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Journal:	*Poultry Science*
Manuscript ID	Draft
Manuscript Type:	Full-Length Article
Key Words:	broiler, lameness, gait score, animal welfare, welfare indicator

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ABSTRACT

Lameness and impaired walking ability are major welfare issues that cause economic losses in rapidly growing meat-type broiler chickens. This study analyzed the prevalence of impaired walking and its associations with production data, abattoir registrations and post-mortem tibia measurements in Norwegian broiler chickens. Gait score (GS) was used to assess walking ability in 59 different commercial broiler flocks (Ross 308) close to the slaughter day, 5900 broilers in total, in 3 different geographical regions. In each flock, 100 random broilers were gait scored and 10 random broilers were culled to harvest tibias. Abattoir registrations on flock level were collected after slaughter. A total of 24.6 % of the broilers had moderate to severe gait impairment. The broilers were sampled in two stages, first slaughterhouse/ region, and then owner/flock. The final models showed that impaired gait is associated with region ($P < 0.001$), first week mortality ($P < 0.05$), DOA ($P < 0.05$), height of tibias mid-shaft ($P < 0.05$) and calcium content in the tibia ash ($P < 0.05$). The prevalence of impaired gait indicates that this is a common problem in the broiler industry in Norway, although Norwegian production conditions differ from EU standards with regards to slaughter age and animal density. Impaired walking ability could not be predicted by the welfare indicators foot pad lesion score, total on-farm mortality and DOA. Further studies are needed to identify feasible animal-based welfare indicators that reliably predict walking ability in broiler chickens.

Key words: broiler, lameness, gait score, animal welfare, welfare indicator

INTRODUCTION

Lameness and impaired gait is a major welfare issue that cause economic losses in rapidly growing meat-type broiler chickens (SCAHAW 2000). The causes for lameness and gait impairment are multifactorial and are comprised of infectious, developmental and degenerative afflictions (Bradshaw, et al. 2002, SCAHAW 2000, Wideman, et al. 2012, Williams, et al. 2000). Rapid growth rate and high body weight are considered the main underlying causes (Angel 2007, Bessei 2006, Kestin, et al. 2001, Knowles, et al. 2008, Pompeu, et al. 2012, SCAHAW 2000) which also leads to increased susceptibility to bacterial bone infections due to excessive shear stress on immature cartilage (Wideman 2016).

Gait scoring (**GS**) is a standardized method to evaluate lameness and walking ability in broilers on farm (Kestin, et al. 1992). Internationally, it is estimated that 14-30 % of broilers suffer from lameness that affects maneuverability, speed, accelerations and gives an impaired gait (Bassler, et al. 2013, Berg & Sanotra 2001, Kestin, et al. 1992, Knowles, et al. 2008, Sanotra, et al. 2003, Sanotra, et al. 2001 a). As such, gait impairment may be associated with pain, less activity and less expression of motivated behaviors, which causes compromised welfare (Bessei 2006, Caplen, et al. 2013 , Danbury, et al. 2000, Dawkins, et al. 2009, McGeown, et al. 1999, Nääs, et al. 2009, Skinner-Noble & Teeter 2009, Vestergaard & Sanotra 1999, Weeks, et al. 2000). Therefore, gait scoring is an important tool to screen broiler flocks for gait impairment and, thus, animal welfare. However, the method is time-consuming and therefore not feasible as a systematic registration tool for farm level evaluation of broiler welfare. A potential strategy for simplification of the assessment of walking ability is to replace time-consuming on-farm gait scoring with other measures that reliably predict impaired walking ability. For this purpose, potential associations between gait score measures and other relevant animal-based measurements should be identified.

