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ANIMAL-BASED INDICATORS FOR ON-FARM WELFARE ASSESSMENT IN POULTRY

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“To everyone who would brush up their own knowledge on welfare in turkeys using this study.”

"E que a adversidade seja contrariada com o
sorriso verdadeiro do esclarecimento
e da humildade espírito."
Anonymous

"It always seems impossible until it's done."
Nelson Mandela

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GENERAL ABSTRACT

Improvements in poultry welfare are essential to ensure the quality of bird's lives, but also may have fruitful outcomes to industry for minimizing the economic losses impacts under better bird performance, and carcass quality with reductions of mortality and condemnations. In fact, adopting a valid, reliable, and feasible welfare assessment protocol on-farm is fairly valuable method not only addressed to poultry welfare, but also, to industries interests, and consumer demands. Several array internal and external identified factors can largely influence the welfare and health evaluation of turkey flocks. The present study focuses mainly on the development of a welfare assessment protocol on-farm based on animal-based indicators. These parameters were carefully reviewed and critically tested. In Chapter 1 and 2, the aim was to do a literature review on animal-based indicators for turkeys (*Meleagris gallopavo*), gathering information about promising indicators that could be included into an on-farm welfare assessment protocol. Social, environmental variables and pathological findings were pointed out as factors affecting behavior and welfare of turkeys that may be relevant economically to the commercial production systems. In Chapter 3, the walks through following line transect methodology used in wildlife studies was adapted to explore their feasibility as a welfare assessment tool. The aim of this study was to compare broiler welfare assessed by individual sampling and transect walks. Six managed flocks were evaluated. Measures on 150 birds were carried out for the individual sampling.

Observers walked slowly on random order paths and recorded the incidence of immobility, lameness, dirtiness, sick, agony and dead birds. It was possible to detected small variations across farms ($P < 0.003$) in the prevalence of most welfare indicators and consistency in inter-observer reliability ($P \geq 0.05$). Surprisingly, both methods differed greatly in the frequency of the studied parameters. Possibly, the transect walks might have overlooked walking impairs due to a large number of birds. Another hypothesis may be related to biased individual sampling procedures. In Chapter 4, the study investigated the feasibility of the transect walks method as a novel approach to on-farm welfare assessment of male and female commercial turkey flocks in Italy. This is the first report of welfare assessment using the transect walks method in Europe at turkey farms. A total of 25 commercial [B.U.T.] - Big 6) turkey flocks (15 male and 10 female) with similar management standard procedures were evaluated. Incidence of birds falling into any of the welfare categories was recorded. The studied indicators were: immobility, lameness, wounds, featherless, small size and serious health issues in the flocks, for instance, sick, terminally ill and dead. In addition, behavioral variables as aggression towards mate, interaction with humans and mating were also considered. Sensitivity of the method was noted by effect of sex ($P < 0.001$) for immobility, lameness, wounds and dirtiness indicators. In addition, inter-observer reliability ($P \geq 0.05$) was also consistent for almost the studied variables. Male birds showed high incidence of immobility (0.14% ± 0.02% vs. 0.02% ± 0), lameness (9.06% ± 0.41% vs. 4.34% ± 0.20%), wounds (3.54% ± 0.19%

vs. 1.38% ± 0.09%) and dirtiness (0.20% ± 0.02% vs. 0.07% ± 0.01%) than female flocks, respectively. Transect walks approach is an essential component, indeed, to improve the welfare assessment on-farm level. It showed to be a valuable alternative method at commercial strains of meat turkeys. Thus, this innovative method fulfills some industries and producers requirements, such as, no bird disturbing or animal catching with decreased of time-consuming and personnel involved, and no extra costs required, at its application at commercial practices. Some further research should be done to fill the complex gaps and improve the welfare protocol introduced herein.

Keywords: animal welfare, animal-based indicator, on-farm assessment protocol, turkey

GENERAL INTRODUCTION

Turkey production in the European Union reached 1.9 million tons in carcass weight equivalent (tcwe) in 2012 mainly thanks to France (414,000 tcwe) followed by Germany (400,000 tcwe) and Italy (288,000 tcwe). One of the highlights in this production chain worldwide is with 442,000 tcwe in 2012 and an outstanding growth of +26.8%, whereas the EU had slightly increased of +2.15% (Forthorn, 2013).

The production, performance, behavior, health and welfare could be modified on modern meat poultry industry with meaningful effects by several fundamental aspects (Dawkins et al., 2004; Beaumont et al., 2010; Marchewka at al., 2013a; Watanabe et al., 2013). Underlying care and management tools, for instance, manipulation of day length and intensity (Sherwin et al., 1999; Moinard et al., 2001; Prescott et al., 2004; Blatchford et al., 2012); ventilation and temperature (DEFRA, 2009); stocking density and group size (Sherwin and Kelland, 1998; Martrenchar, 1999a; Buchwalder and Huber-Eicher, 2004; DEFRA, 2009) are decisive factors at the intensive commercial production. But also, feeding (Hocking et al., 1999b; Hocking et al., 2002; Mirabito et al., 2003; Tatara et al., 2006); floor and litter (Andrews et al., 1974; Bilgili et al., 2009; Abd El-Wahab et al., 2011; Abreu et al., 2011; Youssef et al., 2011; Garcia et al., 2012); and handling, transportation, and slaughter (HFAC, 2008; Petracci et al., 2006; Wichman et al., 2010) are considered critical factors.

The livestock industries handle and cope with animal well-being concern and production interests; they are steadily looking for ways to establish a common agreement between productivity and ethical reasons, and to guarantee that husbandry and welfare requirements are sought. Furthermore, consumers increasingly demand animal products with optimal quality originated from husbandry systems closely attached to outstanding standards of animal welfare. In this regard, a breakthrough welfare assessment protocol for turkeys at farm level appeared to be an essential element to be applied at intensive commercial rearing systems to accurately determine the welfare and health status of the birds. Farmers, managers, birds caretakers' personnel, veterinarians, official technicians, and external welfare assessors can implement this method focused on different purposes, such as for internal appraisal or even for legally assignment. Finally, being a suitable tool to support the industries' decision-making process. Likewise, aiming to establish the appropriate method to assess welfare in different species with commercially relevance, Battini et al., (2014) developed a welfare protocol for dairy goats and Dalla Costa et al., (2014) for horses, and Marchewka et al., (2013b) for broilers, all based on the Principles and Criteria indicated by Welfare Quality® (2009a,b,c).

Especially at the end of the rearing period, due to the fact that many elements are generally acting concurrently, an increase of locomotory problems under intensive poultry production occurs (Sanotra et al., 2001; Knowles et al., 2008). Skeletal problems, such as impaired gait

(Skinner-Noble and Teeter, 2009), bones and cartilages deformations (Cook, 2000), and foot pad dermatitis (Shepherd and Fairchild, 2010; Krautwald-Junghanns et al., 2011) cause severe loss implications on the global market. Locomotory impairs are a widespread abnormality in commercial turkey flocks and may lead to cause pain or discomfort for the birds (Duncan et al., 1991; Broom and Reefmann, 2005; Buchwalder and Huber-Eicher, 2005; Hocking and Wu, 2013). On the contrary, Hocking et al. (1999a) concluded in their experiment that male turkeys with musculoskeletal disease do not demonstrate evidence of pain. In this context pain assessment at on-farm level is a challenge to verify, however worth to pursuit. The risk factors for locomotory impairs can be divided mainly into two categories: (i) genetic background selection (Martrenchar, 1999a), and (ii) environmental factors, for instance photoperiod duration (Martrenchar, 1999a; Brickett et al., 2007), litter quality (Bessei, 2006; Mayne et al., 2004, 2007; Hocking and Wu, 2013), stocking density (Martrenchar et. al., 1999b; Sørensen et al., 2000; Bessei, 2006). Similarly, the bird welfare conditions can be compromised by the simultaneous presence of these different elements or even by their interaction. Additionally, the turkey flocks have a tremendously massive numbers of birds which lead to a particular challenge for health and welfare evaluation, as well as the fast turnover of the production cycles in meat poultry.

The indicators used to evaluate the animal welfare state on-farm are classified into two major groups: (i) animal-based and (ii) resource-based guidelines (Bartussek, 1997; Hörning, 2001; Main et al., 2003).

Although resource-based parameters are far more used rather than animal-based indicators for being quirkier and easier to measure, they are considered an indirect way for assessing animal welfare status. Moreover, they do not indicate necessarily a positive correlation between good management and environmental aspects with high standard of animal welfare (Broom 1996; Sandøe et al., 1997; Mollenhorst et al., 2005; Winckler, 2006). Therefore, the appropriate welfare approach adopted on field for animal husbandry should be based mainly on animal-based descriptors (Webster, 2005).

There is a need of welfare protocol applied in turkey productions that can address consistently the animal welfare topics and can cover the attention and concerns of governmental organizations, industries, consumers and other stakeholders. It is necessary the engagement of all parts including the scientific community to fill the gaps and answer fundamental questions that still exist in this matter, as well as, identifying solutions for the currently and foreseen barriers in the production chain to improve animal welfare needs while ensuring the animal production valuable perspective.

The aims of this currently study[1] were (i) investigate the repeatability and on-farm feasibility of animal-based welfare indicators on turkeys; (ii) develop a welfare assessment protocol for turkey commercial farms to be applied at the end of production cycle. The outcome findings and

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[1] This thesis was one of the several studies that belonged to the Animal Welfare Indicators (AWIN) project financed by The European Union II Framework Program (FP7-KBBE-2010-4).

the obtained conclusions may contribute towards the turkey intensive production with higher concepts of animal welfare considering the industries interests, the consumer demands, and the food safety.

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CHAPTER 1

In this chapter, the article entitled "Review of the social and environmental factors affecting the behavior and welfare of turkeys (*Meleagris gallopavo*)" was published in Poultry Science, 92(6): 1467-1473, 2013. doi: 10.3382/ps.2012-02943

Review

Review of the social and environmental factors affecting the behavior and welfare of turkeys (*Meleagris gallopavo*)

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ABSTRACT In modern rearing systems, turkey producers often face economic losses due to increased aggression, feather pecking, cannibalism, leg disorders, or injuries among birds, which are also significant welfare issues. The main underlying causes appear to relate to rapid growth, flock size, density, poor environmental complexity, or lighting, which may be deficient in providing the birds with an adequate physical or social environment. To date, there is little information regarding the effect of these factors on turkey welfare. This knowledge is, however, essential to ensure the welfare of turkeys and to improve their quality of life, but may also be beneficial to industry, allowing better bird performance, improved carcass quality, and reduced mortality and condemnations. This paper reviews the available scientific literature related to the behavior of turkeys as influenced by the physical and social environment that may be relevant to advances toward turkey production systems that take welfare into consideration. We addressed the effects that factors such as density, group size, space availability, maturation, lightning, feeding, and transport may have over parameters that may be relevant to ensure welfare of turkeys. Available scientific studies were based in experimental environments and identified individual factors corresponding to particular welfare problems. Most of the studies aimed at finding optimal levels of rearing conditions that allow avoiding or decreasing most severe welfare issues. This paper discusses the importance of these factors for development of production environments that would be better suited from a welfare and economic point of view.

Key words: turkey behavior, welfare, production, social behavior, density/group size

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INTRODUCTION

Turkey production is considered small compared with broiler production; however, this industry has achieved a relevant increase since 1980, escalating from 122 million to 226 million turkeys produced in 2006 within the European Union countries (Food and Agriculture Organization, 2012), whereas the value of turkeys produced in the United States during 2010 was $4.37 billion (US Poultry and Egg Association, 2013). Despite the growing relevance of turkeys, the scientific literature regarding the welfare of intensively reared turkeys is scarce compared with other poultry species. There is a major need for more insight into the factors influencing turkey welfare, not only due to public demands to ensure a sustainable production system that foments management practices that take in consideration the welfare of turkeys, but also because this information is needed to reduce losses due to poor bird performance.

A recent study showed that 60% of female and 33.8% of male 16-wk-old turkeys in commercial German facilities showed some degree of footpad lesions (Krautwald-Junghanns et al., 2011). Lupo et al. (2010) indicated that in the French turkey industry the average condemnation rate was 1.8%, whereas condemnation rate for broilers was lower and reached 0.87% (Lupo et al., 2008). These are only some examples of relevant animal welfare issues that also have important implications for the economic return of turkey production. Knowledge of the main factors affecting the welfare of turkeys and the means to minimize this impact can not only improve their quality of life, but may also be beneficial to industry by achieving better bird performance, improving carcass quality, and reducing mortality and condemnations.

