Correlation between broiler lameness and anatomical measurements of bone using radiographical projections with assessments of consistency across and within radiographs

M. J. Toscano,*[1] M. A. F. Nasr,†[2] and B. Hothersall†

*ZTHZ, Division of Animal Welfare, VPH Institute, University of Bern, Burgerweg 22, Zollikofen 3052 Switzerland; and †School of Clinical Veterinary Science, University of Bristol, Langford House, Langford BS40 5DU, United Kingdom

ABSTRACT Lameness represents a major welfare and production issue in the poultry industry with a recent survey estimating 27% of birds lame and 3% unable to walk by 40 d of age. A variety of factors may induce lameness and are typically grouped into 2 broad classes on the basis of being infectious or skeletal in nature with the latter accounting for the majority of cases. The current work sought to build upon a large body of literature assessing the anatomical properties of bone in lame birds. Our specific objectives sought to identify relationships between relevant anatomical properties of the tibia and metatarsus using digital quantification from radiographs of legs and a measure of walking difficulty. Resulting output was statistically analyzed to assess 1) observer reliability for consistency in placing the leg during the radiograph procedure and quantification of the various measures within a radiograph, 2) the relationship between the various measurements of anatomical bone properties and sex, bird mass, and gait score, and 3) the relationship between each measurement and leg symmetry. Our anatomical bone measures were found to be reliable (intra-rater and test-retest reliabilities < 0.75) within radiograph for all measures and 8 of the 10 measures across radiographs. Several measures of bone properties in the tibia correlated to difficulty walking as measured by gait score ($P < 0.05$), indicating greater angulations with increasing lameness. Of the measures that manifested a gait score × bird mass interaction, heavier birds appeared to exhibit less angulation with increasing difficulty walking with lighter birds the opposite. These interactions suggest possibilities for influencing effects of activity or feed intake on bone mineralization with the bone angulation observed. Our efforts agree with that of others and indicate that angulation of the tibia may be related to lameness, though subsequent efforts involving comprehensive measures of bird activity, growth rates, and internal bone structure will be needed if the validity of the measures are to be accepted.

Key words: broiler, lameness, welfare, radiograph, tibia

2013 Poultry Science 92:2251–2258
http://dx.doi.org/10.3382/ps.2012-02904

INTRODUCTION

Lameness in broilers represents major welfare (Bessei, 2006) and production (Bradshaw et al., 2002) issues for the poultry industry as birds with severe forms of the condition are likely suffering from pain (McGeown et al., 1999; Danbury et al., 2000) and may have limited mobility, which reduces access to feed and water (Weeks et al., 2000; Knowles et al., 2008). Lameness remains a widespread problem with a recent survey of 176 commercial broiler flocks indicating more than 27% of birds lame and 3% unable to walk (Knowles et al., 2008) at a mean age of 40 d. The etiology of broiler lameness is unclear, though likely affected by a range of factors and their interactions, including genetic predispositions, growth rates, movement frequency, stocking density, and nutrition (Bradshaw et al., 2002). In general, the causes of lameness originate from infectious or skeletal factors, where the latter is viewed as being responsible for the majority of lameness seen in commercial flocks (Kestin et al., 1994; Bradshaw et al., 2002). Within the category of skeletal problems, there is a broad range of skeletal abnormalities with both individual and common pathologies (Cook, 2000) that appear to associate with the rapid growth rates of modern broilers (Julian, 1998, 2005). Walking ability has been related to specific anatomical bone properties using a categorical scale, although this required birds to be euthanized for examination (e.g., lesion size in tibial dyschondroplasiais; Riddell, 1975a, 1983), whereas other efforts assessing

Received November 12, 2012.
Accepted May 10, 2013.
[1]Corresponding author: Mj.toscano@bris.ac.uk
[2]Permanent address: Department of Animal Wealth Development, Faculty of Veterinary Medicine, Zagazig University, Sharkia, Egypt.

live birds were unable to demonstrate a relationship (Sorensen et al., 2000). Leterrier and Nys (1992) used several bone anatomical properties measured as continuous variables (i.e., femur angulation), which correlated to the appearance of valgus/varus, though they did not relate the measures to walking ability. The use of continuous measures to assess bone properties can provide several benefits to assessments if used appropriately, including improved resolution in identifying correlations, or independence from subjective gradings (e.g., severe, mild, and so on).

The objectives of the current experiment were to quantify various parameters of the metatarsal and tibia bones of broilers and their relation to gait score as an indication of walking ability. We hypothesized that bone angulation would be increased in birds manifesting poorer walking ability. We also compared symmetry between legs against gait score hypothesizing that poorer gait would relate to decreased symmetry (i.e., greater within-bird differences between legs). Tibiotarsus width and length were also measured to indicate gross changes in bone anatomy.

