Differences in prevalence of welfare indicators in male and female turkey flocks (*Meleagris gallopavo*)

Valentina Ferrante,[*] Susanna Lolli,[*,1] Lorenzo Ferrari,[*] Tatiane Terumi Negrão Watanabe,[†] Carlo Tremolada,[‡] Joanna Marchewka,[§] and Inma Estevez[#,¶]

[*]*Department of Environmental Science and Policy, Università degli Studi di Milano, Via G. Celoria 10, 20133 Milan, Italy;* [†]*Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana Animal Disease Diasgnostic Laboratory and Louisiana State University, 1909 Skip Bertman Drive, Baton Rouge, LA 70803-2804, USA;* [‡]*Department of Veterinary Medicine, Università degli Studi di Milano, Via G. Celoria 10, 20133 Milan, Italy;* [§]*Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, 05-552 Magdalenka, Poland;* [#]*Neiker-Tecnalia, Arkaute Agrifood Campus, Animal Production P.O. Box 46, E-01080 Vitoria-Gasteiz, Spain; and* [¶]*IKERBASQUE, Basque Foundation for Science, Maria Diaz de Haro 3, 48013 Bilbao, Spain*

ABSTRACT Previous research has shown that the transect walks (**TW**) method provide a practical and effective approach to welfare assessment in broiler and turkey farms. This method for turkey welfare assessment is reasonable in terms of time demands within minimal costs. Furthermore, TW approach resembles the routine checks used by farmers. The overall aim of this study was to verify the feasibility of the TW method as potential practical tool for on-farm welfare assessment in turkeys during the fattening period. A total of 14 commercial turkey farms (8 male and 6 female flocks) of the same genetic strain (British United Turkeys [B.U.T.] - Big 6) with similar management standard procedures were evaluated. Bird ages at evaluation ranged from 122 to 138 D and 90 to 103 D old, for males and females, respectively. Two independent assessors walked slowly on randomized longitudinal paths (transects) within each house, while recording the prevalence of birds showing any of the 12 welfare and health indicators considered: 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 assessor, gender, and interaction assessor by gender was evaluated by using ANOVA. Reliability of the method was noted by the effect of gender ($P < 0.001$) for immobility, lameness, wounds, and dirtiness indicators. Male flocks showed higher prevalence of immobility (0.035±0.004% vs. 0.004±0.001%), lameness (2.269±0.108% vs. 1.253±0.051%), wounds (0.288±0.014% vs. 0.127±0.009%), and dirtiness (0.050±0.004% vs. 0.022±0.004%) as compared to female flocks. Differences among assessors were relatively minor, with differences detected only for back and tail wounds, dirtiness, aggression towards mate, and interaction with humans. This study reports advantages and limitations of this method for welfare assessment on-commercial turkey flocks and it is the first description of the Italian welfare profile of turkey's commercial flocks.

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

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INTRODUCTION

Consumers currently demand livestock and poultry products originated from animals raised under optimum welfare conditions (Bartussek, 1999; Special EUROBAROMETER, 2016). In addition, an increasing number of farmers recognize the importance of full compliance with animal welfare standards that can play an important economic role in commercial intensive productions.

Animal welfare assessment protocols provide the bases for legal verification at the farm level in order to promote and guarantee high animal welfare standards. The classic indicators that have been used on-farm to assess the welfare of animals can be divided into two major groups (Bartussek, 1997; Hörning, 2001; Main et al., 2003): (i) resourced-based measurements which include, parameters describing the influence of the housing system and management practices on animal welfare, and (ii) animal-based indicators such as behavior, health, and physiological traits (EFSA, 2012). Although resource-based indicators are important, they are considered to be an indirect measurement of animal welfare. Recording animals' reactions to the specific features of the environment is consider more direct, since

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[1]Corresponding author: susanna.lolli@unimi.it

it relates to the state of the animal itself (Sandøe et al., 1997; Mollenhorst et al., 2005; EFSA, 2015). As indicated by Broom (1996), welfare refers to the state of the animal rather than to the level of resources provided. Thus, the assessment of animal-based indicators is considered a more direct assessment of their real welfare state. For this reason, a validated, reliable, and feasible assessment protocol is needed in order to be able to assess the influence of complex, sometimes crucial, factors which can have negative effects on poultry welfare (e.g. genetics, husbandry, housing and management), bird performance, and post slaughter product quality (Winckler et al., 2003). In addition, welfare assessment should be practical in order to be applied to a wide variety of production systems while ensuring a certain required standard for animal welfare (Bartussek, 1999).