Unfortunately, there is a lack of studies conducted under commercial settings, on the effects of the social and physical environment over the behavior, welfare, and performance of commercial turkeys. Most of these

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studies have been conducted under particular experimental situations (Martrenchar, 1999); therefore, the application of results to commercial practice is difficult. In this paper, we review the available scientific literature regarding fundamental factors affecting behavior and welfare of turkeys; this literature is relevant to consider the establishment of science-based management practices and to ensure animal welfare.

MAIN FACTORS AFFECTING THE BEHAVIOR AND WELFARE OF TURKEYS

Density and Group Size

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

Density and group size are factors which effects are often confounded, together with space availability, because only 2 parameters can be controlled simultaneously. Therefore, individual effects of each contributing factor are difficult to differentiate. Although it is possible to minimize the confusion to a certain extent by using specific experimental designs (i.e., Leone and Estévez, 2008), it is not always a practical approach, especially in applied research, in which the size of the commercial housing is fixed. Keeping in mind those issues, in the current review we treat the effects of group size and density, as well as the space availability, as were described in the original study.

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

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

Small groups of familiar toms seem to be able to distinguish nongroup members toward whom they display aggressive interactions, but the frequency of interactions appears to be modulated by enclosure size (Buchwalder and Huber-Eicher, 2004). More pecks toward newly introduce unfamiliar toms were observed in small (2 × 3 m) compared with large pens (6 × 13 m). Buchwalder and Huber-Eicher (2004) explained these results in terms of a minimum critical distance requirement between opponents, which would be essential to avoid chances of aggressive interactions. Therefore, the newly introduced bird would have been able to keep a larger distance in large pens, resulting in fewer aggressive encounters. These results differed somewhat from other scientific evidences that suggest that aggressive interactions, at least in broilers, occur at a higher frequency in open areas rather than in more crowded regions of the enclosure (Pettit-Riley and Estevez, 2001).

Nevertheless, in another study Buchwalder and Huber-Eicher (2003), found that the response toward nonfamiliar conspecifics mainly depended on the size of the group in which the foreigner was introduced. The smaller the group (minimum of 6 up to 30 birds), the more intense the aggressive reaction was, with more fights being initiated and more aggressive pecks being delivered. These results seem to be in accordance with other poultry studies, without aggression-enhancing introductions of foreign individuals to the group, where a reduction in the frequency of aggressive interactions with increased group size was also reported (Estevez et al., 1997, 2002, 2003).

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

Feather pecking is, together with aggressive encounters, an important welfare and management concern in large poultry flocks. They are commonly considered to be linked to large groups, as found for laying hens (Bilčík and Keeling, 2000). No study has looked over the effects of group size over feather pecking in turkeys, but in an experimental study Busayi et al. (2006) compared feather pecking rates of a commercial male line selected for growth and breast yield with a traditional Nebraska Spot turkey coming from small experimental flocks. A higher frequency of pecks and pulls occurred in males (32%) compared with females (15%) of the commercial line, but were not observed in the traditional one. However, differences in time budgets across sexes were small. Some differences were also observed with regard to age, where males showed stronger feather pecks and pulls at 3 wk of age, whereas females showed the highest frequency at 9 wk.

Space Availability and Spatial Distribution

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

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

Aging and Maturation

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

Similar results were obtained by Hughes and Grigor (1996) studying time budgets of beak-trimmed turkey poults up to 12 wk, kept in small groups of 10 to 11 birds. Percentage of sitting/sleeping behavior increased over time, whereas standing/walking behavior primarily declined, and rose at the end of the study. Beak-related behaviors (feeding, drinking, preening, environmental and bird pecking) rose to the peak of 45% in wk 2 and then declined gradually to around 28% by the end of the study. The general decline in activity with age have been found even when the effects of high stocking density and group size were minimized, and sufficient space was provided to the birds (Sherwin and Kelland, 1998). Reduction in activity also reflected on the distances covered: 27.5 m/30 min at 7 wk to 11.9 at 12 wk (Buchwalder and Huber-Eicher, 2005b).

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

Photoperiod and Lighting

Lighting has profound effects on the physiology and behavior of poultry (Manser, 1996). In modern poultry production, photoperiod and light intensity are strictly controlled to promote growth and to avoid excessive feather pecking and cannibalism. Interestingly, even under artificial low light intensity, time budgets seem to follow a photoperiod rhythm, with higher proportion of resting, and low standing and walking occurring during midday (Busayi et al., 2006). At night, turkeys appear to be mostly inactive, although they may stand up 2 to 12 times during the dark period, usually turning around slowly and lying down again (Sherwin and Kelland, 1998).

Although low lighting intensity (1/10 lx) is used to reduce the risk of undesirable behaviors such as feather pecking and cannibalism, it can also inhibit walking, foraging, exploration, and social behaviors (Hughes and Grigor, 1996; Barber et al., 2004). In general, turkeys prefer bright environments, as Sherwin and Kelland (1998) demonstrated that turkeys avoided chambers with less than 1 lx light intensity compared with 5, 10, or 25 lx. But additional studies indicated that turkeys may prefer different light intensities to perform different activities. In this line Barber et al. (2004) demonstrated that in an experimental situation where birds were given continuous access to 4 rooms with different light treatments (below 1, 6, 20, and 200 lx), at wk 2 birds spent most of time in the brightest environment, whereas at 6 wk the authors observed partition of behaviors between the 2 light environments. Resting and perching were only observed in the environment below 1 lx, whereas the rest of the behaviors were performed in the 2 brightest environments. Although environmental enrichment through variation in light intensities may be interesting to improve health and welfare of turkeys, this has never been tested under commercial conditions. From a management point of view, it should be considered that a sudden and temporary increase in light intensity, for bird inspection for example, may lead to fear reaction among birds (Appleby et al., 1992).

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

Another aspect that should be considered in turkey management is that turkeys are known for having potential for vision in the UV-A spectral range, and it is possible that plumage may contain visual information detectable only under in UV-A wave bands (Hart et al., 1999). In fact, results from Hart et al. (1999) and Moinard and Sherwin (1999) suggest that turkeys preferred a UV-A-enriched environment to one illuminated by fluorescent light alone. In modern housing, the use of fluorescent or incandescent lamps that emit low levels of the UV-A spectrum may limit the natural communication conveyed by the plumage of turkeys. In fact, Hart et al. (1999) suggested that provision of supplementary UV light may reduce the incidence of visually mediated, aberrant behaviors.

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

Feeding

The number of studies dedicated to the effects of diet composition, the form in which is presented, and how its availability may influence behavioral patterns and welfare in turkeys is very limited. Turkey poults at 6 to

12 wk fed with pellets spent less time feeding compared with their behavior at the younger age of 1 to 5 wk, when fed with crumbs (Hughes and Grigor, 1996). On the contrary, Hale and Schein (1962) found that 12-wk-old pellet-fed birds spent more time feeding; less time drinking, preening, and resting; and had higher engagement in other behaviors compared with mash-fed ones. The main differences between these results may relate to genetic factors due to 30-yr difference between them, the age of the birds, and how the feed was presented.

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

Feed restriction is a commonly used management practice in the breeder turkey industry to control male BW for optimal semen production and to manage risk of heat stress or musculoskeletal lesions. However, food deprivation can have a negative impact on the welfare of turkeys, which may manifest through changes in their behavior patterns. Hocking et al. (1999) compared the behavior of ad libitum and feed restricted commercial Large White turkey male line from 8 to 28 wk. Ad libitum fed birds mainly showed standing, walking, and preening behavior (44 to 77% of the time budget), whereas feed-restricted birds showed high frequencies of oral activities such as pecking on pen walls and furnishings (20 to 59% of the time budget depending on the week). It was emphasized by the authors that first signs of the increased oral activity and reduction of sitting was observed already 2 wk after restriction began.

Transport

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

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

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

DISCUSSION

Scientific studies on the effects of the characteristics of the physical and social environment of turkeys' behavior and their implications from a welfare standpoint are still scarce. In general, studies have demonstrated that turkeys may show large behavioral adjustments as a response to inadequate environmental conditions. For example, studies focused on the effects of density, group size, or both have shown that high densities led to gait deterioration and decreased activity, insufficient space availability related to a higher frequency of injuries, especially wing breakages, as well as increased aggression levels, whereas large group size led to feather pecking occurrences (Sherwin and Kelland, 1998; Martrenchar et al., 1999; Buchwalder and Huber-Eicher, 2003, 2004, 2005a; Busayi et al., 2006). Similar to other poultry, a general decline in activity was found with increasing age (Hughes and Grigor, 1996; Sherwin and Kelland, 1998; Hocking et al., 1999; Martrenchar et al., 1999; Busayi et al., 2006), with first signs of decreased locomotion becoming apparent generally from 4 wk of age onward (Sherwin and Kelland, 1998), whereas the injurious pecking may occur already after wk 3 of life (Busayi et al., 2006).

Feed presentation has also the potential to alter turkey activity; the provision of feed in pellets compared with crumbles has been associated with longer feeding bouts (Hughes and Grigor, 1996), which could be beneficial to divert the birds from other undesirable activities such as feather pecking. However, these results are in opposition to the increased feeding time when provided with crumble feed in turkey studies conducted

30 yr ago (Hale and Schein, 1962). Also, the addition of fodder enrichments in the form of whole wheat was found to increase eating time; however, it did not influence birds from 6 wk onward (Mirabito et al., 2003). Similar to broiler breeders, in turkeys feed restriction increased oral activity paralleled with increments in standing, walking, and preening behavior (Hocking et al., 1999), which is typically interpreted as a sign of hunger and frustration (Bokkers and Koene, 2004). However, as for broiler breeders, it is required to maintain a BW balance to avoid other health and welfare problems associated with excessive BW.

The issue that has perhaps received the most attention in turkeys is lighting. Turkeys preferred fluorescent over incandescent lighting (Lewis et al., 2000; Moinard et al., 2001), probably because is perceived by them as less intense, and they showed better walking ability when provided with dark periods (Classen et al., 1994; Lewis et al., 1998; Sherwin et al., 1999). Young birds showed clear preferences for brighter environments to perform all activities, whereas adults rested and perched preferably under dim light but conducted all active behaviors in brightness (Barber et al., 2004). Some studies have also shown that birds may benefit from UV-A light-enriched environments by reducing visually mediated aberrant behaviors (Hart et al., 1999; Moinard and Sherwin, 1999).

However, of all the factors that may influence turkey health and welfare, catching and crating (Prescott et al., 2000), as well as transportation to slaughter (Wichman et al., 2010), have been shown to be some of the most detrimental procedures for welfare, with the potential of causing not only major carcass damage and lost profitability, but also the death of the birds if procedures are conducted in an inadequate manner.

Current studies have shown that changes in activity, such as locomotion, and time budget schemes, and exhibition of aggression, feather pecking, or cannibalism are behavioral indicators that can be largely influenced by the conditions of the physical and social environment. However, it is essential to consider that the results presented in this review were mostly based on studies conducted under strict experimental conditions, and therefore, it is difficult to extrapolate the conclusions to what would happen under commercial systems in which several thousand birds are reared simultaneously. Additionally, variability between flocks, farms, and even countries caused by different management systems and environmental conditions can determine to a great extent the variability in behavioral and welfare outcomes. It is also important to remark that in some experimental studies the effects of density, group size, and pen size were often confounded because of the difficulties of separating those effects, and furthermore biological events often do not follow a linear pattern. In broilers, differences between experimental and commercial situations were found to cause uncertainty in welfare risk estimation and hazard consequences (De Jong et al., 2012). This uncertainty could be reduced by further studies, expert opinions and their judgments, and obviously by studies conducted under commercial scenarios. The use of mathematical models for complex analysis may also be relevant to find the optimal balance between flock productivity and welfare (Estevez, 2007).

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CHAPTER 2

In this chapter, the article entitled "Review of the physiological and pathological welfare indicators applied in turkeys (*Meleagris gallopavo*)" was published in Biotechnology in Animal Husbandry, 29(4):727-740,2013.doi:10.2298/BAH1304727W

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REVIEW OF THE PHYSIOLOGICAL AND PATHOLOGICAL WELFARE INDICATORS APPLIED IN TURKEYS (*MELEAGRIS GALLOPAVO*)

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Communication

Abstract: Concern on animal welfare has become an important issue in Europe for a decade now. In commercial poultry husbandry, there are many trials to standardize the production in order to reduce the economic loss caused by poor welfare at marketing age. As it known, factors such as density, group size, space availability, maturation, lighting, feeding, and transportation can have effects on welfare of turkeys. However, to ensure a better quality of live for the birds as well as the industry as good performance, reducing the mortality and condemnations it is important to have another point of view as different kind of indicators. This paper reviews the available scientific literature related to the turkeys' welfare according to the main relevant physiopathology indicators by taking into a count whether they are feasible or not for being used. We addressed foot pad dermatitis and breast skin lesions as being the most relevant indicators so far. They may be relevant to improve the welfare assessment indicators of turkeys. However, measurements of corticosterone, enzyme activities, cytokines, and hematological profile seemed to be flourishing indicators to be applied more often. In this way associating the indicators that were previously studied to these new ones, it is assumed that animal, producer, industry and consumer may have a suitable bond between them (poultry chain) according to their different interests.

