and food consumption, and total incidence of mortality, culls, pododermatitis, and hock burns were calculated for the rearing period, and were included in the regression model. The model successfully predicted total flock mortality at 15 days of age, gait score became significant from day 13 and was capable of predicting the occurrence of hock burns at one or two days of age. Thus, Roberts et al. (2012) showed the powerful predictive power of OF combined with the Bayesian regression model. Recent work indicates that OF technology can even be useful to detect *Campylobacter* infected flocks (Colles et al., 2016), which has a strong welfare impact as strong inflammatory conditions can lead to diarrhoea, poor litter quality, and deteriorated walking ability in affected birds. In their study, based in 31 commercial flocks, Colles et al., (2016) showed that flocks likely to become positive for *Campylobacter* were identified in the first seven to 10 days of life and were characterized by having a lower mean flow rate and consistently higher kurtosis in comparison to non-infected flocks.

Although most OF studies focused in broilers, OF was also used to predict plumage damage in laying hens (Lee et al., 2010) and to identify the management and/or environmental risk factors involved in plumage deterioration. OF data of 18 commercial laying hen flocks were collected by video recordings at different ages and processed with OF algorithms. A hidden Markov chain was used to identify disturbance periods in the OF dataset. To validate the method, an expert observer visualized the video recordings and scored a variety of disturbances like birds running or pecking each other. Measures of disturbances were combined with management, environmental, production, and feather damage (scored according to Bright el al. (2006)) data for each farm to improve the predictive power of the model. Feather scores in later weeks were predicted using Gaussian linear and nonlinear regression models. The model showed improved prediction of feather damage and a good identification of high prevalent damaged flocks during following weeks.

Considering the positive results obtained with OF analysis, the method seems to be a sensitive tool for the assessment of the health and welfare status in commercial broiler flocks as well as in an experimental setting for laying hens. If positive results continue to be supported by research, this technology may have a major impact on poultry management as it benefits the animals, producers, and consumers by reducing economic losses and improving food safety. Besides, this methodology is non-invasive and it is relatively easy to apply in large flocks. It is probably just a matter of time before OF technology is applied to commercial laying hens or other poultry species.

3.3. Infrared Thermal Imaging

Preventing heat stress is crucial to poultry welfare as it may impact behaviour, immunity, and physiological processes and can cause major mortalities (Estevez et al., 2002; Lara and Rostagno, 2013). Infrared thermal imaging (IRTI) technology creates infrared images showing the body's superficial temperature distribution from the infrared radiation emitted by objects that is converted into electrical signals. In the thermal image, each colour expresses a specific temperature range related to the defined scale (Naas et al., 2014), thus it is a practical, non-invasive tool to study welfare aspects related to thermoregulation.

Yahav et al. (2004) used IRTI to determine optimal air velocity (AV) for broilers' thermoregulation, while maintaining adequate temperature and relative humidity. Body weight, feed intake, and faecal excretions were collected to estimate the energetic demands for body maintenance, while body heat loss was calculated by radiation and convection using IRTI. With this methodology, the authors showed that 2.0 m/s was the optimal air velocity, allowing the birds to control body temperature with no detrimental effects on performance. It has also been shown that it is possible to monitor changes in the metabolism of broilers associated to thermal variation by analysing body surface temperature through IRTI. Ferreira et al. (2010) indicated that IRTI was sensitive enough to identify a reduction in metabolic heat production in birds fed with an oil supplemented diet, which was suggested as a nutrition alternative to minimize heat stress. Work by Giloh et al., (2012) corroborated the reliability of using facial surface temperature (measured with IRTI) as an indicator of heat stress by correlating it with changes in body core temperature, corticosterone, thyroid hormones, and arginine vasotocin that are indicative of increased stress levels. Giloh et al. (2012) proved the existence of a strong correlation between facial surface temperature and core body temperature and indicated its usefulness as a determining factor to support decisions in a climate-controlled environment farm. In fact, the authors indicated that under experimental conditions facial surface temperature recorded by IRTI was more informative than ambient temperature regarding the birds status and, thus, has great potential to be applied at a commercial level to monitor thermal stress levels.

IRTI has also been used in other production phases such as incubation and pre-slaughter. Studies conducted by Shinder et al. (2009) using IRTI during the final phase of incubation showed that body weight and body temperature were significantly higher in chicks that were exposed to short periods of cold stress during incubation (days 18 and 19) and had 13% to 18% lower incidence of ascites in comparison to control birds. On the other hand, Naas et al. (2010) used IRTI technology to estimate heat exchange between broilers and their environment at pre-slaughter. These estimations were later used to develop a mathematical model to predict broiler surface temperature as a function of air temperature in order to evaluate the effects of pre-slaughter handling and environmental conditions on welfare and mortality. Naas et al. (2010) showed that featherless body areas reacted rapidly to environmental changes, affecting homeostasis and increasing deaths on arrival at the slaughterhouse. This non-invasive method can, therefore, help to improve flock management during the pre-slaughter phase, as it permits assessing the birds' welfare conditions and facilitates taking remedial actions to guarantee welfare and to reduce mortalities at the end of the rearing period.

In laying hens, IRTI has been experimentally tested as an assessment method for bumblefoot and plumage condition. Wilcox et al. (2009) used IRTI to diagnose subclinical bumblefoot, finding a high correlation between thermal images and the visual score. This correlation was 86.7% in hens classified as clinical, but only 26.7% in hens classified as mildly clinical at day seven post-inoculation with *Staphylococcus aureus*. The authors suggested that IRTI was more sensitive than the visual scoring to detect subclinical cases of bumblefoot, which would facilitate early detection of the inflammation and would reduce associated pain. The method is clearly efficient to detect bumblefoot, even though it is invasive, and collecting feet infrared images requires holding the hens several times during the experiments which is a source of stress and it is detrimental to their welfare (Lay et al., 2011). A potential alternative would be assessing a small, representative bird sample to predict the incidence of bumblefoot in the flock in order to take any preventative or remediating step.

Zhao et al. (2013) used IRTI to determine its potential to assess feather coverage. In their work they considered three feather coverage categories: excellent feather (EF), fair feather (FF) and no feather (NF) in six different body areas (head, dorsal neck, front neck and crop, back, breast, and belly). For all body areas, the EF surface determined by IRTI was positively correlated with the feather scoring, while FF and NF areas were negatively correlated with feather scoring. The IRTI method also confirmed that feather coverage deteriorated in older hens, which lead to a higher feed to egg conversion rate because of higher sensible heat loss.

3.4. Kinematic Analyses

Kinematics is a branch of classical mechanics that describes the geometry of motion without consideration of the masses and the forces that may have caused the motion (Beggs, 1983). One of the main advantages of the kinematic technology is that it offers the possibility to perform a three-dimensional (3D) evaluation of the motion in a rapid and non-invasive way. In broilers, kinematic analysis was used to identify gait abnormalities (Caplen et al., 2012). Spherical retro-reflective markers, infrared cameras, and 3D kinematic data processing software were used to compare the gait characteristics of broiler chickens with their ancestral line, the red jungle fowl, and to find a link between broiler gait parameters to better define lameness scores. Caplen et al. (2012) found that, while jungle fowl increased their velocity by taking strides of comparably longer duration and length, lame broilers took shorter strides and reduced stride duration to accelerate. They also found that the larger pectoral muscle mass of lame broilers displaces their centre of mass, and this requires their feet to be positioned further forward under the body for support. These findings explained the existing differences between both genetic lines and the consequent impact on broilers' welfare. Kinematic analysis has been also used to study spacing behaviour in laying hens in order to calculate minimum space requirements (Mench and Blatchford, 2014).

4. Mobile Apps for Welfare Assessment

A novel approach has been recently proposed for the welfare assessment of commercial broiler and turkey flocks that is based on line transects (Marchewka et al., 2013; 2015). The transect method assesses the frequency of broilers or turkeys showing signs of impaired welfare by noting their incidence while walking along predefined paths or transects that are established among drinkers and feeder lines.

The i-Watchbroiler and i-Watchturkey software applications for mobile devices based on this methodology, allow assessors to easily record the frequency of birds showing any of the defined welfare incidences by pressing on the touch screen menu (Figure 1A, B), which is standardized by the expected number of birds within each transect.

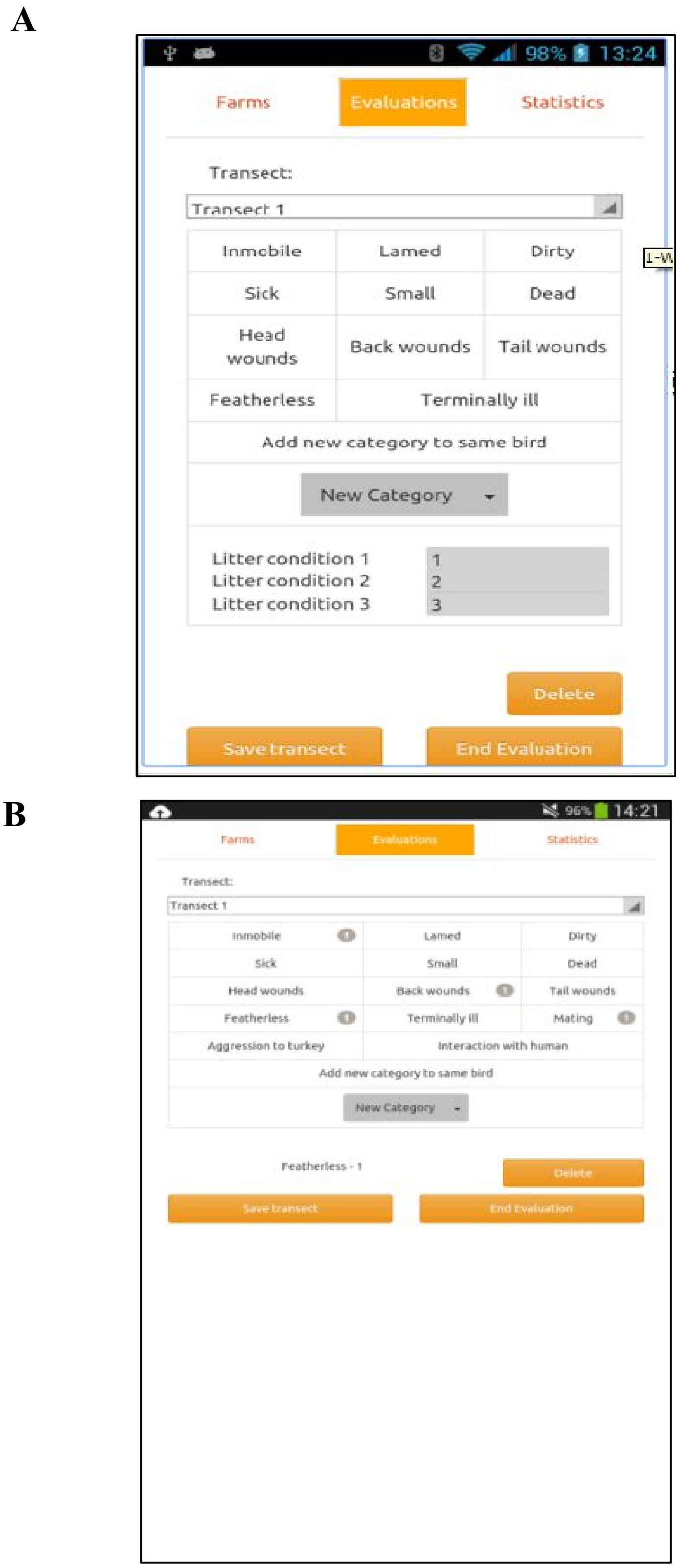
Both apps permit the inclusion of main features of the housing conditions, flock specific characteristics, and age at assessment so that data files, when exported, contain complete information relative to independent variables and the corresponding assessment results for further statistical analysis. These apps include basic statistics tools so that they are able to provide the mean incidence of each welfare indicator immediately after flock assessment and will calculate potential deviations from previously collected data. The results obtained by Marchewka et al. (2013; 2015) showed a good inter-observer reliability of the methodology for both broilers and turkeys and the method was validated for turkeys (Marchewka et al., 2015).

5. Mathematical Modelling

Modelling approaches can be used to enhance the application of technology in commercial poultry farms. Indeed, large sets of data produced by sensors and video recordings can be analysed by complex modelling or artificial intelligence algorithms to generate predictions or risk assessment models. Modelling techniques are essential to interpret data from real time monitoring devices in order to develop control systems or to establish risk alerts.

5.1. Environmental Conditions

A good monitoring of temperature, humidity, ventilation and lighting within poultry houses is essential to guarantee optimal rearing conditions and environmental standards for good welfare. Computational fluid dynamics (CFD) is a branch of fluid mechanics that provides a cost effective means of simulating real flows by using governing equations (Chung, 2010). CFD techniques are based on the resolution of a set of partial differential continuity equations (conservation of mass, conservation of energy, and conservation of momentum) (Bustamante et al., 2013). CFD models have been used to evaluate ventilation efficiency on broiler thermal stress and mortality (Chung, 2010). Predictions were validated with real environmental data collected with a multisensory system composed of 24 air velocity and temperature sensors and two differential pressure sensors (as explained in earlier sections).



Transect: Transect 1

Litter condition 1 1
Litter condition 2 2
Litter condition 3 3

Transect: Transect 1

Featherless - 1

Figure 1: (A) The i-Watchbroiler mobile app menu screen. Major welfare indicators assessed include: lame, immobile, sick, small, dirty, terminally ill, featherless, and wounded birds; (B) The i-WatchTurkey mobile app menu screen, including specific welfare indicators for turkey assessment.

According to Bustamante et al. (2013), this technique not only permits the improvement of thermal comfort and overall welfare of broilers, but also reduces the electric energy consumption.

CFD has been recently used by Rojano et al. (2015) to model and predict climate and air quality parameters (temperature, absolute humidity, and CO_2) in naturally ventilated broiler houses by investigating sensible and latent heat, as well as mass transport and heat transfer emitted by animals, litter, and heaters. The analysis was carried out at two distinct time periods: at the beginning of the growing cycle to assess the influence of heaters and with the birds maintained at low ventilation rates, and at the end of production to assess the influence of stoking density under high ventilation rates and heaters turned off. In the latter case, the effect of sensible and latent heat was examined by simulating three different animal densities in the model. To validate the CFD model, spatial variation of temperature and humidity were collected every 10 min during the production cycle using calibrated sensors as well as indoor and outdoor CO_2 concentrations using photo-acoustic infrared spectrometry. Rojano et al. (2015) indicated that for both phases, the predictions of temperature, absolute humidity and CO_2 were, in general, in agreement with experimental data, but recommended taking into consideration the effect of animals, litter, and by-products generated by the heaters to improve CFD model accuracy. CFD modelling not only appears to provide solutions for optimal poultry farm design, but also may enhance future applications in creating a real time automated system capable of controlling house conditions to avoid mortalities due to thermal stress, thus improving animal welfare.

5.2. Spatial Distribution and Activity Modelling

According to the World Organization for Animal Health (OIE, 2015), "Changes in the spatial distribution of birds may indicate thermal discomfort or the existence of areas of wet litter or uneven provision of light, food or water". Birds' spatial distribution may evidence problems occurring in a poultry house, thus, recent studies have focused on technological approaches that consider birds' spatial distribution in the context of PLF technologies.

Kashiha et al. (2013) used the eYeNamic systems (Costa et al., 2009), which is an image pre-processing tool, to calculate the number of object pixels in ratio to the background from images captured every five minutes by three cameras placed above a commercial flock. From the pixel ratio, a zone occupation density (ZOD) was calculated (60 ZOD per camera as every image captured was divided into 60 zones), together with the mean occupation rate for the flock; this information was used to calculate an activity index. The authors manipulated lighting periods experimentally in order to design a mathematical model based on the variation of the activity index capable of predicting the response during the next light period. When the measurements deviate from the predicted response calculated by the model it indicates that an event might have been occurring in the house (malfunctioning of feeders, drinkers, heating, ventilation, or a visiting human). The model was validated using the farmer's logbook, where all problems occurring within the house were registered. The comparison of predicted and measured distributions showed that the method could report successfully 95.24% of events in real time during a complete growing period while generating no false alarms. This fully automated technology has been already introduced at a commercial level allows for the identification of problems in broiler flocks and helps farmers to conduct real time monitoring of their animals more efficiently.

Lately, Youssef et al. (2015) aimed to predict the behavioural response (activity levels) of broilers under different micro-environmental conditions by introducing a model-based predictive controller (MPC). The dynamic MPC should be able to predict the system output, broiler activity in this case, in response to changes in the control variable (inlet temperature and ventilation rate). In this study, 45 seven-day old broiler chicks were housed in a test chamber where 30 temperature and air velocity sensors as well as CCD cameras were installed to measure temperature inlet, air velocity, and chickens' activity. During the experiment, combinations of ventilation rate and inlet temperature increases were applied. The airflow pattern was estimated to investigate the spatial temperature distribution in relation to the local velocity distribution in the test chamber. This estimation was later compared with the bird's zonal occupation and activity level. A dynamic activity index was calculated on the basis of the variations in the pixel intensity between consecutive frames. Finally, a dynamic modelling of the activity index was calculated to describe the static and dynamic responses of the chicken's activity index in response to variations in air temperature and ventilation rate. With this system, Youssef et al. (2015) were able to detect that non-homogeneous airflow patterns in the test chamber resulted in a heterogeneous spatial distribution of the chickens, with those undergoing heat stress tending to occupy high air velocity areas, and vice versa (Youssef et al., 2015). Even though this technology is still at an experimental stage, it might be useful to correct environmental parameters according to real time behavioural bird response.

5.3. Precision Feeding

The detrimental effects of elevated stocking densities, suboptimal environmental conditions, or inadequate lighting regimes reflect on broilers' feed intake (Ferket and Gernat, 2006). In addition, the use of different feeding strategies can help birds to better cope with different sources of environmental stress, and to prevent the onset of skeletal disorders (Meluzzi and Sirri, 2009). Consequently, the control of feed intake through the use of precision feeding tools would be of great interest to improve flock management and bird welfare.

Gates and Xin (2008) developed two algorithms to determine the feeding behaviour of broiler chickens and laying hens, with the aim of assessing the impact of environmental stressors. The algorithm was used to predict feeding patterns such as the number of meals, time at feeder, and meal size, as well as to discriminate between feeding bouts and stereotyped pecking. While laying hens were subject to heat stress, feeding behaviour of broilers was assessed when presented with a specialized sesame diet. The study was validated using video observations of the birds' behaviour. Both algorithms showed robustness in providing parameters like meal size, time at the feeders, and were able to discriminate between eating at the feeder versus stereotyped pecking, all of which were in agreement with the video recorded observations.

5.4. Monitoring Performance, Stress and Health Status

It is well known that the welfare status of laying hens has a direct impact on egg quality (Lin et al., 2004; Ebeid et al., 2012; Barbosa-Filho et al., 2006), therefore, a drop in egg production or quality may be indicative of ongoing welfare problems.

Mertens et al. (2008) used statistical process control, a technique that permits the formulation of quality limits based on natural process variability, to develop an intelligent control chart to monitor hens' variation on egg weight. Large scale experimental flock data were first used to construct and train the model in order to detect the natural increment in egg weight with increasing age. In a second phase, average egg weight was daily registered and all occurring events (technical failure, mortality, treatments) were recorded in a log file for the validation process. In order to investigate the ability of the control chart to detect drops in egg weight, two main stressors were tested: heat stress and red mite infestation. Results showed that the model was able to detect egg weight losses caused by heat stress, red mite infestation, and other management problems even though some false alarms were registered. The authors could detect abnormalities within two days after the onset of the tested challenges. A more sophisticated algorithm was later developed by Mertens et al. (2009) to control daily egg production. The system was able to detect feed intake decline resulting in reduced egg production. Similarly, an error in feed formulation produced an alarm soon after feed administration.

Transmission colour value (TCV) of the egg shell measured by visible-near infrared transmission spectroscopy was used by Mertens et al. (2010) to monitor flock stress and health in laying hens. TCV was calculated as the ratio between the transmission at 643 nm (maximum absorbance of the pigmentation molecule protoporphyrin IX) and the transmission at 610 nm (a reference wavelength). In addition, the algorithm based on Mertens et al. (2008; 2009) was used to construct a control chart to monitor the course of TCV and to investigate if changes could relate to stressful events. This technique was successful at detecting a significant variation on eggshell pigmentation due to heat stress, infectious bronchitis, and after an abrupt transition to phase two feeding that caused a decline in feed intake. Variation in TCV values warned about the occurrence of a problem four days earlier than the consequent drop of the average egg weight. The authors concluded that tracking daily variations in eggshell colour might be useful as a relevant stress and health status indicator.

Another technique that may have future applications is Support Vector Machines (SVM), a type of machine learning algorithm, used by Hepworth et al. (2012) to identify risk factors for hock burn incidence. SVM are a set of supervised learning algorithms which perform classification by finding the hyperplane that maximizes the margin between two classes of variables (Vapnik, 1995), and it is used in epidemiology for classification, diagnosis, and risk factor identification. Hepworth et al. (2012) recorded data relative to farm management conditions (stocking density, number and age of parent flocks, sex, and rearing system) together with daily water consumption, average weekly weight, mortality, and slaughterhouse outcomes (rejections, downgrades, and hock burns). Test and training data were performed by repeating random division of the collected data in two halves. After ten repetitions, the hierarchical structure was retained in each half of the data. Then, SVM classifiers with linear kernels were built and compared to manually build logistic regression models in order to test SVM classifiers' reliability on predicting hock burn prevalence. As indicated by Hepworth et al. (2012), this technique has an enormous potential to improve poultry health and welfare as it has proved robustness for a broad range of complex data sets. Furthermore, SVM does not rely on restrictive assumptions about the distribution and independence of data, in contrast to logistic regression modelling.

6. Discussion

A wide range of technical developments, complex data processing, and modelling tools have emerged in the past few years with the potential to assess, control, and improve poultry welfare. Although some technologies are still in a developmental phase, others have already been implemented under commercial conditions. In fact, many of the technologies here presented could be integrated in farm management processes to enhance poultry welfare and farm efficiency while facilitating the decision making process during the growing cycle. As one of the fastest-growing production species, with very similar management strategies around the world and with a high level of integration, poultry production and especially broilers' offers the ideal conditions for the application of the latest technological developments. Indeed, as the production cycle of broiler chickens is short (40 to 45 days), large data sets containing a substantial variety of information are relatively easy to acquire, which facilitates testing and implementing such technologies and a continuous improvement of welfare condition in next flocks. The objective of PLF technologies is to address and prevent major poultry welfare issues while providing farmers with better and faster management solutions that would result in higher efficiency and economic profit. This review highlighted the most important technological advances that have the potential to be applied to improve the welfare of broilers and laying hens as well as of other poultry species.

It is clear that environmental conditions and noxious gas concentrations have a major impact on the birds' welfare, health, and performance (Dawkins et al., 2004; Olanrewaju et al., 2008; Wang et al., 2010). Thus, a better and faster control of the environmental conditions along the production process would permit the improvement of birds' health and welfare as well as productive efficiency. Environmental sensors permit real time monitoring of the production conditions in a relatively simple and efficient manner at an affordable cost. However, large environmental data sets are not helpful unless data are processed adequately in order to extract relevant, meaningful information for the end users. Customized algorithms and other complex mathematical techniques allow processing of the collected information to detect variations and their potential consequences, leading to the development of quite precise alert or risk assessment systems. Alerts allow the end user to easily detect when a threshold has been reached, thus facilitating the application of control measures to resolve the problem or to minimize its impact. Hence, automated data collection, processing, and interpretation would permit farmers to fulfil the PLF challenge of improving animal welfare, health, and environmental sustainability (Banhazi et al., 2012) as higher performance will be obtained from a set amount of resources.

Regarding the value of technological advances to address specific poultry welfare issues, research has shown that piezoelectric sensors (Naas et al., 2010) and kinematic technology (Caplen et al., 2012) can be useful to investigate locomotion characteristics and gait abnormalities in broilers, while wireless acceleration sensors can be used to determine the effect of height on the incidence of bone breakage in laying hens (Banerjee et al., 2014). Even though these examples of technological developments are still at an experimental phase and would need further research for commercial implementation, these approaches can be helpful to understand bird locomotion characteristics and to detect locomotion abnormalities, at least in experimental studies.

Analysing and processing data derived from different imaging technologies appears to be suitable to assess gait (Aydin et al., 2010), walking ability (Dawkins et al., 2012), and footpad dermatitis (Vanderhasselt et al., 2013) in broilers, or to detect bumblefoot incidence in laying hens (Wilcox et al., 2009). The Dawkins et al. (2012) study conducted on commercial broiler flocks showed a great potential for a fast detection of abnormal walking behaviour and for a consequent implementation of mitigating strategies. Likewise, SVM classifiers' modelling techniques have been refined to identify risk factors causing hock burn in broiler flocks (Hepworth et al., 2012). At the health level, initial studies in optical flow and wireless sensors indicate that such technology can also be applied to detect infectious diseases that have a major economic and social cost such as *Campylobacter* (Colles et al., 2016) and avian influenza (Okada et al., 2014) before the appearance of the first signs of the disease. Considering welfare issues that are more specific to laying hens, a focus on feather pecking was undertaken using optical flow (Lee et al., 2010), image radio telemetry imaging (Zhao et al., 2013), and sound sensing (Bright, 2008) technologies. A future implementation of these technologies at a commercial level could be efficient to prevent the development of health issues that have major implications for the welfare and performance of meat and egg producing poultry. Production diseases are estimated to cause a 10%–15% reduction in performance in poultry farming (ProHealth Project, 2016). Imaging and sensing technologies able to detect changes in birds' behaviour, health, and welfare would be of great help to minimize economic losses due to this cause.