Several indicators are known to be intimately related to bird welfare, and are highly relevant for the farm economic returns and food safety (FAWC, 2011). For example, breast skin lesions (Kamyab, 2001; Mitterer-Istyagin et al., 2011), hock burns (WQ®, 2009), and foot pad dermatitis (Krautwald-Junghanns et al., 2009, 2011) are reliable indicators of bird welfare (Haslam et al., 2007) but are also major post-mortem indicators of the impact of housing conditions on birds´ health. These indicators show substantial reduction on turkey welfare status associated with a significant economic loss due to increased culling on farm, downgrades and condemnations at processing.

The transect method has been used since last Century in wild animal populations studies (Gates et al., 1968; Buckland, 1985; Buckland et al., 2010) and Bright et al., (2006) used this method for assessing plumage conditions in laying hens. Transect walks (**TW**) for welfare assessment was already tested in commercial broiler and turkey flocks (Marchewka et al., 2013, 2015). In particular, the results for turkey (Marchewka et al., 2015) supports the idea of the TW as a reliable, practical, efficient, and easy to apply method for on-farm assessment of turkey welfare. Besides the advantages of this new approach regarding its reasonable costs, less time consuming and minimally invasive for the birds, the method 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 (Marchewka et al., 2013, 2015). Furthermore, a major advantage is that the method is non-invasive and does not involved bird manipulation, which would be a major challenge in turkey rearing.

The aim of this study was to verify if TW method could be an appropriate approach to assess differences in prevalence on welfare indicators among male and female turkey flocks. Moreover, this study aimed to assess inter-observer reliability and feasibility of TW for sampling of both turkey genders and assessing their welfare during fattening period in Italian management conditions.

MATERIALS AND METHODS

Facilities and Birds

This study was conducted on 14 turkey flocks (8 males and 6 females), that were raised under similar standard management practices. The farms were located in the Lombardia and Veneto regions, in Northeast Italy. Two flocks per farm were included in this study. The size of the flocks ranged from 3,100 to 10,558 beak-trimmed females (90 to 103 D of age) and from 2,250 to 4,000 beak-trimmed males (122 to 138 D of age). All birds were of the same strain (British United Turkeys [B.U.T.] - Big 6) and were reared at a density of 6.0 to 6.3 female/m^2 and 2.7 to 4.1 males/m^2. All flocks had similar management except for the litter substrates: 12 had wood shavings and husk, 1 wood shavings with chopped straw, and 1 only wood shavings. Automatic feeders, drinkers, and ventilation systems were present in all houses. The data collection was 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).

Transect Walks

The houses used in this study were rectangular, 14 m wide and variable length ranging from 70 to 120 m. Each house was divided into 4 longitudinal transects (3.5 m wide paths) covering the length of the house and were delimited by the feeder and drinker lines (Figure 1). All paths were assessed and were numbered from 1 to 4. Two previously trained assessors on the data collection method and welfare assessment of the selected indicators, evaluated paired houses (within the farms). The observations of the assessors were conducted sequentially and independently within the same day. Data collection was performed by walking through the predefined transect paths (1 to 4) in random order, in both directions, starting from the entrance wall and alternating the starting point for each transect. The assessors walked slowly while recorded the number of birds showing any of the welfare indicators according to AWIN, (2015; Marchewka et al., 2015) in a spreadsheet (Polaris Office, Infraware, Seoul, South Korea) installed in a handheld tablet (Samsung Galaxy Tab2 10.1, GT-P5110 Android 4.2.2, Seoul, South Korea). All flock assessments took place at the end of the production cycle, approximately 1 wk before slaughter. Definition for each parameter assessed is included in Table 1.