MATERIALS AND METHODS

Birds

All research was performed in accordance with appropriate UK Home Office approval (PPL: 30/2685). Twenty-seven (n = 27) standard-reared broilers of a commercial strain were reared at a local farm and brought to the University of Bristol's School of Veterinary Science campus at Langford at 31 d of age and housed in 2 groups of 12 and a single group of 3 (originally conceived to serve as spares). The birds were part of a larger study assessing the impact of the analgesic Butorphanol (trade name Alvegesic, manufactured by DechraVeterinary Products, Shropshire, UK, and administered as butorphanol tartrate) on naturally occurring lameness and pain sensation. Birds were selected using a gait score measure (described below) performed on farm to provide equal numbers of lame and nonlame birds. For the purposes of the present study, gait scores were reassessed 3 d after a single administration of drug to ensure the administration of analgesic did not affect gait scores for the purpose of the present study. Effects of Butorphanol are understood to last less than 8 h in poultry (Machin, 2005) and thus were unlikely to affect results of the present work.

All birds were individually labeled with commercial stock marker in a unique color and pattern arrangement to allow for identification. Pens measured 3.05 × 1.22 m, were provided wood shavings approximately 6 cm deep, and were checked daily by animal care staff who ensured ad libitum access to feed (the same provided on-farm) and water via open-top feeders and bell drinkers, respectively. Lights were maintained on a schedule of 12L:12D (~40 lx at ground level).

Gait Score Testing

All birds were assessed for gait score once at 35 d of age by 2 researchers experienced in assessments. One researcher gently encouraged the bird to walk the length of the pen while the other watched from outside. Both wrote down the gait score independently using the 5-point system described by Kestin et al. (1992) but with the inclusion of half-scores. In this system, 0 represents no difficulty walking with increasing values indicating increasing difficulties walking with a 5 associating with complete inability to walk. Scores were compared, and for any bird where the researchers were not in full agreement, additional observations of walking were made until the score was agreed.

Euthanasia

All birds were euthanized at 35 d of age with an intrahepatic injection of euthotal (approximately 1.5 mL/bird), weighed, and then hung by their necks for 12 h at room temperature to allow uniform development of rigor mortis. Both legs were labeled with an identifying tag and then removed from the bird, placed in plastic bags, and frozen at −20°C until radiographs were taken.

Radiographic Assessment

Radiographic assessments were performed to assess observer reliability in generating consistent measures from the same radiographic image (objective 1a) and repeated attempts to radiograph the same bone (objective 1b), the relationship of each parameter measured on the right leg with the individual birds' gait score (objective 2), and the relationship between each parameter and leg symmetry (objective 3). Measurements used in the current work were based on previous efforts and sought to identify curving of bones (directly and indirectly), which have been shown to be altered in birds manifesting lameness (Riddell, 1975b; Leterrier and Nys, 1992).

For the procedure, collected legs were thawed approximately 2 h before radiographs were taken and then placed on ice. Radiographs were made from 2 orientations for each leg (mediolateral and craniocaudal) by a single researcher and held in place using sandbags and foam cushions. Radiographs were taken by placing the leg on a digital cassette [24 × 30 cm Fujifilm (Type CC), Tokyo, Japan] positioned approximately 100 cm below a Xograph machine (Indico 100 RAD 50 kw, Georgetown, Ontario, Canada) after adjusting for the mediolateral (50 mV @ 5mAS) or craniocaudal (60 kV @ 6.3 mAS) orientations. Images were processed digitally (Fuji FCR Capsula XL II digital processor, Tokyo, Japan). For objectives 1a and 1b, 3 sets of radiographs were done for each orientation of the left leg of birds 1 through 12. To improve independence of repeated mea-

