Validated Broiler Welfare Measures Recommended to Researchers

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Meaghan Meyer, Graduate Research Assistant, Anna Johnson, Professor, Elizabeth Bobeck, Assistant Professor, Department of Animal Science, Iowa State University

Summary and Implications

Broiler welfare research has steadily increased to reflect consumers' and producers' concerns with the health and well-being of commercial flocks, as well as the increase in pledges to improve broiler welfare in the restaurant and grocery industries. Six hundred Ross 308 broilers, with a subset exposed to environmental enrichment, were used as an example flock to test welfare measures. Data associated with treatment outcomes are purposefully omitted with the intention to present a purely methodological report that may be used as a reference for poultry researchers. The methods presented here were validated in a research setting to further unite measures of broiler welfare, specifically in research, as they may be in some cases impractical for commercial producers or auditors to implement. Measures described here include video-recording broilers for behavior analysis, which may be too time-consuming and costly to use realistically in full-scale commercial barns, and other postmortem measures that require the use of legs that would commercially be harvested for human consumption. A companion report titled "Validated methods for producers to measure on-farm commercial broiler welfare" can be referenced for simplified measures recommended for commercial welfare audits on-farm. Thus, the methods validated here to measure broiler bird welfare are recommended for broiler research to measure the effects of treatments, environmental enrichment, or general bird welfare.

Introduction

A combination of animal (lameness, behavior), and resource- (feeder, water access) -based measures have been used in broiler research to quantify bird welfare. Understanding how animal-based measures can be used to effectively answer the research question is fundamental. For example, bird activity is an animal-based measure that could provide context to the effectiveness of an enrichment device, or a researcher could test how the addition of omega-3 to the diet affects lameness. A variety of methods used in the literature in poultry and other species, and one novel measure, were tested here to validate the usefulness of different animal-based methodologies in a research setting. Therefore, the objectives of this study were to provide methods and overall recommendations on six separate measures that could be used in a broad range of broiler research.

Materials and Methods

All animal procedures were overseen by the project Principle Investigator, Laboratory Animal Resources attending veterinarian, and the Iowa State University (ISU) Poultry Research and Teaching Farm manager. All protocols were approved by the Iowa State University Institutional Animal Care and Use Committee.

Animals and housing: Six-hundred straight run Ross 308 broiler chicks were transported from a commercial hatchery the day of hatch to the ISU Poultry Research and Teaching Farm where they were housed in 4 ft by 8 ft pens of 30 (1.07 ft²/bird) with *ad libitum* access to hanging poultry feeders and nipple water-lines for a 6-week grow-out. Birds were housed on fresh pine shavings. Five randomly selected "focal" birds from eight "camera" pens (four pens in each room of the barn) were wing-banded and marked with unique colors (red, blue, purple, green, and black) of animal-safe food coloring for identification on day 0 (n=40).

Behavioral recording: For the eight designated camera pens, a Sony HDR-CX440 Handycam (Sony Corp. of America, New York, New York) was positioned over the pen. Each pen was recorded for four 4-minutes periods beginning at 0530, 1130, 1730 and 2330 for the first 9 days of the trial and once weekly for the remaining weeks. Five undergraduate students were trained to analyze video clips using a pre-determined ethogram and walking distance methods (details below) to 90% agreeability by an individual with previous animal behavior experience.

Animal Based Measures:

Broiler behavior: Focal birds were scored using a predetermined behavior ethogram (Table 1). Bird activity was categorized into seven mutually exclusive behaviors including: inactive, active, at feeder, at drinker, preening, other, or out of camera view (Figure 1).

Walking distance: Walking distance in the home pen was measured using the same video recordings/days but a different randomly selected "focal bird" was used. The student observer used a clear sheet to draw a line with a marker each time the focal broiler moved forward during the 4-minute clip, then measured and summed all "walking lines" using the custom ruler tool on Adobe Photoshop (Adobe Systems Inc, San Jose, California). The tool was set to convert x number of pixels the bird walked (measured in a pen template image) to real-life inches, set to a standard length measured on-farm (nipple water-line).