Providing birds with the possibility to express their basic behavioural repertoire is an important welfare aspect. Spatial requirements for basic behaviours and use of space in laying hens have been addressed using kinematic analysis (Mench and Balachford, 2014) and geographic information systems sensors (Daigle et al., 2014). These studies, undertaken at the experimental level, aimed to better understand hens' behaviour and use of space in order to adjust building design and to enrich hens' environment according to scientific knowledge. Continuous real time automatic monitoring of flocks' spatial distribution index (Kashiha et al., 2013) and activity (Youssef et al., 2015) should allow a good control of the flocks' behavioural and welfare state, permitting the detection of deviations from the "normal flock behaviour" in a timely manner in order to prevent, or at least minimize, major behavioural and welfare problems that should lead to better farm management, production efficiency, product quality, energy consumption, and, therefore, should improve long term sustainability.

Sound sensors, that have been around for a number of years can be used for a wide range of applications such as to estimate feed intake (Aydin et al., 2014) and to predict growth (Fontana et al., 2015) in broilers, or to detect environmental conditions leading to heat stress in broilers (Moura et al., 2008) and laying hens (Lee et al., 2015), with the possibility of notifying farmers regarding such conditions. On the other hand, infrared image technology appears to be suitable to provide modelling tools to calculate optimal air velocity rate for good thermoregulation and growth rate (Yahav et al., 2004), as well as for establishing incubation programs that may improve the ability of broilers to cope with heat stress and reduce the incidence of other health issues later in life (Shinder et al., 2009).

Given the advances in technology and their application to the field of animal health and welfare, it seems that, in the near future, the existence of plug-in equipment containing a full range of environmental and sound sensors, image processing, and analytic capabilities might be a reality, allowing a precise automatic welfare assessment and intelligent management at a commercial level. However, different technologies are still facing major limitations for their implementation at a commercial scale. Indeed, data collection and processing refinement is still needed, robust equipment must be developed to resist the harsh farm conditions and must be cost-efficient. Major technical and software advances have yet to take place in order to develop plug and play systems that provide reliable results. Although some technologies are already being used under commercial conditions, flock welfare assessment is generally carried out by applying existing welfare protocols. In order to facilitate the practical application of welfare protocols in meat poultry, mobile apps based on the transect methodology (Marchewka et al., 2013; 2015) have been recently developed. This non-invasive technique may provide a good depiction of the welfare condition within meat poultry in a simple and affordable manner. This method of assessment, if applied on a regular basis by producers, should be able to provide an early warning of the main broiler welfare and health issues and, thus, would also permit reducing economic losses and improve sustainability of the production.

It is crucial from a welfare perspective to validate such technologies before their implementation and to be mindful of their added value to poultry welfare (Daigle, 2014). Indeed, it is stressed that the main goal of using technology in poultry production is not only to facilitate improvement in farmers' lives and enhance production but also to develop our capacity of understanding birds' behaviour in commercial conditions and to improve their quality of life. A better quality of life will also mean that birds will grow healthier and more efficiently, thus, improving welfare is a direct road towards sustainable poultry production systems.

7. Conclusions

Despite of all the technological developments achieved in the last ten years, the challenges for full achievement of PLF goals are still important. Further research is needed to improve the data processing and modelling accuracy and to integrate all the suitable sensing, image processing, and data analysis in a plug-in system that is reliable, simple to understand, and economically viable for widespread use. Once available, PLF technologies will certainly provide added value to farmers, especially in regard to improved welfare and reduced environmental impact and long term system sustainability. Indeed, one of the objectives of installing technological devices in a poultry farm is an efficient and early detection tool of potential abnormal situations. Yet, an initial economic investment would be required to acquire and install such devices. Some of the technologies already used at the commercial stage are resilient and affordable. However, demonstrating and verifying the economical, welfare and environmental advantages of the technology in the medium and long term are critical (Banhazi et al., 2011). Proving the coherence of advanced technology and modelling tools to reduce costs by improving welfare should be a clear convincing argument to facilitate the implementation of these technologies at a commercial level.

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II

CHAPTER 2

The potential of the transect method for early detection of welfare problems in broiler chickens

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Abstract

The potential of the transect method was tested for early detection of welfare problems associated with bird age and genetic line, litter quality, and transect location. On-farm welfare impairment and its consequences on slaughter outcomes were evaluated to test the method's predictive ability. Thirty one commercial Ross, Cobb and mixed RC broiler flocks were evaluated at 3, 5 and 6 weeks of age. Two observers evaluated two transects each, simultaneously and in the same house by detecting welfare indicators including lame, immobile, sick, small, dirty, tail wounds, other wounds (head and back wounds), featherless, terminally ill, and dead birds. Increasing lame, immobile, sick and terminally ill birds according to bird age ($P<0.001$) was detected. Higher incidences of small and sick birds were detected in C and RC ($P<0.001$) as compared to R flocks while more dead and tail wounded were observed in RC compared to R and C flocks at week 5 ($P<0.001$). Dirty incidence increased as litter quality deteriorated ($P<0.001$). A higher incidence of immobile, small, sick, dirty and dead was registered near house walls ($P<0.001$). Differences across observers were detected for lame, immobile and terminally ill birds ($P<0.001$). For the observer by bird age interaction, differences were detected for dirty, tail wounds and other wounds ($P<0.05$). Pearson correlations between welfare indicators at week 3 and those at final weeks of age ($P<0.05$) ranged between r values of -0.2 and 0.654 ($P<0.05$). Correlations between welfare indicators and slaughter outcomes showed a relationship between flock mortality and dead on arrival, footpad dermatitis, leg problems and illness ($P<0.05$). Litter quality positively correlated with downgrades ($P<0.001$). This study showed the potential of transects to detect differences in welfare indicators according to factors which effects were previously reported. It demonstrated the transect potential for detecting and predicting the consequences of welfare impairment on slaughter outcomes. This would make the transect method a useful tool for notifying and rectifying welfare deterioration as early as at 3 weeks of age.

1. Introduction

Broiler chickens' world production reached 22.705 billion birds in 2016 (FAO, 2018), mostly reared in intensive systems. Housing conditions are designed to maximize performance by providing chickens with the adequate physical environment and resources to fulfill their basic needs. Environmental conditions, however, may deteriorate, compromising birds' health, welfare (Dawkins et al., 2004, Estevez, 2007), and farm profitability (Meluzzi and Sirri, 2009). Fast growing broiler chickens are prone to develop welfare problems which consequences are high mortality and low body weight due to lameness (Wideman et al., 2012), that ultimately impact the farm economics (Bassler et al., 2013). Thus, assuring birds´ welfare is not only an ethical responsibility essential to todays´ agribusiness, but it is essential to assure farm revenues and long-term sustainability of the broiler industry.

Assessing animal welfare is not a trivial matter. The Welfare Quality (WQ) protocol (Welfare Quality®, 2009) for on-farm assessment of commercial broiler flocks assigns an overall flock score (not classified, acceptable, enhanced or excellent) based on different health and welfare parameters that were established according to scientific criteria. Nonetheless, there are constraints on the feasible sample size to be analyzed due to time constrains per flock (de Jong et al., 2012), and the protocol requires bird handling which might be stressful in itself. In the past few years, new technologies have been proposed for a better livestock management (Wathes et al., 2008; Berckmans, 2014; Ben Sassi et al., 2016). In this context, the transect method (Marchewka et al., 2013; 2015) and associated mobile apps (i-WatchBroiler, 2018; i-WatchTurkey, 2017) were developed as effective, non-invasive tools for the on-farm welfare assessment of commercial broiler and turkey flocks. The transect method is based on walks conducted along predefined paths, or transects, established between drinker and feeder lines. Along these walks data on previously validated broiler welfare parameters (EFSA Panel on Animal Health and Welfare, 2012) are collected. Good inter-observer reliability, reduced personnel requirements and fast implementation on commercial farms were reported in broilers when compared to the WQ protocol (Marchewka et al., 2013). The transect method was validated for turkeys by evaluating the entire flock during loading before their transport to slaughter plant and two days after being assessed with the transect method (Marchewka et al., 2015). Due to the large size of broiler flocks (20000 to 40000 birds/flock) as compared to turkeys’, and the differences in the loading process, the same validation method would be hard to implement in broilers. As an alternative, testing some of the known effects of the broiler rearing cycle may provide insights of the transect method detection ability of welfare impairment caused by such aspects.

Until now, assessment has been carried out by two observers visiting flocks once towards the end of the rearing period (Marchewka et al., 2013), but welfare problems start developing earlier. Leg problems start developing between 3 and 5 weeks of age causing lameness and immobility (Bradshaw et al., 2002). The incidence of sickness increases with age (Northcutt et al., 2003; Talebi et al., 2005), along with scratches and wounds which seem to occur especially towards the end of rearing when birds are more likely to step on each other (Wideman, 2016). On-farm mortality and dirty feathers are also affected by the deterioration of environmental and management conditions (de Jong et al., 2015). Some of the early signs of lameness, sickness or any of the above mentioned welfare problems might be detected, and perhaps controlled, if a reliable and easy to implement assessment method was developed. Therefore, flock assessment at different time points may provide a practical estimation of the transect method for this purpose.

Differences in welfare and performance sometimes relate to the birds´ genetic makeup, such as ascites and sudden death syndrome in the case of fast growing lines (SCAHAW, 2000; EFSA, 2010). Broiler lines may differ in their immune profiles or immune response (Manzoor et al., 2003), resulting in differences in resistance to necrotic enteritis (Hong et al., 2012; Jang et al., 2013), in their response to heat stress (Azad et al., 2010), or in the prevalence of lameness (Nelson et al, 1992; Dinev et al., 2012). A relationship between genetics and mortality was also established (Kalmar et al., 2013) showing that lines with higher risk of developing welfare problems tend to have higher mortality rates (Rekaya et al., 2013). Thus, broiler chickens´ genetic background should be considered when assessing health and welfare of commercial flocks.

Litter quality usually deteriorates along rearing due to the combination of the effect of stocking densities, insufficient environmental control (Petek and Orman, 2013) and excreta accumulation (van der Hoeven-Hangoor et al., 2013). Consequently, poor litter quality increases the risk for leg problems and sickness towards the end of the rearing period (Sorensen et al., 2000; de Jong et al., 2014). Hence, testing the effect of litter quality on welfare indicators is a pivotal aspect in any on-farm welfare assessment. Furthermore, broilers are more likely to crowd near walls when resting (Newberry and Hall, 1990; Cornetto and Estevez, 2001a, Buijs et al., 2010), where dead chickens are more often seen (Tabler et al., 2002). Birds with poor health or deteriorated leg conditions would be more likely to seek the protection of walls (Newberry and Hall, 1990).

Due to the complexity of on-farm welfare assessment, data from slaughter plants have been used to predict on-farm welfare status. For instance, de Jong et al. (2015) predicted on-farm footpad dermatitis, hock burn, cleanliness and gait scores from slaughterhouse measurements of footpad dermatitis and hock burns. Dead on arrival (DOA) at slaughter can also be used as an indicator of on-farm welfare, as it correlates well with the flock’s health status (Jacobs et al., 2017a). Welfare outcomes at slaughter are also affected by the catching and transportation process (Leandro, 2001; Baracho et al., 2006; Jacobs et al., 2017b). Hence, it may be difficult to separate the impact of the rearing conditions from the effects of the catching, transportation and processing when assessment is only performed at the slaughter plant.

The development of an effective and practical on-farm welfare assessment method with the use of the i-WatchBroiler app, facilitating data collection and analyses, may allow to the identification of early indicators for welfare risk assessment. Identifying these indicators at early stages would allow the implementation of mitigation strategies increasing bird health and performance. This goal is aligned with the aim of the technological advances developed under the umbrella of precision livestock farming and its application to welfare assessment (Berckmans, 2014; Ben Sassi et al., 2016).

The goal of this study was to test the potential of the transect method for early detection of welfare problems, and to determine its variations according to the effect of birds age and genetic line, litter quality and transect position (central/wall). We hypothesized that the method would detect differences in the incidence of leg and health problems according to age, genetic line and litter quality. We predicted higher incidences of welfare problems near walls than in house central locations. We predicted that these outcomes would be associated with slaughter plant results which therefore would suggest the method's potential to predict slaughter outcomes from on-farm welfare impairment.

2. Materials and methods

2.1. Farms and birds

The study was conducted from April, 2015 to July, 2016 in thirty one commercial broiler flocks located in Northern Spain and all being part of the same integrating company. Because of the distance to our Institute, three of the initially assessed farms were replaced with three other farms. The initial flock sizes and bird densities ranged from 17952 to 41561 birds and 15 to 19 birds/m^2, respectively. Bird lines used were Ross 308 (R), Cobb 500 (C), or a mix of both (Ross 308/Cobb 500; RC), being all mixed gender flocks. All houses were provided with automatic drinkers, feeders, and ventilation systems, although the type of ventilation systems did vary. Bird management was similar across flocks, and followed the integrating company guidelines. This study complied with the Spanish legislation regarding the use of animals for experimental and other scientific purposes (Real Decreto 1201/2005).

2.2. Data collection

Farm Data: Data on welfare indicators were collected at 3, 5, and 6 weeks of age. Data collection was based on the transect methodology for welfare assessment for commercial broiler and turkey flocks as previously described (Marchewka et al., 2013; 2015). The method consists on a set of walks (transects) conducted in random order within the areas of the house delimited by the feeder and drinker lines. Marchewka et al. (2013) showed that sampling a minimum of 20% of the house area using the transect method provided a reliable mean of the flock welfare status. Considering this, in our study two previously trained observers simultaneously assessed two transects each, during each observation day and flock. Observers walked one transect starting from the entrance of the house until reaching the opposite wall and returned by a different transect. Transects were randomly chosen, with the precondition that each observer walked one central and one wall transect. Central transects were delimited by two successive feeder and feeder/drinker lines, while wall transects were delimited by one of the house walls and the adjacent feeder line. Assessment of two transects per observer normally lasted 45 to 60 minutes. Sequential observation of contiguous transects was avoided to minimize the occurrence of double-counting birds (Marchewka et al., 2013).

The welfare assessment was performed using the i-WatchBroiler mobile application (i-WatchBroiler, 2015) installed on an Android tablet. Most relevant broiler welfare indicators (EFSA , 2012) were evaluated, and included: lame, immobile, sick, small, dirty, terminally ill, tail, back and head wounded, featherless, and dead birds (see definitions in Marchewka et al., 2013). Assessment was conducted by slowly walking along the transect and clicking on the app screen each time a bird showing one of the above mentioned indicators was observed. This assessment was conducted similarly to the farmers' daily routine, causing minimal disturbance to birds that slightly moved away as approached. Collected data were transformed to percentage of occurrence of each welfare problem per transect, relative to the estimated total number of birds in each specific transect. The estimation of the number of birds per transect was calculated as: flock size on the assessment day * (transect width/house width). The average number of birds per assessed transect was 3572±1553 (Mean ± SE).

In addition, litter quality was evaluated along the observed transects in three different locations (beginning, middle, and end), based on a 5-point scale (being 0 = dry and loose litter, and 4 = caked litter) according to the WQ protocol for poultry (Welfare Quality, 2009). An average litter quality score per transect was calculated. At 5 and 6 weeks of age, a sample of 50 birds was also evaluated for footpad dermatitis (FPD) according to the WQ protocol 5-point evaluation scale (Welfare Quality, 2009). This was not implemented during week 3 as FPD incidence is very low at this age (Bilgili et al., 2006). Flock mortality was collected for each flock.

Slaughter Plant Data: Slaughter plant data of each assessed flock were obtained from the integrating company. Carcass quality and production parameters included: DOA, downgraded carcasses, hematomas, broken wings, and average weight gain per day.

2.3. Statistical Analysis

Frequencies of occurrence of each welfare indicator per transect, calculated as explained above, were analyzed assuming a binomial distribution. Generalized linear mixed model, repeated measures ANOVAs were carried out using the GLIMMIX procedure in SAS 9.3 (SAS Institute Inc., 2011) software. Due to their low occurrence, back and head wounds were pooled and analyzed together by creating an "Other wounds" variable. The experimental unit was the house, and each flock was uniquely identified. Statistical models included age of the birds when assessed (3, 5 and 6 weeks), genetic line (R, C and RC), transect location (central, wall), observer and the two-way interactions observer by bird age, transect location by bird age, and genetic line by bird age as fixed factors. All models included the mean litter quality score corresponding to each transect as a covariate. Farm, nested within each data collection round, was included as a random factor in all models, and the week of age at the assessment was included as the repeated measures unit. A first order autoregressive covariance structure was assumed to account for any linear dependence of measures of each flock over time. For statistically significant effects ($P < 0.05$), least squares means differences were computed, with P-values adjusted for multiple comparisons using Tukey tests. For significant interactions, tests of simple effects (Winer, 1971) were performed to detect differences between levels of each factor at each specific age point ($P < 0.05$).

Pearson partial correlations were calculated using the CORR procedure in SAS to test the relationship between welfare indicators assessed at 3, 5 and 6 weeks, and between these and slaughter plant outcomes for thinning (around the end of the fifth week of age) and final transports. The observer effect was taken into consideration in the partial statement. As correlating many variables could lead to false positive correlations, welfare indicators corresponding to sick, terminally ill and dead were pooled into an "Illness" variable. Lame and immobile were pooled into a "Leg problems" variable and tail wounds and other wounds into a "Total wounds" variable. In addition to the average value of foot pad dermatitis (Av. FPD) calculated for each week (weeks 5 and 6), the percentage of birds with FPD superior to 1 (%FPD> 1) for each week was calculated and both variables were used for the correlation analyses.

3. Results

The effects of the main factors on each welfare indicator are presented in Table 1 and the means (±SE) for these main factors are included in Table 2. Given the low incidence of featherless chickens statistical models did not converge, but overall mean value (±SE) for this variable was 0.0067% (±0.001).

Changes in the incidence of lame, immobile, sick and terminally ill birds were detected with age, with a consistent increment in the frequency for almost all indicators from weeks 3 to 6. Genetic differences were detected only for small and sick birds (Table 2), while the interaction of genetics by bird age was significant for dead (P=0.0005) and tail wounded birds (P<0.0001; Table 3).

The incidence of welfare issues was generally higher along wall transects, with differences for immobile (P=0.015), small (P=0.013), sick (P=0.01), dirty (P=0.0009) and dead (P<0.0001) birds as compared to central transects. No effects of location by age interaction for all welfare indicators was detected (P>0.05) and therefore this interaction was removed from the models. Poorer litter quality caused a higher incidence of dirty birds (P=0.0004) (Table 2). Differences in the incidence of lame, immobile and terminally ill birds were detected according to observer (Table 1), as well as of the observer by age interaction on small (P=0.0041), dirty (P=0.0105), tail wounds (P=0.0001), and other wounds (P=0.0102). Mean values are presented in Table 4.

Relevant correlations between on-farm welfare indicators collected during the growth period are shown on Table 5 in detail. For example the incidence of leg problems observed at 3 weeks of age positively correlated with results at 5 weeks (P<0.0001), and similar positive correlations were observed between incidences of leg problems observed at weeks 5 and 6 (P<0.0001). Positive correlations were also observed between the incidence of leg problems and small birds (P<0.0001), and between small and ill birds (P<0.0001). Litter quality assessed at 3 weeks consistently and positively correlated with that of weeks 5 and 6 (P<0.0001).

Table 1: Effects of bird age, genetic line, transect location, litter quality, and observer (F and P value) for welfare indicators evaluated by the transect method.

Welfare indicator	Bird age		Genetic line		Transect location		Litter quality		Observer	
	F $_{(2,334)}$	**p-value**	**F $_{(2,334)}$**	**p-value**	**F $_{(1,334)}$**	**p-value**	**F $_{(1,334)}$**	**p-value**	**F$_{(1,334)}$**	**p-value**
Lame	144.65	<0.0001	0.08	0.9236	0.61	0.4366	0.02	0.8989	114.07	<0.0001
Immobile	151.46	<0.0001	1.96	0.1423	5.97	0.0151	1.46	0.2272	113.79	<0.0001
Small	26.45	<0.0001	6.07	0.0026	6.16	0.0136	3.22	0.0737	2.66	0.1041
Sick	9.9	<0.0001	7.82	0.0005	6.69	0.0101	0.04	0.8425	0.57	0.4495
Dirty	20.35	<0.0001	2.28	0.1036	11.13	0.0009	14.9	0.0001	16.25	<0.0001
Dead	11.07	<0.0001	0.07	0.9297	31.32	<0.0001	0.01	0.9104	0.52	0.4718
Terminally ill	6.46	0.0018	0.19	0.8247	0.87	0.3508	2.88	0.0905	8.67	0.0035
Tail wounds	28.19	<0.0001	5.79	0.0034	1.45	0.2287	1.26	0.2617	2.57	0.1096
Other wounds	2.89	0.0571	2.16	0.1174	0.01	0.9112	0.31	0.5795	2.32	0.1287

*Bird age: at 3, 5 and 6 weeks; Genetic lines: Ross, Cobb, Mixed Ross/Cobb flocks; Transect location: central and wall transect

Table 2: Mean values (SE) of incidence of birds within each welfare indicator expressed as percentage for each main factor[1].

Welfare indicator	Bird age			Genetic line			Transect position		Litter quality[2]	Observer	
	3 weeks	5 weeks	6 weeks	Cobb	Ross	Cobb/Ross	Central	Wall		1	2
Lame	0.080^{c}	0.172^{b}	0.422^{a}	0.183	0.182	0.175	0.176	0.184	0.006 (0.080)	0.268^{a}	0.121^{b}
	(0.010)	(0.019)	(0.044)	(0.024)	(0.021)	(0.022)	(0.019)	(0.020)		(0.027)	(0.013)
Immobile	0.033^{c}	0.124^{b}	0.301^{a}	0.091	0.113	0.119	0.097^{b}	0.118^{a}	0.114 (0.092)	0.065^{b}	0.175^{a}
	(0.006)	(0.019)	(0.044)	(0.016)	(0.018)	(0.019)	(0.015)	(0.018)		(0.010)	(0.026)
Small	0.079	0.095	0.167	0.109^{ab}	0.086^{b}	0.134^{a}	0.097^{b}	0.119^{a}	-0.187 (0.101)	0.115	0.101
	(0.018)	(0.022)	(0.038)	(0.028)	(0.020)	(0.032)	(0.022)	(0.027)		(0.026)	(0.023)
Sick	0.013^{b}	0.017^{b}	0.030^{a}	0.022^{a}	0.011^{b}	0.025^{a}	0.015^{b}	0.022^{a}	- 0.017 (0.177)	0.018	0.019
	(0.003)	(0.003)	(0.006)	(0.005)	(0.002)	(0.005)	(0.003)	(0.004)		(0.003)	(0.004)
Dirty	0.002	0.0007	0.012	0.001	0.003	0.004	0.001^{b}	0.005^{a}	1.010 (0.284)	0.001	0.005
	(0.001)	(0.0005)	(0.005)	(0.0008)	(0.002)	(0.002)	(0.0007)	(0.002)		(0.0007)	(0.002)
Dead	0.024	0.028	0.048	0.031	0.031	0.033	0.023^{b}	0.044^{a}	-0.022 (0.148)	0.031	0.033
	(0.004)	(0.005)	(0.008)	(0.006)	(0.005)	(0.006)	(0.004)	(0.006)		(0.005)	(0.005)
Terminally ill	0.004^{a}	0.0008^{b}	0.007^{a}	0.003	0.003	0.003	0.003	0.003	0.518 (0.315)	0.005^{a}	0.002^{b}
	(0.001)	(0.0005)	(0.002)	(0.001)	(0.0009)	(0.001)	(0.0009)	(0.001)		(0.002)	(0.0007)
Tail wounds	0.005	0.039	0.032	0.014	0.040	0.011	0.018	0.019	0.162 (0.164)	0.021	0.016
	(0.002)	(0.013)	(0.011)	(0.006)	(0.015)	(0.005)	(0.006)	(0.007)		(0.008)	(0.006)
Other wounds	0.003	0.006	0.003	0.004	0.002	0.006	0.004	0.004	0.125 (0.340)	0.005	0.003
	(0.001)	(0.002)	(0.002)	(0.002)	(0.0009)	(0.003)	(0.001)	(0.001)		(0.002)	(0.001)

[a, b, c] For each parameter, the row means followed by different superscript letter are significantly different ($P < 0.05$).

[1] Significant interactions are not shown in this table for indicators where correspondent simple factors are also significant (See tables 3 and 4).

[2] Values presented are regression coefficients (SE) for this variable estimated with statistical model. A positive coefficient value means that the incidence of each welfare indicator is estimated to increase in the magnitude of the regression coefficient as litter quality value increases (that is, litter quality decreases) one unit.

Table 3: Mean values (SE) of the incidence of birds within each welfare indicator category expressed as percentages for genetic by bird age interaction.

