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Is the rotarod test an objective alternative to the gait score for evaluating walking ability in chickens?

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Abstract

*Walking ability is related to motor co-ordination which, in rodents, can be assessed by an established test in pharmacological studies — the rotarod test. The purpose of this study was to evaluate a modified rotarod test for chickens and its relation to the often-used gait score system. At the end of their rearing period, we tested 138 male chickens (*Gallus gallus domesticus*) from three differing growth performance strains: Ross 308 (fast-growing; n = 46), Lohmann Dual (medium-growing; n = 46) and Lohmann Brown Plus (slow-growing; n = 46). First, the chickens' gait scores were assessed and, immediately following this, they were placed gently onto a steady rod. The velocity of the rotating rod gradually increased, and the latency to leave the rod was recorded. By using a linear mixed model, we were able to show that the latency to leave the rotating rod was significantly predicted by the gait score. Fast-growing chickens had shorter durations on the rotating rod, and these durations were associated with gait score. We conclude that the rotarod test provides an objective alternative method for assessing walking ability in chickens without the need for intense observer training or the risk of observer biases and propose that this novel methodology has the potential to function as a precise, objective indicator of animal welfare.*

Keywords: *animal welfare, broiler, domestic fowl, gait score, motor co-ordination, walking ability*

Introduction

Reduced walking ability and leg weakness are major welfare problems in modern strains of meat chickens (*Gallus gallus domesticus*) (Bessei 2006; Caplen *et al* 2013) and can cause pain (Weeks *et al* 2000). In fast-growing broilers (daily growth > 44 g), more than 30% of the birds can show gait abnormalities, whereas the walking ability of slow-growing broilers — under 5% of birds — (Keppler *et al* 2009), is comparable to that of laying hens (Knowles *et al* 2008). Clearly, the prevalence of leg weakness is related to growth rate and live weight of broiler chickens (Kestin *et al* 2001).

Walking ability can be assessed using a gait score system as described by Kestin *et al* (1992). This method is often proposed for welfare assessment protocols (eg Welfare Quality® 2009). In the gait score system, walking ability is assessed in six categories ranging from 0 (fluent locomotion, no detectable abnormality) to 5 (unable to walk). In order to achieve sufficient intra-observer reliability, assessors are required to train and evaluate the criteria of the scoring scheme. Subjectivity, as regards score assessment, not to mention different training protocols in laboratories and countries around the world has meant that results from different observers are often difficult to compare, ie inter-observer reliability is often low (Butterworth *et al* 2007). Furthermore, observer position (ie, from the front, rear or side of the assessed chicken) can directly affect assessment results (Garner *et al* 2002). It has thus been suggested that broiler gait score should be assessed by two observers (Martrenchar *et al* 1999), which is often not possible in on-farm assessments due to limited personnel resources.

Several attempts have been made to increase inter-observer reliability in assessment of walking ability via alternative methodological approaches. For example, pedobarography or force plates have been assessed as possible barometers of walking ability based on foot pressure and torque characteristics (Corr *et al* 1998; Sandilands *et al* 2011). Other studies have used an automatic treadmill with or without weight relief to measure proxies of the gait (Djukic 2007; Reiter & Bessei 2009), three dimensional kinematic techniques (Caplen *et al* 2012), or automated monitoring systems based on activity levels (Aydin *et al* 2010). Furthermore, tests such as the latency-to-lie tests use the chickens' avoidance of sitting in water to measure leg weakness (Berg & Sanotra 2003). However, many of these methods are difficult to implement commercially due to their technical requirements or expenditure of time.

Health problems, such as skeletal disorders from high muscle mass growth, are widespread among broilers (Su *et al* 1999) and can cause a cranial shift in their body's centre (Duggan *et al* 2017). This can have a negative effect on balance (Cavagna *et al* 1977; Corr *et al* 2003) and, thus, on walking ability. LeBlanc *et al* (2016) investigated the maintenance of balance in adult, Shaver White Leghorn hens. Balance skills were tested on a static perch or on a round-edged, square rod with altered swaying speed. Here, the number of drops and jumps from the perch were recorded, and latencies to leave the rod measured. Laying hens showed no significant differences in the latency to leave the static or swaying perches (LeBlanc *et al* 2016).