Keywords: welfare, pathological and physiological indicator, turkey

Introduction

For many years, commercial turkey husbandry has been practiced in many countries. In order to increase its profitability, and meet the consumers' demands, research is conducted and regulations are put in place. In poultry chain production, welfare concerns were already pointed out (*Voris et al., 1998; Bessei, 2006; Department for Environment Food and Rural Affairs, 2009; Welfare Quality®,* *2009; Beaumont et al., 2010*); nonetheless, causes of downgrades and condemnations are still the industries' main worry (*Petracci et al., 2006; Shepherd and Fairchild, 2010*).

Studies have been developed regarding better management factors influencing domestic turkey flocks, such as diet (*Hocking et al., 1999; Mirabito et al., 2003*), light (*Hart et al., 1999; Sherwin et al., 1999a; Moinard et al., 2001*), animal stocking density and group size (*Martrenchar et al., 1999; Buchwalder and Huber-Eicher, 2004, 2005a*), environmental enrichment (*Sherwin et al., 1999b; Martrenchar et al., 2001)*, and transportation (*Wichman et al., 2010*). Thus, turkey behavior could be modified by variations in such factors (*Hughes and Grigor, 1996; Sherwin and Kelland, 1998; Buchwalder and Huber-Eicher, 2003*) while welfare issues could be accomplished (*Martrenchar, 1999*). The effect of social and rearing conditions on the behavior of turkeys has recently been reviewed by *Marchewka et al. (2013)*.

The solution to what is still being considered as a problematic welfare issue will be found by dual-way cooperation between consumers, farms and the complex animal production-slaughterhouse industries (*Waiblinger et al., 2006; Humane Farm Animal Care, 2008*).

In broiler chickens, among the welfare issues, high susceptibility to metabolic disorders and low locomotor activity were considered the most important (*Bessei, 2006*). It is inferred that similar issues are seen in turkeys.

Using well-defined indicators to assess welfare is extremely important in order to enhance their welfare evaluation. In this case, changes in behavior, haematological profile, plasma hormones concentration, immune measures and enzymatic activities could be mentioned (*Duncan, 1981; Hocking et al., 1999*).

The purpose of this review was to analyze, based on the existing literature, the physiological and pathological indicators that could be associated with welfare in turkey production. Therefore, improvement of the birds' health status, carcass quality, management, economic issues, and providing satisfactory welfare, would lead to better conditions for turkey production.

Pathological Indicators

Foot pad Dermatitis

Foot pad dermatitis (FPD) is defined as a necrotic lesion and inflammation of the foot pad. The main concern is that it could be the route of entry for other microorganisms potentially leading to the appearance of new diseases (*Shepherd and Fairchild, 2010*).

Lesions related to FPD are important not only for food safety and product downgrading, but also for animal welfare; therefore, decreasing them has been considered one of the main goal for the poultry industries (*Shepherd and Fairchild,* *2010*). In addition, FPD was classified as the key welfare indicator in the United Kingdom in turkey production (*Clark et al., 2002*), when considering the criteria of welfare assessments in Europe and the United States (*National Chicken Council, 2010; Shepherd and Fairchild, 2010*).

Severe FPD in commercial turkey flocks are common lesions, whereas, they are unlikely in broilers (*Clark et al., 2002*). In addition, poor litter moisture seemed to be a good example of management deficiencies and the most likely cause of the FPD in turkeys; consequently, decreasing animal welfare (*Mayne et al., 2004; Shepherd and Fairchild, 2010*).

These kinds of lesions in female turkeys were more severe than males during the fattening period according to *Krautwald-Junghanns et al. (2011)*, even though Clark et al. (2002) reported higher frequency in males. It could be associated with the differences in animal density in different countries, (e.g. in Germany, the turkey's density for males is approximately 2.8 birds/m^2, while for females is approximately 5.1 birds/m^2). Nevertheless, the severity of these pathological lesions is associated with other flock management factors such as litter moisture. Moreover, *Grosse Liesner (2007)* stated that genetic predisposition is also an important element of FPD.

Mayne et al. (2007b) demonstrated that FPD could be most likely associated with a rapid inflammatory response, rather than an allergic response to an environmental stimulus in the litter.

Wu and Hocking (2011) conducted an experiment with female growing turkeys in order to observe the effects of litter and animal age on FPD. The most important conclusion was that high moisture litter is the main cause of FPD, which affected the severity and the prevalence of these lesions (*Mayne et al., 2007a; Youssef et al., 2011*). Accordingly, short exposure to wet litter (4h/day; *Abd El-Wahab et al., 2012*; 8h/day; *Youssef et al., 2010*) could be enough for the development and marked increase in severity of FPD.

Thus, to reduce FPD in the flock, good litter management is essential to maintain the level of moisture under the 30% (*Wu and Hocking, 2011*); this result may be reached by using floor heating, or even providing soft litter such as lignocelluloses rather than wood shavings (*Abd El-Wahab et al., 2011*). Furthermore, there is no significant difference in litter pH between different bird groups, suggesting no relation between organic material and FPD, such as bird's droppings and bedding material (*Wu and Hocking, 2011*). On the other hand, according to *Abd El-Wahab et al. (2013)*, when the electrolyte in the diet changes, mainly the sodium/ potassium rate, indirectly interferes with the litter quality due to the increase of water intake and the increases of the excreta moisture, causing the occurrence of the 'wet litter conditions'.

Breast Skin Lesions

Breast skin lesions such as breast buttons, blisters, and purulent bursitis observed in conventional intensive turkey farming are still considered as the most frequent problems and as a result, they are one of the main reason of economic loss (*Krautwald-Junghanns et al., 2009*).

Mitterer-Istyagin et al. (2011) found the prevalence of these alterations in turkey tom than in turkey hen flocks. Although the origin is not clear and probably multifactorial, it was possible to infer that the differences between fattening period regarding body weight and the laying time may be the cause. They also conclude that sex-related influences and age status were important reasons for these results, but could also be determinate by breeding, litter moisture, and management quality.

Muscular and Skeletal Lesions

As occurs in broilers, turkeys also have skeletal abnormalities associated with higher body weight (*Wyers et al., 1991*). In this case, tibial dyschondroplasia (TD) has been described as the cause of enormous economic losses and also an animal welfare problem (*Pines et al., 2005; Tatara et al., 2006*). According to *Hocking et al. (2002),* there is no predisposition for this disease related to diet with higher or lower calcium: phosphorus ratios. Furthermore, the turkeys affected by TD have not been observed to have any locomotion problems as occurs in broilers (*Simsa et al., 2007*). Therefore, the welfare issue of TD could in this case be primarily related to location on the body where osteomyelitis may develop (*Wyers et al., 1991*). Additionally, *Tatara et al. (2006)* discovered the beneficial effects of orally administrating ornithine alpha-ketoglutarate in turkeys with skeletal disorders, which resulted in increased amino acid synthesis. It was shown that the quality of the bone is improved by higher bone mineral density of trabecular and cortical bone as well as the maximum elastic and ultimate strengths were increased.

Hocking et al. (2005) evaluated the efficacy and optimum doses of non-steroidal anti-inflammatory drugs in domestic fowl suffering from articular pain. This model could be useful when applied to different avian species such as turkeys, due to the lack of studies presently available.

In order to demonstrate the efficiency of treatments using analgesics such as synthetic opioids (butorphanol) and anti-inflammatory steroids (betamethasone) in adult turkeys with degenerative join disorders, studies were carried out with regarding locomotion, spontaneous and sexual activities (*Duncan et al., 1991; Buchwalder and Huber-Eicher, 2005b*). It was demonstrated that in all the treatment groups, an increase in the natural function activities was observed.

Physiological Indicators

Corticosteroid Evaluation

It is known that stress events which could be defined as a trigger or stressor that causes a stress response, such as catching and transport of live turkeys (*Marchewka et al., 2013*), may affect animal behavior, decrease the immune system when fighting disease and change population performance (*Korte, 2001; Shini et al., 2010*).

Glucocorticoid hormones are synthesized and released through activation of the hypothalamic-pituitary-adrenal axis complex. Conventionally, it is possible to evaluate glucocorticoid levels in the blood; however the components only remain in circulation for a short period of time (minutes). Nonetheless, another way of measuring them is by analyzing animal feathers, which has been used in order to determine more chronic stress experience indicators (activity durations of the hormone is days-to-weeks) (*Bortolotti et al., 2009*). In addition, corticosterone (CORT) is the main avian glucocorticoid that could be quantified by feather analysis (*Botolotti et al., 2008*).

According to *Botolotti et al. (2009)*, CORT is a stable hormone in feathers that could be used individually or in the flock, which gives the information about the time when the stress event happened and how the bird responded. It is a non-invasive and feasible method (*Botolotti et al., 2008*). Furthermore, this analysis used samples that were collected over many years and stored by taping the calamus to a sheet of paper in a binder kept at room temperature. Therefore, it is a good way to track stress, which is one of the most important factors that influences animal welfare. However, *Lattin et al. (2011)* reported that caution is necessary in the interpretation of CORT results extracted from feathers, due to the effect of the sample and effectiveness of the antibodies used in the feather assay.

Hocking et al. (1999) demonstrated that food restriction in commercially turkeys' production causes increase of plasma CORT during the rearing period, even if the traditional birds at 4 and 8 weeks of age have a relative high levels of this hormone.

Corticosterone metabolites' levels in faeces-urine also could be evaluated, however, many limitations have been discussed, for instance, artifacts caused by sample age, storage and transportation, diet, captivity and biological status (*Millspaugh and Washburn, 2004; Tempel and Gutiérrez, 2004; Möstl et al., 2005; Cabezas et al., 2007; Hayward et al., 2010*).

Stoyanchev et al. (2007) gathered accurate data about natural humoral immunity in turkeys, which had muscular dystrophy, reared under conditions of poor welfare and stress. In general, lysozyme concentrations (LC) were higher in sick animals than in healthier ones due to the body attempt to go through the disease. Nevertheless, under stress challenging the blood serum LC were decreased due to the presence of cortisol.

Furthermore, *Franciosini et al., (2011)* showed LC was lower in broiler turkeys reared in the backyard group (pen measuring 6 m long X 4 m wide that were subdivided into 2 connected spaces, one of which opened) when compared with industrial (13,500 birds with natural light and ventilation) and experimental flocks (optimized light, ventilation, temperature and density according to *Anonymous, 2000*). These results suggest that stress situations possibly caused by predators could be a reason for those findings as well as weather conditions for instance. Moreover, there are difference between different rearing systems and natural immune parameters.

Cytokines belong in many ways of the immune response path and are important in leucocytes development, and their roles. Interleukins (IL) are a type of cytokines that are produced by leucocytes and influence other leucocytes (Snyder, 2007).

Shini et al. (2010) carried out an experimental study in chickens regarding the effects on the expressions of the proinflammatory cytokines (e.g. IL-1β, IL-6) and chemokines (CC) (e.g. CCLi1, CCLi2) of leucocytes and heterophils under CORT oral administration. During a chronic treatment of CORT (1 week), there were a down regulation of cytokines and CC which suggest that there was suppression in the immune response. However, expositing birds to acute stress (until 24h) can cause the increase of immune system. They cited as well that IL and CC could be important markers in order to assess the influence of stress factors in their immune system.

Wu et al. (2007, 2008) reported that IL-1β and IL-8 proteins in chicken, duck, goose, turkey and pigeon have significantly structural and functional homology, which could be used as adjuvant in vaccine for all these species. It is considered as an important tool regarding to modulating the immune system.

Hematological Profile

According to *Maxwell (1993),* the increase of heterophil to lymphocytes ratio (HLR) and basophils are well-known variables indicating stress, such as heat and incorrect transportation. However, there was no evidence of the HLR changes by food restriction in turkeys (*Hocking et al., 1999)*, neither with the crate height during short-term confinement (*Wichman et al., 2010*).

HLR has been used for many years as the method to evaluate the stress, such as, in birds. When the immune system releases CORT, which elevates its blood concentration, afterwards, there is an increase of nonlymphocytes leukocytes (heterophil) and decrease of lymphoid leucocytes (lymphocytes), thus the HLR changing occurs (*Shini et al., 2010*). However, understanding of these mechanisms according to the molecular point of view should be more detailed. It was observed that during an exogenous application of CORT in 7-wk-old chicken experiment, the HLR markedly increased during 1h, 3h, and 24h post administration, which can help the innate immune response (*Shini et al., 2010*).