	Week of age	Cobb line	Ross line	Cobb/Ross line
		(%)	(%)	(%)
Dead	3	0.028 (0.007)	0.024 (0.005)	0.020 (0.005)
	5	0.017^{b} (0.005)	0.028^{ab} (0.006)	0.046^{a} (0.009)
	6	0.063 (0.014)	0.044 (0.009)	0.039 (0.009)
Tail wounds	3	0.006^{ab} (0.003)	0.012^{a} (0.006)	0.002^{b} (0.001)
	5	0.029^{b} (0.012)	0.102^{a} (0.037)	0.020^{b} (0.009)
	6	0.016^{b} (0.007)	0.051^{a} (0.020)	0.042^{a} (0.017)

$^{a, b, c}$ For each parameter, the row means followed by different superscript letter are significantly different ($P < 0.05$).

Table 4: Mean values (SE) of the incidence of birds within each welfare indicator category expressed as percentages for observer by bird age interaction.

	Week of age	Observer 1	Observer 2
		(%)	(%)
Small	3	0.099^{a} (0.024)	0.063^{b} (0.016)
	5	0.099 (0.023)	0.091 (0.022)
	6	0.154 (0.037)	0.182 (0.043)
Dirty	3	0.001 (0.0009)	0.004 (0.002)
	5	0.0005 (0.0004)	0.0008 (0.0006)
	6	0.004^{b} (0.002)	0.035^{a} (0.013)
Tail wounds	3	0.003 (0.002)	0.008 (0.003)
	5	0.055^{a} (0.019)	0.028^{b} (0.010)
	6	0.059^{a} (0.021)	0.018^{b} (0.007)
Other wounds	3	0.002(0.001)	0.003 (0.002)
	5	0.006 (0.002)	0.007 (0.003)
	6	0.008^{a} (0.003)	0.002^{b} (0.0009)

$^{a, b, c}$ For each parameter, the row means followed by different superscript letter are significantly different ($P < 0.05$).

Table 5: Pearson correlation between welfare indicators assessed at weeks 3, 5 and 6 using the transect method[1].

		Week 3						Week 5						Week 6					
Variables[2]		Litter	LP	Small	Illness	Dirty	TW	Litter	LP	Small	Illness	Dirty	TW	Litter	LP	Small	Illness	Dirty	TW
Week 3	Litter	1																	
	LP	0.049	1																
	Small	-0.075	0.333***	1															
	Illness	0.2**	0.263***	0.435**	1														
	Dirty	0.076	-0.166*	-0.15*	-0.074	1													
	TW3	-0.161*	0.132*	-0.052	-0.095	0.059	1												
Week 5	Litter	0.421***	0.097	0.044	0.07	0.033	-0.027	1											
	LP	-0.028	0.435***	0.254***	0.232**	-0.106	-0.067	0.071	1										
	Small	-0.163*	0.277***	0.618***	0.309***	-0.147*	-0.125	-0.065	0.453***	1									
	Illness	0.039	0.176**	0.634***	0.4***	-0.007	-0.084	0.018	0.215**	0.542***	1								
	Dirty	-0.056	0.051	0.246***	0.168*	0.077	-0.066	0.175**	-0.024	0.127	0.283***	1							
	TW	-0.183**	-0.015	-0.142*	-0.222**	0.108	0.396***	0.012	-0.079	-0.21**	-0.12	0.071	1						
Week 6	Litter	0.365***	0.107	-0.014	0.064	0.075	-0.061	0.495***	0.025	-0.121	0.06	0.056	0.013	1					
	LP	-0.092	0.405***	-0.265***	0.207*	-0.116	-0.129	0.011	0.478***	0.429***	0.196**	0.047	-0.107	0.043	1				
	Small	-0.025	0.297***	0.654***	0.345***	-0.082	-0.101	0.017	0.396***	0.619***	0.599***	0.219***	-0.204**	0.064	0.546***	1			
	Illness	0.095	0.183**	0.437***	0.225**	-0.03	-0.168*	0.003	0.091	0.3***	0.374***	0.054	-0.197**	0.156*	0.424***	0.463***	1		
	Dirty	-0.2**	0.113	0.133*	0.121	0.155*	-0.141*	-0.152*	0.1	0.154*	0.075	0.334***	-0.035	0.068	0.121	0.262***	0.017	1	
	TW	-0.015	-0.123	-0.078	-0.094	0.106	0.111	0.11	-0.203**	-0.094	-0.077	0.035	0.568***	0.128	-0.142*	-0.101	-0.048	0.035	1

[1] Significance of the correlation is indicated as follows: * for $p<0.05$; ** for $p<0.01$; *** for $p<0.001$.

[2] Variables presented in this table are: Litter: Litter quality; LP: Leg problems (sum of immobile and lame incidences); Illness: sum of sick, terminally ill and dead incidences; TW: Total wounds (sum of head, back and tail wounds incidences.

Pearson partial correlations between welfare indicators collected from weeks 3 to 6 and slaughter outcomes are presented in Table 6 for thinned flocks and in Table 7 for final flocks. Correlations were generally low to moderate with some negative values, but there were some interesting results. For example, for both thinning and final transports, moderate positive correlations were found for litter quality with Av.FPD and %FPD>1. Litter quality consistently and positively correlated with downgrades and average slaughter weight (Table 6 and 7). For final transports, flock mortality positively correlated with Av. FPD and %FPD>1 ($P<0.0001$), and with the incidence of leg problems during weeks 5 ($P=0.0334$) and 6 ($P=0.0053$) of age. Positive correlations were observed between average slaughter weight and Av. FPD ($P=0.0003$) and %FPD>1 ($P=0.0002$). DOA was positively correlated with illness for both thinning ($P=0.007$) and final transports ($P=0.02$), and with on-farm mortality at final transports ($P=0.02$).

Table 6: Pearson correlations between welfare indicators collected with the transect method (at weeks 3 and 5) and slaughter outcomes of the thinning transport[1].

	Week 3			Week 5			
Variables [2]	Litter	LP	Small	Litter	Illness	Av.FDP	%FDP>1
Av.Weight	0.348**	-0.008	0.106	0.203	0.263*	0.100	0.179
Downgrades	0.260*	-0.016	0.101	0.035	0.251*	0.341**	0.246*
DOA	0.332**	-0.151	0.148	0.225	0.321**	0.176	0.307*
Hematomas	0.318**	-0.078	0.193	0.144	0.35**	0.331**	0.315**
Brokenwings	0.029	0.324**	0.28*	0.429***	0.442	0.027	0.106
Av.FDP	0.467***	-0.078	-0.013	0.135	-0.01	1	0.960***
%FDP>1	0.520***	-0.069	0.038	0.170	0.06	0.960***	1

[1] Results are only shown for variables where at least one correlation is significant. Significance of the correlation is indicated as follows: * for $p<0.05$; ** for $p<0.01$; *** for $p<0.001$.

[2] Variables included are: Av.weight: Average slaughter weight; Av.FDP: Average value of footpad dermatitis at week 5; %FDP>1: Percentage of birds with footpad dermatitis superior to one at week 5; DOA: birds dead on arrival; litter: Litter quality; LP: Leg problems (sum of immobile and lame incidences); Illness: sum of sick, terminally ill and dead incidences.

Table 7: Pearson correlations between welfare indicators collected with the transect method (at weeks 3, 5 and 6) and slaughter outcomes of the final transport.[1]

	Week 3				Week 5					Week 6						
Variables[2]	Litter	LP	Illness	TW	Litter	LP	Small	Illness	TW	Litter	LP	Illness	TW	Av.FDP	%FDP>1	Mortality
Av.Weight	0.128	0.19*	-0.073	0.253**	0.240*	0.171	-0.238	-0.021	0.075	0.295**	0.048	-0.017	0.011	0.337**	0.350**	0.303***
Downgrades	0.335***	0.245**	0.014	0.221*	0.404***	-0.046	-0.309***	0.107	0.051	0.423***	-0.047	0.18	0.050	0.428***	0.371***	0.214*
DOA	-0.094	-0.118	0.055	-0.058	0.008	0.095	0.036	0.211*	0.064	-0.109	-0.05	-0.021	-0.077	-0.037	-0.005	0.218*
Hematomas	0.256**	0.122	0.022	0.296**	0.375***	-0.057	-0.211*	0.168	0.058	0.317***	-0.161	0.154	-0.068	0.232*	0.212*	0.033
Brokenwing	0.275**	0.31***	0.052	0.07	0.29**	0.159	-0.193*	0.085	-0.161	0.304**	0.129	0.166	-0.215*	0.486***	0.450***	0.357***
Av.FDP	0.510***	0.17	0.08	-0.149	0.291**	0.095	0.048	0.133	-0.18	0.386***	0.106	0.161	0.216	1	0.970***	0.417***
%FDP>1	0.552***	0.165	0.097	-0.166	0.3**	0.164	0.078	0.121	-0.196*	0.357***	0.123	0.119	0.033	0.970***	1	0.461***
Mortality	0.359***	0.134	0.299**	-0.251**	0.107	0.201*	-0.024	0.141	-0.144	0.036	0.261**	0.19*	-0.092	0.417***	0.461***	1

[1] Results are shown only for variables where at least one correlation is significant. Significance of the correlation is indicated as follows: * for p<0.05; ** for p<0.01; *** for p<0.001.

[2] Variables included are: Av.weight: Average slaughter weight; Av.FDP: Average value of footpad dermatitis at week 6; %FDP>1: Percentage of birds with footpad dermatitis superior to one at week 6; DOA: birds dead on arrival; litter: Litter quality; LP: Leg problems (sum of immobile and lame incidences); TW: Total wounds (sum of head, back and tail wounds incidence.

4. Discussion

Our goal was to test the potential of the transect method for early detection of welfare problems, and to determine the influence of age and genetic line, litter quality and transect position. We also focused on the potential of the method to predict slaughter outcomes from on-farm welfare impairment. The results of this study indicate that the transect method is effective to detect changes in the welfare status of broiler chickens during the growing period. The results also showed that flock welfare condition is reflected in the slaughter outcomes.

The incidence of almost all variables and specifically lame, immobile, sick and terminally ill birds increased with age, as would otherwise be expected in commercial flocks given the fast growth rate of modern broilers and its implications on skeletal, cardiovascular and immune development (Kestin et al., 1999; SCAHAW, 2000). Although leg problems may be affected by bird weight, deteriorated walking ability was previously reported to increase with age (Sorensen et al., 2000; Cordeiro et al., 2012), which is in agreement with our results. In addition, results of the correlation between welfare indicators and slaughter plant outcomes showed no relationship among the incidence of leg problems and body weight, at least for the data collected in this study. However, contributing factors to the incidence of leg problems include the lack of activity of the birds (Reiter and Bessei, 2009), and the gradual deterioration of litter quality throughout the rearing period (Bessei, 2006; Nääs et al., 2010). High bacterial cell counts and bacterial diseases are reported in older birds (Northcutt et al., 2003), which might explain the development of leg problems and sick birds with age, as for the latter, incidence at week 6 was higher than the double compared to the incidence observed during week 3.

Smaller than expected differences among genetic lines were detected for small and sick birds, with C and RC flocks showing slightly higher incidences. Intensive genetic selection is known to predispose modern broiler chickens to cardiovascular disease, sudden death syndrome, and ascites (Julian, 1998; Bessei, 2006; Hocking, 2014), with a heritability value of 0.3 for sudden death syndrome (Moghadam et al., 2005). Some studies have shown that performance of R lines is worse than that of C lines (Chepete and Mareko, 2008; Marcato et al., 2008), although this might only apply to healthy birds, making the reasons for differences among genetic lines unclear. However, the higher incidence of problems for RC flocks might be easier to explain. Mixed flocks are usually present when no sufficient birds of the same genetic line are available to reach the desired stocking density, which implies filling houses by using two lines. It is likely that remaining birds of at least one of the lines corresponded to the very end of the hatching period which are usually smaller than average (Ulmer-Franco et al., 2010). Thus, it is quite possible that mixed flocks are at disadvantage in comparison to flocks composed by one genetic line as management requirements are no identical for both lines. Higher frequencies of dead and tail wounded birds were found for RC when compared with R flocks at week 5. The higher incidence of dead birds can also be explained by potential competitive disadvantages of mixed flocks. Indeed, increased mortalities are usually observed during the final weeks of the growing period due to the gradual impairment of flock health and welfare, among other aspects. Besides, farm management could have interfered with the results of small and dead birds, since the decision to cull and remove these birds ultimately depends on the individual farmer. On the other hand, the higher incidence of tail wounds in R compared to C flocks at week 5, which was also numerically higher for R during weeks 3 and 6, is probably related to a higher activity or reactivity of R birds. Given that aggressive interactions are unusual in broiler chickens (Estevez et al., 1997), the higher incidence of tail wounds for R birds could be related to higher activity, resulting in more running and jumping on each other, which might increase the risk of injuries. This is a hypothetical explanation to our results, as bird activity was not measured.

Dirty birds were detected more frequently as litter quality deteriorated. Leaking drinkers (Jones et al., 2005), ventilation problems, higher stocking density, and older age at slaughter (Dawkins et al., 2004; Petek and Orman, 2013) contribute to increased moisture, and to the gradual deterioration of the litter, resulting in increased feather dirtiness. In our study, dirtiness was evaluated on the side and back feathers. Feather dirtiness was previously shown to deteriorate in the breast (de Jong et al., 2014) and back areas (Petek and Orman, 2013). As birds grow, the gradual reduction in activity levels would also increase the duration of contact with the litter (Cornetto and Estevez, 2001a; Alvino et al, 2009; Reiter and Bessei, 2009), increasing the risk of plumage dirtiness. This result supports the relevance of assessing litter quality at the three locations of each transect as litter quality can vary considerably among house locations.

A higher incidence of immobile, small, sick, dirty, and dead birds was detected along wall transects as compared to central transects. It is not uncommon to find the worst litter quality around the house periphery where birds tend to sit as they feel more protected against potential predators (Newberry and Hall 1990; Cornetto and Estevez, 2001b; Buijs et al., 2010; Aydin, 2016). Thus, litter quality in this area is likely to deteriorate faster, with negative effects on birds′ plumage and health. In addition, while un-well birds are moving in central areas, they will be disturbed resulting in random movement with constant changes in direction until they find the protection of walls. Once close to a wall, un-well birds are less likely to move away, a phenomenon referred to as "wall trapping effect" (Estevez and Christman, 2006). This might explain why broiler chickens with reduced mobility are more likely observed along wall transects. Other studies such as Tabler et al., (2002) reported higher mortalities along sidewalls, which concurs with our results and shows that impaired birds tend to move to the periphery of the house, and are later found dead there.

Although Marchewka et al. (2013; 2015) reported almost perfect concordance between observers when scoring broilers and turkeys with the transect method, in this study a significant observer effect was found for lame, immobile and terminally ill birds, as well as for small, dirty, tail wounds, and other wounds considering the interaction of observer by bird age. Despite this, taking into consideration that over 3,000 birds were assessed per transect, the numerical magnitude of the difference between observers was not high although statistical difference was reached. In addition, in many cases differences referred to the interaction with age. When pooling the incidence of lame and immobile into a single 'leg problems' variable, the observer effect was no longer detected (results not presented), indicating that statistical differences were due to differences on how to distinguish lame from immobile, which were not uniform among observers, but an overall agreement was reached regarding the detection of total leg problems. In this study, the observers scored two different transects each simultaneously and within the same house, while in Marchewka et al. (2013; 2015) the entire house was assessed separately by both observers. Thus, differences observed in the individual transects may probably be explained in part by the differences found among observers. However, and without denying the fact that differences reached statistical significance, immobile and lame incidences were comparable with those previously found when testing the transect method at 31 and 35 days of age (Marchewka et al., 2013). Previous studies reported 0.9% of lameness (Dawkins et al., 2004), 0.3 to 3.1% of severe lameness, respectively at 28 and 42 days of age (Sorensen et al., 2000), and 0.21% of immobility (Knowles et al., 2008). Our results are within the range of values of these studies. Considering dirty chickens, tail and other wounds, differences between observers might be due to difficulties in detecting these problems. This would be the particular case of tail wounds when stocking densities are high or house lighting is low. We aimed at minimal intervention on the flock during assessments to minimize bird disturbance, and in some cases this might have made visual detection of welfare problems difficult. Preliminary practice of observers and knowledge of species specificities are required before starting data collection.

Correlations between litter scores at different weeks suggest that litter quality evaluation at week 3 can be used as a predictor of litter quality to be expected at 5 and 6 weeks. This is critical given the association between poor litter quality, welfare issues and slaughter results (Dawkins et al., 2004; de Jong et al., 2014). The incidence of leg problems, including severe lameness and immobile birds at 3 weeks correlated quite well with the incidences observed at 5 and 6 weeks. These results suggest a potential of the method for early risk assessment and application of corrective measures which could include improving litter quality, the addition of more bedding material, and adaptations of the ventilation system, or providing better lighting program to promote activity. The frequency of illness and small birds at 3 weeks also correlated positively with the frequencies of both problems at 5 and 6 weeks. It was previously demonstrated that incidence of illness and small birds are associated with poor environmental and management conditions, parental flock age, temperature and humidity conditions of the incubator, or to hatching time (Reis et al., 1997; Tona et al., 2004; 2005).

On-farm welfare indicators also correlated with several relevant slaughter outcomes, which could have some implications. For example, positive correlations between litter quality and FPD for thinning and final transports were found. These results are in agreement with those from a number of previous studies demonstrating the effect of poor litter quality on FPD incidence (Dawkins et al., 2004; Bessei, 2006; Haslam et al., 2007; Meluzzi et al., 2008; Bassler et al., 2013; de Jong et al., 2014).

Litter quality also positively correlated with the incidence of downgraded carcasses, especially for final transports. A clear relationship was established between litter moisture and the incidence of hock burns, breast blisters, and dirty feathers, which are known to increase the incidence of downgraded carcasses (de Jong et al., 2014; Jacobs et al., 2017b). Higher Av.FPD and %FPD>1 values positively correlated with higher average slaughter weight for final transports, which is in line with studies like Kristensen et al. (2006) who showed a positive correlation between body weight, gait score, and occurrence of footpad lesions in heavy birds. Our results show a relationship between litter quality, FPD, and downgrades, corroborating once again that on-farm management and birds' welfare conditions have a critical impact on slaughter results.

DOA is known to be a good indicator of slaughtered flocks´ sanitary condition (Chauvin et al., 2011; Kittelsen et al., 2015; Jacobs et al., 2017a). However, DOA can be seriously affected by transportation practices and climatic conditions (Baracho et al., 2006; Chauvin et al., 2011; Jacobs et al., 2017b). Our results showed that illness at week 5 positively correlated with DOA at thinning transports, and that flock mortality rates correlated with DOA at final transports, along with a positive correlation between mortality and illness. This indicates that illness might have led to higher on-farm mortality, both leading to higher DOAs at slaughter, which was previously reported (Kittelsen et al., 2015). Further, flock mortality at week 6 positively correlated with Av.FPD and %FPD>1 at final transports, suggesting that on-farm problems leading to increased flock mortality would also influence slaughter outcomes. The positive correlation between flock mortality and the average slaughter weight is reflecting what was already shown in previous studies (Haslam et al., 2007), suggesting that faster growth rates might have negative consequences leading to higher on-farm mortality. The results of our study support the already established relationship between on-farm welfare and slaughter results (Dawkins et al., 2004; Dozier et al., 2005; Thomas et al., 2011; Abudabos et al., 2013; Marchewka et al., 2015), and indicate that the transect method can be used to detect on-farm welfare problems that will later translate into poor slaughter outcomes.

This study, although very intense for the resources at our disposal, only monitored 31 flocks, which is a modest number in order to determine the full potential of this method as a predictive tool. In spite of this, the initial results suggest that transects may be useful to improve bird management by providing farmers with specific quantitative information about the flocks’ issues, so precise mitigation strategies could be implemented to correct or minimize on-farm problems. This would translate into better slaughter outcomes, thus permitting a more efficient production system.

5. Conclusion

The transect method, applied with the i-WatchBroiler app, appears to be a practical and effective tool for on-farm assessment of commercial broiler flocks. In this study, we demonstrated that this method, implemented in about 45 minutes per flock, allows the quantitative assessment of the potential impact on welfare status caused by factors such as age and genetic line, litter quality, or the transect location within the house. This method could be considered as a valuable tool to support farmers' decisions and reduce welfare-related problems and their corresponding losses in economic returns. Although discrepancies relative to the observer effect are yet to be improved, our results show that the transect method is a suitable and practical tool for a rapid assessment of on-farm welfare in commercial broiler flocks. If welfare assessments are performed as early as during the third week of age, the transect method could be a valuable tool to anticipate and correct welfare issues at later stages, that will result in improving performance at the slaughter house (DOA and downgrades).

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CHAPTER 3

On-farm broiler chicken welfare assessment using transect sampling reflects environmental inputs and production outcomes

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Abstract

To evaluate the utility of transect sampling for assessing animal welfare in large chicken flocks, we quantified relationships between environmental inputs, welfare problems detected using transect sampling, and production outcomes. We hypothesised that environmental inputs including environmental complexity (i.e. number of environmental enrichment types provided), space allowance, underfloor heating (presence or absence), and photoperiod regimen (18 h continuous vs 16 h intermittent) would correspond to variations in welfare assessment findings, which would predict production outcomes. We conducted on-farm welfare assessment of Norwegian broiler flocks at approximately 28 days of age. We sampled four transects (rows between feeder and drinker lines) per flock to determine litter quality and the proportions of chickens with compromised welfare as indicated by visual signs of walking difficulties, illness, skin wounds and small bird size. Production outcome measures included mortality, reasons for carcass rejection at slaughter, footpad dermatitis, growth rate, feed conversion and an integrated production index. Greater environmental complexity was associated with a reduction in skin wounds and total welfare problems on the farm, lower mortality, fewer rejections due to wounds and underweight birds, and fewer rejections overall. Higher space allowances within levels of environmental complexity were associated with fewer walking difficulties and welfare problems overall, a reduction in rejections due to wounds, and a higher growth rate and production index. Underfloor heating was associated with a reduction in rejections due to leg deformity, and intermittent light was associated with lower illness and skin wound rates on the farm, and lower mortality. Furthermore, fewer welfare problems and better litter quality on the farm were associated with fewer carcass rejections at slaughter. Thus, data from transect sampling varied with environmental inputs and production outcomes, supporting the validity of transect sampling for practical, animal-based on-farm welfare assessment.

1. Introduction

In modern animal agriculture, animals are kept in large groups, with flocks, schools, and herds numbering in the thousands. Keeping track of the welfare of individual animals in such large groups presents challenges. It is, thus, common to base animal welfare assessment on adherence to engineering standards (i.e. *a priori* resource-based rules such as the provision of a certain amount of space/animal). However, because animal welfare is about quality of life, animal welfare assessment has greater face validity when based on observation of animals than inference based on resource provision (e.g. Main et al., 2007; Knierim and Winckle, 2009). Assessment of living animals also has greater face validity than welfare assessment based on *a posteriori* review of animal-based production records and slaughter plant health inspection outcomes. Yet, existing on-farm welfare assessment protocols (e.g. Welfare Quality®, 2009) tend to be time-consuming (Knierim and Winckle, 2009; Marchewka et al., 2013; de Jong et al., 2015), making them more suited for detailed research investigations than as practical industry management tools. In addition, animals can be stressed by protocols that require them to be handled for close examination of their physical condition, which may affect results (Marchewka et al., 2013). There is a need, therefore, for simple and efficient, low-stress tools for welfare assessment on the farm.

Practical welfare surveillance methods for use in large commercial poultry houses are particularly needed. Poultry kept for meat production can be afflicted by a variety of welfare challenges including wounds, infections, and cardiovascular and skeletal metabolic disorders that compromise normal development (Martrenchar, 1999; Marchewka et al., 2015; Meluzzi and Sirri, 2009; Jacob et al., 2016). To assess the prevalence of such welfare problems in a practical, low-stress but systematic manner, a transect sampling procedure has been developed (Marchewka et al., 2013) and validated against the "gold standard" of evaluating every bird in the flock (Marchewka et al., 2015). According to the transect sampling method, an observer walks slowly along successive transects in the house, defined as rows between lines of feeders and drinkers. In each transect, birds located in the space immediately ahead of the observer are scanned as they move out of the path of the approaching observer. The observer records all birds within the transect that are observed to be clearly afflicted by specific welfare-relevant conditions as identified by the European Food Safety Authority Panel on

Animal Health and Welfare (EFSA, 2010). For broiler chickens, these welfare "red flag" indicators include: lame, immobile, head, back and tail wounds, small, dirty, featherless, sick, terminally ill, and dead birds. To optimise surveillance time and minimise the risk of missing or recounting birds, only clearly evident "iceberg" cases that can be rapidly categorised are recorded, rather than attempting to score every bird on a graded scale of severity. Litter quality is also rated at three points along each transect. A free android smartphone application (i-WatchBroiler) has been designed for use in entering the data collected during transect walks in broiler houses (i-WatchBroiler, 2017).

Transect sampling is intended for implementation by stakeholders including farmers, veterinarians, animal welfare auditors and advisors to obtain a quick but quantitative snapshot of the current welfare status of the flock. However, previous research indicates the potential for some variation in results from different observers and sampled transects (Marchewka et al., 2013; 2015; BenSassi et al., 2018), which could mask differences in welfare between flocks. It has not yet been established whether transect sampling can reveal differences in flock welfare according to environmental conditions (e.g. environmental enrichment, space allowance, heating systems, lighting programmes), or offer a useful forecast of production outcomes.