Table 1. Descriptions of measurements made on radiograph projections

No.	Reference	Full description
1a	CC-TibFibAngle	On a craniocaudal radiographic projection, a line was drawn from the most proximo-lateral aspect of the fibula to the most disto-lateral aspect of the tibia. A second line was drawn parallel with the distal articular surface of the tibia and the angle between these lines defined.
1b	CC-TibAngle	On a craniocaudal radiographic projection, a line was drawn from the most proximo-medial aspect of the tibia to the most disto-medial aspect of the tibia. A second line was drawn parallel with the distal articular surface of the tibia and the angle between these lines defined.
2	CC-TibCortDist	On a craniocaudal radiographic projection, a line was drawn between the most medial extent of the proximal and distal epiphyseal region of the tibia (line a-b). A second line was then drawn bisecting line a-b at a point corresponding to the largest distance between line a-b and the medial cortex of the tibial diaphysis.
3	CC-TibCortArea	On a craniocaudal radiographic projection, the area of the radiograph delineated by line a-b and the medial aspect of the tibial cortex.
4	MetAngle	On a craniocaudal radiographic projection of the metatarsal bone, a line was drawn bisecting the width of the proximal aspect of the bone and the trochlea for digit III. A second line was drawn parallel to the proximal articular surface of the metatarsal bone. The angle between these lines on the medial aspect was defined.
5	MetWidth	On a craniocaudal radiograph, the greatest width of the proximal metatarsal epiphyseal region as defined on a dorso-plantar radiograph.
6	ML-TibCortDist	On a mediolateral radiograph, a line was drawn from the most caudal aspects of the proximal and distal tibial epiphyses (line c-d). A second line was then drawn bisecting line c-d at a point corresponding to the largest distance between line c-d and the caudal cortex of the tibial diaphysis.
7	ML-TibCortArea	On a mediolateral radiographic projection of the tibia, the area of the radiograph delineated by line c-d and the caudal aspect of the tibial cortex.
8	ML-TibAngle	On a mediolateral radiographic projection of the tibia, lines were drawn at the widest point of the proximal and distal tibial epiphyses, respectively. The angle measurement tool was then used to define the angle between respective tibial epiphyses where increased angulation would reflect a greater angle in the dorsal direction.
9	ML-TibWidth	The width of the mid-diaphyseal region of the tibia orthogonal to the long axis of the bone at a mediolateral radiographic projection was measured.
10	ML-TibDist	The distance between the most proximal and distal extent of the tibia as defined on a mediolateral radiographic projection was measured.

sures on the same bone and reduce the influence of previous radiograph efforts of that bone (e.g., differences in the resulting angle between the x-ray projections and individual leg due to variations in positioning), radiograph projections for all bones were performed, generating a complete set before beginning the next set. Each radiograph image was assigned a unique code by a second researcher to ensure that subsequent digital assessment (performed by the first researcher) would be performed blind to bird identity. Both researchers were blind to bird-related data (e.g., gait score, sex, and weight) subsequently used for analysis.

Anatomical Quantification

Radiographic images were assessed by a single researcher using commercial software (VPACS, Visbion, Chertsey, UK) that allowed for digital quantification of angles, lengths, and areas of specified regions, which were then recorded to a database for collation of all data relative to individual birds. Eleven specific parameters (Table 1) were made based on a methodology adapted from Leterrier and Nys (1992). Representative images of the collected measurements are provided in Figure 1. To increase measurement consistency (for each parameter), each radiograph was measured in triplicate and a CV was calculated (SD divided by average × 100%). All measurements that exceeded a threshold of 5% were remeasured until compliant.

Statistical Analysis

Subsets from the larger data set were generated for specific analysis to assess intra-rater reliability (objective 1a; consistency within the same radiograph) and test-retest reliability (objective 1b; consistency across repeated radiographs of the same bone). For objective 1a, the subset consisted of the 3 measurements made on the first radiograph of the left leg for birds 1 through 12. For objective 1b, the subset consisted of the first of the triplicate measurements made on the first radiograph and the single measurement taken from the second and third radiographs for birds 1 through 12. From each subset, reliability measures were made based on methods described by Rousson et al. (2002). In brief, the model consisted of

$$Y_{ij} = \mu + s_i + r_i + \varepsilon_{ij},$$

where μ is a fixed parameter and s_i, r_i, and ε_{ij} are independent random effects for the subject, systematic (rater) error, and measurement error, respectively. Each is normally distributed with mean 0 and variances of σ_s^2, σ_r^2, and σ_ε^2. Estimates of intraclass correlations ρ_u and ρ_c were calculated (R_u and R_c, respectively) for intra-rater and test-retest reliability. Values above 0.75 are judged as being reliable (Lee et al., 1989). Code within SAS (v9, SAS Institute Inc., Cary, NC) was written to perform the calculations.