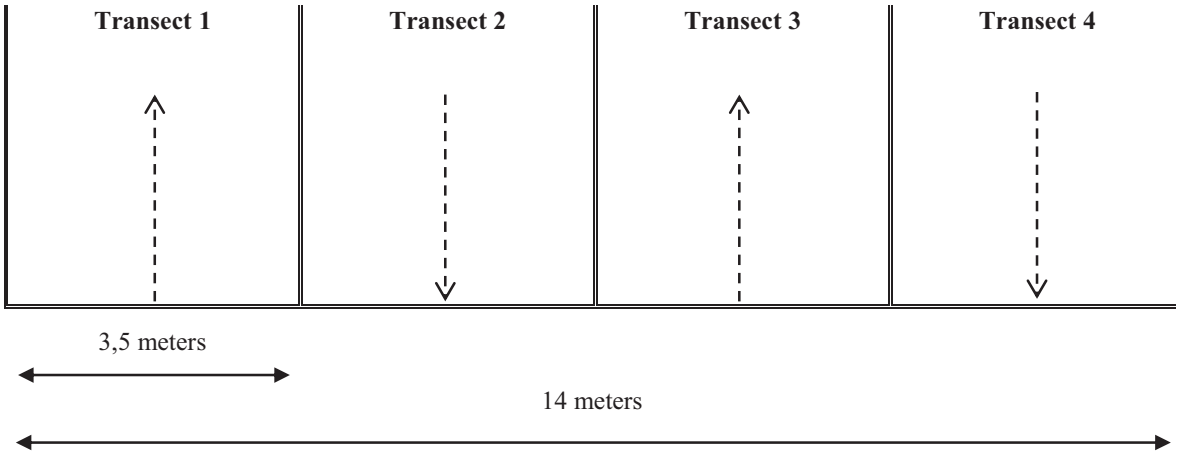

Figure 1. Design of the transect walks of 3.5 m within a 14-m-wide production house. The solid lines show: walls and/or lines of feeders and drinkers. The dashed lines indicate the walking paths along which transect walks were conducted.

Statistical Analysis

The frequencies detected for each welfare indicator were transformed into proportions per transect according to the expected number of birds per transect, assuming that the birds were randomly distributed in the house. All variables were transformed into arc sin square root to meet to normality and homogeneity of residual variance. The effect of assessor and turkey gender and their interaction was determined for all variables by means of a mixed model analysis of variance using the SPSS 2017 software (Version 24.0, IBM Corp. Armonk, NY). Farm was included in all models as a random factor. Least square means were computed in case of statistically significant effects ($P < 0.05$), with P-values adjusted for multiple comparisons by Tukey range tests.

RESULTS

The effect of assessors, turkey gender, and their interactions are summarized in Table 2. There was an effect of the interaction between assessors and gender for head/neck and total wounds. The differences across assessors were significant for back wounds; tail wounds were close to significance ($P = 0.0584$). Nevertheless, difference among assessors disappear when considering total wounds, as a sum of head, back and tail wounds (Table 2). The interaction among assessors and turkey gender was significant for head/neck wounds and total wounds (Table 2).

The mean values of almost all the considered variables were no different for the two assessors; back and tail wounds, dirtiness, aggression towards mate, and interaction with humans were significantly different for the assessors (Table 3).

The results showed clear differences between male and females flocks ($P < 0.0001$). The prevalence of immobility, lameness, wounds, and dirtiness showed a much higher occurrence in male as compared to female flocks (Table 4).

DISCUSSION

One of the aims of this study was to verify the feasibility of the new method approach for welfare assessment in Italian turkey commercial farms, and specifically to determine if differences such as those related to gender can be detected with this new method of assessment.

Under the conditions of this study, the results indicated that the transect walk was highly sensitive to detect differences in the prevalence of welfare indicators among male and female flocks. Male flocks, as expected, were more affected by immobility, lameness, head and tail wounds, and dirtiness than female flocks ($P < 0.0001$). These are considered critical indicators for assessing the welfare status of commercial turkeys.