Lameness scoring: Once weekly, the same focal birds each week (n=40) were removed from their home pens and assessed for walking lameness using a 0-2 scoring system. Zero indicated the ability to walk 5 ft with no signs of lameness, 1 indicated a bird able to walk 5 ft but stopped at least once or showed unevenness in gait, and 2 indicated a broiler not able to walk 5 ft. The lameness assessments were made in groups of five on week one and groups of two or three on weeks 2-6 on a custom-designed plywood walking lameness platform. The structure was 6 ft long by 1.5 ft wide with 1 ft high walls on all sides. 5 ft of walking space was delineated and there were 6 in start and stop sections. Once placed in the starting section, birds were encouraged to walk by (1) a researcher slowly moving their hand back and forth directly behind the bird (2) a researcher gently tapping the bird on their vent region with a gloved hand or (3) a researcher waving behind the bird or gently tapping it with a ping-pong paddle (Figure 2). Individual birds were considered to have completed the task when both feet had crossed into the end section.

Breast blisters and footpad dermatitis: A total of 40 birds (same that were assessed for lameness) were examined for breast blisters and footpad dermatitis once per week by the same researchers. Breast blisters were scored present/absent, with a blister considered present when it was equal to or larger than 0.197 in², when there were one or more breast burns, or when there were scabs on the breast. Footpad dermatitis was scored pass/fail using the American Association of Avian Pathologists (AAAP) Paw Scoring system where erosions, ulcerations, scabs, hemorrhages, and/or swelling on an area greater than one half of the footpad was considered a fail (Figure 3).

Bone quality: On day 42, the 40 focal birds used for lameness scoring and breast and feet examinations were euthanized using carbon dioxide, and the right tibia was collected from each bird and frozen (-20°C). Tibia were thawed overnight, then the following morning each were weighed and scanned using dual energy x-ray absorptiometry (DXA, Hologic, Marlborough, Massachusetts) for bone mineral density (g/cm²) and content (g).

Bone breaking strength of the tibia was measured using the tensile test and compression method on an Instron 3367 Universal Test Machine (Norwood, Massachusetts). The machine had a 30 kN load capacity and two platons controlled to fracture the tibia between them. Each tibia was kept individually in a plastic bag and wrapped in cheesecloth to prevent contamination of the platons or slippage of the bag. Tibia were placed on the lower platon with the lateral/medial condyle end of the bone intentionally placed out of reach of the upper platon and the bend of the bone facing down. The test was run so that the upper platon moved vertically downwards toward the bone at a rate of 10 mm/min and a 15% rate of load. The machine was stopped at the distinct decline in force (visualized on the monitor) and sound of the bone fracturing. Load (kgf) was recorded at the point of break and divided by area (cm²).

Air & litter quality: Ammonia (ppm) level in the air was measured in three separate pens (at the front, middle, and back of the barn) at bird height weekly using a handheld ammonia reader (GasAlert Extreme, BW Technologies, Schaumburg, Illinois) and ammonia test strips. The ammonia reader was titrated weekly with an ammonia tank and provided an exact value, and the strips provide a possible range in values of 5 ppm. Litter quality was likewise measured weekly in three additional pens by gathering litter from three sections of each pen and squeezing it in the hand. According to National Chicken Council (NCC) guidelines, to pass the litter must be "loosely compacted when squeezed in the hand. If the litter remains in a clump when squeezed in the hand, it is too wet".

Statistical analysis: Data in this example (not presented here) were checked for normality using PROC UNIVARIATE and analyzed using PROC GLIMMIX, a generalized linear mixed model, on SAS 9.4 due to most of the behavior data being abnormally distributed.

Results and Discussion

Broiler behavior: Broiler bird behavior has been thoroughly researched and established, and ethograms set up like the one described here have been successfully used to test treatment effects (dietary, environmental, diseasechallenge, etc.). However, the example used here is largely designed and simplified to categorize "active" vs "inactive" behavior rather than specifying several different forms of movement. In this way, the effects of different treatments on promoting or hindering broiler motion can be detected. However, if the researcher wishes to observe complex behavioral patterns, i.e., dust bathing or social interactions, then this ethogram would be less useful.

Recommendation: Creating active and inactive categories is strongly recommended to researchers when interested in physical movement. This measure could be fine-tuned even more by further simplifying the ethogram and only observing for either "in motion" or "not in motion" depending on the research question.

Walking distance: The novel walking distance measure proved to be very successful in quantifying the forward physical activity in inches walked. Since commercial broilers spend the majority of their time inactive in the later stages of production, and this sedentary behavior contributes to leg lameness, it is in the interest of researchers from a welfare standpoint to increase bird physical activity.