Another method to test balance or, more precisely, the physical ability to regulate body balance, ie the motor co-ordination, is the rotarod test. This is an established, standardised paradigm for laboratory rodents (Hamm *et al* 1994; Lalonde *et al* 1995; Lynch & Mittelstadt 2017). In general, the experimental set-up of the rotarod test consists of a frame with a rotatable horizontal rod and assembly device to enable turning. Following a short period of habituation (eg 10 s in mice [*Mus musculus*]), rodents are encouraged to walk counter to the direction of the rod (Hamm *et al* 1994). The rotating speed of the rod is set either at a fixed value or to accelerate over time. When a test animal falls from the rod, it is replaced up to five times. In rodent tests, the most commonly measured variables include latency to fall, duration on the rod for a single trial, total duration on the rod in a session, and number of falls or replacements back on the rod (Hamm *et al* 1994; Monville *et al* 2006; Shiotsuki *et al* 2010).

In this study, the rotarod test was modified in order for use with chickens. For evaluation of this as a possible alternative technique for assessing the walking ability of rearing chickens, three strains differing in growth performance were tested. In order to validate the rotarod test results, the classic gait score of the same chickens was also assessed. It was our assumption that the latencies to leave the rotating rod would correlate with the gait scores of chickens.

Materials and methods

Ethical statement

All investigations were carried out with the approval of the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES, Oldenburg, Germany, file number 33.19-42502-04-16/2108). This study was performed in compliance with national regulations (TierSchNutztV) at the research station of the Institute of Animal Welfare and Animal Husbandry (FLI, Celle, Germany). The chickens showed no injuries after performing the rotarod test.

Study animals and housing

We used three different strains of male chickens that differed in terms of growth performance: Ross 308 (Ross; meat strain, fast-growing), Lohmann Dual (Dual; dual purpose strain, medium-growing), and Lohmann Brown Plus (LB; layer strain, slow-growing). In two successive trials, chickens of each strain were randomly assigned to four experimental pens in groups of 50 animals of the same strain (total number of pens: 24 = 2 trials × 3 strains × 4 pens). In both trials, six chickens were randomly selected for testing from three pens plus another five chickens from a further pen. This resulted in 46 test chickens from each of the three strains. The number of tested animals were calculated *a priori* using a power analysis based on data from preliminary tests.

The animals were obtained as day-old chickens from commercial hatcheries. The rearing period lasted five weeks for Ross (weight gain per week = [bodyweight at slaughter – weight of the day-old chicken]/total number of weeks of the rearing period = 345.4 [± 68 g × week^{-1}]), and ten weeks for both Dual (weight gain: 222.5 [± 19.4 g × week^{-1}]) and LB (weight gain: [129.1 ± 19.4 g × week^{-1}]).

A stable climate was maintained via an automatic ventilation and heating system (Equal pressure ventilation system, Pooch Klimatechnik GmbH, Willich, Germany) with an intermediate programme and temperature started at 36°C on the first day, decreasing continuously to 18°C at 36 days of age. The artificial light regimen included dimming phases of 15 min and started with 24 L (light period): 0 D (dark period) for the first three days and reduced to 16 L: 8 D from day four onwards. Lighting was provided at a minimum of 20 lux by flicker-free tube bulbs (Newlec cold white, HFT 18/840, REXEL Germany GmbH & Co KG, Munich, Germany). Each pen (3 × 2 m; length × width) was covered with wood-shavings (in the case of wet litter, chopped straw with a length < 8 cm was added). One round water dispenser and two round feeding troughs were provided with feed offered as single-phase pellets at an energy content of 12.90 MJ ME kg^{-1} (21% crude protein) which met the broiler and layer chickens' feed requirements. Feed and water were available *ad libitum*.

To potentially further increase variability in walking ability, chickens from three different housing conditions were utilised. Housing conditions varied with respect to enrichment, with elevated platforms and perches offered at different heights in the first trial (for a detailed description, see Malchow *et al* 2018). In the second trial, half of the pens were furnished with grids at three different heights, and the chickens in the other pens were kept without additional structure. These housing conditions were distributed equally between chicken strains.