Huff et al. (2005) compared the effects of 2 different stressors (such as transport and dexamethasone treatments) on the measure of HLR and resistance to *Escherichia coli*, from 3 different turkeys' genetic lines, (according to their rate of growth). Their data supported the concept of lighter and slower growing line birds are more resistance to stress. In addition, HLR were increased in both stressors that were used.

Proportion of basophiles seemed to have no changes into its values during food-restrict event (*Hocking et al., 1999*).

Activities of Plasma Enzymes

Creatine kinase (CK), aspartate aminotransferase (ASAT), alkaline phosphatise (APL) and lactate are some examples for physiological measures of animal welfare which could be considered as turkey welfare indicators.

Wichman et al. (2010) showed that there was no difference between the size of crate and the activity of CK or ASAT; however, frequently changes in turkey behavior were noticed. Moreover, lactate levels were significant lower with male birds in the 55cm crates than in 40cm.

Hocking et al. (1999) observed that the activity of lactate dehydrogenase (LDH) in turkeys from 12 to 24 weeks of age was higher in male turkeys that were fed *ad libitum*. In addition, the performance of ASAT was similar to one found in LDH. However, APL was inversely of the LDH.

Conclusion

In summary, it may be concluded from this review that foot pad dermatitis (FPD), breast skin alterations, corticosterone measure analyses, immune measures (e.g. cytokines and chemokines), hematological profile, and enzyme activities (e.g. creatine kinase, aspartate aminotransferase, alkaline phosphatase and lactate) are suitable indicators of the welfare in the turkey rearing methods and in agreement with the bird protection; hence, the fittest welfare protocol using theses indicator should be built and applied. On the contrary, it is important to notice the real feasibility of those indicators. The aims should be well-understanding of their usability in order to select the proper indicators for the assessment of the turkey welfare.

The proper welfare assessment cannot be done considering solely each factor as singular; it should be evaluated based on the factors that are also involved, such as the rearing methods for turkeys, management, and breeding.

The FPD and breast blisters are for now considered the most practical welfare indicators in turkeys regarding the feasibility for collecting this data scoring. Furthermore, it has a great importance if consider how practical, no time consuming and reliable welfare indicator(s) could be applied on the complex farm-slaughterhouse. Therefore, the real welfare picture could be defined.

Further studies are needed in order to obtain the useful tools in order to figure out which is the best way to deal with these challenges: intensive animal production chain versus poultry protections concerns. Perhaps, the main scope is the equilibrium between the research, farms, industries and consumers.

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Pregled fizioloških i patološkim indikatora dobrobiti ćurki (*Meleagris gallopavo*)

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Rezime

Pitanje dobrobiti životinja je preko jedne decenije jedno od važnijih pitanja u Evropi. U komercijalnom živinarstvu, bilo je mnogo pokušaja da se standardizuje proizvodnja u cilju smanjenja ekonomskih gubitaka izazvanih lošom dobrobiti u uzrastu kada se živina plasira na tržište. Kao što je poznato, faktori kao što su gustina naseljenosti, veličina grupe, dostupnost prostora, sazrevanje, osvetljenje, ishrana i prevoz, mogu imati uticaj na dobrobit ćurki. Međutim, da bi se obezbedio bolji kvalitet života za ptice, kao i za industriju dobre performanse, smanjenje smrtnosti i gubitaka, važno je imati još jednu tačku gledišta kao različite vrste indikatora. Ovaj rad razmatra raspoloživu naučnu literaturu koja se odnosi na dobrobit ćurki "u skladu sa glavnim relevantnim fizičko-patološkim pokazateljima uzimajući u obzir da li su oni izvodljivi ili ne. U radu je pažnja usmerena na dermatitis nogu i lezije kože grudi kao najrelevantnije indikatori do sada. Oni mogu biti od značaja za poboljšanje indikatora ocenjivanja dobrobiti ćurki. Međutim, merenja kortikosterona, aktivnosti enzima, citokina i hematološki profil su indikatori za koje se čini da će se u budućnosti češće primenjivati. Na ovaj način, povezivanjem indikatora koji su ranije ispitivani sa novim, pretpostavlja se da će postojati odgovarajuću vezu između životinja, odgajivača, industrije i potrošača (lanac proizvodnje živine) u skladu sa njihovim različitim interesima.

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CHAPTER 3

In this chapter, the article entitled “Welfare assessment in broiler farms: Transect walks *versus* individual scoring” was published in Poultry Science, 92(10):2588–2599, 2013. doi: 10.3382/ps.2013-03229

Welfare assessment in broiler farms: Transect walks versus individual scoring

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ABSTRACT Current scientific approaches to welfare assessment in broilers are based on individual sampling that can be time consuming under field conditions. On the other hand, farmers conduct routine checks based on walks through the house to screen birds' health condition. We adapted the walks through following line transect methodology used in wildlife studies to explore their feasibility as a welfare assessment tool. The aim of this study was to compare broiler welfare assessed by individual sampling and transect walks. We evaluated 6 identically managed flocks. For individual sampling, we collected measures on 150 birds, including weight, breast dirtiness, hock and footpad dermatitis, lameness, and immobility. Transect observations were conducted by slowly walking on randomized paths within each house recording: immobility, lameness, back dirtiness, sickness, agony, and dead. Transect walks allowed detection of small variations ($P < 0.003$) in the prevalence of most welfare indicators considered with consistency in interobserver reliability ($P \geq 0.05$). In addition, assessments across transects were highly consistent ($P \geq 0.05$). Individual sampling was also sensitive to differences across houses ($P < 0.01$) with the exception of immobility ($P = 0.783$). No differences were found across sampling locations ($P \geq 0.05$). However, both methods differed greatly in the frequency of the incidence of the parameters considered. For example, immobility varied from 0.2 ± 0.02% for transect walks to 4 ± 2.3% for individual sampling, whereas lameness varied between 0.8 ± 0.07% and 24.2 ± 4.7% for transect and samplings, respectively. It is possible that the transect approach may have overlooked walking deficiencies because a large number of birds were scored, although if this was the case, the consistency obtained in the scoring across observers and transects would be surprising. Differences may also be related to possibly biased individual sampling procedures, where less mobile and passive individuals may be more likely to be caught. Furthermore the procedure may cause fatigue and fear reactions reducing mobility. Current study provides new insights into constraints and advantages of broiler on-farm assessment methods, which should be considered for designing on-farm welfare assessment protocols.

Key words: welfare indicator, on-farm assessment, broiler, sampling, transect

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INTRODUCTION

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

Currently, most scientists agree with the need for designing protocols based on the animal (Main et al., 2007). The use of animal-based welfare indicators is

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

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

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

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

Walk-throughs performed by bird caretakers are, to a certain extent, a similar strategy for data collection to line transect methodology, which has been successfully used for years in wildlife studies (Buckland et al., 2010). Some aspects of this approach, such as distance evaluation, were used in a nonintrusive method of plumage condition assessment (Bright et al., 2006). However, the differences in methodology and results between the approach for welfare evaluation closer to the methods used routinely by bird caretakers or flock supervisors and the classical scientific approach of individual sampling have never been compared. The ideal welfare assessment protocol for on-farm conditions should be a method that provides the dynamism of walk-through inspections but is conducted in a way that provides veracity, interobserver reliability, and quantitative results that can be compared across flocks and farms. To our knowledge, none of the available methodologies developed to date would fulfill the requirements of what would be considered a "gold standard."

The goal of this study was to compare the welfare assessment results of broiler flocks evaluated according to 2 different approaches: the transect walks and the individual scoring. The transect walk methodology is based on the idea of walk-through used for broiler care and line transect methodology used in wildlife biology, adding the evaluation of the methodology for interobserver reliability and within- and across-house sensitivity. We compared the results with the individual sampling scoring conducted following the guidelines provided by the Welfare Quality (2009). This is a preliminary study aiming to develop a scientifically sound and practical methodology, combining current scientific findings with the transect approach, for on-farm broiler welfare assessment, with perspective for application in other poultry species.

MATERIALS AND METHODS

Facilities and Birds

The study was conducted from April 30 to May 8, 2012, at 3 farms, located in the same geographical region in Northern Spain belonging to the company Grupo AN from the Navarra region (Spain). Each of the studied farms had paired houses, with flock sizes/house ranging from 13,220 to 27,540 broilers (Cobb 500) reared at a density of 17 birds/m^2. All houses had identical management, other than for the fact that 4 of the houses used chopped straw as litter substrate, whereas 2 used wood shavings. All houses were provided with automatic drinkers, feeders and ventilation systems, artificial light, and windows allowing natural lighting.

Data Collection

We collected data by using 2 methodologies: the transect walk approach that we developed, and the individual sampling assessment based in the protocols developed by Welfare Quality (2009).

Transect Walks. The transect walk approach is based on the methodology widely and successfully used in wildlife studies for decades (Gates et al., 1968; Buckland et al., 2010). Transects walks for bird welfare assessment in our study consisted of standardized walks divided in randomly set paths covering the full area of the house (Figure 1a).

Broiler houses normally have a rectangular shape, although dimensions may vary across companies and countries. The houses in our study were around 13 m wide (variable length) and were divided in five 2.5-m-wide bands. Transects were numbered from 1 to 5: 1 and 5 being wall and 2, 3, and 4 central transects. Transect widths were limited by the location of feeder and drinker lines (for central transects), or the wall and adjacent drinking line (for wall transects), which appeared to create invisible barriers to birds' movements, caused by a human moving forward along the transect (personal observation). Paired houses at each farm were assessed sequentially by 2 observers within the same day, when birds were 31 to 35 d old (birds' welfare may deteriorate in a day toward the end of rearing). This age range, instead of the end of production cycle, was chosen for assessments because it is a common procedure in Spain to depopulate 25% of the flock at this age. A later evaluation may have provided biased results due to the impact of catching during depopulation, which is considered a major cause of stress, therefore providing misleading information about the welfare status of the birds during the production cycle.

Observers conducted the data collection independently in each house. Transect walks were performed in random order, in both directions, starting at the entrance wall and at the opposite of the entrance wall, alternatively. We avoided sequential observations of contiguous transects to minimize the possibility of double-counting birds that may have moved from adjacent scored transects minutes before. The observers walked slowly through the set transect (Figure 1b) while recording in a spreadsheet (Polaris Office, Infraware, Seoul, South Korea) installed in a handheld tablet (ASUS Eeepad TF 101 Transformer, Taipei, Taiwan). Observations of all occurring incidences of birds within the following categories were recorded: immobile (no attempt to move, even after slight encouragement), limping (visible signs of severe uneven walk), dirty (side and back feathers visibly dirty), sick (bird showing clear signs of impaired health with small and pale comb, red-watery eyes, and occasionally unarranged feathering usually found in resting position), agonizing (the bird lies on the floor with closed eyes, breathing with difficulty), and dead. These are validated welfare indicators, which are considered critical parameters in terms of broiler welfare (EFSA Panel on Animal Health and Welfare, 2012), which can be clearly described and identified for data collection in broiler flocks, making them ideal for purpose of methodology validation.

Individual Sampling. During individual sampling, a group of 3 trained scientist collected birds in 6 random locations within the house, with at least one of them collected in 1 of the 5 predefined transects. Each sample consisted of 25 randomly collected birds that were gently pushed to a mobile pen and were kept enclosed during sampling. Each bird was handled gently and individually, weighed on an automatic scale (PCE-WS 30, PCE Instruments, Southampton, Hampshire, UK), and evaluated for footpad dermatitis (score 0 to 4), hock burns (0 to 4), and breast dirtiness (0 to 2). Afterward each bird was released away from the scoring area and observed to evaluate gait scoring (scale 0 to 5) when receding. If not showing willingness to move, we used slight encouragement by touching the bird. For each indicator, a lower score meant a higher welfare status of the individual. After scoring all birds in the sample, the procedure was repeated in the next location. Although under ideal circumstances we should have had 2 teams performing dual individual sampling to check for interobserver reliability, this would require a total of 7 people, and unfortunately we did not have sufficient personnel to do this. In addition, because the individual scoring took half a day per house, the 3-person team could only evaluate 2 houses in a day. Therefore, there was not sufficient time to repeat the scoring a second time in each of the paired houses.