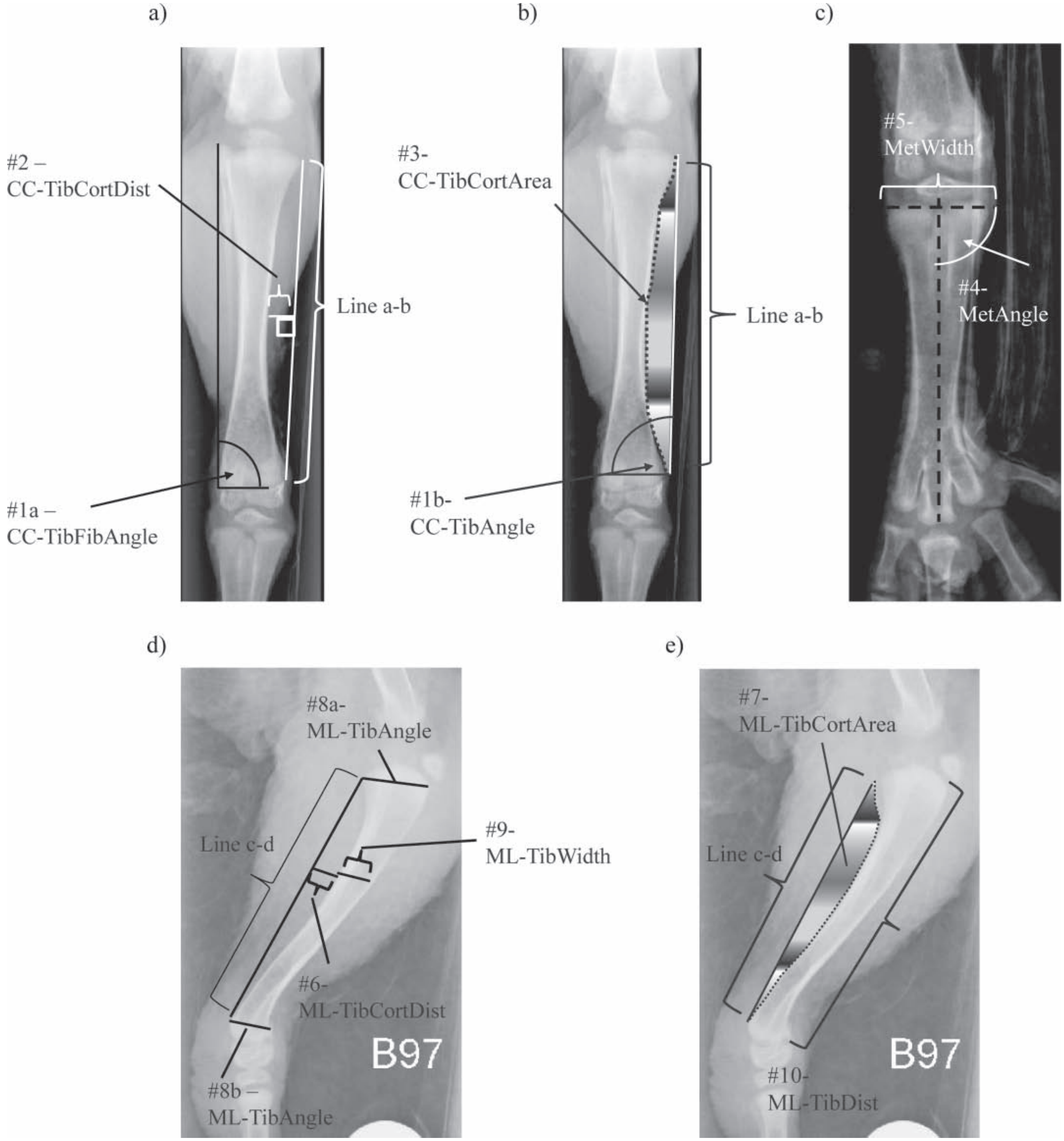

Figure 1. a–e) Visual descriptions of reference lines described in Table 1 and associated measurements.

For objective 2, analysis to assess correlations between the radiograph-derived measurements and gait score was performed with MlwiN (Rasbash et al., 2009), a statistical software package designed for data grouped in hierarchical structures. A random intercept model was constructed for each parameter where the triplicate measurements (i) were nested in the appropriate radiograph of each bird (j). The prediction variables, all fixed effects, included sex, gait score, mass, and the interaction of gait score and mass because bird mass is known to influence bone mineral content (Schreiweis et al., 2003). A separate model was constructed to directly identify the correlation between mass and gait score. All prediction variables were initially included in the model and then removed individually when presence of that model component was deemed ineffective ($P > 0.05$) in a backward step-wise manner by means of a χ^2-test on the deviance in log likelihood between the full and simplified models where the degrees of freedom was the difference in components or terms between the 2 models (Rasbash et al., 2009).

For objective 3, the triplicate measurements for each radiograph were averaged to generate a single value for each parameter. The difference between the left and right side for each bird was calculated and used as the response variable in a single level model with the same prediction variables and method to eliminate variables employed as with objective 2.

RESULTS

Objective 1

Analysis of intra-rater reliability indicated the consistency of the assessor was extremely reliable within radiographs with all measures exceeding the 0.75 threshold and the lowest being 0.86 (Table 2). The assessor also performed well in test-retest reliability where 8 of the 11 measures exceeded the reliability threshold indicating relative consistency in placement of the bone for radiograph imaging (Table 2).