Experimental set-up of the rotarod test

The experimental set-up of the rotarod test consisted of two parts: a wooden framework within which the chicken was placed, and electronic control equipment (see Figure 1). The wooden framework consisted of a rod (50 × 1,000 mm [diameter × length]) and a frame (121 × 211 cm [width × height]). The rod was positioned between the two vertical pillars at a height of 85 cm and, for extra grip, the surface of the rod was covered with a thin layer of black rubber. Two soft foldable mats (185 × 78 cm [length × width]) were placed approximately 17 cm beneath the rod as padding for when chickens left the rod. A wooden shield (50 × 50 cm) was attached at one side of the frame to limit visual distraction and protect chickens from contact with the motor next to the rod.

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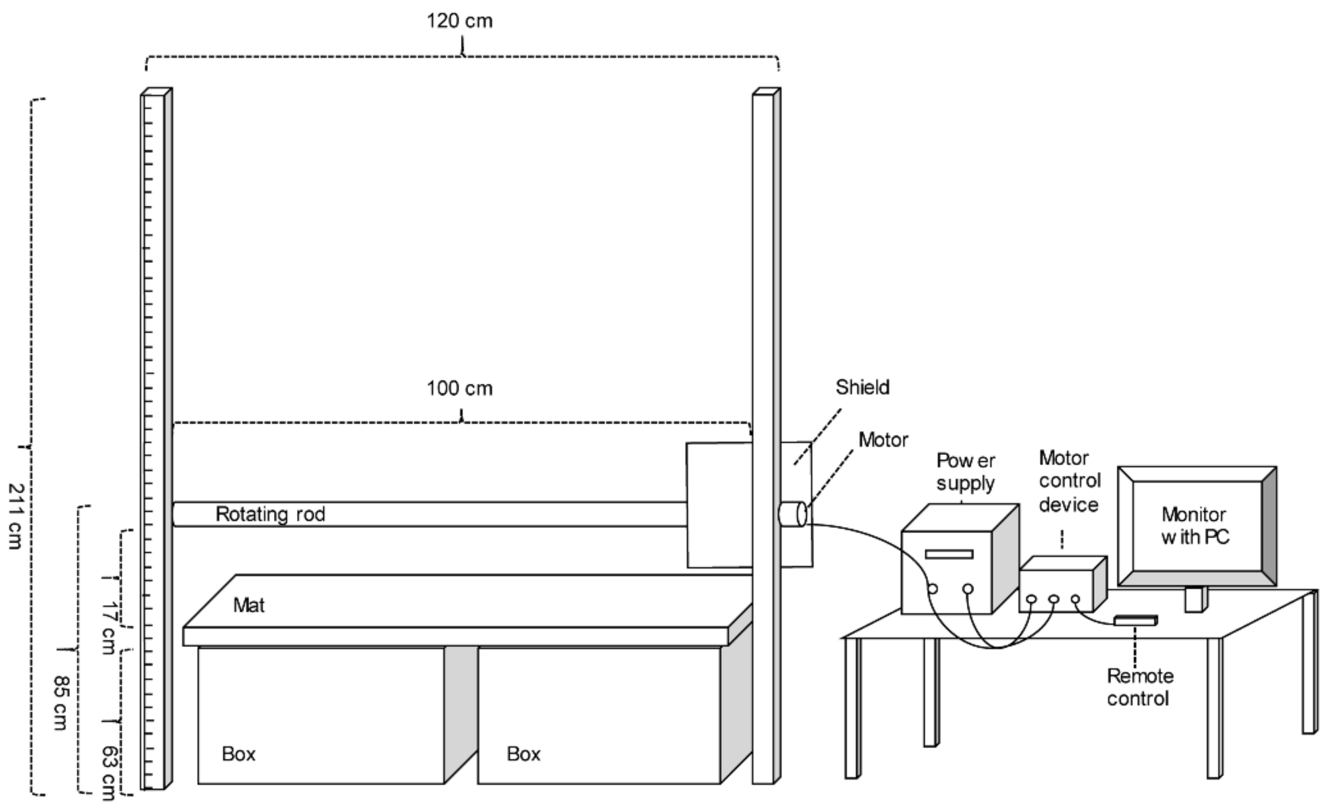
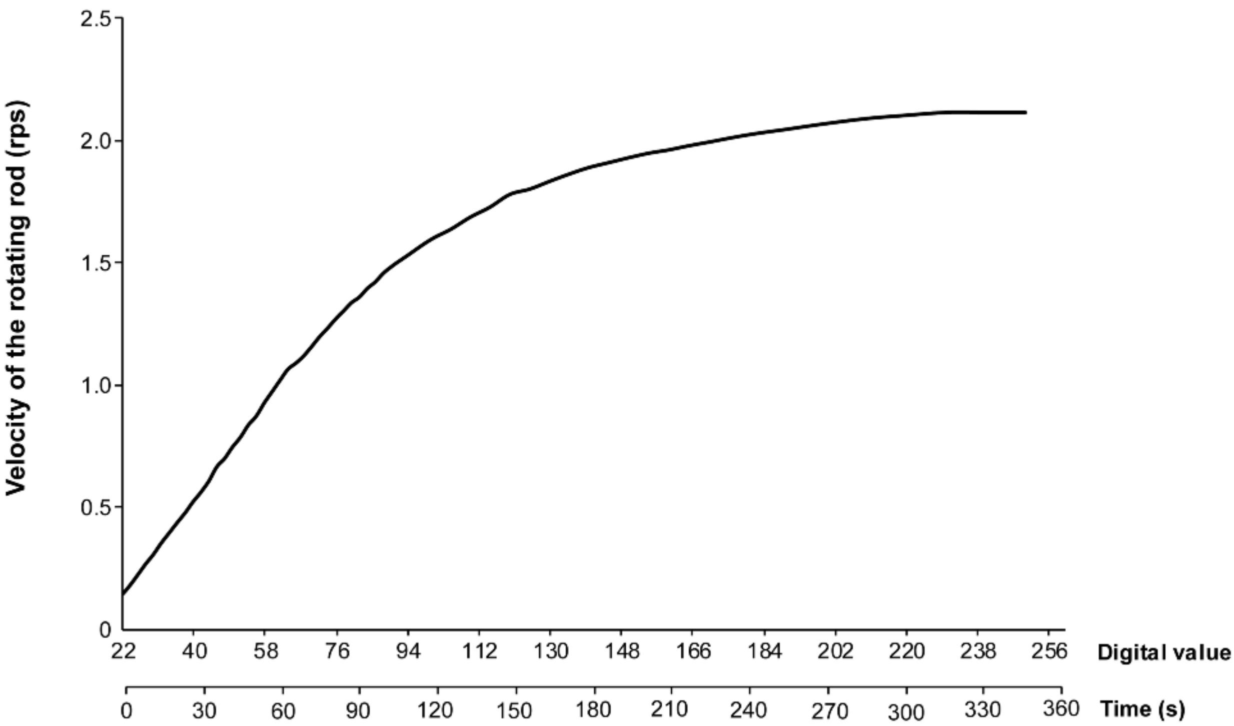
Figure 1