Statistical Analysis

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

To test interobserver reliability and sensitivity of transect evaluation, resulting percentages were checked for normality and homogeneity of residual variance. From the whole set of variables, immobility, agony, and death were nonnormally distributed and were subjected to logarithmic transformation, allowing fulfillment of normality requirements. We performed independent mixed-model repeated measures ANOVA for each of the 6 welfare indicators defined above. The model included transect as a repeated measure, with house and observer as fixed factors. We included farm as a random statement because the between-houses comparison was the main point of our interest. We included interactions between observer by transect and observer by house, as well as house by transect. Least squares means differ-

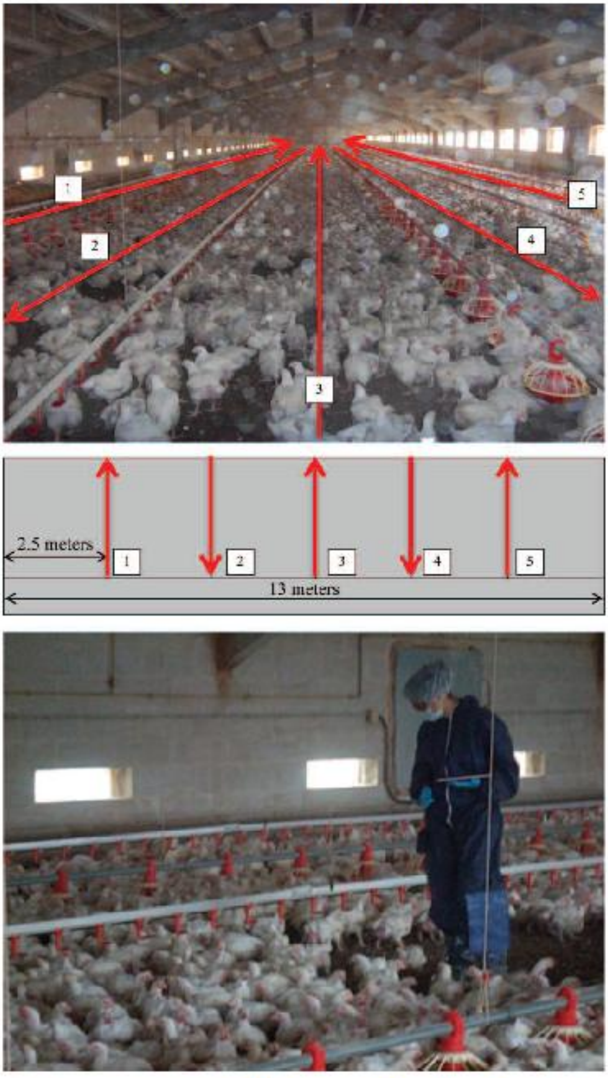

Figure 1. a) Design of the transect walks of 2.5 m within a 13-m-wide production room. The dashed lines (red in color version) show the pathways along which the transect walks were conducted. Arrows show the walking path of the observer between lines of feeders and drinkers. b) Data collection during transects (note the short distance to the observer). Color version available in the online PDF.

Table 1. Effect of house, transect, observer, and the interactions of transect with observer and observer with farm for welfare indicators collected by transects

	ANOVA component					
Welfare indicator	House	Transect	Observer	Transect × observer	House × observer	House × transect
Immobile	<0.00010	0.9033	0.0208	0.1915	0.1235	0.3163
Limping	0.0029	0.7996	0.8496	0.2447	0.0502	0.6451
Dirty	0.0005	0.1003	0.6832	0.1089	<0.0001	0.2046
Sick	0.6293	0.6994	0.6009	0.8107	0.4978	0.9391
Agonizing	<0.0001	0.3656	0.0479	0.7908	0.0604	0.3580
Dead	<0.0001	0.0068	0.0502	0.6666	0.0015	0.0020

ences were adjusted for multiple comparisons by post-hoc Tukey comparison.

We applied bootstrapping techniques to test the precision of the method by taking simulated random samplings combinations from the original data set (Dixon, 1993). Bootstrapping has been used to estimate the accuracy of ecological indices (Stein, 1989; Dixon, 1993) and more recently in a wide range of scientific areas, from genetics (Yang and Rannala, 2012) to economic sciences (Clark and McCracken, 2012). In short, this methodology defines the appropriate model for the observed data, from which it generates n sample data sets using Monte Carlo methods, to finally construct the bootstrap distribution (Efron, 1979, 1987). Expected mean and SE of the data set for each welfare indicator was calculated by taking random samples of one transect (20% of the information), or combinations of 2, 3, and 4 transects (40, 60, and 80% of information, respectively). Simulations were run 10,000 times per house and welfare indicator. All variables, except for immobility, were averaged per house due to lack of differences ($P \geq 0.05$) across observers. Independent bootstrapping was calculated for the indicator immobility for each observer. We used PROC SURVEYSELECT to perform the bootstrap.

Individual Sampling

Data collected in individual samplings were also checked for normality and homogeneity of residual variance. Hock burns, immobility, and dirtiness were nonnormally distributed, and were subjected to logarithmic transformation. The variables were analyzed by independent mixed-model repeated measures ANOVA. The model included transect as a repeated measure and house as fixed factor. However, for this analysis the interaction among both factors could not be included because of the lack of sufficient degrees of freedom. We included farm as a random statement, as for the transect walk analysis. Least squares means differences were adjusted for multiple comparisons by post-hoc Tukey comparison.

All analyses were conducted with SAS 9.3 statistical package (SAS Institute Inc., Cary, NC).

RESULTS

Transect Walks

Sensitivity. Our results showed that transect walk methodology allowed detection of small variations across the studied flocks on the prevalence of the studied welfare indicators. Differences across houses ($P < 0.003$) were found for the incidence of immobile, limping, dirty back, agonizing, and dead birds (Table 1, Figure 2). Only incidence of sick birds remained invariable across the studied houses (Table 1, Figure 2).

Interobserver Reliability. Welfare assessment across observers, or the interaction of observer and transect, and observer by house remained consistent for most variables as indicated by the lack of differences ($P \geq 0.05$) in the assessment (Tables 1 and 2). The effect of observer was only detected for the incidence of immobile and agonizing birds (Table 1); however, the interobserver difference for both variables was not observed ($P \geq 0.05$) for the interaction between house and observer. On the other hand, the house × observer interaction had an effect ($P < 0.0015$) on dirty and dead birds. Nonetheless, the differences across observers (Table 2) ranged between 0.18 ± 0.02% and 0.22 ± 0.03% for the incidence of immobile birds, whereas maximum range of variation across farms and observers for dirty birds was ±0.5%.

Transect Effect. No effect ($P \geq 0.05$) of transect location was detected (1 to 5; 1 and 5 being wall transects, and remaining being central transects) for almost all variables (Tables 1 and 2) studied, except for dead for transect ($P = 0.0068$) and transect × house effect ($P = 0.002$). Applying bootstrapping techniques showed that the mean for each house was similar to the observed mean value by using as little as 20% of the information for all the variables (representative example in Table 3 and Figure 3).

Individual Sampling

Sensitivity. By using individual sampling method, we found differences ($P < 0.01$; Table 4) between houses for limping and dirty birds, footpad dermatitis, and BW (Figures 4 and 5).

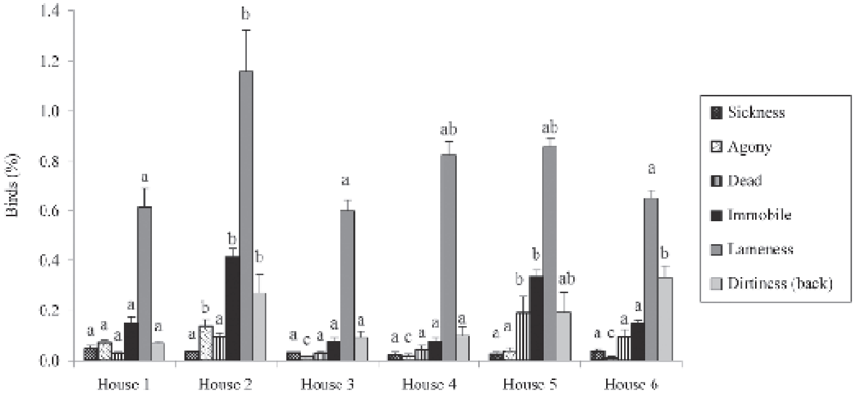

Figure 2. Mean values (±SEM) of each welfare indicator expressed as percentages for each house obtained by transect walks. Differences for each specific measure across houses are indicated by letters; means lacking a common letter (a–c) differ ($P \leq 0.05$).

Transect Effect. We did not find any effect of transect ($P \geq 0.05$) on any of the variables collected by individual sampling, nor any effect of interaction between transect and the house on the variables (Table 4).

DISCUSSION

Transect Walks

The aim of our study was to explore the soundness of a new approach to welfare assessment for broiler flocks, considering the scientific validity, time, and personnel requirements. We also considered the potential acceptability by assessors and producers, which might have an interest in self-assessment. The transect walk approach is based on the routine daily checks conducted by farmers and flock supervisors during inspections, combined with line transect methodology commonly used for evaluating wildlife populations (Buckland et al., 2010). The transect walk approach implies surveying birds throughout the entire production house, registering all individuals falling within each welfare indicator category, established in this study within each transect.

In this study, we homogenized field conditions as much as possible by assigning to the study only houses using birds of identical genetic background (Cobb 500) raised under identical standard management practices and within the same geographical region. All houses were sampled when birds were at similar ages (31 to 35 d) and were assessed in less than a month to minimize variations in environmental conditions that may affect the birds' welfare status (Dawkins et al., 2004). Despite the homogeneity in housing conditions, our results showed that the transect walk approach was highly sensitive and allowed detection of small variations in the incidence of the welfare indicators used in this study such as immobility, birds with severe limping, with dirty back, agonizing, or dead (Table 1, Figure 2). These indicators are known be critical for the welfare status of broilers (Dawkins et al., 2004; Estevez, 2007), but also have a tremendous economic impact. For example, skeletal problems causing immobility in the United Kingdom are responsible for losses estimated in 2 million pounds per year (Walker, 2012). Another indicator used, such as back dirtiness (as used in this study) is considered an important welfare indicator connected to litter quality or stocking density (Berg, 1998; Estevez, 2007).

Welfare assessment with the transect walk approach remained consistent across observers for limping, dirty, sick, and dead birds (Tables 1 and 2, Figure 3). However, minor differences were detected for the incidence of immobility across observers and for the interaction of observer with house for dirty and dead birds (Table 1). The differences across observers ranged between 0.18 ± 0.02 and 0.22 ± 0.03 for the incidence of immobile birds (Table 2). Considering the scope of the sampling (several thousand birds per flock) and the randomized procedures we used for data collection, it is actually quite remarkable that we only found minor effects across observers, whereas house assessment results remained consistent with other studies conducted in broilers under commercial (Sanotra et al., 2003; Dawkins et al., 2004; Knowles et al., 2008) and experimental conditions (Kestin et al., 1992). For example, averages of 0.9% birds unable or with impaired walk were found when using a noninvasive method to evaluate walking ability (Dawkins et al., 2004). These results

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

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

are similar to the values obtained in this study when adding the categories defined as immobile and severe limping. Our results are also comparable with another study (Knowles et al., 2008) in which 0.2% of immobile birds were detected using a method that involved bird handling (Kestin et al., 1992), and using the same methodology (Kestin et al., 1992), averages of 0.3 and 2.7% severely lame birds were noticed for 28- and 42-d-old broilers, respectively (Sørensen et al., 2000). On the other hand, the observer × house effects detected for dead birds can be explained by the fact that we were working under commercial conditions and in 2 of the houses the farmer removed the mortalities in between the data collection of the 2 observers.

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

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

This idea is further supported by the results obtained from applying bootstrapping techniques that allows testing the precision of an estimate by calculating the bias and SE by taking simulated random samplings combinations from the original data set (Dixon, 1993). The resulting expected mean for each house was similar to the observed mean value by using as little as 20% of the information (Table 3, Figure 3). These results indicate that, under the conditions of our study, the assessment of an area covering 20% of the house surface is theoretically sufficient to obtain a reliable mean estimation on welfare status of a broiler flock, based on the parameters we used. If there is an interest in getting the closest to real value of the SEM, then our results suggest that a minimum 60% of the house area should be evaluated. Given that, in this initial study assessing a complete broiler flock by conducting 5 randomly de-

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

		% of information used									
		20		40		60		80		100	
Variable	House	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Limping	1	0.6167	0.1485	0.6171	0.1036	0.6163	0.0850	0.6161	0.0735	0.6160	0.0828
	2	1.1600	0.3300	1.1617	0.2326	1.1605	0.1889	1.1586	0.1645	1.1583	0.1834
	3	0.6009	0.0838	0.6005	0.0595	0.5998	0.0480	0.6003	0.0419	0.6000	0.0469
	4	0.8260	0.1045	0.8255	0.0736	0.8249	0.0597	0.8249	0.0520	0.8250	0.0686
	5	0.8558	0.0755	0.8541	0.0540	0.8546	0.0433	0.8547	0.0376	0.8548	0.0395
	6	0.6508	0.0668	0.6507	0.0469	0.6508	0.0387	0.6507	0.0337	0.6505	0.0373
Dirty	1	0.0676	0.0223	0.0677	0.0158	0.0679	0.0129	0.0680	0.0112	0.0680	0.0124
	2	0.2731	0.1401	0.2736	0.0996	0.2734	0.0816	0.2727	0.0697	0.2723	0.0778
	3	0.0914	0.0476	0.0914	0.0337	0.0916	0.0273	0.0915	0.0237	0.0914	0.0267
	4	0.0983	0.0785	0.0983	0.0557	0.0983	0.0457	0.0982	0.0392	0.0984	0.0440
	5	0.1937	0.1611	0.1928	0.1141	0.1922	0.0924	0.1927	0.0807	0.1929	0.0900
	6	0.0936	0.3337	0.3344	0.0659	0.3341	0.0531	0.3340	0.0462	0.3340	0.0620

termined transect walks took around 4 h (due to location data collection measuring distances from observer location to the front wall, results of which were not presented in this manuscript), we calculate that if the method is proven for its validity, then farm assessment could be conducted in a time lapse ranging between 30 to 60 min and with minimal interference with the daily farm routines.