Footpad lesions (**FPL**) scored at the abattoir, mortality rates during transports (i.e. dead on arrival; **DOA**) and mortality rates on farm are examples of welfare indicators that are widely used. FPL is a necrotic dermatitis on the plantar surface of the foot that is assumed to be painful (De Jong, et al. 2012) and thus, may constitute a welfare issue (Bassler, et al. 2013, De Jong, et al. 2012 b, Ekstrand, et al. 1997 a, Ekstrand, et al. 1998, Marchewka, et al. 2013). FPL is scored routinely at Norwegian abattoirs to assess welfare. However, the relationship between GS on farm and FPL scored at the slaughterhouse differs between studies (De Jong, et al. 2014, Haslam, et al. 2007, Kestin, et al. 1999, Sørensen, et al. 2000), which makes it an unreliable welfare indicator for general leg health, however little is known about the situation in Norway regarding FPL and GS.

An impaired gait may indicate infectious conditions (Wideman 2016) and leg weakness has been found to be associated with mortality both on farm and during transport. For instance, Kittelsen *et al.* (2015) recently found osteomyelitis in 10 % of DOA broilers and in 14 % of broilers dead on farm, close to slaughter age, which may reflect a significant portion of the total on-farm mortality rates (Bradshaw, et al. 2002, Wideman, et al. 2012). However, currently no link has been established between walking ability assessed on farm (gait score), and other animal based welfare indicators such as DOA, FPL, and on-farm mortality. In addition, it is claimed that rapidly growing broilers have a poorer bone quality than slowly growing birds, which results in weak skeletal properties, measured by lower effective breaking strength of the tibial bone (**BS**) (Williams, et al. 2000). Although walking ability has not been associated with breaking strength of the tibia (Brickett, et al. 2007, Ruiz-Feria, et al. 2014, Yalçin, et al. 1998), little is known of bone mineralization in relation to FPL, on farm mortality and DOA.

The maximum animal density allowance in Norwegian broiler production is 36 kg/m^2 during the last days of the production cycle versus the allowed maximum 42 kg/m^2 in EU legislation (European Union Council Directive 2007/43/EC 2007). Furthermore, in accordance with Norwegian standards, broilers are slaughtered at a younger age and slaughter weight than in most other countries; mean slaughter weight and age in Norway is 1.8 kg at 31.5 days (personal communication from industry staff, Hilde Bryhn) whereas in the EU it is typically 2.5 kg by 42 days of age (EFSA 2011). Several studies have indicated that stocking density may be associated with leg weakness, reduced leg strength, poor walking ability and decreased activity level (Buijs, et al. 2009, Hall 2001, Lewis & Hurnik 1990, Sanotra, et al. 2001 b, Sanotra, et al. 2001 a, Sørensen, et al. 2000). Furthermore, high body weights affect the ability to walk (Kestin, et al. 2001, Knowles, et al. 2008, SCAHAW 2000). Therefore, it could be suggested that the lower slaughter weight and lower stocking density in general may result in better walking ability in Norwegian broilers compared to broilers produced according to European legislation. However, little is known about walking ability of broilers under Norwegian production conditions.

In order to determine if there is a potential to replace time-consuming on-farm gait scoring with other animal-based measures that could reliably predict impaired walking ability, and to gain more knowledge about broiler leg health and welfare under Norwegian production conditions, a cross sectional study was designed to investigate the associations between walking ability, production data, abattoir registrations and post mortem tibia measurements in Norwegian broiler chickens.

MATERIAL AND METHODS

Birds

A cross sectional study was set up to explore 59 broiler flocks at different farms. The farms were enrolled according to a predefined sampling frame, based on inclusion terms; type of boiler hybrid (Ross 308), location, mixed gender, ad libitum feeding and slaughtering dates. A sample of 100 birds was arbitrarily selected for gait scoring on each farm. All farms included in the study used fresh wood shavings as litter. The flocks were slaughtered at one of three abattoirs, each representing three distinct production regions. Approximately 20 flocks from each region were included in the study.