Schematic view (top) of the rotarod test set-up including the wooden frame with rotating rod, mats, boxes under the mats, visual shield and motor, and electronic control device consisting of a motor box, power supply, monitor with PC and remote control. Photograph (bottom) of the rotarod set-up with a Dual chicken placed on the rod.

Figure 2



The velocity course of the rotated rod from 0 to 360 s. Velocity was controlled automatically via software that sent an increasing digital value (diva) every 1.545 s to the motor. The maximum speed reached after 360 s was 2.1 rps.

The electronic control equipment consisted of a motor rotating the rod, a power supply, a motor control device, and a computer. A direct-current, 24-V motor (Model DSMP 420-24-061, gear reduction: 61:1, Drive-System Europe Ltd, Werther, Westfalen, Germany) was driven with an accelerating speed controlled by self-customised software (this can be provided upon request) written in Visual Studio C++ 2010 (Microsoft Corporation, Redmond, USA). The motor control device received a digital value command (hereafter referred to as diva) from 0 to 255 (8-bit) from the computer and controlled the motor with a 28-V pulse width modulation supply. When the programme began it counted the command value and the motor accelerated. The motor started with a diva of 22 since below this value, the motor power was insufficient to turn the rod. To accelerate from 0 to a maximal rotation within 360 s, every 1.545 s the diva was increased by one (from 22 to 255 diva; 360 s/[255 diva – 22 diva] = 1.545 s). The maximum speed reached after 360 s was 2.1 rps (revolutions per second) (Figure 2). An accelerating speed of rotation has been suggested to be more suitable for the assessment of motor co-ordination than a fixed speed (Monville *et al* 2006). The observer was able to start and stop the motor of the rotating rod via remote control. The animal number, time and latency until a chicken left the rotating rod was recorded with the software.