Environmental enrichment refers to additions and modifications to the housing environment that increase environmental complexity, stimulate species-specific behaviour and facilitate biological functioning, thereby improving animal welfare (Newberry, 1995; Young, 2007). Elevated resting surfaces such as perches, platforms and boxes can serve as environmental enrichment for broilers (Estevez and Newberry, 2017). Use of such structures may strengthen muscles and joints and enhance the blood supply to the legs, wings and heart (Ventura et al., 2010). Their provision has been associated with greater tibial diaphysis diameter (Bizeray et al., 2002), a reduction in tibial dyschondroplasia (Kaukonen et al., 2017), reduced severity of footpad dermatitis (Kiyma et al., 2016), lower mortality due to heat stress (Pettit-Riley and Estevez, 2001), and a lower heterophil to lymphocyte ratio (Campo et al., 2005; Ohara et al., 2015). Peat moss is an environmental enrichment material that stimulates ground scratching and dustbathing behaviour (Petherick et al., 1990; Olsson and Keeling, 2005; Guinebretiere et al., 2014). A negative association between lameness score and dustbathing frequency has been reported (Vestergaard and Sanotra, 1999), suggesting that peat could improve leg health. Moreover, peat can have beneficial effects on the digestive tract mucosa (Trckova et al., 2005), and its use as a feed supplement has been associated with increased weight gain, and feed efficiency (Kocabagli et al., 2002; Ozturk et al., 2011; Kim et al., 2015). Bales of litter material provide enrichment by stimulating exploratory pecking and foraging behaviour as well as acting as platforms (Kells et al., 2001; Vasdal et al., 2018). Collectively, increased environmental complexity resulting from provision of multiple types of enrichment simultaneously may have additive welfare benefits.

Space allowance is also of relevance to broiler welfare and production outcomes. In different studies, increasing the space allowance from 0.044 - 0.083 m^2/bird (Sørensen et al., 2000), 0.046 - 0.074 m^2/bird (Dozier et al., 2006), or 0.066 - 0.500 m^2/bird (Arnould and Faure, 2003), reduced the prevalence or severity of footpad dermatitis. An increase in space allowance from 0.044 - 0.083 m^2/bird (Sørensen et al., 2000), or reduction in the stocking density from 44.8 - 15.9 kg/m^2 (Knowles et al., 2008), also improved walking ability. In addition, more space/bird has been associated with lower mortality, contact dermatitis and carcass bruising (Hall, 2001), and increased growth and feed efficiency (e.g. when comparing 30-35 kg/m^2 with 40 kg/m^2 (McLean et al., 2002; Dozier et al., 2005; Dozier et al., 2006; Abudabos et al., 2013) and 50 kg/m^2 (Benyi et al., 2015)).

Underfloor heating is reported to improve temperature uniformity and efficiency of temperature distribution in comparison to hood heating (de Leval et al., 2013). It may also reduce litter moisture and ammonia, resulting in reduced cardiovascular disease and ascites (Owada et al., 2007), leg problems (Knowles et al., 2008), footpad dermatitis and feather dirtiness (de Jong et al., 2014). Underfloor heating has been associated with a reduction in footpad dermatitis in turkeys (Abd El-Wahab et al., 2011; 2013), and with lower mortality, greater weight gain and lower feed consumption in broilers (Nawalany et al., 2010).

The photoperiod regimen can affect body temperature and the immune system (Zheng et al., 2013), and regulate feed intake (Olanrewaju et al., 2006) and physical activity (Lewis and Morris, 1998). Long photoperiods (20-24 h) have been associated with susceptibility to leg problems in broilers (Knowles et al., 2008; Brickett et al., 2007) and increased mortality and leg problems in Japanese quail (Moore and Siop, 2000). An intermittent photoperiod regimen alternating between 2 h light and 2 h dark has been associated with less footpad dermatitis and higher body weights in broilers when compared to a short (8 h) continuous photoperiod (Olanrewaju et al., 2015). Provision of at least 6 h of darkness/day is now required in the European Union, with at least one uninterrupted dark period of at least 4 h (EU, 2007). In Norway, the latter provision is limited to two uninterrupted dark periods of at least 4 h (Norwegian Animal Welfare Legislation, 2013). These requirements have resulted in two typical forms of photoperiod regimen in Norway, one comprising 18 h of continuous light and the other having two 4-h dark periods/day (i.e. 16 h intermittent light).

In the current study, we used the transect sampling method to collect data from Norwegian commercial broiler flocks. Our aim was to investigate relationships between environmental factors, transect data and production data (including health inspection findings from the slaughter line where every bird in the flock is evaluated). Based on previous reports, we hypothesised that environmental complexity (defined as the number of environmental enrichment types provided), space allowance, underfloor heating, and photoperiod regimen would correlate with both transect and production findings. Specifically, we predicted that greater environmental complexity, greater space allowance, presence (vs absence) of underfloor heating, and 16 h intermittent (vs 18 h continuous) lighting would be associated with indications of improved welfare both on the farm and at slaughter. Further, we expected to find positive associations between on-farm welfare problems and causes of rejection at slaughter, and a negative association between litter quality and footpad dermatitis. Additionally, we examined the consistency of transect data between observers, and between transect locations within the house (left vs right side, beside wall vs more central).

2. Materials and methods

2.1. Ethics statement

The study was conducted between February and May, 2017, on 15 farms located in southeast Norway. All farms belonged to the same cooperative, and functioned in accordance with Norwegian animal welfare legislation governing poultry production (Norwegian Animal Welfare Legislation, 2013). Farm owners gave their consent to participate in the research, participation was voluntary, and no personal details were collected. No biological samples were collected for research purposes. Because no experimental manipulations were made and observations were non-invasive, the study did not require approval of animal use by the Norwegian Food Safety Authority (Norwegian Regulations on Use of Animals in Research, 2015).

2.2. Environmental inputs

At each farm, we evaluated two consecutive Ross 308 mixed sex flocks kept in the same house (Table 1). All houses were well-insulated, with concrete floors and automatic drinkers, feeders and ventilation systems. Ten houses had underfloor heating. Houses were thoroughly cleaned and supplied with a thin layer of fresh litter material (generally softwood shavings) before placement of each flock. Initial flock size ranged from 9,600 - 34,050 broilers (mean ± SE, 19,480 ± 809) and initial space allowance ranged from 0.056 - 0.073 m^2/bird. Artificial lighting was provided by LED lights, with either a single 6-h dark period or two 4-h dark periods daily from 7 days of age until 3 days before slaughter. Farmers checked their flocks at least twice daily. They removed any birds found dead, humanely culled any moribund or severely disabled birds, and kept records on these numbers. On the day of slaughter (between 32 and 35 days of age), the mean stocking density (± SE) was 32.08 ± 0.63 kg/m^2.

The cooperative had a recommended environmental enrichment programme, which involved providing boxes for perching by 7 days of age (1 box/50 m^2), and peat (10 l/50 m^2) and wood shavings bales (1 half-bale/100 m^2) at 7, 14, 21 and 28 days of age. Farmers supplied these enrichments to varying degrees in the different flocks (Table 1). The boxes were either cardboard or plastic, ranging from about 0.2 - 0.25 m high and with an upper surface area of about 0.2 - 0.3 m^2. Some boxes had openings allowing birds to go under them when young, and some were stacked in pyramids. The boxes remained in the house throughout rearing. Peat was provided over the whole floor (as litter), loose in piles, contained in low surrounds, or as bales (200 l bales, presented whole or cut in half). Wood shavings bales (25 kg) were cut in half and presented with or without removal of their plastic wrapping. Because the added peat and wood shavings bales became integrated into the litter, they were renewed weekly.

Table 1: Environmental provisions to each flock.

Farm	Under-floor heating	Lighting regimen (18 h continuous vs 16 h intermittent)	Flock	Space allowance (m^2/chick started)	Environmental enrichment type (X indicates provision)		
					Boxes	Peat	Wood shavings bales
1	Yes	18 h	1	0.070	X	X	X
			2	0.061		X	
2	Yes	18 h	1	0.066	X	X	X
			2	0.073	X	X	X
3	No	16 h	1	0.057			
			2	0.073	X	X	X
4	Yes	18 h	1	0.056			
			2	0.072	X	X	X
5	Yes	16 h	1	0.057			
			2	0.072	X	X	X
6	No	18 h	1	0.057	X	X	X
			2	0.072	X	X	X
7	Yes	16 h	1	0.068	X	X	X
			2	0.058			
8	Yes	18 h	1	0.061	X	X	X
			2	0.074	X	X	X
9	Yes	18 h	1	0.063	X		
			2	0.071	X	X	
10	No	16 h	1	0.072	X	X	X
			2	0.060	X	X	
11	No	16 h	1	0.062			
			2	0.071	X	X	X
12	No	18 h	1	0.069	X	X	X
			2	0.063		X	
13	Yes	16 h	1[1]	0.058	X		X
			2	0.060	X		
14	Yes	18 h	1	0.060	X		X
			2	0.066	X		
15	Yes	18 h	1	0.061	X	X	
			2	0.063			

[1] On-farm welfare assessment data missing from this flock.

2.3. On-farm welfare assessment

Two trained observers visited each flock once at 26 - 30 days of age, shortly before slaughter when welfare problems were most evident. Before starting the data collection, we determined the dimensions of the house (mean ± SE, 1284 ± 53 m^2), and width of each transect (mean ± SE, 2.06 ± 0.04 m). Transects were defined as wall or central transects. Wall transects comprised the area demarcated by a side wall and the nearest feeder or drinker line (whichever was closest to the wall, typically a drinker line), extending the length of the house from one end wall to the other end wall. Central transects comprised the area bounded by adjacent feeder and/or drinker lines (typically one of each), extending the length of the house. Transects were numbered consecutively starting with the wall transect on the left side of the house, as viewed when standing at the end of the house closest to the entrance door.

On farm visits, each observer assessed the prevalence of the welfare indicators (Table 2) within one central transect and one wall transect, for a total of four transects/flock. One observer sampled two transects on the left side of the house, walking up one transect and returning down the other, and the second observer sampled two transects on the right side of the house. While one observer sampled a wall transect, the other simultaneously sampled a central transect, and vice versa. We randomised the side of the house evaluated by each observer, and alternated the order of observing wall and central transects. Both wall transects were included in the flock assessment because observations of Spanish broiler flocks indicated that immobile, small, sick, dirty and/or dead birds were more likely to be found in wall than central transects (Marchewka et al., 2013; BenSassi et al., 2018). We selected the two observed central transects pseudo-randomly, avoiding contiguous transects to minimise double counting of the same birds, and any atypically wide (> 3 m) or narrow (< 1 m) transects. Evaluation of two transects took an average of 30 - 35 min depending on house length. Birds were assigned to the welfare indicator best describing their condition based on rapid visual assessment.

Table 2: Ethogram of broiler welfare problems recorded during transect sampling, and subsequently pooled categories.

Indicator	Description	Category
Lame	Walks with obviously uneven strides or unsteady steps. May exhibit outward or inward twisting of one or both legs leading to severe limping. Lameness is clearly advanced rather than in early stages.	Walking difficulties
Immobile	Does not move away when approached or moves by propping on wings or crawling. If gently nudged, moves with difficulty, no more than three steps before sitting down again.	
Sick	Signs of impaired health, including small and/or pale comb, red, watery or closed eyes, retracted neck and disarranged/raised feathers. Usually found in a resting position. Includes wry neck.	Illness
Terminally ill	Lying with head resting on ground or lying on back, with signs of being close to death (e.g. laboured breathing, half-closed eyes). Excludes panting related to heat stress.	
Dead	No signs of life.	
Head wounds	Skin scratches on head or neck indicated by the presence of fresh or dried blood/scabs visible from 1 - 2 m away.	Skin wounds
Back wounds	Skin scratches on back (between neck and tail) and/or wings indicated by the presence of fresh or dried blood/scabs visible from 1 - 2 m away.	
Tail wounds	Skin scratches around tail and hips indicated by the presence of fresh or dried blood/scabs visible from 1 - 2 m away.	
Small	Stunted growth. Approximately half average size of flock mates. May have yellow downy feathers, especially on head.	Small
Dirty	Extensive dark staining of body sides, wings, chest, back, and/or tail feathers due to prolonged contact with wet litter. Excludes light soiling or discolouration of feathers caused by dust, peat or excrement.	Not observed
Featherless	Lacking feathers on majority of back and wings. Excludes moulting.	Not observed

We observed no dirty or featherless birds in the flocks visited. Due to low numbers, we assigned the transect counts for the remaining indicators to four broader categories: walking difficulties, illness, skin wounds, or small (Table 2). The counts in each welfare category were summed across the four assessed transects and expressed as a proportion of the total number of birds estimated to be present in those four transects. This denominator was calculated based on the total number of birds present in the house on the assessment day and the dimensions of the transects, assuming a uniform distribution of birds across the house. We also calculated an overall welfare problems index (i.e. summed counts across all categories as a proportion of the estimated number of birds in the observed transects). We evaluated litter quality at the beginning, middle, and end of each walked transect on a 5-point scale, from 0 (dry and loose litter) to 4 (caked litter) based on the Welfare Quality® (Welfare Quality, 2009) protocol for poultry, and calculated the average litter score/flock.

2.3. Production outcomes

Flocks were slaughtered at a mean age of 33.6 days, all at the same slaughter plant following 2-phase CO_2 gas stunning. Production data on each flock were provided by the farmers and the slaughter plant. We calculated total mortality on the farm up to the day of slaughter as [(found dead + culled)/number of chicks started]. Reasons for carcass rejection were routinely recorded by health inspection personnel stationed along the slaughter line. They recorded the primary reason for rejection of each bird though multiple reasons could exist. We categorised these reasons as: perosis (any pronounced leg deformities), illness (sum of liver disease, heart disease, ascites, persistent egg yolk, and discolouration/suspicious smell), wounds (scratches, bruises, hematomas, fractures and dislocations), and underweight (below marketable weight). We expressed the numbers rejected in each category, and total number rejected, as a proportion of the total number of birds slaughtered. Footpad dermatitis was evaluated by slaughterhouse personnel according to standard procedure for Norwegian flocks, whereby 100 feet/flock were assessed on a 3-point scale (0 = no lesions, 1 = mild lesions, 2 = severe lesions), and points were summed to give a flock score ranging from 0 - 200. Further flock data included growth rate [mean g eviscerated carcass weight/days of age at slaughter], and the feed conversion ratio [total kg feed provided/((number slaughtered - number rejected) * mean kg eviscerated carcass weight)]. We also calculated an integrated production index value for each flock [mean g eviscerated carcass weight * (number slaughtered - number rejected)/(days of age at slaughter * number of chicks started)].

2.4. Statistical analysis

We analysed all data using SAS 9.4 (SAS Institute, NC, USA). Associations of the four environmental inputs (environmental complexity, space allowance, underfloor heating, and photoperiod regimen) with each on-farm welfare assessment variable and each production outcome variable were investigated using generalised linear mixed models (GLIMMIX procedure). Environmental complexity (i.e. number of environmental enrichment types provided) was treated as a continuous variable ranging from 0 – 3 to explore linear trends irrespective of the specific combinations of enrichment materials used. This approach recognised the underlying continuity of complexity despite imprecise quantification. Due to collinearity between space allowance and environmental complexity, the residuals of space allowance regressed on environmental complexity were included in the model as a continuous variable describing the variation in space allowance around the regression line at each level of environmental complexity. Underfloor heating (absence vs presence) and photoperiod regimen (18 h continuous vs 16 h intermittent) were categorical factors. We used additional GLIMMIX models to estimate associations of the on-farm welfare assessment variables (1) walking difficulties, illness, skin wounds, and small birds, (2) the welfare problems index, and (3) litter score, with the production outcomes (total mortality, reasons for rejection at slaughter, total rejections, footpad dermatitis score, growth rate, feed conversion ratio, production index). Farm was included as a random effect in all models (see S1 Appendix for model specification details and covariance estimates for farm).

Response variables comprising counts expressed as proportions were analysed according to the binomial distribution with logit link, maximum likelihood estimation and Laplace likelihood approximation. Because flock footpad dermatitis scores were heavily right-skewed, with a majority of flocks receiving a score of 1, we compared flocks receiving scores of 1 vs > 1 based on the binary distribution with logit link. We analysed the remaining response variables (mean litter score, growth rate, feed conversion, production index) according to the gamma distribution with log link and residual pseudo-likelihood estimation (see S1 Appendix for details). We applied the inverse link to back-transform estimated values (continuous factors) and least squares means (categorical factors) to their original scale for graphical presentation. The absence of underfloor heating, and a continuous photoperiod of 18 h, served as the reference levels for least squares means estimation. We also evaluated the degree of agreement in findings on the proportion of birds with walking difficulties, illness, skin wounds, and small birds between pairs of transects within flocks. We compared the differences between the pairs of transects that were (1) assessed by different observers, (2) located on the left vs right side of the house and (3) located in wall vs central transects, using the Wilcoxon signed-ranks test in the UNIVARIATE procedure.

3. Results

3.1. Descriptive data

The welfare problem indicators occurred at low levels (Table 3; S1 Appendix), with lameness contributing most to the welfare problems index. Skin wounds were most common in the tail region. The litter scores at sampled locations varied from 0 - 2, with no scores of 3 or 4 being recorded. Culling by the farmer accounted for about 30 % of the mortality. Ascites and liver disease were the most common reasons for rejection at slaughter, resulting in the pooled illness category accounting for the majority of rejections. Footpad dermatitis occurred at low levels, with flock scores ranging from 1 - 13 of a possible 200.

3.2. Associations between environmental inputs, welfare assessment findings and production outcomes

With increasing environmental complexity (Table 4), we detected fewer birds with skin wounds during the transect walks ($P = 0.004$; Fig 1A), and the overall welfare problems index was lower ($P = 0.002$; Fig 1B). Increasing environmental complexity was associated with lower mortality ($P < 0.001$; Fig 1C), a lower proportion of rejections due to wounds ($P < 0.001$; Fig 2A) and underweight birds ($P = 0.002$; Fig 2B), and a lower overall rejection rate ($P < 0.001$; Fig 2C).

Table 3: Prevalence of welfare problems detected by transect sampling during on-farm welfare assessment, and production outcomes.

Welfare indicator[1]	Mean	SE	Production outcome[2]	Mean	SE
Lame (%)	0.22	0.02	Mortality (%)[3]	3.59	0.35
Immobile (%)	0.07	0.01	Culled (% of mortality)[4]	30.31	3.91
Walking difficulties (%)[5]	0.29	0.03	Rejection due to perosis (%)	0.01	<0.01
Sick (%)	0.03	<0.01	Rejection due to liver disease (%)	0.35	<0.01
Terminally ill (%)	<0.01	<0.01	Rejection due to heart disease (%)	0.07	0.01
Dead (%)	0.04	0.01	Rejection due to ascites (%)	0.71	0.06
Illness (%)[6]	0.07	0.01	Rejection due to persistent egg yolk (%)	0.01	<0.01
Head wounds (%)	0.01	<0.01	Rejection due to discolouration/smell (%)	0.05	0.01
Back wounds (%)	<0.01	<0.01	Rejection due to illness (%)[7]	1.19	0.11
Tail wounds (%)	0.08	0.01	Rejection due to wounds (%)	0.14	0.04
Skin wounds (%)[8]	0.08	0.01	Rejection because underweight (%)	0.20	0.08
Small (%)	0.12	0.04	Total rejections (%)[9]	1.66	0.15
Dirty (%)	0	0	Footpad score[10]	2.37	0.46
Featherless (%)	0	0	Growth rate (g/day)[11]	43.20	0.42
Welfare problems index (%)[12]	0.57	0.06	Feed conversion (ratio)[13]	2.22	0.02
Litter score[14]	1.17	0.06	Production index (g/day)[15]	40.86	0.42

[1] On-farm welfare indicator data from transect sampling on 15 farms (2 flocks/farm; n = 29 flocks). Counts expressed as % of estimated number of birds in four walked transects per flock.

[2] Flock production data (n = 30 flocks). Reasons for rejection counts expressed as % of total number of birds slaughtered.

[3] [(Found dead + culled)/number of chicks started] up to day of slaughter, expressed as %.

[4] Number culled, as a % of mortality up to day of visit (n = 26 flocks due to missing data).

[5] Includes lame and immobile.

[6] Includes sick, terminally ill and dead.

[7] Includes liver disease, heart disease, ascites, persistent egg yolk and discolouration/suspicious smell.

[8] Includes head, back and tail wounds.

[9] Includes perosis/leg deformity, rejection due to illness, rejection due to wounds and rejection because underweight.

[10] 100 feet/flock scored on 3-point scale (0 = no lesions, 1 = mild lesions, 2 = severe lesions), giving a maximum possible flock score of 200.

[11] [Mean g eviscerated carcass weight/days of age at slaughter].

[12] Includes lame, immobile, sick, terminally ill, dead, head, back, and tail wounds, and small.

[13] [Total kg feed provided to flock/((number slaughtered - number rejected) * mean kg eviscerated carcass weight)].

[14] Scored from 0 (dry, loose litter) to 4 (caked litter) in three locations / transect.

[15] [Mean g eviscerated carcass weight * (number slaughtered - number rejected)/(days of age at slaughter * number of chicks started)].

Table 4: Regression coefficient estimates for associations of environmental complexity and space allowance with welfare problems detected by transect sampling, and production outcomes.

Variable	Environmental complexity[1]						Residuals of space allowance[2]					
	Mean	SE	Lower 95% CL	Upper 95% CL	F[3]	P	Mean	SE	Lower 95% CL	Upper 95% CL	F[3]	P
On-farm welfare assessment indicators												
Walking difficulties	-0.09	0.04	-0.18	-0.01	4.50	0.055	-57.95	11.09	-82.11	-33.79	27.31	<0.001
Illness	-0.04	0.08	-0.20	0.13	0.24	0.632	5.77	19.48	-36.66	48.21	0.09	0.772
Skin wounds	-0.26	0.08	-0.43	-0.10	12.46	0.004	-31.27	20.41	-75.73	13.19	2.35	0.151
Small	-0.09	0.07	-0.23	0.05	1.94	0.189	34.11	17.25	-3.49	71.70	3.91	0.072
Welfare problems index	-0.12	0.03	-0.19	-0.06	16.29	0.002	-26.79	7.93	-44.07	-9.51	11.41	0.006
Litter score	-0.09	0.05	-0.20	0.03	2.62	0.131	3.16	14.32	-28.03	34.35	0.05	0.829
Production outcomes												
Mortality	-0.05	0.01	-0.06	-0.03	35.81	<0.001	1.09	1.91	-3.03	5.21	0.33	0.577
Rejection due to perosis	-0.19	0.15	-0.52	0.14	1.48	0.245	-56.67	38.87	-140.63	27.30	2.13	0.169
Rejection due to illness	0.03	0.01	<0.01	0.06	4.45	0.055	-2.92	3.50	-10.47	4.64	0.70	0.419
Rejection due to wounds	-0.35	0.04	-0.44	-0.27	88.99	<0.001	-78.88	13.51	-108.05	-49.70	34.11	<0.001
Rejection because underweight	-0.16	0.04	-0.25	-0.07	15.08	0.002	17.49	9.83	-3.74	38.72	3.17	0.099
Total rejections	-0.06	0.01	-0.08	-0.04	29.35	<0.001	-1.28	2.96	-7.66	5.11	0.19	0.673
Footpad score (binary)	0.10	0.34	-0.64	0.84	0.09	0.774	121.44	92.39	-78.15	321.03	1.73	0.211
Growth rate	-0.01	0.01	-0.02	0.01	2.17	0.164	4.89	1.50	1.65	8.12	10.63	0.006
Feed conversion	<0.01	0.01	-0.01	0.01	0.01	0.927	-1.64	1.45	-4.78	1.50	1.27	0.280
Production index	-0.01	0.01	-0.02	0.01	0.46	0.511	4.68	1.85	0.68	8.67	6.40	0.025

[1] Number of environmental enrichment types (boxes, peat, wood shavings bales) provided (0-3).

[2] Residuals of space allowance (m^2/bird) regressed on environmental complexity.

[3] $F_{1,12}$ for welfare assessment variables; $F_{1,13}$ for production variables. See Methods and Table 3 footnotes for explanation of variables.