Objective 2

All lame birds were found to be bilaterally lame. Gait scores, which ranged from 0 to 4, proved to be an effective predictor of several measures directly including measures 2 (CC-TibCortDist), 7 (ML-TibCortArea), and 9 (ML-TibWidth), as well as indirectly with interactions between gaitscore and mass (Table 3). Model responses where interaction effects where identified—measures 6 (ML-TibCortDist) and 8 (ML-TibAngle)—manifested similar values at low gait scores independent of bird mass. With increasing gait score, responses increased or decreased with relatively greater or lesser bird mass, respectively. The specific thresholds of mass for the change in the response was 2.26 and 2.14 kg for measures 6 (ML-TibCortDist) and 8 (ML-TibAngle), respectively. Sex effects were found for measures 1b (CC-TibAngle), 4 (MetAngle), and 5 (MetWidth) where each manifested greater values in males with the exception of measure 4 (MetAngle). Bird mass by itself (i.e., without a gait score interaction), affected measures 1a (CC-TibFibAngle), 2 (CC-TibCortDist), 5 (MetWidth), and 10 (ML-TibDist), where mass correlated positively with gait score with the exception of measure 2 (CC-TibCortDist). Weight as a response variable showed a positive correlation with gait score.

Objective 3

No response variables were found to be effective predictors for any of the leg symmetry measures (data not shown).

DISCUSSION

The current study was conducted to assess continuous measurements of bone anatomical properties that are likely related to broiler lameness. Our effort served 2 broad purposes, the first being to assess the reliability of an individual to consistently conduct measurements on bone from a radiograph as well as place the limb over repeated attempts, a broad objective that parallels similar efforts in various fields (Drinka, 2006; Dawoodi and Perera, 2012; Kose et al., 2012). The second purpose was to assess the relationship between the bone measurements and gait score in broilers.

Our first objective indicated a high level of reliability in scoring repeated measurements from a single radiograph, a success rate likely benefiting from the guidelines establishing where reference points were positioned and the ability to alter image resolution and scale to ensure their consistent placement. Despite the success found in the current work, additional study is warranted to demonstrate that the tested methods are superior to traditional measurements used by others (Leterrier and Nys, 1992) as digital quantification is not necessarily superior (Gstoettner et al., 2007). There did not appear to be a pattern of intra-rater reliability

Table 2. Results of intra-rater (objective 1a) and test-retest (objective 1b) reliability with associated MS for the various parameters measured from radiographs

	Objective 1a				Objective 1b		
	Intra-rater reliability				Test-retest reliability		
Reference	$MS_{subject}$	MS_{rater}	MS_{error}	R_u[1]	$MS_{subject}$	MS_{error}	R_c[1]
CC-TibFibAngle	2.979	0.096	0.093	0.912	2.671	0.244	0.768
CC-TibAngle	3.030	0.074	0.069	0.934	2.547	0.313	0.704
CC-TibCortDist	3.144	0.022	0.017	0.984	2.455	0.356	0.663
CC-TibCortArea	3.116	0.110	0.023	0.972	2.585	0.249	0.757
MetAngle	2.972	0.027	0.103	0.909	2.704	0.204	0.803
MetWidth	3.135	0.046	0.019	0.980	2.746	0.202	0.808
ML-TibCortDist	3.127	0.035	0.024	0.976	3.018	0.046	0.956
ML-TibCortArea	3.108	0.087	0.029	0.968	2.936	0.122	0.885
ML-TibAngle	3.175	0.000	0.003	0.997	2.440	0.362	0.657
ML-TibWidth	3.052	0.093	0.057	0.944	2.917	0.129	0.878
ML-TibDist	3.164	0.001	0.009	0.992	3.101	0.035	0.967

[1]R_u and R_c values above 0.75 are considered reliable.

Table 3. Model components that proved to be effective predictors for each of the quantified measures of the right leg as well as bird mass[1]

	Constant			Sex (male)		Mass		Gait score		Gait score × mass	
Reference	Estimate	SE	Variation (%)	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
CC-TibFibAngle	82.09	2.51	19.2			3.46**	1.31				
CC-TibAngle	76.89	0.82	21.7	3.361**	1.18						
CC-TibCortDist	11.51	1.60	27.9			−2.605**	0.92	0.57**	0.204		
CC-TibCortArea	407.11	14.26									
MetAngle	85.65	0.85	31.8	−4.670**	1.23						
MetWidth	15.35	1.55	66.8	1.403**	0.52	2.672**	0.9				
ML-TibCortDist	4.86	3.13	41.3			1.381	1.8	3.004	1.194	−1.284***	0.66
ML-TibCortArea	385.46	22.89	18.4					25.215***	10.16		
ML-TibAngle	−4.66	7.33	56.0			4.439	4.22	10.047	2.797	−4.434*	1.55
ML-TibWidth	6.56	0.22	15.3					0.219***	0.096		
ML-TibDist	81.75	3.57	27.1			5.924**	1.86				
Mass (kg)	1.71	0.04	25.6	−0.062	0.1			−0.009	0.023	0.198*	0.04

[1]For each measure, the effect of that component on the overall response (i.e., being male for the sex term or a 1-unit change in the other, continuous measures) is provided by the estimate with respective SE as well as the variation explained by the inclusion of the predictors.

