

## Wooden breast myopathy links with poorer gait in broiler chickens

Marianna Norring<sup>1\*</sup>, Anna Valros<sup>1</sup>, Jarmo Valaja<sup>2</sup>, Hanna-Kaisa Sihvo<sup>3,4</sup>, Kaisa Immonen<sup>3</sup>, Eero Puolanne<sup>3</sup>

<sup>1</sup> *Research Centre for Animal Welfare, Department of Production Animal Medicine, University of Helsinki, Helsinki, Finland.*

<sup>2</sup> *Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland.*

<sup>3</sup> *Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland.*

<sup>4</sup> *Veterinary Bacteriology and Pathology Research Unit, Finnish Food Safety Authority Evira, Helsinki, Finland.*

\* Corresponding author Marianna Norring

*E-mail: Marianna.Norring@Helsinki.fi*

Short title: **Myopathy and gait of broilers**

## **Abstract**

Wooden breast myopathy, a condition where broiler breast muscles show a hardened consistency post-mortem, has been described recently. However, it is not known how wooden breast myopathy affects the bird activity or welfare. Altogether, over 340 birds of five commonly used commercial hybrids were housed in 25 pens, and sample birds killed at ages of 22, 32, 36, 39 and 43 days. Their breast muscle condition was assessed post-mortem by palpation. The birds were gait scored and their latency to lie was measured prior to killing. For further behavior observations one affected and healthy bird in 12 pens were followed on five days for 20 minutes using video recordings. The connection of myopathy to gait score and activity was analyzed with mixed models. A higher gait score of wooden breast affected birds than of unaffected birds (2.9 vs.  $2.6 \pm 0.1$ ,  $P < 0.05$ ) indicated a higher level of locomotor difficulties over all age groups. The wooden breast affected birds had fewer crawling or movement bouts while lying down compared with unaffected ( $P < 0.05$ ). Wooden breast myopathy affected birds were heavier (2774 vs.  $2620 \pm 91$  g;  $P < 0.05$ ) and had higher breast muscle yield (21 vs.  $19 \pm 1$  %;  $P < 0.05$ ) than unaffected birds over all. Older birds had longer lying bouts, longer total lying time, fewer walking bouts, more difficulties to walk and to stand compared with younger birds ( $P < 0.05$ ). Birds with poorer gait had longer total lying time and fewer walking bouts ( $P < 0.05$ ). Birds with greatest breast muscle yield had the largest number of lying bouts ( $P < 0.05$ ). It was concluded that wooden breast myopathy was associated with an impairment of gait scores, and may thus be partly linked to the common walking abnormalities in broilers.

**Keywords:** activity; animal welfare; *Gallus gallus*; lameness; poultry

### **Implications:**

Wooden breast myopathy is a newly discovered meat quality defect in fast growing meat type chickens that is only observed post mortem. The animal welfare effects of wooden breast myopathy has not been studied earlier. In this study signs of wooden breast myopathy was detected in 38 % of the broilers during the study. Subtle behavioural changes observed with wooden breast affected individuals suggest a link with poor gait of broilers. These findings raise a concern for possible animal welfare effects of wooden breast myopathy that needs to be addressed.

## Introduction

Broiler meat consumption is increasing globally and the meat is produced in large and efficient plants with hybrids of uniform breeding goals. Leg weakness in fast growing chickens grown for their meat is a common, yet unresolved animal welfare problem (Knowles *et al.*, 2008). This issue has also been emphasized in a recent report for the European Parliament (Broom, 2017). Gait score is a good predictor for broiler mobility (Caplen *et al.*, 2014), and walking ability is usually weaker in heavier (Sørensen *et al.*, 1999; Kestin *et al.*, 2001) and faster growing birds (Kestin *et al.*, 2001). Another measure for leg weakness is the willingness of birds to maintain a standing posture, as quantified by the latency to lie test (Bailie *et al.*, 2013). Multiple risk factors for lameness has been proposed, but no clear causative mechanism for the wide spread prevalence (Knowles *et al.*, 2008) has been identified, nor has the issue been resolved.