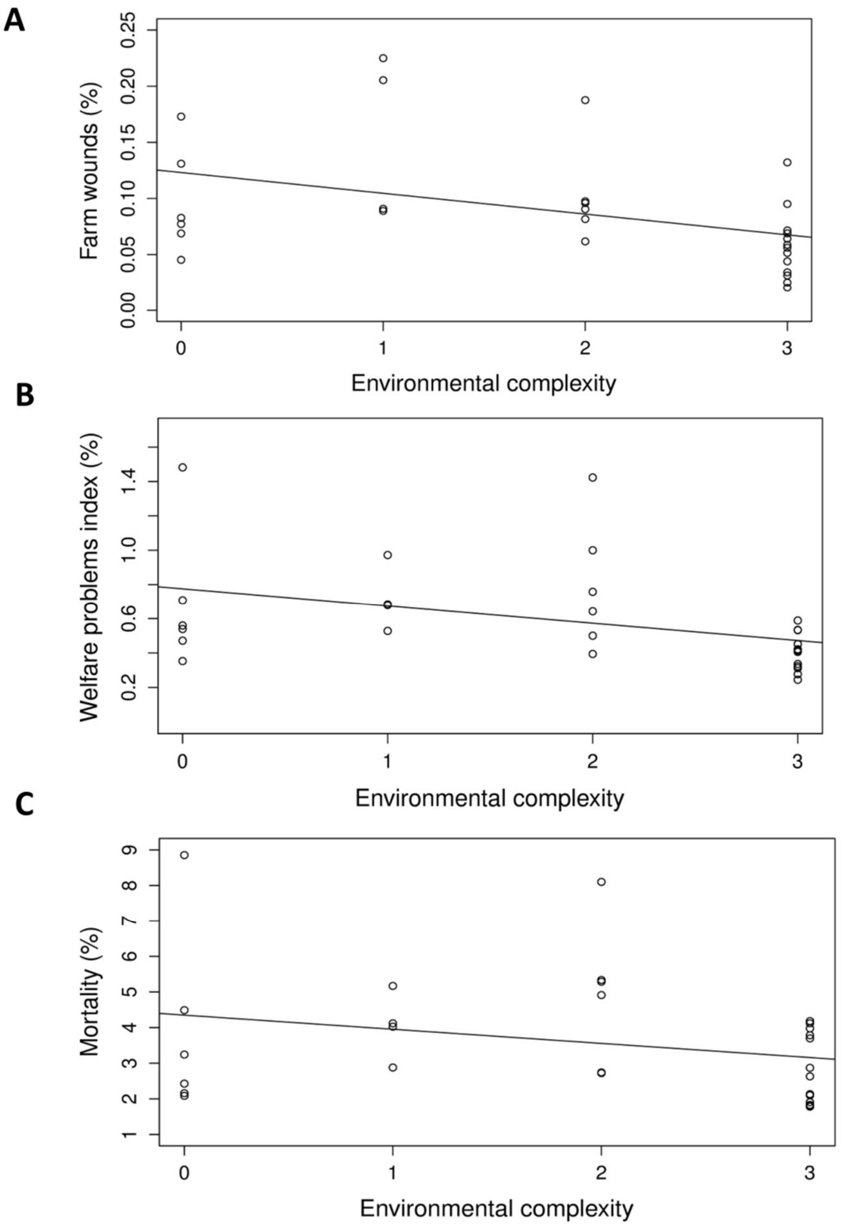

Figure 1: Associations of environmental complexity with (A) skin wounds ($r^2 = 0.183$), (B) welfare problems index ($r^2 = 0.164$), and (C) mortality ($r^2 = 0.074$).

Environmental complexity is based on the number of environmental enrichment types (boxes, peat, wood shavings bales) provided (from 0 - 3). Data points are back-transformed estimates. (A, B) Birds detected with skin wounds, and sum of birds detected with welfare problems (walking difficulties, illness, skin wounds, small size), as a % of the estimated number of birds in 4 assessed transects. (C) Number found dead and culled on the farm up to the day of slaughter as a % of number of chicks started.

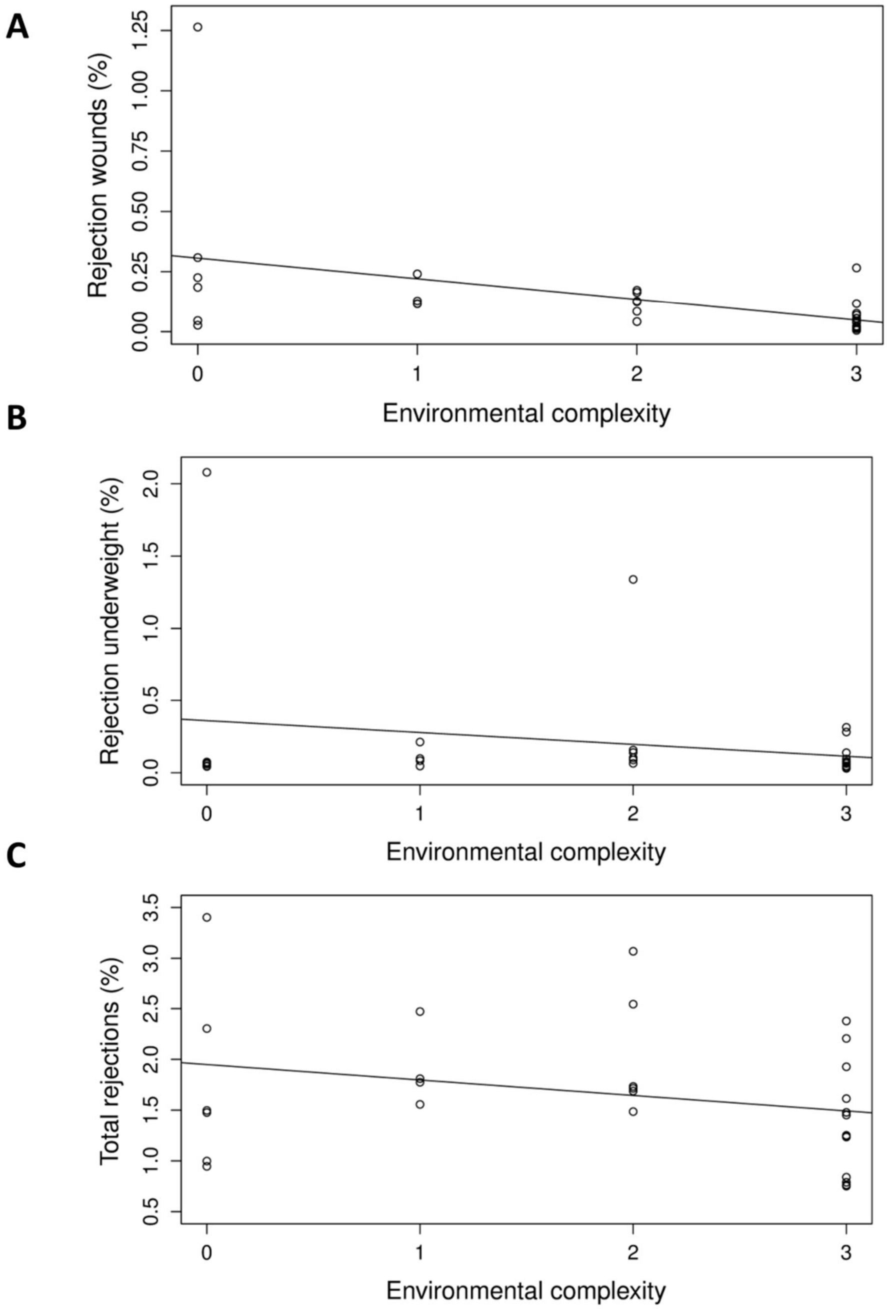

Figure 2: Associations of environmental complexity with rejections due to (A) wounds ($r^2 = 0.206$), and (B) underweight birds ($r^2 = 0.053$), and (C) total rejections ($r^2 = 0.076$). Environmental complexity is based on the number of environmental enrichment types (boxes, peat, wood shavings bales) provided (from 0 - 3). Data points are back-transformed estimates. (A, B, C) Carcasses rejected as a % of total number of slaughtered birds.

As space allowance residuals increased (Table 4), fewer birds with walking difficulties were detected ($P < 0.001$; Fig 3A) and the welfare problems index was lower ($P = 0.006$; Fig 3B). Higher space allowance residuals were also associated with a lower proportion of slaughter rejections due to wounds ($P < 0.001$; Fig 4A), a higher growth rate ($P = 0.006$; Fig 4B), and a higher production index overall ($P = 0.025$; Fig 4C).

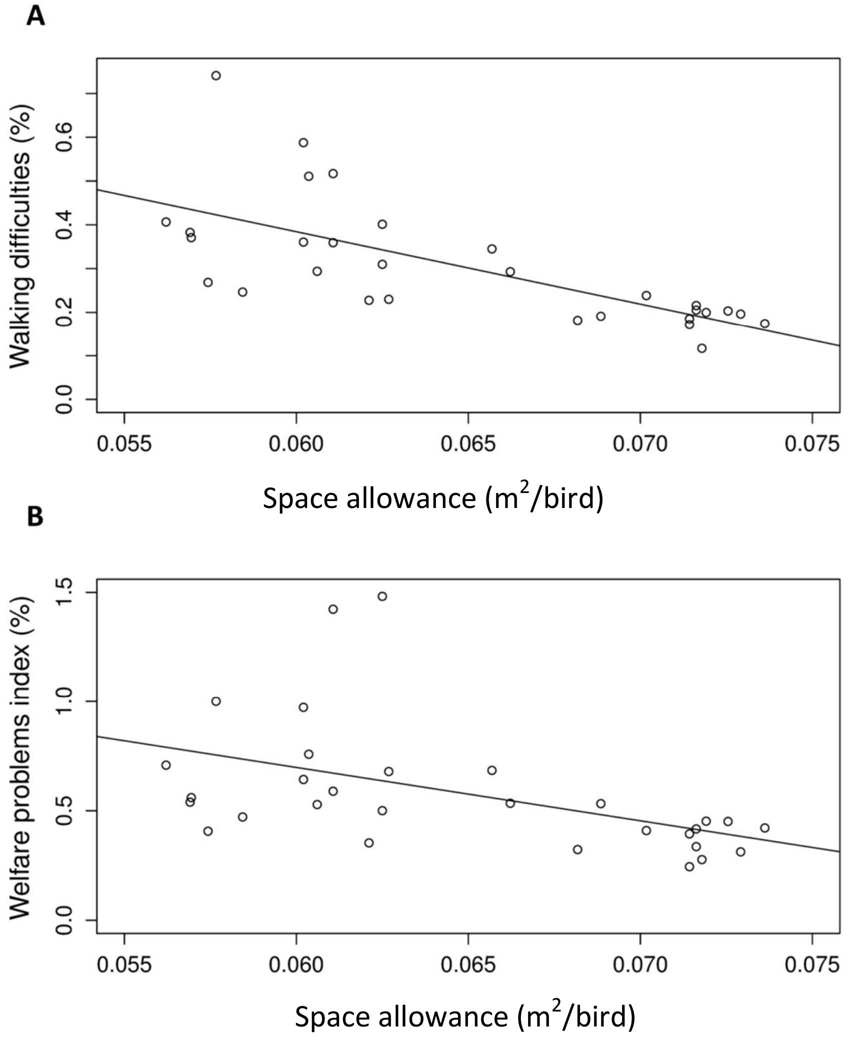

Figure 3: Associations of space allowance (m^2/bird) with (A) walking difficulties ($r^2 = 0.480$) and (B) welfare problems index ($r^2 = 0.233$). Data points are back-transformed estimates from analysis of residuals of space allowance regressed on environmental complexity. (A) Birds detected with walking difficulties, (B) Sum of birds detected with welfare problems (walking difficulties, illness, skin wounds, small size). Both variables are calculated as a % of estimated number of birds in 4 assessed transects.

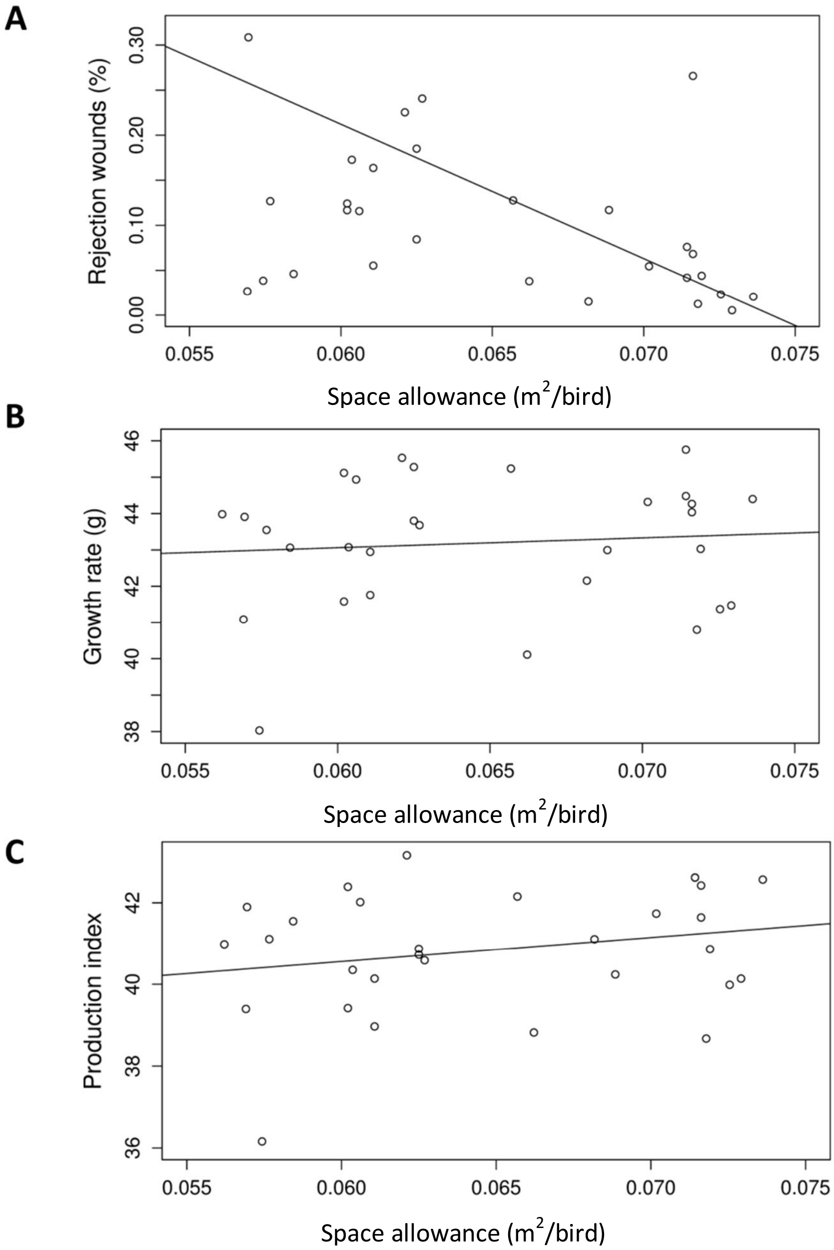

Figure 4: Associations of space allowance (m^2/bird) with (A) rejections due to wounds ($r^2 = 0.151$), (B) growth rate ($r^2 = 0.007$), and (C) production index ($r^2 = 0.048$). Data points are back-transformed estimates from analysis of residuals of space allowance regressed on environmental complexity. (A) Carcasses rejected due to wounds as a % of total slaughtered birds. (B) [Mean g eviscerated carcass weight/days of age at slaughter]. (C) [Mean g eviscerated carcass weight * (number slaughtered - number rejected)/(days of age at slaughter * number of chicks started)].

The significant associations of environmental complexity and space allowance withwelfare assessment findings and production outcomes had low to modest r2 values ranging from 0.007 to 0.480 (Fig 1-4).

The presence of underfloor heating (Table 5) was associated with fewer rejections due to perosis (P = 0.037; Fig 5A). The 16 h intermittent photoperiod regimen (Table 5) was associated with lower rates of illness (P = 0.015; Fig 5B) and skin wounds (P = 0.026; Fig 5C) on the farm than the 18 h continuous photoperiod regimen, as well as lower mortality (P = 0.022; Fig 5D).

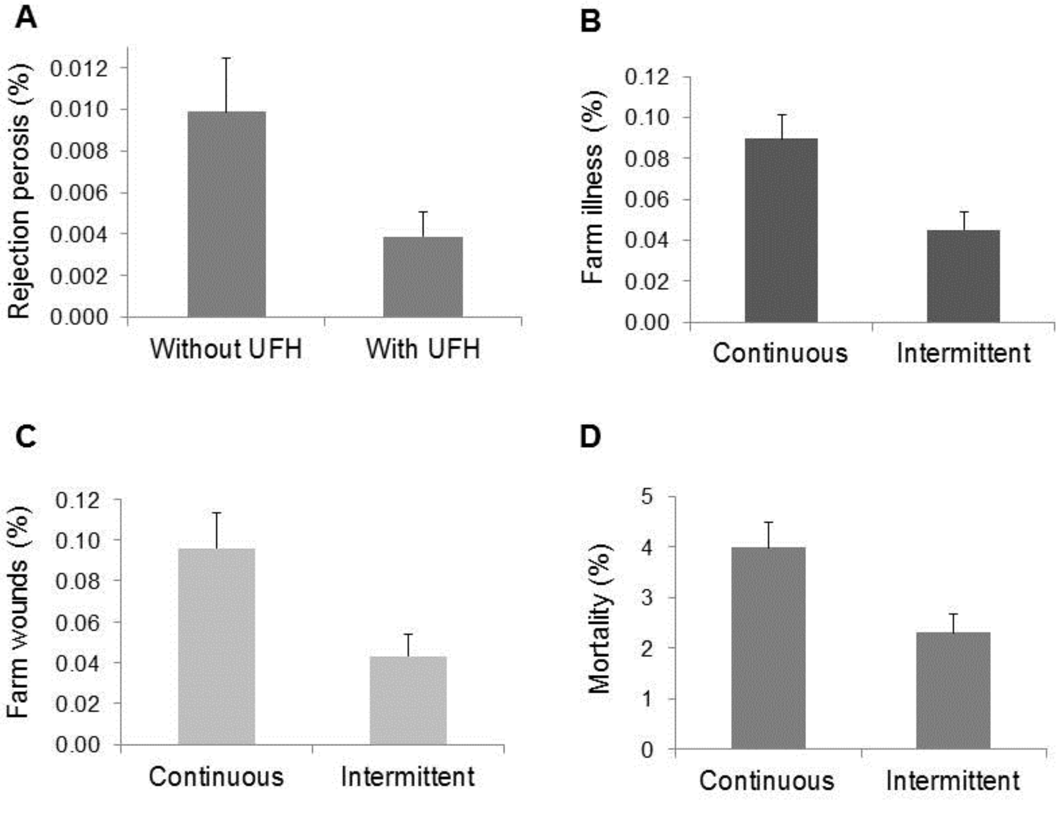

Figure 5: Associations of underfloor heating with (A) rejection due to perosis, and of photoperiod regimen on on-farm (B) illness, (C) skin wounds, and (D) mortality.

Table 5: Back-transformed least squares means for associations of underfloor heating and photoperiod regimen with welfare problems detected by transect sampling and production outcomes.

Variable	Underfloor heating										Photoperiod regimen									
	Without				With						18 h continuous				16 h intermittent					
	Mean	SE	Lower 95% CL	Upper 95% CL	Mean	SE	Lower 95% CL	Upper 95% CL	F[1]	P	Mean	SE	Lower 95% CL	Upper 95% CL	Mean	SE	Lower 95% CL	Upper 95% CL	F[1]	P
On-farm welfare assessment indicators																				
Walking difficulties (%)	0.23	0.03	0.17	0.31	0.29	0.03	0.23	0.37	1.66	0.221	0.27	0.03	0.21	0.35	0.25	0.04	0.18	0.33	0.27	0.616
Illness (%)	0.08	0.01	0.05	0.12	0.05	0.01	0.04	0.07	3.62	0.081	0.09	0.01	0.07	0.12	0.05	0.01	0.03	0.07	8.11	0.015
Skin wounds (%)	0.07	0.02	0.04	0.12	0.06	0.01	0.04	0.09	0.14	0.717	0.10	0.02	0.07	0.14	0.04	0.01	0.03	0.08	6.47	0.026
Small (%)	0.05	0.02	0.02	0.10	0.10	0.03	0.05	0.17	2.22	0.162	0.08	0.02	0.04	0.14	0.06	0.02	0.03	0.13	0.18	0.678
Welfare problems index (%)	0.44	0.07	0.32	0.62	0.51	0.06	0.40	0.70	0.55	0.472	0.57	0.07	0.44	0.74	0.40	0.06	0.29	0.56	3.10	0.104
Litter score	1.34	0.16	1.02	1.75	1.08	0.11	0.87	1.34	1.72	0.214	1.13	0.11	0.91	1.41	1.27	0.16	0.97	1.66	0.44	0.518

[1] $F_{1,12}$ for welfare assessment variables; $F_{1,13}$ for production variables. See Methods and Table 3 footnotes for explanation of variables.

Table 5: Back-transformed least squares means for associations of underfloor heating and photoperiod regimen with welfare problems detected by transect sampling and production outcomes.

Variable	Underfloor heating										Photoperiod regimen									
	Without				With						18 h continuous				16 h intermittent					
	Mean	SE	Lower 95% CL	Upper 95% CL	Mean	SE	Lower 95% CL	Upper 95% CL	F[1]	P	Mean	SE	Lower 95% CL	Upper 95% CL	Mean	SE	Lower 95% CL	Upper 95% CL	F[1]	P
Production outcomes																				
Mortality (%)	3.22	0.51	2.29	4.53	2.86	0.37	2.17	3.77	0.33	0.578	3.98	0.50	3.03	5.23	2.31	0.37	1.63	3.26	6.71	0.022
Rejection due to perosis (%)	0.01	<0.01	0.01	0.02	<0.01	<0.01	<0.01	0.01	5.41	0.037	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	0.15	0.709
Rejection due to illness (%)	1.34	0.21	0.96	1.87	0.98	0.12	0.75	1.28	2.33	0.151	1.25	0.16	0.96	1.64	1.05	0.16	0.75	1.46	0.77	0.397
Rejection due to wounds (%)	0.06	0.03	0.02	0.15	0.07	0.03	0.03	0.15	0.12	0.739	0.09	0.03	0.04	0.18	0.05	0.02	0.02	0.13	0.80	0.387
Rejection because underweight (%)	0.08	0.03	0.03	0.19	0.09	0.03	0.04	0.18	0.05	0.823	0.11	0.04	0.05	0.24	0.06	0.03	0.02	0.15	1.37	0.263

[1] $F_{1,12}$ for welfare assessment variables; $F_{1,13}$ for production variables. See Methods and Table 3 footnotes for explanation of variables.

Underfloor heating (UFH, without vs with). Photoperiod regimen (18 h continuous vs 16 h intermittent). Bars show back-transformed least squares means ± SE (differences, $P < 0.05$). (A) Carcasses rejected due to perosis (leg deformity) as a % of total number of slaughtered birds. (B, C) Birds detected with signs of illness, and skin wounds, as a % of the estimated number of birds in 4 assessed transects. (D) Number found dead and culled on the farm up to the day of slaughter as a % of number of chicks started.

3.3. Relationships between welfare assessment findings and production outcomes

An increased prevalence of walking difficulties, illness and small size on the farm was associated with increased mortality, and increased rejections due to illness and underweight birds, and increased total rejections at slaughter ($P < 0.05$; Table 6). A higher prevalence of walking difficulties was also associated with increased rejections due to wounds ($P < 0.001$). A higher welfare problems index on the farm was associated with higher mortality, rejections in the illness, wounds, and underweight categories, and total rejections ($P < 0.001$). Higher litter scores were associated with lower mortality, but higher rejections due to illness, wounds and underweight birds, as well as total rejections ($P < 0.01$; Table 6). Litter scores were not associated with footpad dermatitis scores in this study.

3.4. Consistency between observers and transect locations

There was no difference between observers in the recording of each individual welfare indicator, but observers differed in the overall number of welfare indicators registered ($P = 0.039$; Table 7). A higher prevalence of skin wounds was detected on the right than left side of the house ($P = 0.010$). Illness, small size and welfare problems index values were higher in wall than central transects ($P < 0.05$). The total number of birds estimated to be present in the observed transects did not differ between observers, left vs right side, or wall vs central transects.

Table 6: Regression coefficient estimates for relationships between welfare problems detected by transect sampling, and production outcomes.

		Production outcomes									
On-farm welfare assessment indicators[2]		Mortality	Reasons for rejection				Total rejected	Footpad score	Growth rate	Feed conversion	Production index
			Perosis	Illness	Wounds	Under-weight					
Walking difficulties	Mean	162.53	254.75	38.82	457.45	146.61	78.23	-121.23	0.30	-4.44	-2.74
	SE	10.01	183.17	16.51	53.87	49.68	14.17	306.29	6.31	5.27	6.79
	Lower 95% CL	140.23	-153.39	2.04	337.43	35.92	46.66	-803.69	-13.77	-16.19	-17.86
	Upper 95% CL	184.83	662.88	75.59	577.48	257.30	109.80	561.23	14.36	7.31	12.39
	$F_{1, 10}$	263.76	1.93	5.53	72.11	8.71	30.49	0.16	<0.01	0.71	0.16
	P	<0.001	0.195	0.041	<0.001	0.015	<0.001	0.701	0.963	0.419	0.696
Illness	Mean	-119.00	1085.85	804.52	-234.54	525.02	707.60	-267.01	-4.19	17.80	-18.88
	SE	45.82	596.08	71.29	234.15	200.61	62.55	1076.20	25.28	19.08	26.67
	Lower 95% CL	-221.09	-242.30	645.67	-756.25	78.04	568.24	-2664.94	-60.52	-24.70	-78.31
	Upper 95% CL	-16.92	2414.01	963.37	287.18	972.00	846.97	2130.92	52.15	60.30	40.56
	$F_{1, 10}$	6.75	3.32	127.35	1.00	6.85	127.99	0.06	0.03	0.87	0.50
	P	0.027	0.099	<0.001	0.340	0.026	<0.001	0.809	0.872	0.373	0.495
Skin wounds	Mean	2.15	92.64	18.77	18.16	-74.41	56.61	1644.17	-0.43	-1.80	-0.90
	SE	21.08	384.19	32.20	105.85	125.56	28.33	890.20	15.76	13.44	17.03
	Lower 95% CL	-44.82	-763.39	-52.99	-217.68	-354.18	-6.52	-339.32	-35.55	-31.75	-38.84
	Upper 95% CL	49.11	948.66	90.52	254.00	205.36	119.73	3627.65	34.70	28.14	37.04
	$F_{1, 10}$	0.01	0.06	0.34	0.03	0.35	3.99	3.41	<0.01	0.02	<0.01
	P	0.921	0.814	0.573	0.867	0.567	0.074	0.095	0.979	0.896	0.959

[2] Walking difficulties, illness, skin wounds and small were predictors in one model, and the welfare problems index and litter score were predictors in separate models.