$^*P < 0.001$; $^{**}P < 0.01$; $^{***}P < 0.05$.

assessments associated with particular types of measures such as position of the bone or type of measurement (e.g., angles vs. distances), though the overall range was much narrower than those of the test-retest reliability assessment. The 5% CV threshold used during measurements as an initial barrier to entry would also likely have a critical effect on driving the rater to greater consistency within the same radiograph and increasing R_u. Nonetheless, our intra-rater results suggest that appropriate training, objective criteria for placement of guides, and an internal standard to ensure consistency during measurements can provide reliable data within single samples.

The second purpose of the first objective—assessing variation in placement across multiple radiographs, (i.e., the test-retest procedure)—indicated most measures were able to be replicated with consistency, though at a reduced success rate compared with objective 1a, both in the number of measures reaching threshold as well as the associated correlations. With the exception of measure 8 (ML-TibAngle), test-retest assessments of measures taken from the mediolateral position were numerically greater than those taken from the craniocraudal, a result likely related to the broader base of the leg when placed laterally, which would allow for greater stability and reduce the variation between attempts. Although we can assume that the variation across radiographs is random (i.e., not associated with a particular subset of gait scores), excessive variation reduces the ability to detect treatment effects or correlations between variables. Our results from objectives 1a and 1b indicate that use of radiography with digital quantification is an effective method to assess bone properties, though internal standards are needed to ensure data are used efficiently with the minimal numbers of birds.

Analysis relating to our second objective indicated that several measures—measures 2 (CC-TibCortDist), 7 (ML-TibCortArea), and 9 (ML-TibWidth)—correlated to gait score, either as a main effect or as an interaction with bird mass. The cause of lameness in broilers is multifactorial though rapid growth and the associated skeletal abnormalities are considered to be dominant (Bradshaw et al., 2002) resulting in conditions with angular bone deformity (Julian, 1998). Of the several measures taken from the craniocaudal perspective, measure 2 (CC-TibCortDist) correlated positively to difficulty walking. Despite this finding, measure 2 (CC-TibCortDist) provides only an indirect measure of angulation by quantifying the depth of the groove formed by the medial extents of the proximal and distal epiphysis with the medial cortex. In support of our finding, Leterrier and Nys (1992) assessed various bone parameters similar to those in the present work and found birds manifesting varus deformity to have greater angulation in the distal tibia. Direct measures of angulation in the current effort found no relationship with gait, which may relate to our difficulties in identifying appropriate reference guides. For measure 1a (CC-TibFibAngle), although we were able to consistently quantify the measure across within and across radiographs, the fibula, which was used as a reference point because it offers clear visibility on the radiograph, is nonetheless loosely attached to the tibia and thus is likely a poor reference for angulation. In measure 1b (CC-TibAngle), the angle between the proximal chondyles and distal epiphysis would be dependent on the position of the bone during radiography, which as suggested by the small R_c for that measure, was highly variable. Nonetheless, the correlation between measure 2 (CC-TibCortDist, which could be used as a proxy for angulation) and gait score suggests this may be an effective measure of walking ability; improvements in positioning the bone during radiography to bring consistency across radiographs will likely improve the utility of this measure in assessing gait score.

Within the mediolateral perspective, gait score was found to relate to curving in the tibia (measure 8, ML-TibAngle), though the pattern of the change was dependent on postmortem bird mass. Based on our model, heavier birds, specifically those above 2.27 kg, manifesting a worsening gait (i.e., increasing gait score) would have less angulation, whereas birds below this mass would have increased angulation with worsening gait. Measure 6 (ML-TibCortDist), which indicates curving indirectly by quantifying the depth of the groove on the dorsal side, had a similar modeled pattern with a slightly higher threshold of 2.32 kg. Although our measurements did not include pathological diagnoses, the observed changes may relate to tibial dyschondroplasia, a principal skeletal abnormality causing lameness in broilers. Tibial dyschondroplasia results from rapidly reproducing chondrocytic cells within the metaphyseal plate, which are unable to produce bone due to poor or absent blood flow producing lesions that enlarge the tibia's proximal end (Riddell, 1992). Chrondrocytes typically do not reach a mature size and begin necrosis due to energy depletion (Brighton and Hunt, 1978; Hargest et al., 1985; Pines et al., 1998) and lack of specific nutrients required by the cells (e.g., 1,25-dihydroxycalciferol), which can be depleted in the metabolic acidosis often accompanying rapid growth (Rennie et al., 1993). Bird mass by itself has been suggested as a cause of tibial dyschondroplasia via compression of the epiphyseal growth plate and restriction of blood flow (Thorp, 1988), though causal mechanisms are believed to be related to growth rates as discussed above. In extreme cases of tibial dyschondroplasia, angular deformation will occur as the weakened proximal head becomes pulled by the gastrocnemius muscle (Duff, 1986; Riddell, 1992). Surprisingly, we found that greater deformation occurred in lighter birds where reduced growth rates would be expected. This finding may relate to secondary factors such as increased activity levels in lighter birds and associated greater use of the gastrocnemius muscle in contrast to heavier birds, which would be expected to be more sedentary. We did not measure activity levels to verify whether this mechanism is indeed taking place nor perform histological assessment to verify the occurrence of tibial dyschondroplasia, but future work would benefit from their inclusion. Alternatively, birds that developed a poor gait relatively earlier might have had difficulty accessing feed and water resulting in reduced weight gain and lower weight. Lastly, birds predisposed to reduced feed intake may have had compromised bone mineralization leading to weak bones prone to curving. Given these uncertainties, continuous monitoring of growth rates is also necessary to establish the appropriate chronology of events and whether changes are causally related.