Measurements

Assessment of walking ability

Walking ability was assessed using the gait score system developed by Kestin *et al* (1992). Here, the chicken's gait is classified into one of six distinct categories: Gait score 0 — fluent locomotion, no detectable abnormality, furled foot in the air; Gait score 1 — slightly undefined defect in its gait; Gait score 2 — easily detectable walking abnormality, no restriction in its movement, quick and short steps; Gait score 3 — limited walking ability, visible defective locomotion, limp in one leg, quick steps; Gait score 4 — able to walk for a brief period, strong detectable walking abnormality, quick squats down; and Gait score 5 — unable to walk, locomotion could be achieved with the assistance of wings.

The Ross chickens were tested on the two days prior to slaughter at five weeks of age, and the Dual and LB chickens were tested at ten weeks. Tests were performed in the morning (from 0800 to 1200h) by one observer (JM) in a separate test arena (2.5 × 0.5 m; [length × width]) located in the stable alley. The test chickens were randomly selected from all pens and gently transferred from their home pen to one end of the test arena. To encourage the chickens to walk, three non-tested conspecifics from the same pen were placed at the opposite end of the test arena. Chickens were given 1 min to habituate and typically began to walk independently. If a chicken did not voluntarily start walking after 2 min, it was gently coerced into doing so. The

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observer assessed the chickens from a distance of at least 1 m via a vantage point on the long side of the test arena.

The assessment of walking ability with the gait score system was always carried out before the rotarod test. This was to ensure the observer avoided being biased from the results of the rotarod test. There was a possibility chickens may have had a short latency to leave the rotating bar, which was not necessarily related to a poor gait score. It was also possible that results could have been influenced by the order in which tests were carried out. However, our proposal was approved by the competent authority who would only sanction the minimum number of animals sufficient for statistical analysis. Thus, we decided only to use one test order. A greater number of animals would have been needed in order to test the effect of test order. An additional reason was that the rotarod test probably demands greater muscle strength compared to walking in the gait score system, ie the effect of a rotarod test prior to a gait score test is likely to be more profound than *vice versa*.

Rotarod test procedure

After the individual gait score was assessed, each test chicken was taken to the rotarod apparatus and placed at the middle of the rod. The motor was started when both of the chicken's feet were grasped around the rod and stopped when the chicken actively or passively left the rotating rod. This was registered as the latency to leave the rotating rod(s). Following this, the chicken was weighed (nearest [± 10] g) and returned to its home pen. Additionally, each test was video-recorded (Model VAZ2S, AIPTEK International GmbH, Willich, Germany) in order to analyse the manner in which chickens left the rod, ie active (jumping/flying down from the rotating rod) or passive (falling from the rotating rod).

Statistical analysis

To relate the latencies obtained from the rotarod test to the gait scores, we used a linear mixed effect model (LME). For the initial model, the latency to leave the rotating rod was the dependent variable; gait score (numerical factor), weight gain per week (numerical factor), manner of leaving the rod (categorical factor) and their respective two-way interactions were included as fixed factors. Compartment ID (24 pens) was considered a random factor nested within the random factor strain. Non-significant factors were step-wise excluded by backward selection of the respective least significant factor, while the three main factors remained in the final model, as they were the parameters of interest. Residual plots indicated no deviation from a normal distribution. In the case of significant effects of gait score, a *post hoc* analysis was performed using *post hoc* pair-wise *t*-tests with Bonferroni correction. All models were run in R 3.2.5 (R Core Team 2016 using the nlme package (Pinheiro *et al* 2017).

Table 1 Number of chickens in the three strains assigned to different gait scores (n = 46 per strain).

Gait score	Strain			Total
	Dual	LB	Ross	
0	7	44	0	51
1	36	2	5	43
2	3	0	18	21
3	0	0	19	19
4	0	0	4	4

Results

The three strains of chickens showed differing distributions of gait scores (hereafter GS, as shown in Table 1). Most Ross chickens were assigned to GS 2 and 3, the majority of Dual chickens to GS 1, and almost all LB chickens to GS 0. GS 5 was not assigned to any chicken.