The observed differences in gender might relate to the differences in age and weight of male and female flocks (Kestin et al., 1999; Bradshaw et al., 2002; Knowles et al., 2008). For instance, at the end of the production cycle, males may achieve 20 Kg average body weight at 140 D of age whereas females will reach 9 Kg average at 100 days of age. The fact that adult male turkeys have larger body weight than females, could led to higher prevalence of degenerative hip disorders that will result in a state of chronic pain and reduction of movements (Duncan et al., 1991).

Males spend less time standing or walking showing longer lying periods. This could lead to a higher presence of breast buttons and blisters, as observed in previous studies conducted at the slaughterhouse (Buchwalder and Huber-Eicher, 2005, Mitterer-Istyagin et al., 2011). In addition, male turkeys remain for an average of 40 D longer in the production facility as compared to females. This may cause a larger deterioration of the litter quality, hence increasing the chances for dirtiness and health problems. Moreover, being male

Table 1. Description of the welfare indicator categories. Birds meeting any of the descriptors within a category were counted as belonging to that category. Individual turkeys could be classified as belonging to more than one category (AWIN, 2015).

Indicator	Description	How to assess
Immobility	Turkeys are considered immobile when they do not make any attempt to move. Immobile turkeys may rest in an unnatural position with the legs extended to the front or sideways.	A turkey is considered immobile when approached by the observer or after slight encouragement with a stick does not move.
Lameness	Lame turkeys walk with obvious difficulty. One or both legs are not firmly placed on the ground.	A lame turkey moves away from the assessor but stops to rest after 2 to 3 steps.
Head/Neck wounds	Head wounds refer to all types of fresh or older injuries on the head area which are mostly the result of aggressive pecking by conspecifics.	Head wounds are visible signs of injuries on the head area related to acute or chronic wounds. Head area includes head, beak, snood, and neck.
Back wounds	Back and Tail wounds refer to all types of fresh or older injuries on back area.	Back wounds are visible acute or chronic lesions, including bleeding. Back area is between the end of the neck and the beginning of the tail.
Tail wounds		Tail wounds are visible wounds on the tail area, including acute, chronic and/or bleeding wounds. Tail area includes the vent.
Small size	Turkey is visibly smaller than the average of the flock.	A small turkey is approximately 1\2 the size of an average turkey in the flock.
Featherless	Turkey has one or more visible areas of missing feathers on the body	A turkey is considered featherless when has missing feathers on an extended area on the back, or back and wings.
Dirtiness	Plumage dirtiness may be correlated with hock burn, contact dermatitis and lameness for individual turkeys or may be associated with the environment and production system. Plumage dirtiness can be assessed as part of on farm inspections.	Dirtiness is a very clear and dark staining of the back, wing and or tail feathers, not including light discoloration of feathers from dust, covering at least 50% of the body.
Sick	The sick turkeys are usually found in a resting position, with the pendulous crop hanging in front of the breast or with missing body parts. Usually sick turkeys show clear signs of impaired health.	Sick turkey shows clear signs of impaired health, such as, small and pale snood, red and watery eyes, and occasionally unarranged feathering.
Terminally ill	A terminally ill turkeys cannot be cured and generally die; the situation of a terminally ill turkey is so severe that no treatment with help.	A terminally ill turkey lies on the ground with head resting on the ground or back, usually with half closed eyes with feeble breath.
Dead	There are several factors causing death in intensive turkey production. Mortality surely affects the industry and farm income, but it is also an important animal welfare concern.	During the Transect walks, each dead turkey is noted on the recording sheet or by using the i-WatchTurkey App[1] on a smartphone or tablet.
Aggression towards mate	The pecking behavior is normally used by turkey to establish a hierarchical organization. The so called "peck order" starts at an early age and will be established at different times according to the flock size and complexity. Among the causes of aggression towards mate are: high densities, insufficient space availability for feeding and drinking, and group size.	Aggression towards mate is a clear aggressive attack towards the head of another turkeys or chasing or pecking, including fights and leaps.
Mating	Sometimes, due to the mistake in the turkeys sexing at hatching, males and females are reared together and some toms can show mating behavior. This normal behavior can lead to lesions on the back of females due to the sexual dimorphism.	The tom makes an attempt or "sit" on top of a female.