The area circumscribed between the line connecting proximal and distal epiphyses and the caudal surface (measure 7, ML-TibCortArea) correlated to gait score indicating a deeper groove as bone length (measure 10, ML-TibDist) was not altered. We would expect that deeper grooves would occur in bones with greater deformation as the bone adapts a curved shape supporting the anatomical changes seen in the lighter birds and discussed above. Craniocaudal bone width (measure 9, ML-TibWidth) increased with gait score and may also relate to greater tibial curving via increased mechanical loading (Lynch et al., 1992), which might be expected to compensate for the increased strain on the bone. Because these latter measures did not exhibit a mass × gait score interaction, they may represent effects of curving that are independent or tangential to growth rates such as activity patterns. Barreiro et al. (2009) identified differential radiographic densities between sections of the tibia and suggested the greater values within the proximal head could result from a proportionally greater load exerted by the relatively high number of muscles and ligaments that attach or originate at this location. This differential effect could be interpreted with the current work in that less active birds, or those likely to have poor walking ability (i.e., elevated gait scores) and less use of these muscles and ligaments, would have less bone mineral content in the proximal head leading to greater flexibility and curvature.

We were unable to find any relationship between gait score and anatomical symmetry between the 2 legs. Our hypothesis was based on the assumption that birds with normal gait would have right and left legs that are mirror images of each other, whereas birds with poorer gait would have increasing anatomical differences as stride became altered. Leg pathologies of broilers generally appear bilaterally (Riddell, 1983) as in the current study, though can afflict a single leg or each leg in a different manner, particularly in cases of valgus/varus (Leterrier and Nys, 1992). Our analysis indicates the lack of asymmetry as it relates to gait, though our method of calculating the difference between legs by averaging the triplicate measurements within a single radiograph may have introduced additional variation that impaired our ability to identify a correlation with gait score.

In conclusion, our results offer a novel methodology to assess lameness in broilers and potential mitigating skeletal factors. Our effort to assess consistency of the researcher placing the leg during the radiograph procedure and subsequently measure quantification of various parameters suggests internal controls are needed to ensure an acceptable threshold of reliability. Our measures indicate that curving of the tibia may be related to lameness, though subsequent efforts involving comprehensive measures of bird activity, growth rates, and internal bone structure will be needed if the validity of the measures are to be accepted.

ACKNOWLEDGMENTS

The authors gratefully thank Lindsay Crane and Tora Thomas of the University of Bristol's Langford Veterinary Services for assisting with the radiography equipment, as well as Neil Burton, Clinical Teacher at Langford Veterinary Services, for assisting with editing of the paper.

REFERENCES

Barreiro, F. R., A. L. Sagula, O. M. Junqueira, G. T. Pereira, and S. M. Baraldi-Artoni. 2009. Densitometric and biochemical values of broiler tibias at different ages. Poult. Sci. 88:2644–2648.

Bessei, W. 2006. Welfare of broilers: A review. World's Poult. Sci. J. 62:455–466.

Bradshaw, R. H., R. D. Kirkden, and D. M. Broom. 2002. A review of the aetiology and pathology of leg weakness in broilers in relation to welfare. Avian Poult. Biol. Rev. 13:45–103.

Brighton, C. T., and R. M. Hunt. 1978. The role of mitochondria in growth plate calcification as demonstrated in a rachitic model. J. Bone Joint Surg. Am. 60:630–639.

Cook, M. E. 2000. Skeletal deformities and their causes: Introduction. Poult. Sci. 79:982–984.

Danbury, T. C., C. A. Weeks, A. E. Waterman-Pearson, S. C. Kestin, and J. P. Chambers. 2000. Self-selection of the analgesic drug carprofen by lame broiler chickens. Vet. Rec. 146:307–311.

Dawoodi, A. I., and A. Perera. 2012. Reliability of metatarsus adductus angle and correlation with hallux valgus. Foot Ankle Surg. 18:180–186.

Drinka, P. J. 2006. Inter-observer reliability of radiologists' interpretation of mobile chest radiographs for nursing home-acquired pneumonia. J. Am. Med. Dir. Assoc. 7:467–469.