Individual Sampling

Individual sampling is the most commonly used procedure for bird welfare assessment in broilers (Welfare Quality, 2009; De Jong et al., 2012c), for which a sample between 100 and 150 birds per flock is recommended, due to time and personnel requirements. We were interested in determining how our transect walk ap-

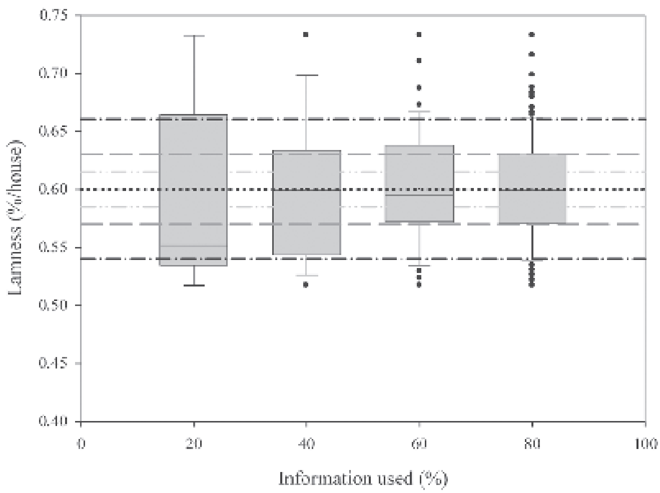

Figure 3. Mean values (±SD) expressed as percentages obtained from bootstrapping from 20, 40, 60, and 80% of information for limping birds in comparison with mean (· · ·), 2.5% (— · · · —), 5% (— — —), and 10% (— · —) of error rates of value obtained from 100% of information for house 3 (example).

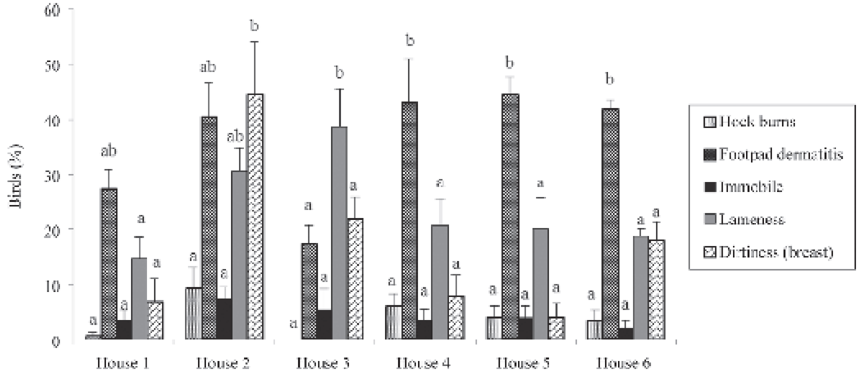

Figure 4. Mean values (±SEM) of each welfare indicator expressed as percentages for each house obtained by individual samplings. Means lacking a common letter (a,b) differ ($P \leq 0.05$).

proach would compare with this well-known and widely accepted methodology.

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

An important advantage of the individual sampling methodology that cannot be overlooked is that it allows scoring the incidence of footpad dermatitis and hock burns, which are important welfare indicators, in addition to their economic relevance to industry. Our results regarding the values for footpad dermatitis ranging from 18 to 48% for birds with scores 3 and 4 were within the values obtained by a recent study conducted in 386 Dutch flocks, in which 26.1 to 38.4% had mild or severe footpad lesions (De Jong et al., 2012b). With regard to hock burns, an evaluation of more than 2,000 birds at the age of 4 wk showed an incidence of 0.5% (Kjaer et al., 2006), whereas in our study the mean incidence was of 3.6%, with farm values ranging between 0 and 8.43%. The much higher upper range of our results might be caused by the older age of birds

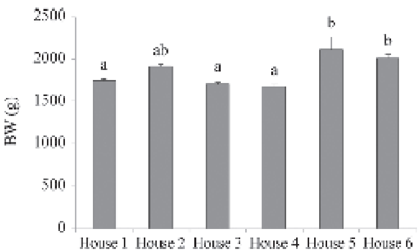

Figure 5. Mean values (±SEM) of BW for each house obtained by individual samplings. Means lacking a common letter (a,b) differ ($P \leq 0.05$).

Table 4. Effect of farm, house, observer, transect, and the interactions transect with observer and observer with farm for welfare indicators collected by individual samplings

	ANOVA component	
Welfare indicator	House	Transect
Immobile	0.7839	0.8495
Limping	0.0017	0.2616
Dirty	0.0002	0.7103
Hock burn	0.0941	0.8095
Footpad dermatitis	0.0112	0.4577
BW	0.0010	0.8676

in our study (more than 30 d old) or due to the fact that the observed flocks were placed at the farms in winter, when the incidence of footpad lesion tends to be more important. Regarding BW, relevant differences were found between houses (Figure 5). However, these particular houses did not appear to be the ones with lower incidence of welfare problems such as lameness or dirtiness.

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

Method Comparison

The results of this study show clear, major discrepancies between both methods of welfare assessment. The results obtained by individual sampling would indicate a substantially reduced welfare status of broiler flocks compared with results obtained by applying the transect walk methodology considering the welfare indicators used in this study. The indicators which could be directly compared across transect walks and individual samplings were severe lameness and immobility. Mean incidence of lameness and immobility was 24.18 ± 4.68% and 4.22 ± 2.3%, respectively, for individual sampling, whereas for transect walks mean frequency for lameness was 0.78 ± 0.07% and 0.2 ± 0.01%.

The discrepancies across the 2 methods may be related to the observers failing to detect birds within the immobile or limping (severely lame) category during transect walks. This is a likely possibility and further studies should be conducted for improvement of the accuracy and reliability of this new methodological approach for on-farm welfare assessment. However, it should be also considered that when using 25 birds as the sample size in each location of the house for individual sampling, the effect of scoring just one bird in a given category would already increase the incidence of such category to a 4% incidence for this sample. Therefore, although individual sampling may be ideal for the assessment of large animals in which herd size may be several hundred (Vasseur et al., 2012), it may be more difficult to apply, or at least bring up some methodological questions, when applied to large poultry flocks.

This issue could be easily overcome by increasing sampling size. However, the assessment of 150 birds in our study took between 3 to 4 h for an experienced 3-person team. The speed of assessment could certainly be improved, but still even if doubling the speed it would take around 4 h to sample 300 birds, that would represent 1% of the population for a 30,000-bird flock.

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

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

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

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

The outcomes of our study revealed large differences between pictures obtained by the 2 methods analyzed in this study. However, much of the discrepancy can be well explained and justified by the arguments stated above. Certainly the transect methodology still needs much testing to ensure that lameness and immobility are not overlooked and that the methodology provides a realistic quantitative assessment of the most relevant welfare indicators. Indeed, behavioral assessment will also need to be considered if the methodology is validated in future studies.

Conclusion

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

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CHAPTER 4

In this chapter, the article entitled "Transect walks as an on-farm welfare assessment method for turkeys (*Meleagris gallopavo*)" will be submitted in Poultry Science.

Transect walks as an on-farm welfare assessment method for turkeys (*Meleagris gallopavo*)

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ABSTRACT

There is increasing consumer demands for poultry products that meet the minimum expectations in terms of animal welfare during their production cycle. Additionally, a growing number of farmers are aware about full compliance with the vital animal welfare standards that could play an important economic role in commercial intensive productions. Transect walks (TW) method appeared to provide a practical approach to welfare assessment in broilers farms. This method could be considered a reasonable approach for turkey welfare evaluation in terms of time demand within reasonable costs. Furthermore, TW approach resembles the routinely checks used by farms. The overall aim of this study was to verify the feasibility of the TW method as potential tool for on-farm welfare assessment in turkeys fattening period. A total of 14 commercial [B.U.T.] - Big 6) turkey farms (8 male and 6 female) with similar management standard procedures were evaluated (1-2 flocks/1-2 houses/farm). Bird's age ranged from 122 to 138 d and 90 to 103 d old, respectively. Two independent observers walked slowly on randomized longitudinal bands within each house and recording the incidence of birds showing among 12 welfare and health indicators: immobility, lameness, wounds, small size, featherless, dirtiness, sick, terminally ill, dead, and behavioral indicators, such as, aggression towards mate, interaction with humans and mating. The effect of observer, sex, and interaction observer by sex were evaluated by using ANOVAs. Sensitivity of the method was noted by effect of sex ($P < 0.001$) for immobility, lameness, wounds and dirtiness indicators. In addition, inter-observer reliability ($P \geq 0.05$) was also consistent for almost the studied variables. Male birds showed high incidence of immobility (0.14% ± 0.02% *vs*. 0.02% ± 0), lameness (9.06% ± 0.41% vs. 4.34% ± 0.20%), wounds (3.54% ± 0.19% vs. 1.38% ± 0.09%) and dirtiness (0.20% ± 0.02% vs. 0.07% ± 0.01%) than female flocks, respectively. Current study reports the limitations and advantages of this method for welfare assessment on-farm and it is the first description of the Italian welfare profile of turkey commercial flocks.

Keywords: animal-based indicators, welfare assessment, on-farm protocol, turkey

INTRODUCTION

Consumers currently demand livestock and poultry products originated from animals raised under high welfare conditions (Bartussek, 1999). Additionally, an increasing number of farmers are aware about importance of full compliance with the animal welfare standards that could play an important economic role in commercial intensive productions (Braghieri et al., 2005).

In 2012, turkey production reached 288,000 metric tonnes in carcass weight equivalent (tcwe) in Italy and 1.9 million tcwe in the European Union (Forthorn**,** 2013). However, concerns regarding increasing risk of poor bird welfare have been raised provided the large production volume of the turkey industry. Indeed, animal welfare assessment protocols have meaningful effects on providing the bases for legal verification at the farm level, in order to promote and guarantee high animal standards. The current welfare legislation and programs, undoubtedly, are of large interest for industry, farmers, scientists, and consumers (Napolitano et al., 2007; Welfare Quality, 2009).

The classic parameters that have been used on-farm to evaluate the welfare state of animals can be divided into two major groups (Bartussek, 1997; Hörning, 2001; Main et al., 2003): (i) resourced-based which include measurements, describing the influence of the housing and management system on animal welfare and (ii) animal-based parameters dealing with behavior, health and physiological traits. Although parameters included in the first category are important, it is considered an indirect measurement of animal welfare. On the other hand, recording animals’ reactions to specific environments is more direct, since it relates to the state of the animal itself (Sandøe et al., 1997; Mollenhorst et al., 2005). As indicated by Broom (1996), welfare refers to the state of the animal rather than the evaluation of the resources provided to it. Therefore, the assessment should be based on such animal-based indicators.

For this reason, a validated, reliable and feasible assessment protocol is needed to assess the influence of different, complex, and crucial factors, such as genetic, husbandry, housing and management system, which can cause negative effects on poultry well-being, bird performance and post slaughter product quality (Winckler et al., 2003). In addition, this evaluation should be possible to be applied to a wide variety of production systems ensuring a certain required standard for animal welfare (Bartussek, 1999).

Few protocols including animal-based indicators exist to assess animal welfare at farm level, and none specifically focuses on turkeys under intensive commercial production. Several important indicators may impair the bird welfare, but also have an important economic impact for industry and food production and safety (Stull and McDonough, 1994). In this respect, breast skin lesions (Kamyab, 2001; Mitterer-Istyagin et al., 2011), hock burns (Welfare Quality, 2009), foot pad dermatitis (Krautwald-Junghanns et al., 2009, 2011) are major post-mortem indicators of house conditions and the general bird welfare (Haslam et al., 2007). These parameters show substantial decrease of turkey welfare status associated with relevance economic loss through culling on farm, and downgrading and carcass condemnations at processing.