Gait scoring

Walking ability was evaluated using the six-point gait scoring scale as described by Kestin *et al.* (1992). The scale is explained in Table 1. Birds with scores ≥ 3 were considered to have an impaired gait since this affects maneuverability, speed and accelerations and is likely associated with pain (Kestin, et al. 1992, McGeown, et al. 1999). Data were collected during an 18 months period and the broilers were gait scored within the last three days prior to slaughter, i.e. mean age at scoring was approximately 29 days (range: 26-30). Prior to sampling, the lights in the barn were switched off and a cardboard wall, approximately 50 cm high and 5 m long, was placed around a random group of broilers before the lights were turned on again. 25-30 broilers were isolated at a time. When the light was switched on, individual animals were taken one at a time out from the cardboard pen and placed carefully on the floor for gait scoring. Scoring of individual broilers took between 5 seconds and 2 minutes. Birds that did not walk away within approximately 2 minutes were encouraged to walk by a person walking slowly behind them. The procedure was repeated at 3-4 different locations throughout the barn in order to avoid resampling and to secure a representative sample. Two trained poultry veterinarians carried out the farm visits and the scoring of all flocks. Final scores were determined by consensus between the assessors on site.

Retrospective Welfare Registrations

The farm and abattoir registrations were collected after slaughter. These registrations included FPL scores, DOA and on farm mortality. FPL were scored on herd level at the abattoir; 100 feet from 100 broilers were examined post mortem, using the so-called Swedish footpad scoring system: 0 = no lesions, 1= mild, superficial lesions, 2 = severe, large or deep lesions (Ekstrand, et al. 1998).

Tibia Measurements

After gait scoring, 10 random broilers were culled on farm by blunt trauma to the head and cervical dislocation. Both tibias were harvested and shipped over-night to the University of Aarhus, Department of Food Science, in Denmark. 600 right tibias were forwarded to breaking strength analyses (BS) and 600 left tibias were sent to ash analysis of ash content (**AC**) at the University of Aarhus, Department of Animal Science. A technical error to the freezer resulted in the decomposition of 300 tibias from 150 broilers, leaving them unfit for examination. This left 450 left tibias and 450 right tibias for bone measurements. Muscles and connective tissues were dissected from the bones. Breaking strength of tibias was determined using a texture analyzer (TMS-Touch, Food Technology Corporation, Virginia, USA) equipped with a Volodkovitch fixture the tibia was fixated and the physical power was applied to the midpoint of the bone by a static load, measured in Newton. In addition the weight and length of the bone were measured, along with the height of the mid shaft. Prior to the chemical analysis of AC, the bones were thawed at 4°C and milled using a knife mill, and the resulting bone powder was stored at -20°C until further analysis. The ash content was determined after incineration at 525˚C for 6 h and P was quantified by the vanadomolybdate colorimetric procedure (Stuffins 1967) and for Ca by atom absorption spectrometry (Model S2AA System, Thermo Electron Corporation Ltd., Cambridge, UK) after hydrochloric acid/nitric acid treatment of the ash fraction.