Duff, S. R. 1986. Effect of unilateral weight-bearing on pelvic limb development in broiler fowls: Morphological and radiological findings. Res. Vet. Sci. 40:393–399.

Gstoettner, M., K. Sekyra, N. Walochnik, P. Winter, R. Wachter, and C. M. Bach. 2007. Inter-and intraobserver reliability assessment of the Cobb angle: Manual versus digital measurement tools. Eur. Spine J. 16:1587–1592.

Hargest, T. E., C. V. Gay, and R. M. Leach. 1985. Avian tibial dyschondroplasia. III. Electron probe analysis. Am. J. Pathol. 119:199–209.

Julian, R. J. 1998. Rapid growth problems: Ascites and skeletal deformities in broilers. Poult. Sci. 77:1773–1780.

Julian, R. J. 2005. Production and growth related disorders and other metabolic diseases of poultry: A review. Vet. J. 169:350–369.

Kestin, S., T. Knowles, A. Tinch, and N. Gregory. 1994. Leg weakness in broiler chickens, a review of studies using gait scoring. Pages 203–206 in 9th European Poultry Conference.

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

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

Kose, O., M. Celiktas, F. Guler, A. Baz, E. Togrul, and S. Akalin. 2012. Inter- and intraobserver reliability of the Crowe and Hartofilakidis classifications in the assessment of developmental dysplasia of the hip in adult patients. Arch. Orthop. Trauma Surg. 132:1625–1630.

Lee, J., D. Koh, and C. N. Ong. 1989. Statistical evaluation of agreement between two methods for measuring a quantitative variable. Comput. Biol. Med. 19:61–70.

Leterrier, C., and Y. Nys. 1992. Clinical and anatomical differences in varus and valgus deformities of chick limbs suggest different aetio-pathogenesis. Avian Pathol. 21:429–442.

Lynch, M., B. H. Thorp, and C. C. Whitehead. 1992. Avian tibial dyschondroplasia as a cause of bone deformity. Avian Pathol. 21:275–285.

Machin, K. L. 2005. Controlling avian pain. Compendium April:299–308.

McGeown, D., T. C. Danbury, A. E. Waterman-Pearson, and S. C. Kestin. 1999. Effect of carprofen on lameness in broiler chickens. Vet. Rec. 144:668–671.

Pines, M., V. Knopov, O. Genina, S. Hurwitz, A. Faerman, L. C. Gerstenfeld, and R. M. Leach. 1998. Development of avian tibial dyschondroplasia: Gene expression and protein synthesis. Calcif. Tissue Int. 63:521–527.

Rasbash, J., F. Steele, W. Browne, and H. Goldstein. 2009. A User's Guide to MLwiN, v2.10. Center for Multilievel Modeling, University of Bristol, Bristol, UK.

Rennie, J. S., C. C. Whitehead, and B. H. Thorp. 1993. The effect of dietary 1,25-dihydroxycholecalciferol in preventing tibial dyschondroplasia in broilers fed on diets imbalanced in calcium and phosphorus. Br. J. Nutr. 69:809–816.

Riddell, C. 1975a. The development of tibial dyschondroplasia in broiler chickens. Avian Dis. 19:443–462.

Riddell, C. 1975b. Studies on the pathogenesis of tibial dyschondroplasia in chickens. III: Effect of body weight. Avian Dis. 19:497–505.

Riddell, C. 1983. Pathology of the skeleton and tendons of broiler chickens reared to roaster weights. I. Crippled chickens. Avian Dis. 27:950–962.

Riddell, C. 1992. Non-infectious skeletal disorders of poultry: An overview. Pages 119–145 in Bone Biology and Skeletal Disorders in Poultry. C. C. Whitehead, ed. Carfax Publishing, Abingdon, UK.

Rousson, V., T. Gasser, and B. Seifert. 2002. Assessing intrarater, interrater and test-retest reliability of continuous measurements. Stat. Med. 21:3431–3446.

Schreiweis, M., J. Orban, M. Ledur, and P. Hester. 2003. The use of densitometry to detect differences in bone mineral density and content of live White Leghorns fed varying levels of dietary calcium. Poult. Sci. 82:1292–1301.

Sorensen, P., G. Su, and S. Kestin. 2000. Effects of age and stocking density on leg weakness in broiler chickens. Poult. Sci. 79:864–870.

Thorp, B. H. 1988. Pattern of vascular canals in the bone extremities of the pelvic appendicular skeleton in broiler type fowl. Res. Vet. Sci. 44:112–124.

Weeks, C. A., T. D. Danbury, H. C. Davies, P. Hunt, and S. C. Kestin. 2000. The behaviour of broiler chickens and its modification by lameness. Appl. Anim. Behav. Sci. 67:111–125.