The latency to leave the rotating rod showed a significant association with the GS (LME, factor gait score, $F_{1, 109} = 9.83$; $P = 0.0022$; Figure 3). Further *post hoc* tests revealed that chickens with GS 0 and GS 1 stayed significantly longer on the rotating rod compared to chickens with GS 2, 3, and 4 (Figure 3).

The latency to leave the rotating rod was significantly affected by weight gain per week (LME, factor weight gain per week, $F_{1, 109} = 11.34$; $P = 0.001$; Figure 4).

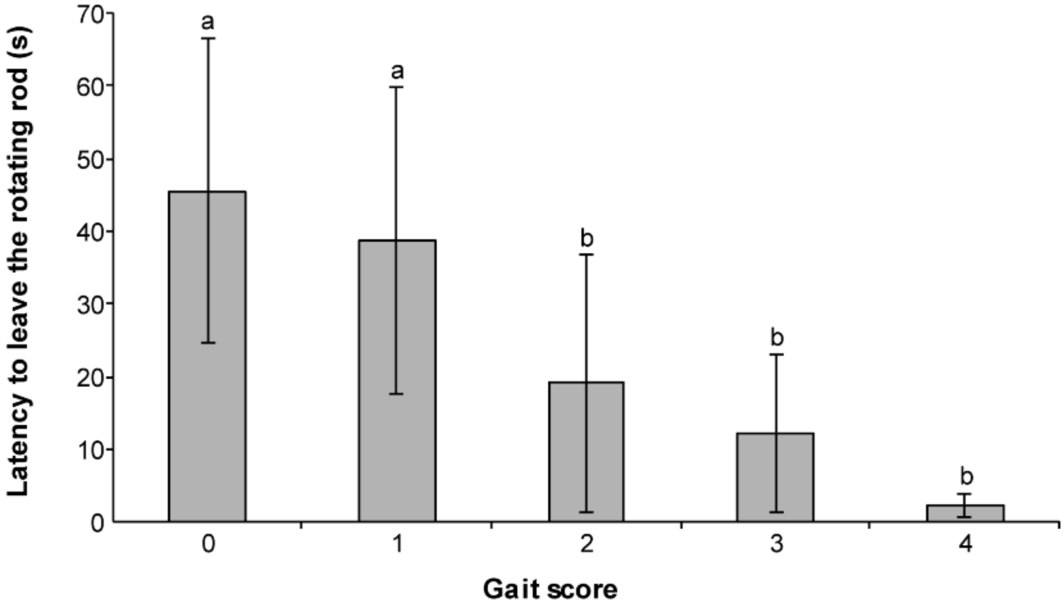
The manner of leaving the rod (active or passive) did not affect the latency to leave (LME, factor manner of leaving the rod, $F_{1, 109} = 0.06$; $P = 0.81$).

Discussion

A modified rotarod test was used to assess the motor abilities of three strains of chicken differing in terms of weight gain per week. Latencies to leave the rotating rod were significantly related to walking ability as assessed by the gait score system. Thus, the rotarod test seems to be a valid method of measuring walking ability on the basis of motor skills of rearing chickens. In addition, this method provides a greater level of objectivity and avoids possible observer bias as has often been reported for the gait score system. Furthermore, a key advantage of the rotarod approach is that walking ability is measured in a more precise and continuous measurement than is seen with the categories of the gait score system.