Statistical Analyses

All data were collected into a Microsoft Office Excel 2013 spreadsheet with the dependent variable (GS) on a six point ordinal scale from 0-5. The preliminary inspection of data was performed in pivot tables and diagrams, using Excel. The complete dataset was transferred to STATA and all statistical analyses, including summary and descriptive statistics, were performed in STATA version 14.1 (StataCorp, TX, USA). Univariable analyses were performed for all possible predictors with inclusion of the random effect of farm (n=59). Two multiple ordered regression models were constructed by forward selection and backward elimination approaches. Variables obtaining a significance level of ≤ 0.2 were included in the model building. Both models were two leveled mixed-effects ordinal logistic regression with robust estimates, adjusting for the nested clusters of random effect at farm level (n=44). The likelihood ratio test was used to determine the better fit in a model with or without the influence of regional effects. The first model controlled for the regional differences of GS with first week mortality as the predictor (fixed effect) covariate. The second model did not include the region as a correction variable as this was not assumed to adjust for the selected covariates. The fixed effects of DOA, tibial mid-shaft height and the calcium content of the tibia were retained in the model. In the first model, a two-way interaction term between region and first week mortality was included. The interaction estimates were not significant and the outcome variable was not influenced by the interaction term, studied by marginal effects. In the second model, two-way and three-way interaction terms were included out of which all were dismissed due to lack of significant influence on the outcome variable, studied by marginal effects and significance of interaction terms. The Akaike information criterion confirmed the decision of excluding the interaction term in the model. The collinearity was weak to low (0.3-0.5) between the covariates. The conditional distribution of the response, given the random effects, was assumed multinomial with success probability determined by the logistic cumulative distribution of the six leveled ordinal outcome variable. The covariate effects were assumed to be the same across all cumulative logits. By predicting the probability of assigning each observation to a given gait score category, the probability distribution was visualized and interpreted by using histograms and matrix plots. The distribution across gait scores reflected the descriptive statistics on raw data. The fixed effects and random effects were visualized and reported as odds ratios with variance components. The cutoff for statistical significance was set at $p \leq 0.05$. The comparison of likelihood-ratio tests was used to determine the benefit of using mixed models to standard ordinal regression models. The analysis concluded that the effect of farm was substantial in both models. Thus there was enough variability between farms to favor a mixed-effect ordered logistic regression. All two-way linear relationships between DOA, breaking strength, total mortality and FPL were analyzed by simple linear regression.

RESULTS

The mean descriptive flock data are presented in Table 2.

Impaired Walking Ability

The overall distribution of gait scores is given in Table 1. At a mean age of 28.9 days, 24.6 % of the broilers in this study had an impaired gait (GS $\geq$ 3). There were differences ($P <$ 0.001) in the observed number of GS $\geq$ 3 between the three regions; 12.95 %, 14.5%, 48.8 %, respectively. Moderate to severe lameness varied substantially between flocks, regions and seasons. The study design did not allow for identification of seasonal effects since it was confounding to the regional effect on the outcome.

Descriptive Statistics

The mean slaughter weight was 1251.6 gram. A difference in slaughter weight between the three regions was found. However, neither the slaughter weight (carcass weight) nor daily weight gain was associated with GS ($P > 0.05$) (Table 3). The mean mortality on farm across the study was 2.94 % (range: 0.80-6.80) (Table 2) and was not associated with GS ($P > 0.05$) (Table 3). The total on farm mortality did not distinguish between culling and animals that died from other causes. The mean FPL score in the study population was 13.03 (range: 0-100) (Table 2). Univariable mixed effect, ordinal logistic regression analysis, showed that FPL score, DOA, region, BS, tibia length, tibia mid-shaft height, tibia weight and tibia calcium percentage were all significantly associated with GS ($P < 0.05$) (Table 3).

Regression Models

Two models were constructed to determine associations of predicting variables to GS. The first model concluded that the region adjusted for the effect of first week mortality on GS. The likelihood ratio of the mixed effect ordinal regression model versus a standard ordinal regression model obtained χ^2= 398.96 (p<0.01) which is considered a strong argument for using the presented model. The model included 5900 observations (n=59 groups). The correlation coefficient was 0.43 between region and first week mortality, indicating a weak co-linear relationship. The interaction term of region and first week mortality was OR=0.8, p=0.61 and OR=0.63, p=0.19 for region 2 and 3 respectively. The second obtained a likelihood ratio of the mixed effect ordinal regression model versus a standard ordinal regression model equal to χ^2= 845.61 which was considered a strong argument for using the presented model. The final model included 4400 observations (n = 44 groups), restricted by missing values for ash analyses. All interaction terms as described in the method section obtained p-values of ≥ 0.2. The pairwise correlation coefficients for the covariates ranged from -0.33 and 0.30, indicating a weak co-linear relationship. The results obtained in the two models are given in table 4 and table 5.