Table 6: Regression coefficient estimates for relationships between welfare problems detected by transect sampling, and production outcomes.

		Production outcomes									
On-farm welfare assessment indicators[2]		Mortality	Reasons for rejection				Total rejected	Footpad score	Growth rate	Feed conversion	Production index
			Perosis	Illness	Wounds	Under-weight					
Small	Mean	148.00	-496.08	207.95	-5.35	312.85	237.95	227.03	2.77	-6.26	-6.93
	SE	10.54	398.05	18.86	59.43	39.10	14.56	421.99	5.59	3.77	5.78
	Lower 95% CL	124.51	-1382.98	165.92	-137.75	225.72	205.51	-713.22	-9.69	-14.67	-19.81
	Upper 95% CL	171.49	390.82	249.98	127.06	398.98	270.40	1167.28.9	15.23	2.15	5.96
	$F_{1, 10}$	197.09	1.55	121.55	0.01	64.01	267.09	0.29	0.25	2.75	1.43
	P	<0.001	0.241	<0.001	0.930	<0.001	<0.001	0.602	0.631	0.128	0.259
Welfare problems index	Mean	118.19	79.18	98.53	243.75	266.67	135.85	184.61	0.78	-3.39	-6.051
	SE	5.74	63.11	11.01	21.17	28.26	8.74	138.77	3.37	2.40	3.56
	Lower 95% CL	105.78	-57.15	74.75	198.01	205.63	116.96	-115.17	-6.51	-8.57	-13.75
	Upper 95% CL	130.60	215.51	122.31	289.49	327.72	154.73	484.40	8.06	1.79	1.65
	$F_{1, 13}$	423.44	1.57	80.14	132.53	89.07	241.48	1.77	0.05	2.00	2.88
	P	<0.001	0.232	<0.001	<0.001	<0.001	<0.001	0.206	0.822	0.181	0.113
Litter score	Mean	-0.16	1.47	0.36	1.62	0.59	0.50	-1.87	0.03	<0.01	0.02
	SE	0.04	0.82	0.06	0.25	0.17	0.06	1.42	0.03	0.02	0.03
	Lower 95% CL	-0.24	-0.31	0.22	1.08	0.22	0.38	-4.93	-0.03	-0.05	-0.05
	Upper 95% CL	-0.08	3.25	0.50	2.16	0.96	0.62	1.19	0.08	0.05	0.08
	$F_{1, 13}$	18.25	3.19	31.70	42.61	12.05	76.19	1.75	0.93	<0.01	0.28
	P	<0.001	0.097	<0.001	<0.001	0.004	<0.001	0.209	0.353	0.988	0.605

[2] Walking difficulties, illness, skin wounds and small were predictors in one model, and the welfare problems index and litter score were predictors in separate models.

Table 7: Mean differences in prevalence of welfare problems (%) between transects according to observer identity and transect location (left minus right; wall minus central), with Wilcoxon signed-ranks test (S) results.

Comparison		Walking difficulties	Illness	Skin wounds	Small	Welfare problems index[1]	Birds (n)[2]
Observers	Mean	-0.04	-0.02	-0.03	-0.05	-0.14	-1.21
	SE	0.03	0.01	0.02	0.03	0.06	96.87
	$S_{n=29}$	-62.5	-71.5	-81.0	-62.0	-94.5	0.5
	P	0.181	0.124	0.064	0.162	0.039	0.992
Left vs right	Mean	-0.01	0.02	-0.04	0.01	-0.03	-35.84
	SE	0.03	0.01	0.02	0.03	0.06	96.63
	$S_{n=29}$	-15.5	37.5	-109.0	61.0	2.5	-85.5
	P	0.744	0.427	0.010	0.169	0.958	0.063
Wall vs central	Mean	0.04	0.04	-0.01	0.05	0.11	-78.27
	SE	0.03	0.02	0.01	0.02	0.05	215.19
	$S_{n=29}$	54.5	94.5	-22.0	116.0	96.5	-9.5
	P	0.245	0.039	0.625	0.006	0.034	0.841

[1] Difference in sum of individual welfare indicator counts as a % of the estimated number of birds in the observed transects.

[2] Difference in the estimated number of birds in the compared transects.

4. Discussion

4.1. Environment inputs

We expected the transect data to be positively associated with increasing environmental complexity based on previous reports suggesting beneficial effects when providing platforms, peat and bales of foraging material alone or in combination (Kells et al., 2001; Ohara et al., 2015; Estevez and Newberry, 2017). Indeed, we found that the overall welfare problems index declined with increasing environmental complexity, accompanied by reduced mortality and fewer rejections at slaughter due to wounds, underweight birds and overall. This might be because the enrichments stimulated multiple behavioural activities (Vasdal et al., 2018) having positive effects on health.

We did not detect changes in growth rate, feed efficiency or production index value with increased environmental complexity. Although consumption of peat has previously been associated with increased weight gain and feed efficiency (Kocabagli et al., 2002; Ozturk et al., 2011; Kim et al., 2015), the amounts provided in the current study were probably insufficient to affect flock growth. Also, provision of elevated structures such as perches and bales has not previously revealed effects on weight gain, feed conversion, or carcass yield (Bizeray et al., 2002; Simsek et al., 2009; Yildirim and Taskin, 2017).

The transect data revealed a reduced prevalence of skin wounds with increasing environmental complexity. The observed wounds were mainly scratches around the tail, most likely resulting from birds accidentally scratching one another with their claws when scrambling to avoid a perceived danger. The reduced skin wound rate is consistent with reports of reduced disturbances (Ventura et al., 2012) and fear responses (Altan et al., 2013) in enriched houses, possibly influenced by more even distribution of birds within the house. Fewer rejections due to wounds suggest that experience with enrichments may have also resulted in calmer birds during catching. The non-significant tendency for reduced walking difficulties in enriched flocks ($P < 0.10$) is consistent with similar findings from other flocks provided with multiple types of enrichment under Norwegian housing conditions (Vasdal et al., 2018). In Norway, farmers are required to promptly cull any birds that become immobile due to leg disorders. The lower mortality in flocks receiving more types of enrichments may, thus, be influenced by a beneficial effect of enrichment on leg health resulting in less culling.

Higher space allowance residuals at each level of environmental complexity were associated with fewer walking difficulties and welfare problems overall, as well as fewer rejections due to wounds and a higher growth rate and production index. These findings are consistent with previous studies reporting better gait scores with increased space allowance (Sørensen et al., 2000; Dawkins et al., 2004; Knowles et al., 2008). The improvement is possibly related to the impact of space on opportunities for locomotor activity (Simitzis et al., 2012), though greater space allowance does not always lead to increased use of space (Leone et al., 2010). More space may facilitate access to feeders and drinkers, contributing to increased feed intake and weight gain with higher space allowance (McLean et al., 2002; Benyi et al., 2015). Furthermore, improved walking ability with increased space allowance may have reduced the risk of injury during pre-slaughter handling (Baracho et al., 2006), which could explain the reduced rejection rate due to wounds.

Collinearity between environmental complexity and space allowance raises the possibility that some results attributed to environmental complexity are at least partially explained by increased space allowance, particularly those in common with results based on the space allowance residuals (i.e. lower welfare problems index, fewer rejections due to wounds). However, differences in results for environmental complexity (i.e. lower mortality, fewer rejections of underweight birds and total rejections) and space allowance residuals (i.e. reduced walking difficulties and skin wounds, higher growth rate and production index) suggest that both factors make important contributions.

Although previous studies on underfloor heating are limited, beneficial effects have been reported in turkey and broilers (Nawalany et al., 2010; Abd El-Wahab et al., 2011; 2013). In the current study, underfloor heating was associated with a reduction in rejections due to perosis (leg deformities), though these were infrequent (range 0 - 5 birds/flock). While underfloor heating affords a high degree of temperature uniformity throughout the house (de Laval et al., 2013), other methods (e.g. use of heat exchangers (Bokkers et al., 2010)) can also be effective. All the houses in the current study were well insulated and equipped with modern automated heating, ventilation and drinker systems, explaining the relatively low litter scores, absence of dirty birds and lack of association between underfloor heating and footpad dermatitis scores. Underfloor heating also has the potential to produce dusty conditions contributing to health problems, but we found no evidence for increased illness due to underfloor heating in the current study.

Illness and skin wounds detected during transect walks, and total mortality, were lower under the 16 h intermittent photoperiod regimen compared to the 18 h continuous regimen. These findings could be related to the shorter overall duration of daily light exposure, given that long photoperiods have been linked to greater fear (Bayram and Ozkan, 2010) and an increased risk of mortality due to metabolic and skeletal diseases Hassanzadeh et al., 2000; 2003; Schwean-Lardner et al., 2013a; b). Further, our findings could be related to providing two daily dark periods instead of one. For example, if the birds under 16 h intermittent light were less hungry when the lights came on after the relatively short dark periods, they may have engaged in less scramble competition at the feeders , resulting in fewer skin scratches. Intermittent photoperiod regimens have been associated with a reduction in leg problems (Moore and Siopes, 2000), higher body weight gain (Sun et al., 2017), and a lower prevalence of footpad dermatitis (Skrbic et al., 2015). However, we did not detect such differences in this study, possibly because the two lighting regimens were more similar to each other than those compared in the previous studies.

4.2. Estimating production outcomes from welfare assessment indicators

We found that a higher welfare problems index was associated with an increase in flock mortality, rejection due to illness, wounds, underweight birds, and total rejections. These results are consistent with previous reports on relationships between on-farm welfare and production outcomes (de Jong et al., 2014; 2015; Saraiva et al., 2016; Jacobs et al., 2017; BenSassi et al., 2018). Further, our results indicate that a higher prevalence of walking difficulties on the farm was related to increased rejections due to wounds at slaughter. This is possibly because birds with impaired walking ability were at greater risk of being trampled by conspecifics during pre-slaughter catching and loading (Baracho et al., 2006). Higher litter scores were associated with higher rejections due to illness, wounds, underweight birds, and total rejections, in keeping with previous reports demonstrating associations between litter quality, welfare issues and production outcomes (Dawkins et al., 2004; de Jong et al., 2014; BenSassi et al., 2018). It was unexpected to find that higher litter scores were associated with lower mortality. Perhaps the rate of culling was lower in flocks experiencing deteriorating litter conditions, resulting in lower mortality on the farm and a correspondingly higher rejection rate at slaughter.

The correspondence of illness, small birds and overall welfare problems on the farm with illness, underweight birds and total rejections at slaughter, respectively, supports the validity of transect sampling for anticipating relative rates of rejections. Walking difficulties on the farm did not forecast perosis rejections, probably because compromised walking can occur for reasons other than leg deformities (Wideman, 2016). Walking difficulties may have precipitated, or been precipitated by, other conditions, explaining associations with rejections due to illness and underweight birds. For example, lameness could cause difficulties in accessing feed and water, thereby increasing vulnerability to illness and impairing growth. Bacterial infections can also produce lameness (Wideman, 2016). The lack of correspondence between skin wounds on the farm and wounds detected at slaughter could be explained by the latter including injuries sustained during pre-slaughter handling. The absence of a relationship between litter scores and footpad dermatitis scores was probably due to the generally good litter quality in this study.

The on-farm welfare assessment indicators were not associated with a reduced growth rate or production index, or a higher feed conversion ratio. Nor were walking difficulties related to growth rate in this study. Culling of disabled birds and the relatively early slaughter age of Norwegian flocks may account for these findings. Considering that welfare problems tend to increase with age (BenSassi et al., 2018), detection of a higher welfare problems index at around 28 days of age could potentially forecast compromised growth and a loss of feed efficiency in flocks kept to greater ages.

4.3. Consistency measures

Wilcoxon signed-rank tests showed consistency between observers in the recording of individual welfare indicators, but an observer effect on the overall welfare problems index. Because each observer sampled a different pair of transects, some of the variation between observers may be attributable to factors other than observer effects. When comparing between house sides (left vs right), results were consistent for all welfare indicators except skin wounds, for which higher levels were detected on the right than the left side of the house. This finding may have been related to bird reactivity to people entering the house, given that the house entrance was located on the right side of the house at a majority of farms. We also observed more illness, small birds, and overall welfare problems in wall than central transects. There are reports showing that broilers tend to sit by the house walls (Buijs et al., 2010; Aydin, 2016), and that more dead birds are found by walls than in central transects (Tabler et al., 2002; Marchewka et al., 2013), consistent with our finding. Uneven distribution of welfare issues within the house highlights the value of sampling both central and wall transects, and transects on both sides of the house, to obtain a representative sample.

Some heterogeneity of results between observers and in different locations in the house suggests that the ease of implementing transect sampling comes at a cost of some loss in precision. Nevertheless, transect sampling allows rapid surveillance of large numbers of birds, providing the cost efficiency that is necessary for widespread implementation (Sørensen et al., 2010).

4.4. Limitations and future directions

Bird movement and double counting might affect the reliability of transect sampling, especially in the presence of environmental enrichments that could alter bird movement patterns and distribution. Further research to assess the detectability and rate of repeated sampling of the same birds would be useful. The welfare indicators used in this study were focused on clearly evident health problems. Because health status does not inform about satisfaction of behavioral motivations (Brake et al., 1999; Botreau et al., 2007), consideration should be given to extending the transect sampling method to include behavioral indicators of positive welfare (Green and Mellor, 2011), facilitating inferences about welfare based on additional dimensions of animal welfare.

Caution is needed in interpreting the detected associations. We conducted 142 tests of significance, of which 43 were significant. At a 5% probability level, we can expect that approximately 7 of the significant findings represent false discoveries. Because animal welfare is affected by complex interactions between genetic background, environmental conditions and management (Fraser, 2008), some of the detected associations between on-farm and slaughter variables may have occurred due to the mutual influence of other, unmeasured factors. Relatively weak r^2 values point to the presence of unexplained variance and possible non-linear effects. Future studies with larger sample sizes would support investigation of non-linear effects as well as evaluation of the robustness of our results from variables with low prevalence and wide confidence intervals. There is also a need for a more quantitative measurement scale for environmental complexity that captures variation in types and amounts of enrichment materials supplied.

Farmers may benefit from implementing transect walks in all of their flocks to aid in benchmarking of welfare indicators in specific houses. Such data would provide a basis for comparing current flock findings with prior house, farm and regional averages, helping to identify the effects of making specific changes. Results rising above established targets could then trigger more detailed investigation to pinpoint causal factors. Since factors that predispose birds to develop welfare problems can continue to affect flock members after others have been culled, detecting emerging problems through transect sampling could spur timely interventions to improve welfare in the current flock. Future research is needed to evaluate the relative value of assessments conducted at different ages.

5. Conclusions

We have demonstrated that data collected using the transect sampling method enabled detection of differences in broiler chicken welfare associated with differing environmental provisions. The transect data were also associated with rejection at slaughter and certain other productivity outcomes. Thus, we have established the utility of this approach for animal-based welfare assessment, which is a prerequisite to widespread adoption. Our results also suggest that flock welfare can be improved by providing multiple types of environmental enrichment, increased space allowance (over the range 0.056 - 0.073 m^2/chick started), underfloor heating and an intermittent lighting programme with two 4-h dark periods. We conclude that the transect sampling approach offers a practical method for acquiring direct, quantitative data on the welfare of chickens on the farm, rather than relying solely on indirect assessment of the farmer's adherence to engineering standards that, alone, cannot assure good welfare (Main et al., 2007; Fraser, 2008; Kinierim and Winckler, 2009). It also offers information about the current welfare status of a flock in a timely manner for enabling interventions instead of waiting for data generated at the end of the production cycle (e.g. footpad dermatitis at slaughter, which occurred too rarely to be informative in the current study). Overall, our results support the soundness of the transect sampling method as a practical tool for swiftly assessing welfare in large broiler flocks.

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IV

CHAPTER 4

Broiler chicken on-farm welfare assessment: Estimating the soundness of the transect method

Manuscript submitted:

BenSassi. N., Averós, X., Estevez, I. Broiler chicken on-farm welfare assessment: Estimating the soundness of the transect method. Submitted.

Abstract

Assessing commercial broiler chickens´ welfare usually comes at the cost of reduced precision due to the large flock sizes and required time commitments. The transect method for on-farm welfare assessment was validated for commercial turkey flocks by comparing transect results with individual bird assessment at the end of rearing. As this validation is not feasible in broilers dues to the large flock size and catching practices, the aim of this study was to evaluate the soundness of the transect method in broiler flocks through a capture-recapture approach of a known subpopulation of 80 birds. Groups of ten chickens were captured in eight locations of the house and individually marked. Birds´ movement was tracked during the two following days by walking paths delimited by feeder and drinker lines called transects. Two observers collected the position of detected marked birds while walking along non-adjacent transects (4 samplings/house/day). Detection and repetition rates per house and within transect were calculated. The effects of flock density, transect number/house (6 *vs* 8) and sampling time (morning *vs* afternoon) were determined. The number of travelled transects was calculated for birds detected more than once and the population random distribution was tested by comparing the number of observed and expected birds/transect. The effect of flock density, transect number/house and position (wall *vs* central) were analyzed. Results showed higher repetition rates in six-transect houses and during morning samplings. The number of travelled transects was higher in eight-transect houses and from birds first detected at walls, indicating longer travelled distances in wider houses. Higher values of the distribution index were observed at walls in eight-transect houses indicating a higher than expected number of observed birds. Bootstrapping transect results showed that a representative assessment could be obtained by sampling only two transects. Overall, our findings suggest that assessments based on two transects provide sound results for welfare assessment of commercial broiler chickens if conducted on a wall and a central transect, separated by three transects in between. Lower repetition rates, required time for assessment and bird disturbance would be achieved with such recommendations while maintaining the robustness of final results.

1. Introduction

Public concern about animal welfare has resulted in the need of developing assessment protocols that can be applied on commercial farms to provide consumers with information on certain welfare requirements. The Welfare Quality® protocols (2009) are the most commonly used welfare assessment methods for cattle, pigs and poultry. In regard to poultry, the protocols are time consuming despite the limited sample size that is assessed (de Jong et al., 2012). Attempts to simplify the protocols included the evaluation of on-farm provided resources (Buijs et al., 2016), or assessment of post-mortem condition (de Jong et al., 2016). The use of technology to assess broiler welfare through precision livestock farming is also emerging (Ben Sassi et al., 2016), even though many of these methods are still at the experimental phase (Berkmans, 2014).

The transect method is a recent approach for on-farm welfare assessment of meat poultry (Marchewka et al., 2013, 2015). The method was initially developed as an alternative for turkey assessment as their capture was not feasible when close to market weights. This method is implemented by walking through transects, which are defined as the areas delimited by feeder and drinker lines, while collecting the incidences of birds showing any of the defined welfare problems: leg problems, sickness, skin wounds and/or dirtiness (Marchewka et al., 2013; Ben Sassi et al., 2018). Data are collected by clicking on the assessment screen of the i-WatchBroiler app (i-WatchBroiler, 2015) or the i-Watchturkey app (i-WatchTurkey, 2014).

Specifically for broiler chickens, the effects of bird age, genetic line, litter quality and transect position (wall vs central) on the incidence of welfare indicators collected were shown with this method (Ben Sassi et al., 2018). Transect results were also effective at discerning the effects of on-farm environmental enrichment (wood shaving bales, boxes, and/or peat moss), increased space allowance, and photoperiod regimen (Ben Sassi et al., submitted). On-farm welfare problems detected with the transect method were correlated with increased rejections at slaughter (BenSassi et al., 2018; 2019), highlighting the link between on-farm collected data and production outcomes. These results suggest the robustness of the transect method, even though there are still questions regarding its accuracy and possibilities of repeating birds that may be moving along and across transects.

In commercial farms, chickens are confined in large houses that allow longitudinal and transversal movement, resulting in freely moving birds. When conducting transects, individuals escaping from visual inspection might represent a potential bias affecting the assessment results. It is also unknown whether assessments may be altered by repetition of birds, even though care is taken to avoid adjacent transects. The transect method was validated in commercial turkey farms by comparing the results with the gold standard of individual assessment of the entire flock during the load out phase (Marchewka et al., 2015). Nevertheless, the much larger group size of commercial broiler flocks impedes the same type of validation. An alternative approach to estimate the soundness of the method may be to track a known subpopulation to determine the detection rate along with possibilities of repetition.

In studies of wild life ecology, estimation of the population abundance and movement patterns is conducted using different marking and tracking methods (White et al., 1982). The capture-recapture method, which consists in marking individuals in a population, releasing, and recapturing (or re-sighting) them later on (Seber, 1982), may be a useful approach to estimate the movement of birds when conducting transects, and particularly to estimate birds that may be repeated during the assessment process. As birds' movement is related also to management aspects (Estevez and Christman, 2006), studying management features might provide insights on its potential effect on assessment results. For instance, broiler chickens are more active in the morning as compared to afternoons (Hocking et al., 1996), which might increase the likelihood of overlooking birds during morning assessments or, on the contrary, could result in a higher rate of repeated observations if they move transversally. Birds' use of space is affected by stocking density (Estevez et al., 1997; 2007). Thinning, a commercial practice consisting in depopulating part of the flock, results in decreased stocking density which may modulate movement patterns of the remaining birds (Leone and Estevez, 2008), and increase the probabilities of overlooking individuals as more space is available. Moreover, birds are reported to travel longer distances when in large experimental pens (Mallapur et al., 2009; Leone et al., 2010). According to the house dimensions and the lock density, birds might be overlooked as they tend to escape from observers by running in front or taking the perpendicular direction to the observer's movement. This could also result in repeating birds when conducting more than one transect/house. Repetition in both longitudinal and transversal directions (i.e. both in the evaluated and neighboring transects) should then be investigated. A higher use of enclosure peripheral areas was reported at higher stocking densities (Aydin, 2016; Buijs et al., 2010; 2011), especially for impaired birds (BenSassi et al., 2018). This suggests an uneven distribution indicating that both central and peripheral areas should be sampled when implementing transects.

The aim of this study was to assess the soundness of the transect method for broiler welfare assessment by determining its capability to detect individuals of a known subpopulation. The probability of repeating birds within and across transects was also tested. For this purpose, a subpopulation of broiler chickens was marked, and then tracked for two consecutive days to estimate the detection and the repetition rates within and across transects (per house). We predicted higher detection and lower repetition rates with high densities, and during lower activity periods. We also estimated the number of transects that repeated birds travelled, the subpopulation distribution and effects of tested management factors, expecting a higher number of travelled transects at lower flock densities and in larger houses.

2. Materials and Methods

2.1. Experimental design and data collection

This study was conducted in Northern Spain from March, 2016 to November, 2017. Eleven commercial broiler flocks placed at three different farms were used for the study. All farms belonged to the same integrating company and followed identical management practices. House dimensions ranged from 1250 to 1950 m^2 (Table 1) with initial stocking densities ranging from 17 to 19 birds/m^2. All houses were provided with automatic drinkers, feeders, automatic ventilation and artificial light. Flocks were all of mixed genders, and genetic lines were Ross 308, Cobb 500, or a mix of both (Ross 308/Cobb 500). Thinning took place during the fifth week of age in some flocks. When assessments were performed, flock densities ranged between 11.36 and 17.84 bird/m^2 (Table 1).

Table 1: Number of sampled houses per farm, house dimensions, number of transects per house and stocking densities of sampled flocks at the time of the data collection.

Farm	Houses/farm	House dimension (m^2)	Transects/ house	Rounds	House	Flock density[1] (birds/m^2)
1	1	1950	6	1	1	16.35
2	2	1250	6	1	1	16.44
					2	16.40
				2	1	11.85
					2	11.90
				3	1	17.19
					2	17.83
3	2	1500	8	1	1	17.52
					2	17.83
				2	1	12.82
					2	11.39

[1] Stocking densities in the day of birds marking, lowest values correspond to thinned flocks prior to the start of the study.

Before data collection, house dimension and transects’ width were measured using a laser meter (Robert Bosch GmbH, GLM 250 VF Professional, Switzerland). The length and width of the house was measured by placing the laser meter in one wall and measuring the distance to the opposite wall. The area between feeder and drinker lines was considered a transect if wider than 1 m. Transects were categorized as “wall transect” if delimited by a wall in one side and as “central transect” if delimited by a feeder and/or drinker lines on both sides. Transect measurements were also taken with the laser meter by two observers, with one observer maintaining the laser meter on a feeder/drinker line, while the second placed a clapboard on the next feeder/drinker line. The number of transects per house were either 6 or 8 (Table 1) depending on the house width (10 to 15 m) and the disposition and number of feeder and drinker lines. In total 4 six-transect and 3 eight-transect houses were used. Mean transect width was 1.83±0.029 m (mean±SE), with a mean number of sampled birds/transect of 3150±56 (mean±SE).