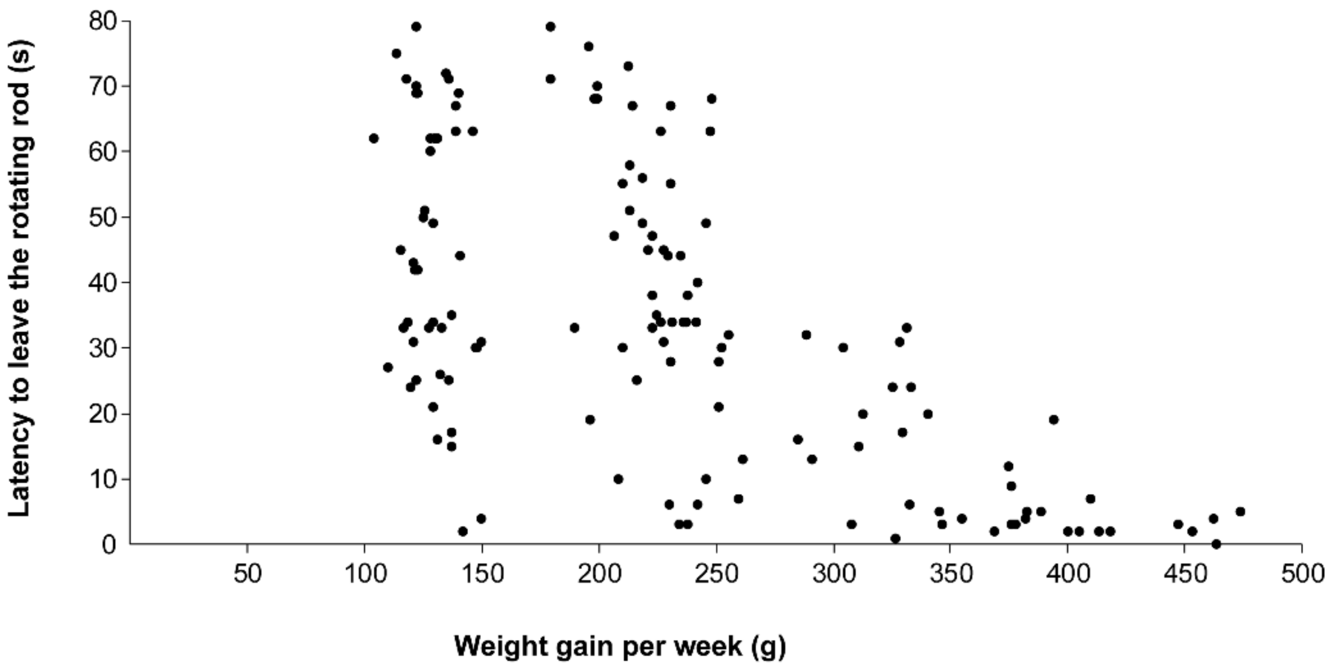
Compared to the slow-growing chickens (eg Dual and LB), fast-growing chickens (eg Ross) displayed a poorer walking ability in both the rotarod test and the gait score system. This result was in accordance with the findings of Kestin *et al* (2001) who compared the GS in 13 broiler genotypes

Figure 3



Effect of gait score on the latency to leave the rotating rod (means ± SD). Significant differences between gait scores are marked by different superscripts ($P < 0.05$).

Figure 4



The relationship between the latency to leave the rotarod and weight gain per week.

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and found that the growth rate affected walking ability. Additionally, the distributions of GS 2 and GS 3 for Ross chickens were comparable to previous studies, which reported that more than 90% of the chickens indicated lameness at slaughter date, and approximately 30% were classified as GS worse than 2 (Kestin *et al* 1992; Sanotra *et al* 2001). The strains differed anatomically, in terms of breast muscle size, tibial length and maximal breaking force (Mueller *et al* 2018). It is likely that these differences affect both the latency to leave the rotating rod and the gait score. However, in this study, different strains from differing housing conditions were deliberately utilised in order to increase the variability of the data. Comparing different strains was not the aim of this study *per se*.

The chickens left the rod either actively or passively, ie either by jumping intentionally or flying from the rod or falling unintentionally. If chickens with good motor co-ordination had jumped off earlier this would have resulted in misleading latency to leave data. In such instances, a short latency to leave would not necessarily indicate a GS of 5 (worst walking ability). Interestingly, neither the manner of leaving the rod nor its interaction with either of the other factors significantly affected the latency to leave the rotating rod. Thus, the data obtained from the rotarod test seem to be robust in terms of how chickens left the rotating bar.