Tibia Measurements

The mean load needed to break tibia was 341.98 N (range: 223.50-436.41). The mean tibia weight was 6.40 grams (range: 3.90-10.20), mean tibia length was 81.12 mm (range: 64.90-88.50) and the mean height of the tibia mid shaft is 7.81 mm (range: 6.81-9.17) (Table 2). The tibia measurements (breaking strength, weight, length and height of mid shaft) are internally correlated (Table 6) and associated with GS (Table 3). However, when corrected for the random effect of farm and included with the covariates calcium content and DOA; only the height of the tibia mid-shaft is associated with impaired gait (Table 5). A negative association between breaking strength and FPL was found ($P < 0.01$). Breaking strength was not associated with first week mortality, total mortality on farm or DOA ($P > 0.05$). The mean ash content of tibia was 31.15 %, mean calcium percentage of the ash content is 35.00 % and mean phosphorus percentage 17.91% (Table 2). The calcium percentage of the ash content is associated with impaired gait in univariable analysis ($P <0.05$) (Table 3), and in model 2 (Table 5), but not in the model that corrects for both farm and region (Table 4). No associations were found between ash content, phosphorus content and impaired gait in the models. The calcium/phosphorus ratio was not associated with gait score (*P* = 0.12). Significant associations were found between the following variables: DOA and phosphorus content, breaking strength and ash content of tibia, breaking strength and calcium content, mortality on farm and ash content, FPL and ash content, FPL and calcium content.

DISCUSSION

This study used gait scoring to describe walking ability in broilers under Norwegian production conditions and to investigate the association between walking ability, production data, abattoir registrations and tibia measurements. Briefly, nearly 25 % of the broilers included in this study displayed a moderate to severe gait impairment. Impaired walking ability was associated with first week mortality, when accounting for regional differences. Furthermore, DOA, height of the tibia mid-shaft and the calcium content in the tibia ash were associated with an increasing gait score.

The prevalence of moderate to severe lameness is in accordance with results reported from several European studies (Kestin, et al. 1992, Knowles, et al. 2008, Marchewka, et al. 2013, Sanotra, et al. 2003, Sanotra, et al. 2001 a). International studies have identified associations between impaired walking ability and leg problems, with increasing body weight and age of the broilers (Kestin, et al. 2001, Knowles, et al. 2008, Nääs, et al. 2009, Sanotra, et al. 2001 a, Sørensen, et al. 2000) as well as stocking density (Buijs, et al. 2009, Dawkins, et al. 2004, Hall 2001, Lewis & Hurnik 1990, Sanotra, et al. 2001 b, Sanotra, et al. 2001 a, Sørensen, et al. 2000, Thomas, et al. 2004). Thus, even though the present study was conducted under Norwegian production conditions with lower slaughter age, slaughter weight and stocking density than the above mentioned reports, the reported number of GS>3 was comparable to findings in other studies where broilers were produced according the European legislations. A direct comparison between studies is however, not possible. The current results indicate that factors other than age, body weight and stocking density contribute to impaired walking ability on flock level.

First week mortality and regional differences were associated with impaired walking ability at the end of the production period (Table 4). Leg disorders and lameness are known to be related to general mortality on farm (Bradshaw, et al. 2002, Wideman, et al. 2012). Furthermore, infectious leg disorders such as bacterial chondronecrosis and osteomyelitis (BCO) have been suggested as important causes for both lameness and on farm mortality (Bradshaw, et al. 2002, Kittelsen, et al. 2015, Wideman 2016). This is the first study to report a link between first week mortality and impaired gait. One possible explanation is that impaired gait close to slaughter age may arise from early infections that persist in the flock throughout the growth period. Rapid growth rate places mechanical stress to growing bones which makes them more susceptible to colonization of bacteria and development of BCO (Wideman 2016). In addition, the BCO risk increases for broilers that remain in a sitting posture for long periods, since this compresses the blood supply to their legs (Wideman 2016). It may also be, that there is a potential impact of regional factors (e.g. climate, season, feed mill, and hatchery) linked to an underlying infectious cause, especially since breeder flocks and hatcheries have been suspected of being the source of *Staphylococcus* spp. and *Enterococcus* spp. that have been isolated from lame broilers (Wideman 2016). In this study there was a negative association between DOA and impaired gait which may indicate that lame birds, in accordance with national regulations, are culled from the flock before transport to the abattoir.