Sihvo *et al.* (2014) describes a new myodegenerative disease in broiler and calls it wooden breast (also referred to as woody breast). This breast muscle myopathy is defined by abnormal microscopic appearance in tissue samples and it is accompanied by hard consistency of the *pectoralis major* muscle post mortem (Sihvo *et al.*, 2014; Sihvo *et al.*, 2017). Wooden breast myopathy possibly exhibits metabolic signs of oxidative stress (Abasht *et al.*, 2016) and it often appears with white striping (Sihvo *et al.*, 2014) which is associated with fast growth rate (Kuttappan *et al.*, 2013a). The incidence of the wooden breast myopathy on farms is unknown, yet white striping is estimated to affect one tenth of broilers (Petracci *et al.*, 2013). Mazzoni *et al.* (2015) observed some degree of structural abnormalities in all sampled heavy sized broilers. However, there are no detectable symptoms on live birds to single out the affected individuals. The affected meat possess no food security risk, however the quality is inferior at least with white striping (Mudalal *et al.*, 2014) and that leads industry in some countries to down grade the most valuable parts of a carcass. There is a need for further studies on signs of wooden breast in live birds and possible links with poor environment (Kuttappan *et al.*, 2016) and there is no knowledge whether wooden breast myopathy affects bird behavior, walking ability or welfare. It was hypothesized that myopathy would have a negative effect on

mobility and thus have welfare implications for the animals. This study explored a possible link between wooden breast myopathy and mobility of birds of five commercial broiler hybrids.

## **Materials and methods**

### *Animals, housing and management*

The study conformed to the European Directive (2010/63) on the protection of animals used for scientific purposes and no harm was expected by the keeping, observing or killing of the animals. Therefore, an internal animal license number KEK13-006 was granted by the University of Helsinki laboratory animal center. The birds were managed according to the broiler welfare directive (2007/43/CE). Male broiler chickens (*Gallus gallus*) of 5 commercial hybrids were acquired from commercial hatcheries and housed in the one experimental room simultaneously. The room consisted of 25 pens of 90\*90 cm. Each pen contained 13 or 15 chicks of the same hybrid on day 1 after hatching. The stocking density did not exceed 37 kg/m<sup>2</sup> during the experiment. Birds of each hybrid were housed in 4 to 5 pens.

A thin layer of fine sawdust litter was added on top of hard rubber mats. The litter was renewed every week and pens were partly cleaned daily, according to need. Broilers were inspected twice a day and injured or sick birds euthanized. Birds were fed a pelleted wheat and soy bean meal based feed 12 (MJ/kg), designed to meet their nutritional requirements, *ad libitum* from pan feeders. Water was offered from nipple drinkers. The room had no windows and two dark phases of 4 and 2 hours each discontinued the lighting. Room temperature was 33°C degrees for the hatchlings and 20°C towards the end of rearing.

### *Procedures*

Two (mean 2.1) birds per pen were evaluated at ages of 22, 32, 36, 39, 43 days. In total, 266 broilers were analysed and the 52 remaining birds were killed at ages of 15, 19 and 50 days (data not included) and 15 birds died or were euthanized due to illnesses on other days. The post mortem examination indicated sudden death

syndrome to be the most frequent cause of death. The bird to be evaluated was chosen by always selecting the second bird clockwise from the west corner of each pen. The gait of these selected birds was evaluated using the scoring system by Kestin *et al.* (1992) where 0 indicates normal bird and 5 bird unable to walk. Gait was scored individually in their home pen while pen mates were herded to the other side of the pen with wire mesh. Gait was independently scored by 2 trained observers and mean scores were used in analysis.

In addition, their spontaneous latency to lie (Baillie *et al.*, 2013) was observed 3 times in a row in the home pen. Water has previously been used (Berg and Sanotra, 2003) to increase the motivation to stand but here the test was completed without it. The test session started by fencing off all other birds in the pen. When the selected bird sat down it was encouraged to stand by approaching or gently touching it with a 1m blunt plastic stick with paper clippings attached to the end to ensure visibility. As soon as the bird stood up, the stick was removed. The time interval from standing to lying was measured. The latency to lie test was repeated 3 times. Gait was evaluated during and after latency to lie testing.

After the mobility testing the birds were individually weighed and then killed by mechanical cervical dislocation followed by phlebotomy of the jugular veins. Immediately post mortem, the birds were necropsied and their feet were evaluated. The presence or absence of footpad and hock dermatitis was recorded. The right *pectoralis major* muscle was removed. One observer scored the muscle for consistency change by manual palpation within 10 minutes post mortem. Muscles with a focal or diffuse area of abnormally hardened muscle consistency were scored to be wooden breast affected. Muscles of normal consistency were scored unaffected. Breast muscle yield was given as percentage of live weight.