Since the last century, transect walks have been a fruitful and worldwide used method in wildlife animals populations (Gates et al., 1968; Buckland, 1985; Buckland et al., 2010). Bright et al., (2006) used this method for assessing the plumage conditions on laying hens. Transect walks for welfare assessment has already been tested in broiler farms (Marchewcka et al., 2013) and could also be a valuable tool for welfare assessment in turkey production. Besides the advantages of this new approach regarding the reasonable costs, less time consuming and not high physically demanding (Marchewka et al., 2013), the methodology has similarities with the walk-through the house performed by turkey caretakers on intensive production as a daily routine procedure to check the health status of the birds. Furthermore, a major advantage is that the method it is a non-invasive method and does not involved bird manipulation, which would be a major challenge in turkey rearing.

The aim of this study was to verify the reliability, feasibility, effectiveness, and how practical the method is on-farm for turkey welfare assessment during fattening period. We hypothesized that the transect methodology could also be an appropriate approach to evaluate turkey welfare on-farm once it has already been tested at broiler farms conditions. Moreover, this non-invasive method should allow evaluating an entire turkey house with a large numbers of birds in real-time observations at a commercial setting. During the assessment, the indicators should mainly be checked for the reliability in assessing the bird welfare conditions. The notation system seeks to identify turkeys flocks based upon a broad range of clearly defined and measurable welfare criteria.

MATERIALS AND METHODS

Facilities and Birds

This study was conducted from March 18 to June 26, 2014, on a total of 6 female and 8 male turkey farms. The farms were located in the Lombardia and Veneto regions, in Northeast Italy. Two flocks per farm were included in this study. Each of the studied farms at least one or a paired houses with flock size/house ranging from 3,100 to 10,558 beak-trimmed females (90 to 103 days of age) and from 2,250 to 4,000 beak-trimmed males (122 to 138 days of age). All birds originated belonged to the same genetic strain (British United Turkeys [B.U.T.] - Big 6) and were reared at a density of 6.0 – 6.3 female birds/m^2 and 2.7 – 4.1 male birds/ m^2. All houses had similar management except for the litter substrates: twelve farms used wood shavings and husk, one used wood shavings and chopped straw and one farm used only wood shavings. Automatic feeders, drinkers, and ventilation systems were present in all houses. The data recording were conducted in one season in each selected farm; spring/summer, in order to minimize the effect of the environment variations.

On-Farm Data Collection

Data were collected by using the transect walk approach methodology developed by Marchewka et al., (2013). This new approach at commercial meat poultry flocks is based on the method used successfully in wildlife studies (Gates et al., 1968; Buckland et al., 2010).

Transect Walks.

The houses used in this study were rectangular, 14 m wide and variable length ranging from 70 to 120 m length. Each house was divided into 4 longitudinal transect (3.5 m wide bands) covering the entire house. Bands were numbered from 1 to 4 and walls, feeders and drinkers lines marked the boundaries between transects (Figure 1).

Two observers, who were previously trained in transect data collection and in welfare assessment of the selected indicators, evaluated at least paired houses (within the farms) sequentially and independently within the same day. The assessment took place at the end of production cycle, approximately one week before slaughter. The data collection was performed by walking through the predefined transect bands (1 to 4) in random order, in both directions, starting from the entrance wall and alternating the starting point for each transect (Figure 2). The observers walked slowly and recorded in a spreadsheet (Polaris Office, Infraware, Seoul, South Korea) installed in a handheld tablet (Samsung Galaxy Tab 2 10.1, GT-P5110 Android 4.2.2, Seoul, South Korea) the number of birds showing one of the following validated welfare indicators (Jong de et al., 2012; Marchewka et al., 2014). Immobility (bird does not make any attempt to move, even after slight encouragement); lameness (bird has clear evidence of limp and uneven walk, with or without any slight encouragement to walk, likely with wings assistance); skin injuries (head/neck, back and tail/vent wounds); missing feather (bird has visible area(s) of missing feather on the body); small size (easily distinguishable females or males with visibly lower body weight or smaller size when compared to the average of the flock); dirtiness (the majority areas of the back and wings is covered by manure); sick (bird showing clear signs of impaired health with pale head, red-watery eyes and occasionally unarranged feathering usually found in resting position. Birds with the pendulous crop hanging in front of the breast or with missing or deformed body parts, with clearly different (pale/yellowish body color), terminally ill (bird lays on the floor showing its weakness with full or half eyes closed. The head might be rested on the body back or even on the floor. The frequency of breath is also reduced and the bird is not alert), aggression towards mates (clear aggressive attack towards other birds head or chasing or pecking, including fights and leaps), interaction with humans (bird performs clear and perceptible hit with the wings, run into, jump onto or peck by the turkey to the human feet, legs or hands), and mating (bird making an attempt or "sitting" on other bird). Furthermore, individual turkeys could be classified as belonging to more than one category. The number of dead birds was also collected. These are considered the critical parameters for turkeys and broiler welfare (Duncan and Mench, 1993; Dawkins et al., 2004; Estevez, 2007; EFSA, 2012).

Statistical Analysis

The observers recorded the incidence of each selected welfare and health indicators affecting birds during the transect walks. Afterwards, the observed frequencies were transformed into proportion per each transect, assuming that the birds were randomly distributed in the house, as well as, knowing of the total number of birds per flock in each evaluated house.

The whole set of variables considered subjected of this analysis was immobility, lameness, wounds, small size, featherless, dirtiness, sick, terminally ill, dead, aggression towards mate, interaction with humans and mating.

To investigate whether the transect walk methodology detect even small variation between houses comparison, farm was considered as a random statement. To test inter-observer reliability and sensitivity of this new welfare assessment approach at turkey commercial conditions system, all variables were transformed into arc sin square root to meet to normality and homogeneity of residual variance. The independent mixed-model repeated measures ANOVA was performed using PROC MIXED procedure in the statistical software package SAS 9.3 (SAS Institute Inc., Cary, NC) for all 12 welfare indicators. Least squares means differences were used as post-hoc Tukey test. It was considered the effects of observer and sex. In addition, the interaction between observer by sex was also included. Descriptive statistics were processed by computing overall data of the surveyed Italian turkey farms. All statements of statistical difference significance were set at $P \leq 0.05$.

RESULTS

Sensitivity.

The results showed clear differences across male and female farms ($P < 0.0001$), as independent groups, for the incidence of immobility, lameness, wounds and dirtiness on turkey birds (Table 1 and 3).

Inter-observer Reliability.

There was no significant effect across observers, or the interaction of observer by sex, for almost all studied variables considering male and female turkey farms. The studied welfare indicators remained invariable ($P \geq 0.05$) as shown in Tables 1 and 2. Nonetheless, the observer effect was found for lameness ($P = 0.0083$), small size ($P = 0.0036$), dirtiness ($P = 0.0001$), sick ($P = 0.0103$), terminally ill ($P = 0.0433$), aggression towards mate ($P = 0.0029$) and interaction with humans ($P < 0.0001$) variables. In addition, the effect of the interaction observer by sex was detected, solely, for wounds indicator ($P = 0.0021$) as demonstrated in Table 1.

Welfare Profile.

The mean values (± SE) of incidence of each single welfare and health indicator is expressed in percentage (%) are presented in Table 3. Overall, the analysis showed that there was a lack of differences ($P \geq 0.05$) for almost all studied variables across male and female farms in the assessment. However, as is apparent from the results, considerable significant differences across turkey farms to immobility, lameness, wounds, and dirtiness indicators were detected (Tables 1 and 4).

DISCUSSION

One of the aims of this study was to verify the repeatability and the feasibility of the new method approach for welfare assessment in turkey commercial system. This new welfare approach is based on the transect walks applied in wild life (Gates et al., 1968; Buckland et al., 2010) merged with the concept that bird caretakers checks the health status of birds routinely walking in the entire house. Transect walks applied in welfare assessment in poultry production is considered as being a new scientifically approach that plays a key role for the short and long-term sustainability of the production (Marchewka et al. 2013). Additionally, this method does not disturb the birds, and no animal handling is necessary to evaluate a massive number of turkeys/flock. It requires only one observer to perform the complete protocol with less time-consuming and economically acceptable, without high additional expenses than previously developed welfare protocols. In addition to the advantages, the method is readily acceptable and applicable by producers.

This study was carried out in 8 male and 6 female turkey farms, a total of 25 flocks (15 and 10 flocks, respectively), by two observers in at least paired houses. The birds were genetically identical (B.U.T. - Big 6) with similar age (122 to 138 d and 90 to 103 d) among male and female groups, respectively. It was assumed that birds had a homogeneous distribution in the house with similar management practices across the farms.

Overall, by adopting the transect walks approach in a complete turkey flock, the length of time lapse ranged from 40 min to 1 h 30 min by conducting 4 randomly transects depending on the length dimensions of the house, quantity of birds and the welfare and health status of the flock.

Under the condition of this study, these results indicated that transect walk method was sensitive and allows variation within farms by considering male and female groups independently. With respect to the traits measured at these studied turkey farms, the incidence of the used welfare variables, such as, immobility, lameness, wounds, and dirtiness were detected statistically different across farms. These are considered important and critical indicators for assessing the welfare status of the birds. The behavioral indicators remained constant under this conditions (Table 1). In this context, these results lead to infer that it would be reasonable to perform that transect walks at male and female turkey farms and that this new approach could be theoretically sufficient to demonstrate the welfare status of the turkeys by assessing each flock.

Regarding the effect of the observers outcomes by assessing the welfare adopting the transect walks; it was observed that the evaluation remained constant for almost all indicators at evaluated farms. Nonetheless, minor differences were found for the incidence of lameness, small size, dirtiness, sick, terminally ill, aggression towards mate, and interaction with humans (Table 1 and 2).

The differences across observers of for the incidence of aggression towards mate at farms ranged between 0 and 0.02 ± 0.01 (Table 2). Surprisingly, taking into account that this assessment was done to evaluate thousands of bird/flock in a randomly procedure of collect the data, only minor differences across observers were found. Likewise, these low incidences of small size, sick and terminally ill birds were also detected as shown in Tables 1 and 2. It might be possible that the farmers' intervention during the transect walks between the data collection of the two observers, could explain why there was a significant difference from observers. Often, bird caretakers pick up small, sick, and terminally ill birds and allocate them at the nursery area inside the house at turkey farms in Italy (Table 1). This is a well-known protocol adopted by farmers of the studied poultry industry.

The differences across observers for the incidence of dirtiness ranged between 0.18 ± 0.02 and 0.10 ± 0.02 at turkeys flocks (Table 2). Correspondingly, similar finding about the effect of observer for the incidence of dirtiness was described at broiler farm (Marchewka et al., 2013). This might have led to a subjectively evaluation of this indicator during the collection of data from turkeys farms when the dirty feathers were mistaken by not measuring indirectly the quality and characteristics of the litter, but only the dust and manure attached. However, it also could be that walking in different day time to perform the transect walks, the intensity of natural luminosity differed in each house, changing the visual perception of the observer regarding this welfare indicator assessment.

The effect of observer was detected for interaction with human indicator (Tables 1 and 2), which might be associated with the daily routine checks performed by the bird caretakers and the height of the observers in this study. The observer 1 was a male with 1.92 m tall, while the observer 2 was a female with 1.70 m tall. The hypothesis for finding the influence of the observer by recording this indicator is that observer with high height could have influenced the visual perception of the birds. Behavior is triggered mainly by external stimuli (Duncan, 1998) and might be that the taller assessor was perceived as a predator; therefore, less interaction was recorded. On the other hand, birds could have been used to this kind of exposition while the walks were performed by a medium-height observer, who had similar height of the routine bird caretakers. In addition, the birds react according to their previously experience. When the farmer conducted the routine daily checks, s/he always starts her/his activity near to the entrance door, which corresponds to transects 1 and 2, closest to the door. This management procedure let the birds used to this kind of approach which could explain partially why these birds were more interactive with human presence than the most distance birds. In this context, when turkeys were controlled in a daily basis by the farmers, they were in a sort of continuously practices by getting used to this kind of human-approach and to these specific management procedures. Furthermore, it is also important to consider the interaction with human indicator as an exploratory behavior. These are likely possibilities that should be further investigated.