Height of the tibia mid-shaft and the calcium content of the tibia ash were associated with impaired gait. The finding of calcium content associated with impaired gait revealed in the current study, stands in contrast to previous studies of GS and ash content with no such association (Brickett, et al. 2007, Ruiz-Feria, et al. 2014, Talaty, et al. 2010, Venäläinen, et al. 2006) . Ash concentration of the tibia is often used to estimate the degree of bone mineralization (Shastak, et al. 2012) since the organic component is important for the breaking strength (Velleman 2000). For instance, bones in rapidly growing broiler strains are found to have lower ash contents and to be more porous than slowly growing controls (Shim, et al. 2012, Thorp & Waddington 1997, Williams, et al. 2000). In our study, there were regional differences in the calcium content from the tibia whereas no effect of phosphorus was observed. We can therefore not rule out a possible influence of confounding variables (feed mill, season etc.). The nutritional abnormalities resulting in avian rickets are usually related to alterations in dietary levels of calcium, phosphorus, or vitamin D and can result from errors in feed formulation or mixing (Long, et al. 1984, Waldenstedt 2006, Wise 1975). It could therefore be hypothesized that the association between calcium content and GS may be due to an unfavorable relationship between the content of calcium and phosphorus of the bones or in the nutrient supply since rickets is a commonly associated with leg weakness in broilers (Long, et al. 1984, Wise 1975). However, no association was found between the calcium/phosphorus ratios and gait score. No feed or blood samples were collected during the study, therefor rickets or a mineral imbalance cannot be verified. Regarding the association between the height of tibia mid shaft and GS it could be suggested that this is related to the content of calcium and phosphorus, or it may be due to a disproportional body weight and tibia properties. However, body weight was not noted for the individual broilers. Breaking strength and ash content were not associated with gait, which is in agreement with published literature that found little or no association between bone strength and GS (Brickett, et al.

2007, Ruiz-Feria, et al. 2014, Yalçin, et al. 1998). It has to be emphasized that tibias were not collected from the birds undergoing gait scoring, and therefore no causal relationship could be established between walking ability and tibia mid shaft height and calcium concentration.

The overall prevalence of FPL in the present study was low compared to available publications (Ekstrand et al., 1997 a; Ekstrand et al., 1998; De Jong et al., 2012 b; Bassler et al., 2013; Marchewka et al., 2013). Furthermore, no association between impaired gait and FPL score was detected, which is in contrast to findings by De Jong et al. (2014) who found impaired gait to be associated with FPLs. The lack of association between FPL and GS may result from the low FPL score found in this study. It has to be emphasized that GS and FPL were not scored in the same individuals, and therefore no causal relationship can be concluded from this study. The favorable footpad health revealed in this study may be the result of using fresh wood shavings, which is presumed to be an optimal bedding (Almeida Paz, et al. 2010). In addition, the lower stocking density in Norway compared to EU (European Union Council Directive 2007/43/EC 2007), may be beneficial for the footpads, since high stocking density may deteriorate the litter quality (Blokhuis & Van der Haar 1990). In Norway, the broiler producers are allowed maximum stocking densities only if they keep their FPL between 0-80 points and thus employ a number of measures to improve environmental factors that affect footpad health, including floor heating and ventilation. Therefore, the low prevalence of FPL may reflect management practices, litter quality and stocking density. However, FPL score is not feasible as a retrospective welfare indicator for overall leg health situation on farm.