A total of 80 birds were captured at random at eight locations in each house. All chickens were marked with numbers (1 to 80) for individual identification using a black permanent, non-toxic marker. To maximize the distance and minimize disturbances for the birds, ten chickens were marked per location (Figure 1A). Marking was performed at 30±2 (mean±SD) days of age. Twenty-four hours after marking, birds were tracked for two consecutive days by two trained observers that collected data in separated houses within the same farm.

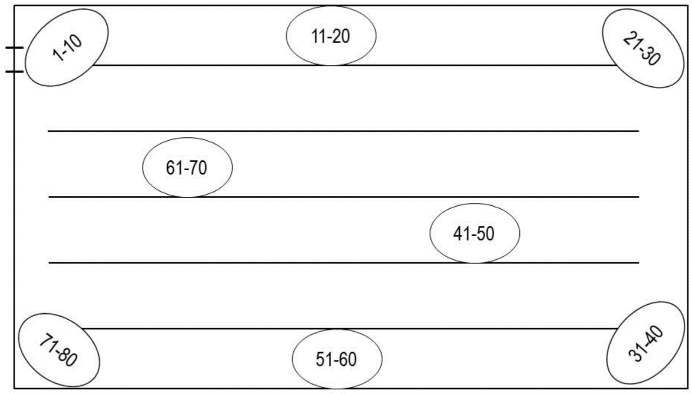
Observations were always performed following the recommendations of Marchewka et al. (2013; 2015) and starting from transect 1, located at the right side of the house, with the house entrance door as a reference (Figure 1B). As standard practice with the transect method, sampling of two adjacent transects was avoided in order to minimize repetition risks. Transect walks were conducted until completing all transects in the house by assessing transects 1, 3, and 5, and returning to assess transects 2, 4 and 6 (Figure 1B). During the transect walks, the id and spatial location of detected marked birds were recorded on a house template that included longitudinal references and the location of all transects. Each of the two observers conducted the assessment simultaneously in one of the houses, swapping houses when finishing. A total of four samplings were collected, two per observer, house and day, being two in the morning and two in the afternoon. A 15 min interval was allowed between house samplings. Data of one of the sampled houses was missing for the second day of data collection due the thinning of the flock.

2.2. Calculation of parameters and statistical analyses

2.2.1. Detection and repetition rates

The detection rate per house sampling was calculated as (N detected marked birds per house sampling/Total N marked of birds* 100). The repetition rate/house sampling was calculated as (N repeated marked birds per house sampling/N detected marked birds in the same house sampling* 100). The bird repetitions within a transect was calculated as (N repeated marked birds in a transect/N detected marked birds within the same transect* 100).

A



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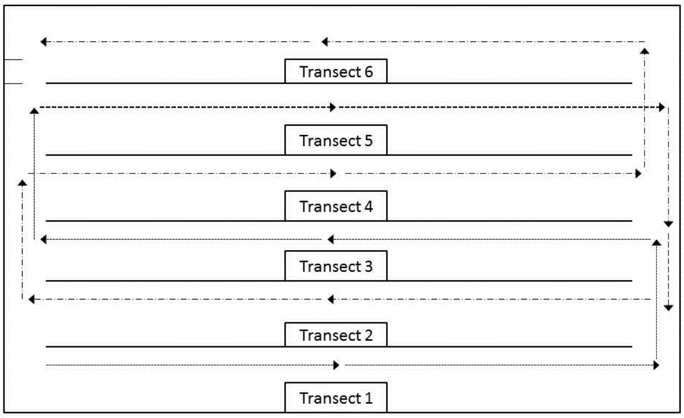

Figure 1: (A) chicken sub-population marking distribution, and (B) tracking patterns. The dashed lines starting in transects 1, 3 and 5 show the first part of data collection while those travelling transects 2, 4 and 6 show the second part.

For repeated birds, the transversal movement was estimated by calculating the number of travelled transects between the first and second observation of each repeated bird in a house sampling. The percentage of repeated birds was calculated according to the distance, in transects, from the first observation. For instance, in a six-transect house, we calculated the percentage of repetition 2 transects away from the first conducted by summing the number of birds that were first detected in transect 1 and repeated in 3, first detected in 2 and repeated in 4, first detected in 3 and repeated in 5, and first detected in 4 and repeated in 6. This number was then divided by the total number of birds detected.

The effect of flock density, number of transects/house (6 *vs* 8 transects) and sampling time (morning *vs* afternoon) on detection and repetition rates/house sampling were tested assuming a Gaussian distribution. The same effects were tested for the repetition rate within transects, variable that was modeled assuming a Poisson distribution. Repeated measures, generalized linear mixed model ANOVAs were carried out with the GLIMMIX procedure in SAS 9.3 (SAS Institute Inc., 2011). All effects were introduced as categorical variables except flock density, which was included as a co-variate. The effect of the observation day was first included in the model and then removed due to non-significance. Flock nested within farm was included as a random effect, and the day by house sampling was the repeated measures in the three models. A first order autoregressive covariance structure was assumed to account for any linear dependence of measures of each flock over time.

For the number of travelled transects in the case of repeated birds, the effects of flock density, number of transects/house and transect position where the bird was first detected (wall *vs* central) was tested assuming a Gamma distribution. The sampling time (morning *vs* afternoon) was first introduced in the model, but then removed due to non-significance. The repeated measures unit consisted in the interaction between the observer and observation day. For statistically significant effects ($P < 0.05$), least squares means differences were computed for all models, with P-values adjusted for multiple comparisons using Tukey tests.

2.2.2. Distribution of the marked subpopulation

To test the differences in distribution between the expected and the observed number of marked birds, we calculated the distribution index according to the formula by Keeling et al. (2017): (N observed marked in transect-N expected marked birds in transect)^2/N expected marked birds in transect. The expected number of marked birds/transect was estimated according to the specific transect dimensions. The distribution index tend to zero when the observed and expected number of marked birds are similar, indicating a random bird distribution. The distribution index was first calculated by transect, then we calculated a mean distribution index per flock and per day for wall and central transects.

The effects of number of transects/house (6 vs 8 transects), transect position (wall *vs* central) and their interaction were tested on the subpopulation distribution index assuming a lognormal distribution. Flock density at day of sampling was first included in the model and then removed due to non-significance.

2.2.3. Bootstrapping simulations

Bootstrap analysis was applied to examine the method's stability when varying the number of sampled transects per house. This method, used to optimize sampling methods, generates a collection of simulated random sampling combinations from the original data set using the Monte Carlo method (Dixon, 2001) to construct the bootstrap distribution (Efron, 1979, 1987). Expected mean and SE of the data set was calculated by taking random samples of one transect, or combinations of 2 to 5 transects in six-transect houses, and 2 to 7 transects in eight-transects houses. Simulations were run 10,000 times using PROC SURVEYSELECT in SAS 9.3 (SAS Institute Inc., 2011) software. Calculations were averaged per farm (given that houses belonging to the same farm were of the same size) and across all rounds of data collection.

This study complied with the Spanish legislation regarding the use of animals for experimental and other scientific purposes (Real Decreto 1201/2005).

3. Results

3.1. Detection and repetition rates, and subpopulation distribution

The detection rate of the marked subpopulation was 64.76% ± 0.87 (mean ± SE), with no effect of any of the tested factors (Table 2). The repetition rate when conducting all transects per house was 23.85% ± 0.77, but was as low as 1.66% ± 0.58 (mean ± SE) within transect. The repetition rate/house was higher in six-transect as compared to eight-transect houses (Table 2), while higher repetitions within transects were found in morning samplings (Table 2).

Table 2: Effects of stocking density, number of transects/house (6 *vs* 8) and sampling time (morning *vs* afternoon) on detection and repetition rates per house and within transects (mean±SE) of a marked subpopulation of broilers assessed using the transect method.

		Variables	Detection rate[2]	Repetition rate/house sampling	Repetition rate within transects
Flock density (birds/m^2)		Mean RC[1]	0.232	-0.339	0.149
		SE	0.339	0.265	0.137
		$F_{1,16}$	0.47	1.63	1.26
		P	0.502	0.220	0.278
Transect number/house	6 transects	Mean[2] (%)	63.735	26.405	1.389
		SE	1.166	0.915	0.537
	8 transects	Mean[2] (%)	66.129	20.531	2.006
		SE	1.335	1.048	1.146
		$F_{1,16}$	1.82	17.84	0.59
		P	0.196	<0.001	0.452
Sampling time	Morning	Mean[2] (%)	65.177	24.054	3.126
		SE	1.262	0.991	1.112
	Afternoon	Mean[2] (%)	64.687	22.881	0.263
		SE	1.233	0.968	0.263
		$F_{1,18}$	0.17	0.72	5.03
		P	0.687	0.406	0.038

[1] Mean RC: Mean regression coefficients estimated for the effect of flock density on detection and repetition rates per house sampling and within transect.

[2] For Repetition rate within transect, P-values and F correspond to the results of the statistical model run with Poisson distribution, whereas Mean and SE are calculated from raw data.

When only considering the observations of repeated birds, 67% and 71% of the repetitions occurred in adjacent transects in six and eight-transect houses, respectively (Table 3). In both house sizes, the percentage of repetitions decreased as the number of transects in between increased. When considering the transect where each bird was first detected and the location where it was observed later on, results showed that on average, birds travelled more than 2 transects if first detected in transect 1 (wall), while they travelled 1 to 2 transects if first detected in any of the other transects (Table 3).

Table 3: Distribution of repeated birds (%) according to the number of transects away, and number of travelled transects (mean±SE) according to where marked birds were first detected.

	Transect number /house		Adjacent transect	2 transects away	3 transects away	4 transects away	5 transects away	6 transects away	7 transects away
Distribution of repeated birds (%)	6		71.212	15.151	10.038	2.841	2.272	-	-
	8		67.372	12.689	9.365	4.230	3.021	2.719	0.302
		First detected in	Transect 1	Transect 2	Transect3	Transect4	Transect 5	Transect6	Transect 7
N travelled transects	6	Mean	2.055	1.319	1.715	1.360	1	-	-
		SE	0.105	0.077	0.104	0.046	0	-	-
	8	Mean	2.402	1.948	2.178	1.864	1.760	1.187	1
		SE	0.205	0.176	0.247	0.177	0.176	0.070	0

Regarding the subpopulation distribution, our results showed that the distribution index was lower in six-transect houses ($F_{1,37}$= 14.43, P<0.001).Mean values are presented in Table 4. Significant differences were detected for the interaction between the number of transects/house and transect position ($F_{1,37}$= 29.22, P<0.001; Fig.2). The number of travelled transects by repeated marked birds was higher in eight compared to six-transect houses, and for birds initially detected on wall transects in comparison with those detected in central ones (Table 5).

3.2. Bootstrapping simulations

The results of the bootstrapping simulations on the percentage of detected marked birds/m^2 showed that the mean value remained stable irrespectively of the number of transects observed (Table 6).

Table 4: Mean±SE of transect width, number of expected and observed marked birds per transect and the distribution index in 6 and 8 transect houses and according to the transect position (wall *vs* central).

Transect number /house	6 transects		8 transects	
Mean house width (m)	11		15	
	Mean	SE	Mean	SE
Transect width (m)	1.773	0.029	1.800	0.055
Expected[1]	13.333	0.215	10	0.284
Observed	10.802	0.236	7.945	0.251
Distribution index	1.538	0.200	6.340	1.171

According to transect position	Central		Wall		Central		Wall	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Transect width (m)	1.850	0.039	1.618	0.034	2.171	0.062	1.136	0.034
Expected	13.957	0.305	12.086	0.133	11.350	0.319	5.950	0.182
Observed	9.972	0.267	12.821	0.381	6.594	0.231	12.00	0.432
Distribution index	2.060	0.216	1.018	0.274	3.252	0.104	9.429	1.771

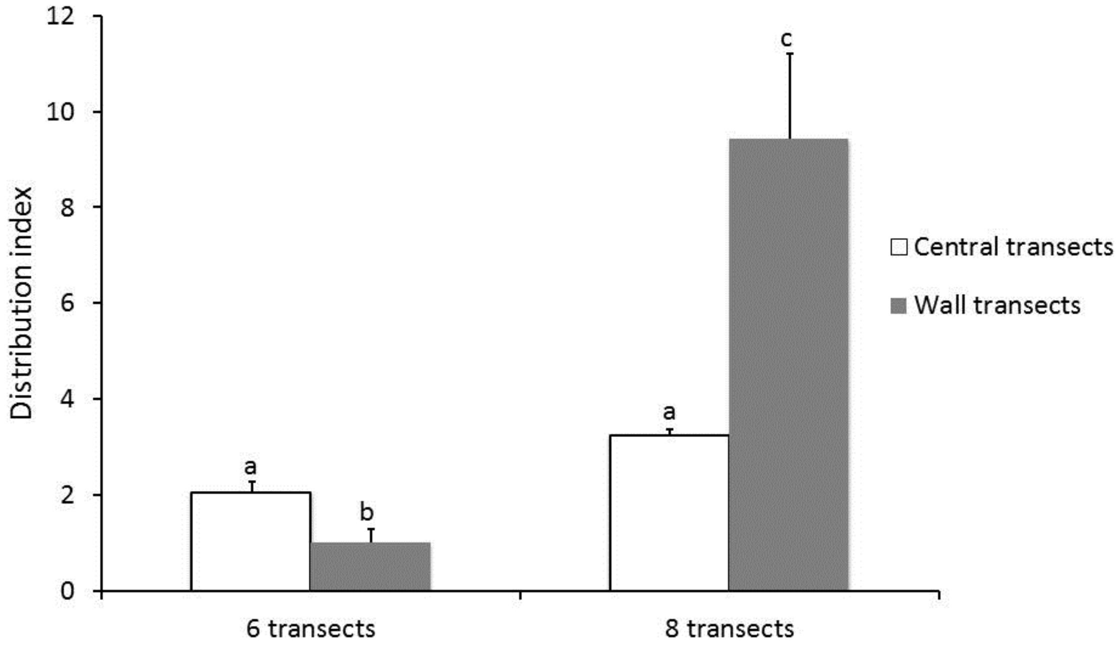

Figure 2: Interaction between transect number/house (6 vs 8) and transect position (central vs wall) on the distribution index of a marked subpopulation of broiler chickens.

Table 5: Effects of stocking density, number of transects/house (6 vs 8 transects) and transect position (wall vs central) on the number of travelled transects over repeated observations of marked bird using the transect sampling method.

			Travelled transects of repeated birds
Flock density (birds/m2)		Mean RC[1]	-0.012
		SE	0.008
		F[2]	2.04
		P	0.153
Transect number/house	6 transects	Mean	1.580
		SE	0.046
	8 transects	Mean	1.881
		SE	0.068
		F[2]	16.21
		P	0.005
Transect position	Central	Mean	1.438
		SE	0.036
	Wall	Mean	2.068
		SE	0.087
		F[2]	56.91
		P	<0.001

[1] Mean RC: Mean regression coefficient estimated for the effect of stocking density on the number of travelled transects, and the marked population distribution index (CI= (Number of observed - Number of expected)^2/ Number of expected).

[2] The number of degrees of freedom was $F_{1,848}$ for the stocking density and $F_{1,7}$ for the number of transect/house and transect position.

Table 6: Bootstrapping simulation results for the percentage of marked birds detected/m^2 (Mean ± SE) according to the number of transects assessed for each farm across sampled flocks.

		Farm 1		Farm 2		Farm 3			
Flock size (birds)		31891		19051		22359			
House dimension (m^2)		1950		1250		1500			
Covered area (%)	Transects number	Mean	SE	Mean	SE	% information	Transects number	Mean	SE
17%	1	0.037324	0.000111	0.051112	0.000185	12.50%	1	0.042460	0.000217
33%	2	0.037320	0.000079	0.050960	0.000126	25.00%	2	0.042626	0.000154
50%	3	0.037272	0.000063	0.051290	0.000104	37.50%	3	0.042660	0.000125
66%	4	0.037222	0.000055	0.051372	0.000092	50.00%	4	0.042467	0.000107
84%	5	0.037313	0.000049	0.051274	0.000082	62.50%	5	0.042649	0.000096
100%	6	0.03729	0.000045	0.051314	0.000075	75.00%	6	0.042595	0.000089
						87.50%	7	0.042739	0.000081
						100.00%	8	0.042617	0.000076

4. Discussion

The soundness of the transect method was tested by applying the capture-recapture approach on a known subpopulation of broiler chickens reared under commercial conditions. Eighty birds were individually marked and tracked across transects during two consecutive days. Detection and repetition rates per house and within transect were calculated. For repeated birds, we estimated the number of travelled transects. The hypothesized subpopulation random distribution was analyzed considering the transect number and position. The recommended number of transects to sample for a representative assessment with the minimum effort was estimated.

4.1. Detection and repetition rates

The transect method intends to be a practical welfare assessment tool for meat poultry reared under commercial conditions. Detection rate of marked chickens, when all transects were observed, reached nearly 65% of the marked subpopulation. Given that detection rates only include detected non-repeated birds, individually locating almost two thirds of a subpopulation of 80 individuals within flocks that ranged between 15000 and 32000 birds can be considered relatively satisfactory, especially if we consider the fast and non-invasive features of the method. Due to the natural tendency of birds to move away when perceiving an approaching human, some of the marked birds might have been overlooked, even though walking through transects was always performed at a slow pace.

It is important to point out that the probability of detecting all 80 marked birds depends on the probability that the observer and each particular bird coincide in time and space in one of the assessed transects. This combination of likelihoods makes it statistically improbable to detect all specific marked birds in one round of house sampling, explaining why the detection rate when assessing the entire house is unlikely to reach a 100%. Almost 24% of the birds were repeated in the house samplings as all transects were conducted. Differences in birds' movement patterns (Preston and Murphy, 1988) may help explaining the results obtained in detection and repetition rates as birds with higher mobility are likely to be repeated later on in one or more transects. Our results on the repetition rate/house sampling suggest that most birds tend to move away laterally as the observer walks along the transect. In fact, lateral movements at an angle of ninety degrees from the potential predator's line of attack have been shown to be the natural escaping strategy in birds' taxa (Kullberg et al., 2000). The tendency to move laterally to the observer trajectory explains the repetition rates obtained when assessing the entire house. Indeed, higher repetition percentages were found especially in the transects adjacent to the one first conducted (see table 3). On the contrary, it was particularly important to note that the repetition rate within transects (birds observed more than once in a particular transect) was low with a mean of 1.66±0.58% (mean ± SE). This is important in practice, given that assessments are advised to be based on 2 transects per house (Marchewka et al., 2013), which means that chances of overestimating welfare problems are actually low.

The regression analyses showed that the risk of repetition within transects was higher in the morning assessments as compared to afternoons (Table 2). This difference is likely due to the higher morning activity levels reported for the domestic fowl (Hocking et al., 1996), usually dedicated to forage (Dawkins, 1989). Even though the repetition rate within transects was overall low, higher activity levels in the morning may have resulted in some birds that did not move in a perpendicular direction but kept moving within the length of the transect, or may have moved away and returned to the observed transect within a short time period. Higher repetition rates/house were detected in six-transect in comparison to eight-transect houses. Because six-transect houses are narrower than eight-transect houses (11 vs 15m, respectively), the probability of observing the same bird increases as a result of the lower number of transects. Besides, when considering the sequence of observations in wider houses, the observer assessed transects 1, 3, 5 and 7 before coming back to transects 2, 4, 6 and 8 providing birds with a longer time lapse and space to redistribute, thus decreasing the risk of repetitions. In addition, our results on the percentage of repeated birds among transects showed very high repetition in adjacent transects in comparison to the following ones. These results not only support the recommendation by Marchewka et al. (2013) of avoiding observations in adjacent transects, but also confirm our suggestion to sample transects that are further away. According to our findings in Table 3, skipping at least three transects would minimize the risk of repetition. This is particularly advisable in narrower houses.

It is clear from our results that birds in wider houses (i.e. eight-transect houses) travelled more transects as compared to six-transect houses. Birds under experimental pen conditions were shown to travel longer distances in larger enclosures (Leone and Estevez, 2008; Leone et al., 2010) with longer total and net distances, and longer mean and maximum step length (Mallapur et al., 2009). Eight-transect houses were not larger in total available area but they were wider as compared to six-transect houses. The higher number of transects in wider houses, and the additional time required to assess eight-transect houses resulted in birds travelling longer distances which explains our results, especially considering birds´ tendency to move laterally when perceiving an approaching human. Our results also showed that the movement patterns are affected by the position of the transect in which the marked bird was observed (wall or central), as birds first detected at walls crossed more than 2 transects in both six and eight-transect houses (Table 3). On the contrary, the mean number of transects travelled by birds located in central transects was 1.5. Birds at walls can only escape towards the central house area. The possibility of moving only in one direction would explain the difference in results.

Our findings on detection and repetition rates and number of travelled transects were demonstrated on a healthy marked subpopulation which ability to move along transects should be better than that of unhealthy birds. When conducting welfare assessments, the interest is focused on birds with impaired welfare (e.g. lame, immobile, sick individuals). Differences in activity levels between impaired and healthy birds were demonstrated (Aydin et al., 2010), not only for chickens with leg difficulties (Dawkins et al., 2012; Roberts et al., 2012; Dawkins et al., 2017), but also for those infected with diseases (Colles et al. 2016). Welfare assessment of impaired birds with the transect method is likely to result in much lower repetition rates as birds with compromised welfare are expected to move less (if at all) and therefore will be less likely to be found again in the following conducted transects. Future studies using the transect method should focus on estimating these variables on an impaired subpopulation, although it is challenging as such birds should be culled by the farmer on the basis of minimizing animal suffering.

4.2. Marked subpopulation distribution

The results on the distribution index suggest that the marked subpopulation was closer to a random distribution in six-transect than in eight-transect houses (see Table 4). When comparing central and wall transects, the distribution index showed opposite patterns in six and eight-transect houses. While it tended to zero at walls in six-transect houses, it was slightly higher in central transects. The distribution index was much higher at walls for eight-transect houses. In fact, the number of observed birds doubled the expected value on walls, altering significantly the distribution index in eight-transect houses. These results may be due to two different factors. On one hand, the wall effect (Newberry and Hall, 1990; Cornetto and Estevez, 2001) may have been much stronger in wider eight-transect houses. Ventura et al. (2012) showed a lower use of central areas in experimental small control pens when compared with pens equipped with barrier perches. Therefore it is suggested that in larger houses the strong preference for walls may have resulted in higher values of distribution index. On the other hand, the layout of eight-transect houses was such that the wall transects were smaller than the average with a mean width of 1.136 m. This might explain the low number of expected birds (estimated according to the transect width), in comparison to the observed number. In addition to the narrower width of wall transects, eight-transect houses were shorter than six-transect houses resulting in a lower wall space available per unit of area (the mean percentage of wall area was 9% and 6.6% in six and eight-transect houses, respectively). Therefore, the lower availability of wall areas might have resulted in a higher demand of wall space due to stronger preference (Newberry and Hall, 1990; Cornetto and Estevez, 2001). Such lower wall availability, combined with the above mentioned preference for walls may have resulted in birds congregating at walls in eight-transect houses. Although this effect was only observed in eight-transect houses, the potential for wall effects suggest that in order to have a more representative sample of the flock the transect method should be conducted by selecting a wall and central transects.

Flock density did not affect any of the tested variables. Although thinning resulted in densities reaching as low as 11 birds/m^2 in some flocks, no differences were shown neither on the detection and repetition rates, nor on the number of travelled transects and distribution index. Even though Ventura et al. (2012) demonstrated higher activity levels at low densities (8 and 13 birds/ m^2), they also reported a significant decrease in activity levels with age. As birds in our study were assessed at 30 days of age, when activity levels is significantly lower (Newberry and Hall, 1990; Cornetto and Estevez, 2001; Alvino et al., 2009), this might explain the lack of effect of flock density on the number of travelled transects. Indeed, the high percentage of repetition between adjacent transects might confirm our assumption, suggesting that due to lower activity levels, birds escaping the observer did not move far away. Our findings suggest that welfare assessments using the transect method are not affected by lowering densities that resulted from thinning flocks.

4.3. Bootstrapping simulations

The bootstrapping technique is an analytical method designed to calculate the minimum sample effort required without losing accuracy of the sampling (Qumsiyeh, 2013). Our results indicate that the mean percentage of detected birds remained stable even when assessing a single transect, as compared to the assessment of the entire house. Variability around the sample mean also remained stable but was slightly lower when assessing two transects in comparison to assessing only one (Table 6). These findings are in agreement with the results from Marchewka et al., (2013) who reported a stable mean estimation and minor changes in SE when evaluating only 20% of the house area, equivalent in their study to two transects. Despite the fact that assessments in our study were conducted on healthy marked birds, which ability to escape from the observer is expected to be higher than that of birds with welfare issues, SE did not increase excessively when the number of sampled transects decreased. Therefore, the results of this study are in agreement with previous studies by Marchewka et al., (2013; 2015) who suggested assessing two transects for a representative assessment of the population. In addition, consideration should be given to the economic cost and time constraints when assessing broiler welfare in commercial flocks. Reducing sampling to two transects (wall and central), would still provide a reliable mean, minimize bird disturbance, and reduce cost and time requirements as compared to sampling the entire house. It also has the benefit of minimizing the risk of repeating birds if conducted transects are separated by at least three transects in between.