Conducting the rotarod experiments requires only the briefest of introductions to master the set-up and electronic control device. Training is unnecessary and the rotarod test easy to handle. Conversely, use the gait score system demands practical training and clear instructions for the observer. In the studies by Garner *et al* (2002) and Webster *et al* (2008), the gait score system was modified from 6 to 3 categories, whereby both inter-observer and the retest reliability were better in the 3- compared to the 6-point scale. An advantage of the gait scoring system is that the observer requires minimal equipment (simply paper and a pen) as only rough categorical measures are taken as opposed to the continuous measures needed for the rotarod test. Also, the rotarod test requires additional space for the set-up and an electrical power source. However, our study device was a first prototype and readily able to be reduced in size for on-farm use. For example, frame height could be reduced down to 120 cm or less, and a 50-cm long rod should offer ample space for the test chicken to manoeuvre along. In addition, the electronic devices can be miniaturised. When compared to other electronic equipment used to assess walking ability, such as video-based techniques (Aydin *et al* 2010), the rotarod method seems less complex and arguably takes less time for preparation.

In our study, only one diameter size was utilised for the rod, and no adjustments were made to this to cater for the foot size of the different strains. Perhaps an association exists between foot size and rod diameter which affects birds' ability to remain on the rod. So it would be advisable to adjust the diameter of the rod to reflect the respective foot size of birds to be tested. Here, we occasionally observed that Ross chickens required several attempts to claw around the rod and tilted forward. Thus, optimum rod diameter may depend not only on the birds' foot size but also their ability to manipulate and control their toes. However, this trait also affects walking ability, which is the crucial parameter tested by the rotarod test. Furthermore, for the rotarod test, chickens must mainly walk when the rod is rotating; however, by moving the feet, grasping and perching are also involved in maintaining balance. The specific surface and anatomic properties of birds' feet are usually regarded as being adapted to allow precise positioning of the feet and maintaining balance (Galton & Shepherd 2012; Sustaita *et al* 2013). However, modern strains selected for different purposes, such as for laying or growth, may differ in their ability to grasp due to different body traits. Thus, these properties should be investigated in more detail.

The aim of the rotarod test is to show different latencies between normal and detectable abnormalities in the walking ability of chickens. In future studies, it should be possible to refine the results and provide a clear link between a range of latencies to leave and categories of walking ability. In our study, each chicken was tested only once in the rotarod test, to avoid possible effects of training or habituation. These effects are potentially more likely to be seen with the rotarod test as opposed to the GS test since, in the latter, an easier and often voluntary movement is assessed. A general problem with repeated testing in fast-growing chickens is that they must be performed within a few days to control for the effects of weight. Nevertheless, future studies should be performed to test the repeatability of the rotarod test within subjects. Furthermore, all tested chickens were assessed for a short time (max 5 mins) in the rotarod test. The test situation was identical for all animals, all were separated visually from their pens yet they always had acoustic contact with conspecifics. However, it might be possible that social isolation affects strains or individuals differently. A previous study has shown differences in social reactions between different layer strains (Dudde *et al* 2018). Even although we have used a standardised test protocol for the rotarod test, we could not fully exclude any potential strain-specific effects of the brief visual separation. However, strain differences were beyond the scope of our study.

When applying the gait score system, the position of the observer can affect the assessment. For example, an observer from the side position could assess limping based on sharp turns from a healthy chicken which could lead to a wrong choice of score (Garner *et al* 2002). The results of the rotarod test are independent of the position of the observer, as the observer has only to note when the chicken leaves the rod.

The latencies to leave the rotating rod are a continuous measure, whereas the GS are categorical data. It is possible to modify the GS data into continuous data (Tuyttens *et al* 2014), but the method is still subjective and highly dependent on the observer. The great advantage of a continuous measure is that the latencies allow a more precise analysis of the walking/locomotor performance of the animals compared to the six GS; thus, more subtle but welfare-relevant differences in walking skills may be detected by the rotarod test. Moreover, continuous data can be subjected to particular types of statistical analyses, compared to categorical data, which provide greater statistical power.

Animal welfare implications and conclusion

The rotarod test provides an objective alternative method for assessing walking ability in chickens without intense observer training or the risk of observer biases. In contrast to the gait score system, the rotarod test is less dependent on observer experience for assessing gait. The results of the rotarod test showed a significant association with normal gait and detectable abnormalities in the gait of the chickens. In its current state, the rotarod test requires a degree of fine-tuning to align it to anatomical differences between strains. This novel methodology of an objective assessment with a precise measure has the potential to be implemented as an indicator of animal welfare.

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