The gait score method has been criticized for its subjectivity in scoring procedure and the poor repeatability (Caplen, et al. 2012, Cordeiro, et al. 2009, Weeks, et al. 2002). In this study, the gait scores could be considered reliable since the scores were determined by consensus between the two observers. Several less subjective methods to evaluate walking ability have been developed, such as latency to lie and kinematic analysis (Berg & Sanotra 2003, Caplen, et al. 2012, Corr, et al. 2007). However, these methods are not feasible for commercial flock-assessments. Another weakness of gait scoring is the lack of discriminatory ability of impaired walking by underlying pathological conditions and physical restraints due to body conformation (De Jong, et al. 2012) which may influence the validity of these measures as welfare indicators.

In conclusion, 24.6 % of the broilers in this study had a moderate to severe lameness at the mean age of 28.8 days. Previous studies have indicated that GS ≥ 3 is a painful condition which is associated with inactivity and compromised welfare (Caplen, et al. 2013 , McGeown, et al. 1999, Nääs, et al. 2009, Weeks, et al. 2000). Considering the prevalence of moderate to severe gait impairment, the welfare implications are substantial. The regression models showed association between increasing gait scores and the following variables: first week mortality, region, DOA, tibia mid-shaft height and tibia calcium content. This shows that the most common welfare registrations in the broiler industry today; FPL, mortality on farm and DOA, are not reliable alone to retrospectively demonstrate a gait problem on farm. However, the association between first week mortality and impaired gait signifies the potential for employing early farm mortality as a predictor for poor leg health towards the end of the production cycle. Finally, the current study advocates the need for improved animal-based registrations that are used along the whole production cycle. This study has identified associations that call for further studies to illuminate the relationship between mortality during the first week of production and how this may result in lameness in the end of production cycle, with a special focus on potential persisting bacterial infections. In addition more studies to support the effect of stocking density and nutritional composition on walking ability and to determine whether early infections can give rise to impaired walking ability later in the production period. In conclusion further studies are needed to investigate the unexplored causes for impaired walking ability.

ACKNOWLEDGMENT

We sincerely thank the 60 farmers who allowed us to evaluate their broilers. We are also very grateful to the abattoir staff that provided us with list of producers with broilers close to slaughter at any given time. Jens Askov Jensen is also greatly acknowledged for his technical assistance on analysis of breaking strength. The study was financed by the Norwegian Research Council (Project no. 207691), Animalia, Nortura, Norsk Kylling and KLF.

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Table 1. The distribution of broilers within the gait score categories 0-5[1] along with scoring criteria

Gaitscore	Criteria	Frequency[2]	Percentage	SD	Min	Max
0	No detectable abnormality, fluid locomotion, furled foot when raised	280	4.75	6.98	0	29
1	Slight defect difficult to define	1 855	31.44	17.40	1	61
2	Definite and identifiable defect, but it does not hinder the broiler in movement	2 298	38.95	13.35	12	65
3	An obvious gait defect which affects the broilers ability to maneuver, accelerate and gain speed	1 340	22.71	18.59	2	72
4	A severe gait defect, the broiler will only walk a couple of steps if driven before sitting down	115	1.95	4.79	0	36
5	Complete lameness, either cannot walk or cannot support weight on the legs	12	0.20	0.43	0	2

[1]In accordance with Kestin et al. (1992).
[2] N = 5900

Table 2. Descriptive data for the gait scored flocks

Variable	Mean	Std.Error	Min	Max
Flock size	16 565.8	4 920.4	4 500	28 000
Slaughter age, days	31.2	0.19	28	35
Age on the day of scoring	28.9	0.13	26	30
Slaughter weight, g[1]	1 251.6	161.6	954	1 631
Daily weight gain, g	39.9	0.48	31.8	50.8
Flock GS	1.84	0.5	1.02	3.21
Flock foot pad lesion score	13.03	2.52	0	100
DOA, %	0.07	0.06	0.01	0.36
Total mortality on farm, %	2.94	0.16	0.80	6.80
First week mortality on farm, %	1.03	0.08	0.1	3.3
Breaking strength, load in N	341.98	6.94	223.5	436.4
Tibia weight[2], g	6.40	1.16	3.90	10.20
Tibia length, mm	81.12	2.33	64.90	88.50
Height of tibia mid shaft, mm	7.82	0.54	6.81	9.17
Tibia ash content[3]	31.15	3.59	24.18	38.92
Calcium[4]	35.00	0.68	33.57	37.06
Phosphorus[5]	17.91	0.16	17.29	18.21