[1]i-WatchTurkey App has been developed in order to facilitate assessment and evaluation of results. https://play.google.com/store/apps/details?id=com.daia.iwatchturkey

turkey heavier and more aggressive than females, the risk to cause severe lesions to their mates' increases (Marchewka et al., 2013). Lameness and other welfare indicators have obvious implications for the welfare of turkeys (Kamyab, 2001; Krautwald-Junghanns et al., 2009), but also have a serious impact on the economic revenue for the farmer. A clear relationship between the prevalence of lameness, immobility, and carcass quality was reported by Marchewka et al. (2015). They found a very high correlation between the prevalence of leg disorder and the prevalence of condemnations and other carcass quality indicators.

The results of the study showed a quite consistent agreement in most welfare indicators used in the study and independently assessed by two assessors over the several thousand birds that compose each of the observed flocks. These results concur with the reliability of the TW as already found by Marchewka et al. (2015). Nonetheless, minor differences were found for the prevalence of back and tail wounds, dirtiness, aggression towards mate, and interaction with humans. The differences across assessors for the prevalence of aggression towards mate ranged between 0 and 0.006. These values are very low if considering the assessment was done upon the evaluation of thousands of birds per flock in a random procedure of data collection.

An effect of the assessors was detected for the ´interaction with humans´ indicator, which might relate to the differences in height of the two assessors that collected the data in this study. Assessor one was 1.93 m

Table 2. ANOVA results with the effects of assessor, turkey gender, and their interactions for all scored welfare indicators.

		Analysis of variance factors		
Indicator		Assessor	Gender	Assessor*Gender
Immobility	F	1.0548	32.2516	0.3775
	P	*0.3057*	*<0.0001*	*0.5397*
Lameness	F	2.3070	51.6078	0.2689
	P	*0.1304*	*<0.0001*	*0.6046*
Head/Neck wounds	F	0.2107	72.6369	4.8308
	P	*0.6468*	*<0.0001*	*0.0292*
Back wounds	F	9.3460	3.1390	0.1533
	P	*0.0026*	*0.078*	*0.6959*
Tail wounds	F	3.6252	33.6008	1.3731
	P	*0.0584*	*<0.0001*	*0.2427*
Total wounds	F	0.0034	67.1698	4.2080
	P	*0.9535*	*<0.0001*	*0.0416*
Small size	F	1.4873	0.9945	0.0097
	P	*0.2241*	*0.32*	*0.9215*
Featherless	F	0.9896	0.2794	0.2500
	P	*0.3211*	*0.598*	*0.6177*
Dirtiness	F	8.2691	19.3069	0.1336
	P	*0.0045*	*<0.0001*	*0.7151*
Sick	F	2.8584	0.4836	0.0279
	P	*0.0925*	*0.488*	*0.8675*
Terminally ill	F	0.4459	0.1777	0.4669
	P	*0.5051*	*0.674*	*0.4953*
Dead	F	0.4570	0.6761	0.2436
	P	*0.4999*	*0.412*	*0.6222*
Aggression towards mate	F	6.9470	2.8197	2.8197
	P	*0.0091*	*0.095*	*0.0947*
Interaction with humans	F	19.7626	0.6542	0.0034
	P	*<0.0001*	*0.420*	*0.9538*
Mating	F	1.3571	2.9830	1.3571
	P	*0.2455*	*0.086*	*0.2455*

Table 3. Mean values (± SEM) for assessor 1 and 2 on the prevalence of each welfare indicator expressed as percentages.