Interestingly, the results presented in Table 1 showed the effect of observer was detected at fattened turkey farms for lameness indicator. It might be assumed that this finding might be correlated to the perception of the observer to assess lame birds which in such way allowed a matter of subjectivity. This could be attributable to the fact that lames birds struggle to walk and stand in different severe degrees and in a range of different scores. Therefore, not only were birds with severe lameness recorded, but birds also, with less evidence of this problem could be seen and registered to the data collection table. The fact that the effect of observer was detected for this important welfare indicator, suggests the importance of an accurate training to enlighten and highlight the minimal differences, which could be found at on-farm level, as being an essential requirement for performing this new method for turkey welfare evaluation. Another hypothesis could be drawn that the density number of male birds per m^2 is half than female turkeys, which implies double number of female birds per m^2. Under this circumstance, to observer lame birds in male might be easier than female turkeys. Only with further studies, these assumptions could be confirmed. With further investigation by adopting co-related studies with transect walks and Cartesian distances (with location data collection measuring distances from the assessor position to the front and lateral walls), the explanation for these assumptions could be achieved in the modern poultry production and the outcomes could reveal why these findings were obtained in this current study.

Overall, the results obtained by transect walks showed that male and female turkeys had substantially difference of welfare concerns by considering the welfare and health indicators in this study as demonstrated in Table 3.

It is clear from the results that male turkeys were much more affected by immobility (0.14 ± 0.02 and 0.02 ± 0.00; P = <0.0001) and lameness (9.06 ± 0.41 and 4.34 ± 0.20; P = <0.0001) than female birds, respectively (Table 3). The values obtained in this study were much higher upper range when compare with another studies in broiler flocks. For instance, using the methodology developed by Kestin et al., (1992), Knowles et al., (2008) found that only 0.2% of immobile birds, Sørensen et al., (2000) observed mean of 0.3 and 2.7% of severe lame birds for 28 and 42 days old. Likewise, Dawkins et al., (2004) detected 0.9% with severe locomotory problem using a non-invasive method. The differences observed in turkey and broiler birds might be caused by having different genetic background, by being different species, by the older age of the birds, and by rearing heavier birds (Kestin et al., 1999; Bradshaw et al., 2002; Knowles et al., 2008). For instance, at the end of the turkey cycle production, male birds achieve an average weight of 20 Kg in 140 days and female of 9 Kg in 100 days old, according to the commercial strategic preference of the company and the demands of the consumers. The fact that adult male turkeys have large body weight than female birds, could led to high incidence of degenerative hip disorders which may cause pain or discomfort complying the behavioral activities. In other words, the birds spent less time standing, walking and few steps were noticed (Duncan et al., 1991; Buchwalder and Huber-Eicher, 2005), increasing the lying times and hence increasing the prevalence of breast buttons and blisters (Mitterer-Istyagin et al., 2011). These very problematic situations cause worrisome economic losses and severe implications to the protection of fattened turkeys (Kamyab, 2001; Krautwald-Junghanns et al., 2009).

Wounds, dirtiness were highly significance effect of sex ($P < 0.0001$) as presented in Tables 1 and 3. In this regard, the effect of sex at these welfare indicators assessment, the duration of the cycle production would be one of the causes. Considering that male fattening turkeys stayed about 40 days more in the rearing system than female birds, there is a loss of litter quality, hence higher possibility of observer dirt feathers. Moreover, male turkey as being heavier than female might cause much more severe lesions to their mates, as well as more time to develop diseases.

Conversely, female turkeys tent to had higher incidence interaction with humans as provided in Table 3, although it was not observed the deference of means statistically ($P = 0.429$). A possible cause for showing this tendency by being more interactive with humans might be that female turkeys seemed to be more curious and willing to explore the environment rather than male as observed in this study, as well as for the reasons described previously. Not even one episode of mating was observed at female farms, which emphasis that this behavior may occur only in male farms due to sexing mistake selection at the hatchery (Table 3). Considering that meat chickens usually reach within 6 weeks the slaughter age, and it is known that the sexual maturity occurs about 18 weeks, it might be imply that mating as behavior indicator is rarely seen also at broiler flock production.

Taking into account the results herein reported (Tables 2 and 3), this study supports another research about transect walks applied in hybrid male turkey flocks by Marchewka et al., (2014). Similar findings were pointed out for sick (0.05%), terminally ill (0.03%) and behavioral indicators, such as, aggression towards mate (0.002%), interaction with humans (0.31%) and mating (0.02%). However, in the same study, immobility (0.60%), small (0.59%) and dead (0.14%) birds had higher incidence of almost 10 times more, surprisingly. On the other hand, lameness (2.36%), wounds (1.43%), featherless (0.04%), and dirtiness (0.07%) were considered less than the [B.U.T.] - Big 6 turkey flocks from this current report. Under this condition, it suggests that birds varying in welfare status are directly related to the turkey strains and management standard protocols.

CONCLUSION

In summary, the conclusions may be drawn from these results that transect walks method was proven to be a feasible and repeatable to assess the welfare and health status of turkey flocks at commercial system, as well as being acceptable in term of time requirements and personnel demands.

The currently results herein might be used for setting value of welfare indicators by giving the status of the bird welfare and health and establishing a database with these information for monitoring the welfare status of turkeys flocks at the end of the production cycle from commercial rearing conditions. In fact, this study reports the first turkey welfare profile within European commercial facilities by adopting transect walks method as welfare assessment protocol. It is highly relevant to take this into consideration to improve the current situation of turkey production by proposing management recommendations for the practical farmer about how to prevent welfare and health treats based on this recording data. Thus, this methodology envisions a complete assessment for welfare concern in turkey commercial production in a meaningful and easy system, as well as being a practical tool for management targets. Finally, further research is required to investigate and worth to pursuit if there may have any positive correlation with economic impact for the producers and the industry by adopting the transect walks approach and its outcome findings from this new welfare assessment protocol at turkeys flocks. This raises an interesting question of how results from this new method could be compared with data collected at the slaughterhouse and how fair payments could be made to a farmer where birds have a better level of welfare measured by these direct animal-based indicators included in the transect walks method. Furthermore, recording continuously the data may likely to be highly effective monitoring to the welfare and health status of the birds with historical and current information during whole production cycle; thus, transect walks methodology is foreseen to be an important and potential asset tool for decision-making process, such as, control strategies or managements changes to be successfully implemented in the meat poultry industries.

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Table 1. Effect of observer, sex, and the interaction of observer with sex for all scored welfare indicators collected during the transects at turkey farms

Welfare indicator	ANOVA component		
	Observer	Sex	Observer X sex
Immobility	0.1465	<0.0001	0.4832
Lameness	0.0083	<0.0001	0.6823
Wounds	0.0615	<0.0001	0.0021
Small	0.0063	0.5931	0.776
Featherless	0.2939	0.5315	0.7012
Dirtiness	0.0001	<0.0001	0.8842
Sick	0.0103	0.8523	0.7128
Terminally ill	0.0433	0.9225	0.5013
Dead	0.173	0.6337	0.546
Aggression towards mate	0.0029	0.1376	0.0778
Interaction with humans	<0.0001	0.4292	0.7545
Mating	0.164	0.0856	0.164

Table 2. Means values (± SE) of incidence of turkeys within each welfare indicator expressed as percentages for each observer and the overall values

Welfare Indicator	Overall	Observer	
		1	2
Immobility	0.0873% ± 0.0101%	0.1044% ± 0.0154%	0.0694% ± 0.0128%
Lameness	7.0293% ± 0.2932%	7.5398% ± 0.4460%	6.4954% ± 0.3723%
Wounds	2.6116% ± 0.1358%	2.8772% ± 0.2220%	2.334% ± 0.1488%
Small	0.0434% ± 0.0070%	0.0585% ± 0.0107%	0.0275% ± 0.0084%
Featherless	4.016% ± 0.2740%	4.3699% ± 0.4656%	3.6456% ± 0.2758%
Dirtiness	0.1449% ± 0.0121%	0.1832% ± 0.0180%	0.1048% ± 0.0152%
Sick	0.0751% ± 0.0104%	0.0962% ± 0.0168%	0.0529% ± 0.0116%
Terminally ill	0.0100% ± 0.0030%	0.0142% ± 0.0045%	0.0055% ± 0.0039%
Dead	0.0125% ± 0.0027%	0.0154% ± 0.0040%	0.0094% ± 0.0037%
Aggression towards mate	0.0102% ± 0.0034%	0.0199% ± 0.0065%	0
Interaction with humans	0.1770% ± 0.0249%	0.2854% ± 0.0440%	0.0637% ± 0.0157%
Mating	0.0041% ± 0.0017%	0.0070% ± 0.0032%	0.0011% ± 0.0011%

Table 3. The overall mean values (±SE) of turkeys within each welfare indicator expressed as percentages for male and female farms obtained by transect walks

Welfare indicator	Male	Female
	Mean ± SE	Mean ± SE
Immobility	0.1369% ± 0.0160%^{A}	0.0218% ± 0.0051%^{B}
Lameness	9.0634% ± 0.4095%^{A}	4.3384% ± 0.1964%^{B}
Wounds	3.5416% ± 0.1899%^{A}	1.3814% ± 0.0936%^{B}
Small	0.0472% ± 0.0108%	0.0382% ± 0.0073%
Featherless	4.3998% ± 0.4374%	3.5079% ± 0.2593%
Dirtiness	0.2019% ± 0.0176%^{A}	0.0695% ± 0.0119%^{B}
Sick	0.0878% ± 0.0158%	0.0582% ± 0.0119%
Terminally ill	0.0123% ± 0.0049%	0.0069% ± 0.0026%
Dead	0.0124% ± 0.0040%	0.0126% ± 0.0035%
Aggression towards mate	0.0157% ± 0.0057%	0.0029% ± 0.0022%
Interaction with humans	0.1813% ± 0.0380%	0.1714% ± 0.0287%
Mating	0.0072% ± 0.0030%	0

Differences for each specific measure across sex are indicated by superscripts letters; means in the same row lacking a common letter (A-B) significantly differ ($P \leq 0.0001$).

Figure 1. Design of the transect walks of 3.5 m within a 14-m-wide production room. The double lines shows: (blue) walls and (yellow) lines of feeders and drinkers. The red dashed lines show the walking pathways along which transect walks were conducted.

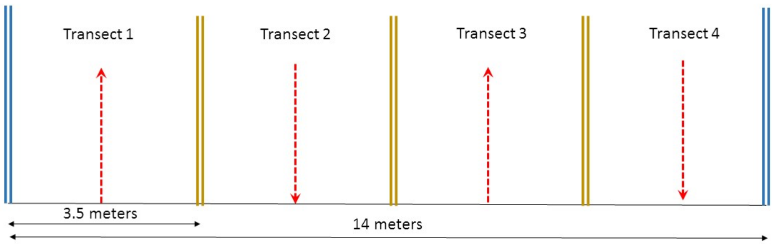

Figure 2. Data collection of welfare assessment during transects walks applied in turkey farm. Observer walking slowly trough the transect band during data collection to reduce the disturbance to the flock. The transects are limited by feeder (left) and drinker lines (right).

GENERAL CONCLUSIONS

In conclusion, this study highlights some important and complex features of turkey welfare at intensive production as well as the trials to accomplish the appropriate assessment implementation. It is likely to be assumed that the transect walks approach aiming the welfare assessment on turkeys is a valid, reliable and feasible welfare protocol on-farm level towards no incensement of production costs. This new scientifically approach plays a key role for the three important goals for the long-term sustainability of poultry production: (i) establish a common agreement between industries, (ii) the interest of the consumers, and (iii) guarantee the well-being of the birds. Additionally, this method does not disturb the birds, and animal handling is not necessary to evaluate a massive number of turkeys/flock. It requires only one observer to perform the complete protocol with less time-consuming and economically acceptable, without high additional expenses than previously developed welfare protocols. The method has the advantage as being readily acceptable and applicable by producers. Thus, this methodology envisions a complete assessment for welfare concern in turkey commercial production in a meaningful and easy system, as well as being a practical tool for management targets.

Specific, effective, rigorous and competence on-going training programs about transect walks approach should be adopt by the stakeholder worldwide, to disseminate, teach, brush up on existing skills, and standardize the data collection from a methodological point of view to achieve high inter-observer reliability. In addition, this will give the integration of outreached research with the targeted community promoting effective exchange of the knowledge,

Immobility, lameness, dirtiness, small size, wounds, featherless, sick, terminally ill, and dead are, indeed, the most clearly identifiable promising animal-based indicators focused on meat poultry welfare problems that have welfare and economic impact. In addition, aggression towards mates, interaction with humans and mating seemed to be considered feasible indicators in behavioral assessment.

However, further investigations are necessary and worthy to pursuit to foster a common understanding addressed on animal-based indicators emphasized in pain assessment and recognition on turkeys at individual and flock levels, in order to fill the gaps that still exist regarding these issues with important and essential additional information. In this context, the improvement and development for more outright on-farm welfare assessment protocols would be achieved successfully, guaranteeing the well-being of the birds and ensuring the concerns of the industry, farmers and consumers.

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