[1] Carcass weight, not included head, internal organs, feather or feet

[2] Weight of fresh bone

[3] Percentage of tibia

[4] Percentage of ash content

[5] Percentage of ash content

566 **Table 3.** Univariable multilevel mixed effects ordinal logistic regression analysis of
567 associations between GS and independent variables

Variable	OR	Std.Error	*P*-value[1]	95 % CI
Foot pad score	0.98	0.007	**0.031**	0.97 - 0.99
Age at scoring	1.26	0.19	0.131	0.93 - 1.69
Slaughter weight[2]	1.00	0.001	0.762	0.99 - 1.00
Dead on arrival	0.002	0.006	**0.027**	0.001- 0.50
Stocking density	0.99	0.00003	0.634	0.99 - 1.00
First week mortality	1.42	0.34	0.145	0.88 - 2.29
Total mortality on farm	1.23	0.149	0.082	0.97 - 1.56
Load at breaking	1.008	0.003	**0.024**	1.00 - 1.015
Weight of tibia	1.24	0.055	**0.000**	1.13 - 1.35
Length of tibia	1.02	0.003	**0.000**	1.01 - 1.03
Height of mid-shaft tibia	1.23	0.044	**0.000**	1.14 - 1.32
Slaughter house 1	Baseline			
Slaughter house 2	2.34	0.496	**0.000**	1.54 - 3.54
Slaughter house 3	10.86	2.352	**0.000**	7.09 - 16.60
Daily weight gain	1.06	0.037	0.100	0.98 - 1.13
Ash content	1.043	0.049	0.368	0.95 – 1.14
Calcium content	1.805	0.44	**0.016**	1.11 – 2.91
Phosphorus content	6.988	7.008	0.053	0.98 – 49.89

568 [1] Numbers in bold are significant in univariable analysis
569 [2] Carcass weight, not included head, internal organs, feather or feet

Table 4. Model 1, multilevel mixed-effects ordinal logistic regression analysis with random effect of farm and fixed effect of abattoir/region with significant associations between GS and independent variables

Variable	OR	Std.Error	*P*-value	95% CI
Abattoir/region 1	Baseline			
Abattoir/region 2	2.37	0.47	<0.01	1.60 - 3.51
Abattoir/region 3	13.81	3.1	<0.01	8.89 - 21.44
First week mortality	0.68	0.1	<0.01	0.51 - 0.90
Random effect	0.36	0.07		0.24 - 0.54

Table 5. Model 2, multilevel mixed-effect ordinal logistic regression analysis with random effect of farm level, leaving regional differences out and significant associations between GS and independent variables

Variable	OR	SD	*P*-value	95 % CI
DOA	0.0002	0.0008	0.043	5.19×10^{-8} - 0.77
Height of tibia mid-shaft	1.89	0.55	0.028	1.07 - 3.35
Calcium content	1.97	0.49	0.003	1.26 - 3.08
Random effect	0.098	0.22		0.63 - 1.52

Table 6. Correlations between GS and bone strength variables[1]

	GS	Load[2]	Weight[3]	Length[4]	Height[5]
GS	-0.96				
Load	0.18	1.00			
Weight	0.16	0.91	1.00		
Length	0.17	0.97	0.95	1.00	
Height	0.18	0.96	0.96	0.99	1.00

[1] The bone used to test was tibia

[2] The strength, in Newton, that is needed to break the bone

[3] Weight of the tibia, gram

[4] Length of the tibia, millimeters

[5] Height of the tibia mid shaft, millimeters