Overall, this study aimed at analyzing the soundness of the transect method using capture-recapture techniques of a marked bird subpopulation and tracking their movements under commercial conditions. Our findings were generally expected and might be considered robust. We found higher repetition rates in six-transect houses and during morning samplings. More transversal movement was registered in wider eight-transect houses and when birds were first detected at walls. These findings are consistent with previous results, confirming that population movement under commercial conditions might potentially influence assessment outcomes. Therefore, it is recommended to skip three transects after evaluating the first one to minimize risks of repetition (if enough transect are available in the house). We found significant differences in the distribution index between central and wall areas especially in wider eight-transect houses, which was related to the higher preference of wall transects. Therefore, it is advisable to sample both wall and central transects for a representative welfare assessment of the impaired population. Bootstrapping transect data showed that assessing two transects provided comparable results to those obtained when assessing the entire house. Lower repetition rates, required time for assessment and bird disturbance would be achieved with such recommendations while maintaining the robustness of final results.

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GENERAL DISCUSSION

The aim of the present Doctoral Thesis was to investigate on the practical applicability and soundness of the transect method for on-farm broiler chicken welfare assessment. Chapter 1 provides an overview of the existing technologies for poultry welfare assessment, and their potential on-farm implementation for real-time detection of welfare problems. The practical applicability of the transect method was studied and reported in Chapters 2 and 3 through testing the prevalence of welfare indicators under varying commercial house management and environmental conditions. In Chapter 2, we explored the effect of bird age and genetic line, litter quality and transect position (central vs wall), while in Chapter 3, the effect of environmental enrichment was investigated, together with additional factors such as the variation in space allowance, underfloor heating and photoperiod regimen. In both studies, the association between welfare indicators obtained with the transect assessments on-farm and the production outcomes at the slaughter house were considered. The results obtained were logical in both experiments, showing the potential of the transect method to detect variations of specific on-farm environmental conditions on the prevalence of broiler chicken welfare indicators, and the association of these indicators with production outcomes. Furthermore, the soundness of the transect method was tested through capture-recapture methods by tracking the movement of a known bird subpopulation while conducting transects. The experiment reported in Chapter 4 showed satisfactory results when estimating the subpopulation detection rate and the number of transects that should be skipped between those assessed in order to minimize risks of bird repetition. The experiment also confirmed the differences in the number of detected marked birds according to the transect position (central vs wall) and provided further recommendations for a robust assessment.

The results of this work suggest the usefulness of the transect method for its implementation as a tool for on-farm welfare assessment and routine data collection during daily farm checks. The present, final chapter will offer a general discussion of the main findings of this Thesis.

1. Technology advances

The incorporation of PLF technologies in livestock farming is paramount, not only to assist farmers but also to improve animal welfare. Technology allows farmers to better control the required environmental parameters with less effort, and provides the possibility of alerting in case of deficiencies. Existing precision livestock technologies have paved the way for innovative thinking and for their introduction in all phases of the poultry production including hatching, growth and slaughter. The application of technology is facilitated by the high level of specialization of poultry farms, permitting the acquisition of specific technologies for each production phase. The availability of internet networks, IT-based management systems and real time data monitoring is making the process of farm control faster, more efficient and reliable. Based on the literature review, the main technologies currently under development for poultry include devices to control the house environment, and devices for direct animal data collection. The first category assures good housing and bird comfort by maintaining air velocity, relative humidity and temperature within adequate values. The devices for animal data collection permit to assess risks of health and welfare impairment through the evaluation of birds' behaviour and physiological parameters. Given that both categories represent valuable sources of information to the farmer, the combination of these two types of devices into a single system would further facilitate data collection and interpretation. For instance, data collected during daily checks or during welfare assessments conducted using the i-WatchBroiler app could be combined with data on the house temperature, humidity, pressure and air velocity. This would facilitate the detection and interpretation of abnormal welfare findings. It would also have tremendous implications for the management and efficiency of broiler farms in terms of time and resources, as the interconnection between all generated information would allow storage on a single device that could be checked at any time and by all involved workers and/or actors in the production chain. If welfare assessment data are included in a combined system that incorporates environmental conditions and welfare indicators, it would facilitate tracing the progression of a flock. Data collection on successive flocks would permit benchmarking, thus allowing the possibility of applying corrective measures to improve flocks welfare. For instance, monitoring temperature distribution within the house combined with continuous assessments of leg problems might serve as an early warning about potential increase in the prevalence of leg problems as the existence of this association was previously demonstrated (Tullo et al., 2017).

However, PLF technologies still face challenges including appropriate data analysis, visualization and interpretation. These challenges coupled with the complexity of some devices may limit the applicability and profit of available systems (Van Hertem et al., 2017). Some PLF devices are more suitable for experimental studies (e.g. to understand the underlying mechanisms of a specific welfare issue) than for use at a commercial level. Future applications of PLF devices would be of great relevance to improve animal welfare if challenges including the complexity and economic viability of the systems are overcome. A study estimated the satisfaction level of European farmers on the use of PLF after a two year experience. It was reported that pig, cattle and broiler farmers are overall satisfied with the use of technology because detection of potential problems takes place much earlier than with conventional methods, even though the high cost of the product is still a limiting factor (Hartung et al., 2017). In addition to the continuous monitoring of farm aspects, the use of PLF was shown to limit greenhouse gas emissions through a more efficient use of energy, reducing farm environmental impact (Tullo et al., 2018). If these challenges are overcome, the spread-out of PLF technologies would enhance farm sustainability through limiting farm environmental impact and improving its economics while maintaining good animal welfare.

2. Associations between environmental conditions and transect assessments

The findings of these studies suggest the practical applicability of the transect method for detecting management and environmental variations at the farm level. In Chapter 2, the effects of genetic line, litter quality and transect position along with variations occurring across age were investigated. Results showed an increased incidence of a majority of the collected welfare indicators (lameness, immobility, sickness, dirtiness, wounded, small and terminal birds) with age, a finding commonly reported in broiler chicken studies (Northcutt et al., 2003; Knowles et al., 2008; Dawkins et al., 2012). Due to the fast growth rate the prevalence of welfare issues increases progressively throughout rearing until slaughter. Such progression of welfare issues was detected with the transect method. We also demonstrated a higher incidence of dirty birds with the deterioration of litter quality, coinciding with previous results (de Jong et al., 2014; Li et al., 2017). Higher incidences of immobile, small, sick, dirty and dead birds were found at wall with respect to central transects, similar to the findings by Aydin et al. (2016). The difference in incidence on welfare issues according to the transect position confirm the need of sampling both wall and central transects in order to obtain a representative calculation of the welfare status (Marchewka et al., 2013). Our results using the transect method seem to be in accordance with previous studies testing the effect of bird age, litter quality and house area which suggests the practical applicability of the transect method for on-farm welfare assessment.

In Chapter 2, the welfare assessment was conducted three times per flock at 3, 5 and 6 weeks of age. According to EFSA (2012), an appropriate assessment method should fulfil certain criteria of robustness, including the frequency of assessment within the same flock. Assessing flocks from 3 weeks of age until slaughter permitted quantifying the worsening of welfare condition and testing the method's potential to clearly detect such effects. However, incidences of welfare issues at 3 weeks were low. The high activity level at this age might impede thorough assessments due to birds escaping from the observer, in addition to the fact that very low incidences of welfare problems are to be expected at this age. On the other hand, welfare assessment at 6 weeks of age might be more difficult due to the reduced activity of heavier birds and the high stocking densities reached towards the end of rearing making the movement through the flock more laborious. In our study, welfare issues went from almost inexistent at 3 weeks to irreversible at 5 and 6 weeks of age. Given the low incidences of welfare issues at 3 weeks of age and the fast increase during last weeks, future studies should focus on finding a threshold where welfare problems start to emerge, but are still reversible. It is also possible that benchmarking of different flocks can help to establish a sensitive threshold. Either strategies would permit the detection of welfare impairment early enough to intervene with strategies to control the problems. The possibility to intervene and reverse the situation is paramount at keeping the farm efficiency and sustainability until final weeks of age.

As explored in Chapter 3, the transect method was also successful in detecting variations in welfare indicators with the provision of environmental enrichment (in the form of peat moss, bales of wood shaving and boxes) suggesting the practical applicability of this method under commercial conditions. The increase in environmental complexity (number of environmental enrichment types provided) resulted in lower incidences of skin wounds (sum of head, back and tail wounds), welfare problems index (sum of all welfare indictors), and mortality rate. These results are in agreement with the studies reporting an improved general welfare condition with the provision of environmental enrichment (see literature reviews of Estevez and Newberry, 2017 and Riber et al., 2018). Even though peat moss and wood shaving bales were provided at least 4 times per flock, and boxes were introduced from the first week of age, these enrichments were supplied at varying degrees in the studied flocks. Besides, environmental enrichment was unevenly distributed across the house resulting in welfare assessments conducted in both enriched and non-enriched transects. Despite these constraints, the transect method permitted the detection of significant variation in flocks provided with environmental enrichment in comparison with control flocks.

In the same study, a lower incidence of leg problems (sum of lameness and immobility) and welfare problems index was detected with increased space allowance. A decreasing incidence of illness (sum of sick, terminally ill and dead birds), skin wounds and mortality rate was reported with intermittent 16h lighting regimen, which is also in accordance with previous published results in commercial broiler chickens (Sørensen et al., 2000; Hassanzadeh et al., 2003; Knowles et al., 2008). We also found higher incidences of illness, small and welfare problems index at wall transects, coinciding with the results reported in Chapter 2.

In relation to the production outcomes, increased environmental complexity resulted in fewer rejections at slaughter due to wounds, underweight birds and total rejections. Higher space allowance was associated with fewer rejections due to wounds. These results suggest that higher environmental complexity and space allowance have beneficial effects to the birds, can result in better performance and lower fear response (Ventura et al., 2012; Altan et al., 2013) which might explain the lower incidence of rejections due to wounds. Higher space allowance was also associated with a higher growth rate and production index, as previously shown in broiler chickens (Estevez et al., 2007). Positive effects of environmental enrichment and space allowance on production outcomes suggest that an improvement of on-farm welfare conditions might have affected production outcomes, which confirm the impact of animal welfare on farm efficiency and sustainability.

Even though significant statistical differences were observed in Chapter 3, the statistical models coefficients of determination (r^2) were relatively low, indicating that the effects of environmental enrichment, space allowance and lighting regimen were significant, but were not the sole factors affecting the incidence of welfare problems and production outcomes. Our findings showed that even though welfare issues are multifactorial as they could be caused by genetic, environmental and management factors (Fraser, 2008), the transect method is detecting previously reported effects of environmental enrichment, space allowance and lighting regimen even when conducted only once per flock. Besides, this experiment was conducted in Norway where welfare standards are high (Miele et al., 2013; Vanhonacker et al., 2014), and farmers are required to promptly cull birds suffering from any welfare issue. Conducting welfare assessments once per flock in Norwegian conditions did not seem to affect the capacity of the method to detect variations in the prevalence of welfare problems under varying environmental conditions.

The result included in Chapters 2 and 3 showed associations between on-farm welfare indicators collected by means of the transect method and production outcomes collected at the slaughter house through regular data collection procedures. In Chapter 2, previously demonstrated relationship between flock illness and the incidence of DOA (Jacobs et al., 2017) was found. In Chapter 3, leg problems, illness, small birds and welfare problems index were correlated with rejections due to illness, underweight and total rejections. In both studies, significant correlations between litter quality and production outcomes were obtained. For example, in Chapter 2 litter quality correlated with downgrades, hematomas, and broken wings, while in Chapter 3 Litter quality was found to correlate with mortality, rejection due to illness, wounds and underweight. These results agree with previously reported associations between litter quality and production outcomes (Dawkins et al., 2004; de Jong et al., 2014). As stated in Chapter 3, such results might have been conditioned by the high number of regression analyses carried out which might have resulted in significant but spurious correlations. However, the number of correlated variables was reduced by summing up the incidence of related welfare indicators into broader categories. These correlations, even if low, were significant, indicating a certain capacity of the method to predict the worsening of the production outcomes due to poor welfare conditions of assessed birds as early as at 3 weeks of age. Considering the great environmental and management variability existing among commercial farms and the simplicity in conducting the transect method, it is quite remarkable that is possible to detect such clear relationships among variables.

Overall, the results reported in Chapters 2 and 3 indicate the practical applicability of the transect method to be conducted under varying on-farm commercial conditions. Future studies should focus on collecting more data related to other management and environmental conditions and testing sharper associations, such as the effect of air quality and variation in water consumption on the prevalence of welfare problems, as previously reported in broiler chickens (Manning et al., 2007a; b). On-farm welfare indicators collected under Spanish and Norwegian conditions using the transect method were associated not only with environmental and management conditions but also with final production outcomes. Even though management and climate conditions were very different in both studies, logical results were obtained indicating, again, the practical applicability of the transect method whether it is conducted several times or only once per flock. Considering the short time needed to implement the transect method, the results obtained suggest that welfare assessment might be conducted easily several times per flock during regular flock inspection in order to detect welfare impairment. Early inspections may permit to implement mitigation strategies to improve animal welfare and farm economic efficiency.

3. Soundness of the transect method

In Chapter 4, we present the results of a study estimating the soundness of the transect method by applying the capture-recapture technique on a known subpopulation of marked broilers in commercial conditions. Almost two-thirds of the marked subpopulation was detected when conducting the transect assessment, although the repetition rate when sampling the entire house reached 23.85%. The repetition rate per transect was 1.66%. Differences in repetitions for the house and the transect levels suggest that the high repetition rate when assessing the entire house was probably due to a high proportion of birds moving across transects. This study was implemented on healthy birds that are able to move freely in comparison to birds with impaired welfare (especially those with leg problems and diseases). Thus, findings of this study probably better represent the detection and repetition rates of birds with welfare problems that do not limit bird mobility such as small, wounded and dirty individuals. For birds with impaired mobility, the repetition rate would be expected to be lower due to their condition. Nevertheless, it is important to indicate that because only two transects are needed for a representative assessment of the flock welfare (as shown by our results of bootstrapping transect data), the expected rate of repetition is expected to be low. Further refinements of the method can be applied to reduce repeatability. In this sense, the data analysis suggest that three transects should be skipped between the two assessed in order to minimize repetition rates. Future studies could focus on the detection and repetition rates of a subpopulation of lame, immobile and sick birds, given that their movement patterns are different from healthy individuals (Dawkins et al., 2012; Dawkins et al., 2017; Colles et al. 2016). In this case, the detection rate is expected to be similar or higher to that of healthy birds, and the repetition rates should be much lower as limping and immobile individuals usually have slow to inexistent movement.

Differences in the prevalence of marked birds between wall and central transects were suggested by our results relative to the distribution index. These results support the necessity to conduct both central and wall transects for a representative flock sampling as suggested in Chapters 2 and 3. Our findings indicates that bird distribution is not totally random within the house due to the preference for house peripheries (Newberry and Hall, 1990; Cornetto and Estevez; 2001), but also due to the wall trapping effect previously described (Estevez and Christman, 2006). In this study, more groups of broiler chickens were marked close to the walls than in central areas, which might have resulted in more birds remaining at walls. Future studies should further test this aspect by marking equal number of birds in central and wall transects and assess their distribution.

Bootstrapping analyses, is a specific data analysis technique designed to optimize sampling effort by calculating the lowest sample size that allows to achieve similar precision as when realizing the entire sampling. This analysis showed that conducting two transects per house would not change the mean and variability of the sample when compared to sampling the entire house. These results are comparable to the findings by Marchewka et al. (2013), who also estimated the minimum number of transects to be assessed. Therefore there is consistency in results showing that welfare assessment could be implemented by conducting only two transects per house, being one central and one wall in order to better capture the diversity that may occur. In addition, with only two transects per house, the risk of repeating birds will be minimized by skipping three or more transects in between. This approach would allow an effective welfare assessment of broiler chicken flocks while saving time and resources and would help to increase available records for a more representative benchmarking.

4. Limitation of the studies and the transect method

Even though the transect method seems to provide sound results and to be practical to implement at commercial conditions due to the low time constraints and the facility of data collection, some limitations should be taken into consideration for further improvement of the method. For instance, significant differences were found between observers for some of the collected welfare indicators. This highlights the relevance of reaching an agreement between observers before starting the data collection, especially when novel observers are involved in the studies. This problem seems to be less relevant in Chapter 3, as differences between observers were found only for the welfare problems index. In this case, other factors may have played a role in the differences found among observers. Indeed, differences might have been the result of an uneven distribution of environmental enrichment within the house, which might have affected the distribution of impaired birds. In both studies, observers conducted the assessment in different sides of the same house taking into consideration to switch sides. Even though transects assessed by each observer belong to the same house, observers did not assess the same sample of birds (as stated in the definition of Martin and Bateson, 2004), which might have been one source of discrepancy among observers. Nevertheless, this argument is probably not the only cause. A recent study on turkey farms using the transect method reported differences between observers in dirtiness, tail and back wounds, along with other turkey specific welfare indicators (Ferrante et al., 2018). These results are comparable to those reported in Chapter 2 of this Thesis, as differences between observers were found in dirtiness, tail and other wounds. This is showing that some indicators might still be relative, intangible or differently perceived by observers. In addition to farm related factors, like light intensity, flock density or feather coverage that could affect the detection of some welfare indicators, the evaluation of such indicators might differ according to the flock. The appreciation of dirty birds, for instance, might vary according to the house area (where litter quality could be worse), as actually different levels of feather dirtiness might exist across the house, which could explain such variation in assessment.

Nevertheless, it is important to emphasize that even though statistical differences were obtained, the numerical difference in the prevalence of welfare indicators between observers reported in Chapter 2 was low. For example, the maximum variability was observed for lame (0.268±0.027 and 0.121±0.013 for observer 1 and 2 respectively) and immobile birds (0.065±0.01 and 0.175±0.026 for observer 1 and 2 respectively). These results suggest that even with these differences, the assessment of the entire house would be similar across observers. In addition, probably if both observers assessed the same sample of birds, most of the discrepancies might have been reduced.

The transect method assesses physical and behavioural indicators, which cover a set of welfare issues that can be experienced by broiler chickens as recommended by EFSA (2012). However, the actual incidence of some welfare problems such as featherless, back and head wounds is quite low in broiler chickens, although they can be relatively common in other poultry species (turkeys and laying hens). Further, footpad dermatitis and hock burns are not included in the transect walks even though they are important welfare indicators in broiler chickens (Hepworth et al., 2011). The assessment of these indicators would involve bird catching and handling, and therefore, they were not contemplated during transect assessments. However, as a link exists between the incidence of specific welfare indicators and litter quality (de Jong et al., 2014), which is evaluated when conducting transects, a prediction of footpad dermatitis and hock burns could be eventually calculated using modelling techniques. These indicators could also be predicted from slaughter results as suggested with the WQ® protocol for broiler chickens (de Jong et al., 2016).

In Chapter 3, we reported differences in the distribution of 'skin wounds' between house sides. Differences might be related to the position of house entrance, increasing bird reaction in that specific area each time a human entered. This increased reaction might result in a higher risk of birds jumping and injuring each other. These results suggest that an even distribution of welfare indicators between house sides should not be assumed as suggested by our results on the difference in incidences of indicators between central and wall transects. These findings reinforce the idea that the sampling has to be conducted in two different areas of the house for a more accurate estimation of the farm assessment.

Overall, the transect method provides evidence of practical implementation and soundness of results when conducted in commercial broiler chicken farms. The present Doctoral Thesis provided insights on the potential of the transect method to be a useful welfare assessment method of commercial flocks of broiler chickens. We demonstrated the capacity of the method to detect association between the incidence of welfare indicators and farm environmental and management conditions. Previously shown relationship between on-farm impaired welfare statue and production outcomes were demonstrated using the transect method. Sound results could be obtained with satisfactory detection and repetition rates. The minimum number of transects to sample for a representative assessment was reported along with the number of transects to skip in between to avoid high repetition rates. Given the low time constraints required to conduct welfare assessments and the practical features of data collection, the transect method has the potential to be implemented by all stakeholders of the broiler chicken industry for an improvement of animal welfare resulting in better farm efficiency and sustainability.

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MAIN CONCLUSIONS

First: The results of the studies conducted with the transect method showed a clear increment of the incidences of lame, immobile, sick, dirty, wounded, small and terminally ill birds with age and a higher incidence of dirty birds with worse litter quality.

Second: An improvement in broiler chicken welfare condition was found when environmental enrichment was provided. Lower incidences of wounds and welfare problems index were detected in enriched flocks, along with lower mortality rates as compared with control flocks. Other management factors, such as increased space allowance and lighting regimen had an impact on flock welfare that was detected with the transect method.

Third: Higher incidences of immobile, small, sick, dirty and dead birds were found at wall with respect to central transects in Spanish conditions. Higher incidences of small, illness and welfare problems index were found at walls in Norwegian conditions.

Fourth: An association between animal welfare indicators collected on-farm with the transect method and production outcomes collected at slaughter was confirmed. Higher incidence of flock illness also correlated with higher dead on arrival. These results reflect the potential of the method to anticipate slaughter outcomes.

Fifth: The soundness of the transect method was tested by a capture-recapture approach of a subpopulation of marked birds. Two-thirds of the marked subpopulation was detected. The repetition rate within transect was low, showing low likelihood of overestimating welfare indicators within each transect. Three transects should be skipped between the two assessed to minimize repetition rates per house sampling.

Sixth: Broiler chickens with welfare issues do not appear to distribute randomly within the house. Both wall and central transects must be conducted when implementing welfare assessments. In the light of the obtained results, the assessment of two transects per house appears to be sufficient to obtain a representative welfare assessment.

Seventh: The transect method applied with the i-WatchBroilers app provides a sound on-farm welfare assessment. The method is practical to be conducted under commercial conditions due to its characteristics and efficiency although the use of clear definitions of welfare indicators and observer training must be implemented to minimize differences in judgement across observers.

CONCLUSIONES GENERALES

Primero: El método de los transectos muestra un aumento significativo en las incidencias de pollos cojos, inmóviles, enfermos, sucios, heridos, pequeños y terminales según la edad. Se encontró una mayor incidencia de aves sucias asociada con una peor calidad de cama.

Segundo: El enriquecimiento ambiental tiene un efecto positivo en lotes de pollos donde se observó una menor incidencia de heridas e índice de problemas de bienestar detectados con el método transecto además de la menor tasa de mortalidad. Otros factores de manejo como el espacio disponible y el régimen de luz tuvieron un impacto positivo que fue detectado por el método transecto.

Tercero: Se observan incidencias más altas de aves inmóviles, pequeñas, enfermas, sucias y muertas en transectos de pared con respecto a los transectos centrales en condiciones españolas. En las condiciones noruegas, se encontraron mayores incidencias de enfermos, pequeños e índice de problemas de bienestar en transectos de pared.

Cuarto: Se confirma, mediante el uso de transectos, una asociación entre los indicadores de bienestar animal recolectados en la granja y los resultados de producción al sacrificio. Una mayor incidencia de enfermos se correlaciona con mayor número de muertos a la llegada al matadero. Estos resultados reflejan la capacidad del método para anticipar los resultados del sacrificio.

Quinto: La solidez del método transecto se probó mediante una técnica de captura-recaptura de una subpoblación de aves marcado en naves comerciales. Se detectaron dos tercios de la subpoblación marcada. La tasa de repetición dentro del transecto fue baja, mostrando una baja probabilidad de sobreestimar los problemas de bienestar dentro de cada transecto. Se deberían omitir tres transectos entre los dos evaluados por muestreo para minimizar las tasas de repetición en la nave.

Sexto: Los resultados indican que los pollos de engorde no se distribuyen al azar dentro de la nave. Al implementar evaluaciones de bienestar, se deberían de realizar tanto transectos de pared como centrales. Dos transectos por nave son suficientes para una evaluación representativa del bienestar.

Séptimo: El método de los transectos realizado con la aplicación i-WatchBroilers proporciona resultados sólidos para la evaluación del bienestar en granja. Es un método práctico en condiciones comerciales que permite una evaluación eficiente y rápida de los pollos de engorde. Sin embargo, una buena definición de los indicadores de bienestar evaluados además de una formación sólida de los observadores son necesarias para minimizar las diferencias en la evaluación.

ABOUT THE AUTHOR

After the engineering studies in the National Agronomy Institute of Tunisia (INAT), Neila Ben Sassi was involved in an International Master of Science in Animal Genetic improvement and Reproduction Biotechnology, which took place in the Autonomous University of Barcelona and the Polytechnic University of Valencia. During the second year of Master, she was in the National Institute for Agricultural Research (INIA) in Madrid, where she worked on bioinformatic tools to identify copy number variants polymorphisms associated with productive traits in Holstein and on the analyses of the genetic variability and predictive ability of SNP.

She always was very curious about animal behaviour and knew that, somehow, she would end up working in ethology and welfare. During the PhD years, deepening her knowledge in this field was a priority which reinforced her beliefs that this is the field where she wants to persuit her career.

Contributions to international conferences

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Research internships

- Department of Animal and aquaculture science, Norwegian University of Life Science, As, Norway. Under the supervision of Prof. Ruth Newberry and Dr. Judit Vas, January-May 2017.

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Courses

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- SAS guide Enterprise workshop, Neiker-Tecnalia, Vitoria-Gasteiz, Spain, September 12th, 2017.
- Publishing workshop, Tecnalia, San Sebastian, Spain, September 21th, 2016
- Statistics and R programming, Neiker-Tecnalia, Derio, Spain, March 7- 22, 2016.
- Advanced course in Precision livestock farming, Mediterranean Agronomic Institute of Zaragoza, Spain, April 13-17, 2015.