Recommendation: Using video recording and a trained observer, this measure can be used to detect differences in distance walked by broilers between treatments during the same exact time of day in different pens. Furthermore, in our research conditions, the birds were left to their own devices and not in human contact while being video-recorded, making this an interesting alternative for researchers.

Walking lameness: The unique walking lameness platform was successful in identifying lame birds that were unable to walk 5 ft without stopping (score 1), and most importantly, broilers who were unable to "follow the leader" on the walking platform and could not walk 5 ft at all (score 2). Broilers tended to flock together while on the platform, and if one "leader" bird could be motivated to walk, the other bird(s) would readily follow. The few lame birds we did assess were easy to identify because the motivation to follow was superseded by the inability to keep up with penmates due to lameness.

Recommendation: While fewer lame birds are seen in clean research conditions than in a commercial environment, lameness may be detected successfully by researchers conducting gait scores in broilers using a platform similar to the one described.

Breast blisters & footpad dermatitis: These conditions are less prevalent in a clean research environment with fresh litter than what occurs in commercial barns with a much higher bird turnover.

Recommendation: Thus, these methods are best applied to research conditions with re-used litter or in treatments aimed to specifically impact these conditions.

Air & litter quality: Environmental measures follow a similar pattern as breast blisters; in research conditions with a much lower turnover of birds and high cleanliness, these measures are less vital than they would be commercially.

Recommendation: However, they are recommended to researchers who plan to re-use bedding or expect treatments to affect litter moisture or ammonia levels in the air. The NCC recommendations to measure litter were easy to follow, and while the ammonia test strips were extremely easy to use, the handheld ammonia reader (titrated weekly with an ammonia tank) was more accurate and provided an exact value rather than a range.

Bone quality: The bone quality measures (DXA and bone breaking strength) were effective in detecting differences in the quality of the tibia. Bone mineral content is a measure of how much bone is present (correlated with weight of bone), while density reflects quality.

Recommendation: Changes in bone mineralization due to alterations in activity level provides the ability compare compression strength between treatments, thus using a compression test in bone breaking is recommended. Tibia quality reflects the overall leg condition of the broiler, and since leg abnormalities are arguably the biggest welfare concern broilers face, this measure is highly valuable to researchers.

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Measure ¹	Defined
Active	Bird legs were in a continuous forward motion (walking or running).
Inactive	Bird stood in one place or rested its abdomen on the litter, head rested or raised
	while any part of its body was or was not in contact with another bird.
At feeder	Bird head over feeder circle, bird in feeder or bird stood on feeder tray.
At drinker	Bird stood beneath drinker line.
Preening	Dust-bathed or head/beak twisted around in contact with feathers.
Other	Any behavior not otherwise identified.
Out of view	Bird was obstructed or not observed due to being under the heat lamp or inside the
	feeder and could not be seen.

Table 1. Ethogram for focal broiler bird home pen behavior

¹All behaviors were collected as frequency (the number of times the behavior occurred during 4-min periods) and duration (defined as length of time behavior was exhibited in seconds)

²Bird behavior was measured continuously during 4-minute periods

Figure 1. Subset of identified broiler bird home pen behavior from a video still including A) inactive, B) at feeder, C) at drinker, and D) preening. Behaviors not identified here include active, other, and out of view.



Figure 2. Five broiler chicks being scored for lameness on the custom platform during Week 1. Platform contained a 15 cm start (A) and stop (B) section, along with 1.5 m of walking space (C).



Figure 3. Adapted from American Association of Avian Pathologist's Broiler Paw Scoring Guide. FAIL (Score Criteria) PASS (Score Criteria)

- Normal color* and skin (*note, skin color may vary from yellow to white due to b
- Slight discoloration or darkened skin •
- Hyperkeratosis (thickening of skin) .
- Lesion covering less than 1/2 of foot pad



Pass (washed paws with no



Pass (paws with no cuticle and normal skin color)



Pass (washed, post-scald paws with scab covering less than 1/2 some color variation, healed the area of the foot pad)



skin and no ulcerations)

Pass (paws with no cuticle &

- Erosions, ulceration, or scab formation that covers more than 1/2 of foot pad and may include the toes
- Hemorrhages or swelling of foot pad



Fail (washed paws) Ulceration is present and lesion is more than 1/2 the area of the foot pad; lesions are also present on the toes



Fail (paws without cuticle) Ulceration is present and the lesion is more than 1/2 the area of the foot pad. Swelling of the foot pad is also visible.

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