	Assessor 1		Assessor 2		
Indicator	Mean	*SEM*	Mean	*SEM*	*p-value*
Immobility	0.026	*0.004*	0.020	*0.004*	*n.s.*
Lameness	1.991	*0.122*	1.759	*0.094*	*n.s.*
Head/Neck wounds	0.210	*0.015*	0.204	*0.014*	*n.s.*
Back wounds	0.041	*0.005*	0.065	*0.007*	*<0.01*
Tail wounds	0.467	*0.053*	0.336	*0.036*	*<0.05*
Total wounds	0.231	*0.017*	0.220	*0.015*	*n.s.*
Small size	0.013	*0.003*	0.008	*0.002*	*n.s.*
Featherless	1.094	*0.131*	0.924	*0.741*	*n.s.*
Dirtiness	0.049	*0.005*	0.030	*0.004*	*<0.01*
Sick	0.03	*0.005*	0.02	*0.003*	*n.s.*
Terminally ill	0.002	*0.001*	0.002	*0.001*	*n.s.*
Dead	0.000	*0.001*	0.000	*0.001*	*n.s.*
Aggression towards mate	0.006	*0.002*	0.000	*0.000*	*<0.01*
Interaction with humans	0.08	*0.013*	0.02	*0.004*	*<0.0001*
Mating	0.001	*0.001*	0.000	*0.000*	*n.s.*

tall male while assessor two was a 1.70 m female, and it is possible that the differences in height might have influenced the visual perception of the birds and their interactions with them. Behavior is triggered mainly by external stimuli (Duncan, 1998) and might be that the taller assessor was perceived more as a threat or might have triggered higher fearful responses on the birds. In addition, it is important to remark that the assessors were not observing the flock simultaneously and small variations of behavioral interactions might have occurred at different times, thus such difference may be a reflection of the differences in behavior of the birds. However, the consistency of our results may lead to infer that the TW method is a reliable method to perform welfare assessment in male and female commercial turkey flocks.

The current results herein might be used for setting values of reference in regards to welfare indicators for monitoring the welfare status of male and female commercial turkey flocks at the end of the production cycle. In fact, this is the first report on turkey welfare profile within Italian commercial facilities by adopting

Table 4. Mean values (±SEM) for male and female turkeys flock and welfare indicator expressed as percentages.

	Males		Females		
Indicator	Mean	*SEM*	Mean	*SEM*	*p-value*
Immobility	0.035	*0.004*	0.004	*0.001*	*<0.0001*
Lameness	2.269	*0.108*	1.253	*0.051*	*<0.0001*
Head/Neck wounds	0.264	*0.013*	0.111	*0.009*	*<0.0001*
Back wounds	0.059	*0.006*	0.043	*0.006*	*n.s.*
Tail wounds	0.538	*0.047*	0.186	*0.021*	*<0.0001*
Total wounds	0.288	*0.014*	0.127	*0.009*	*<0.0001*
Small size	0.012	*0.003*	0.008	*0.002*	*n.s.*
Featherless	1.041	*0.113*	0.959	*0.075*	*n.s.*
Dirtiness	0.050	*0.004*	0.022	*0.004*	*<0.0001*
Sick	0.020	*0.004*	0.020	*0.004*	*n.s.*
Terminally ill	0.002	*0.001*	0.002	*0.001*	*n.s.*
Dead	0.000	*0.001*	0.000	*0.001*	*n.s.*
Aggression towards mate	0.004	*0.002*	0.001	*0.001*	*n.s.*
Interaction with humans	0.040	*0.010*	0.060	*0.011*	*n.s.*
Mating	0.001	*0.001*	0.000	*0.000*	*n.s*

transect walks method as welfare assessment protocol. It is highly relevant to be able to provide a quantitative assessment of the welfare problems to be able to improve the current situation of turkey production. Knowing the precise situation in regard to welfare indicators can help to propose practical management recommendations to the farmer. Further research in the use of the TW may demonstrate the direct link between welfare indicators and economic impact.

CONCLUSION

This method allowed to quantify the differences in welfare status between male and female commercial turkey flocks. The TW seemed to provide a feasible method to assess the welfare of commercial turkey flocks. This study further support that the TW is a reliable and feasible method to assess the welfare of turkey flocks and it is acceptable in term of time requirements and personnel demands. The reliability was confirmed by the results of the evaluation carried out by different assessors.

The transect walks applied for on-farm poultry welfare assessment is considered a new scientific approach that might plays an important role for the short and long-term sustainability of the poultry production systems. Additionally, this method is minimally disrupting to the birds, and no animal handling is necessary to evaluate a massive number of turkeys per flock. It requires only one assessor to perform the complete protocol in about 60 min and it is economically acceptable. In addition to the advantages, the method is readily acceptable and applicable by producers.

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