### *Activity*

For behavior observation 12 pens were video recorded, including 2 or 3 pens per breed. One camera covered 4 pens from above, at an approximate 1.5 m distance from the floor. All the pens were recorded simultaneously on days 12, 17, 25, 34, and 42 for one hour (18:00-19:00). For individual recognition the back feathers of 4 birds per pen were color marked. The selection of individuals for behavior analysis was

done based on post mortem data. One wooden breast affected marked bird and one marked unaffected bird in 12 pens were observed from video recordings from day 12 until killed at age 39 to 42 days. The behavior of these birds was observed continuously during two 10 minute periods from video recordings on each filming days.

Using the video films, the initiation and end of standing and lying posture was observed. In addition, number of movement bouts were observed during episodes of lying or standing. Lying was defined to begin when the body of the bird or the hocks touched the ground. Movement bouts during lying were defined to occur when a bird moved in any direction during lying while its hocks or breast touched the ground; or when it crawled or changed position rhythmically at least 3 times, or dustbathed. Walking bouts were defined as locomotion while in a standing position. One observer analyzed all the videos.

### *Statistical methods*

The average gait scores of an individual bird scored by 2 observers was used in the analyses. The average latency to lie of the 3 consecutive observations was used in analyses. To evaluate the connection of myopathy and age to gait score, latency to lie, body weight and breast muscle yield separate linear mixed models analysis were used. Pen (25) and hybrid (5) were considered random effect. Wooden breast myopathy (affected or unaffected) and bird age (5 ages) were inserted as fixed factors. The interaction between age and wooden breast myopathy was tested but it was non-significant thus omitted from the models.

Data from video recordings was analyzed separately. Total amount of lying, mean lying bout length, number of lying bouts, total number of movement bouts during lying and total number of locomotion bouts during standing were calculated for two daily observation periods for each day and for each animal. The connection of wooden breast myopathy, age, gait score and breast meat yield to lying and activity (total lying time, mean lying bout length, lying bout frequency, frequency of movement while lying and frequency of walking) was analyzed with separate linear mixed models. Age, wooden breast myopathy, gait score and breast meat yield were included in the model as fixed factors. Bird was inserted as random variable nested

within pen (12). Significance threshold was set at  $P = 0.05$ . Statistical analyses were conducted with SPSS 23.

## Results

### *Gait score and latency to lie*

Wooden breast myopathy was detected in 100 of total 266 broilers. The gait score of birds affected by wooden breast myopathy was poorer than that of unaffected birds (Table 1). Wooden breast myopathy affected birds were heavier and had higher breast muscle yield than unaffected birds (Table 1). There was an effect of age on gait score ( $F_{4,238} = 65$ ,  $P = 0.001$ ; Fig 1), indicating impairment of gait score as the birds grew older. Age had a similar effect on latency to lie ( $F_{4,260} = 3$ ,  $P = 0.010$ ; Fig 2). Age also had effect on weight ( $F_{4,256} = 903$ ,  $P = 0.001$ ; Table 2) and breast muscle yield ( $F_{4,256} = 63$ ,  $P = 0.001$ ); older birds grew heavier.

### *Lying and activity*

The total sum of movements per 10 minutes while lying down was associated with wooden breast myopathy; the affected birds having fewer movements while lying down (Table 1). Total lying time, mean lying bout length, number of lying bouts or walking bout frequency were not related with the presence or absence of wooden breast myopathy. Older birds had longer total lying time per 10 minute observation ( $F_{4,223} = 5$ ;  $P = 0.001$ ; Table 3). Older birds also had longer, and fewer lying bouts, and fewer walking bouts compared with younger ( $F_{4,205} = 5$ ;  $P = 0.001$ ,  $F_{4,205} = 5$ ;  $P = 0.001$ ,  $F_{4,223} = 18$ ;  $P = 0.001$ ; Table 3). However, the movement frequency while lying down was not affected by age.

Birds with poorer gait score had longer total lying time; there was a 32 seconds increase in lying per 10 minute observation time (95% Confidence Interval 4 to 61) with an increase of one on the gait score scale ( $F_{1,223} = 5$ ;  $P = 0.027$ ). Birds with poorer gait score also had fewer walking bouts (slope -3 bouts, 95% Confidence Interval -5 to -1;  $F_{1,223} = 7$ ;  $P = 0.007$ ). However, gait score was not linked with mean lying bout length, number of lying bouts or the movement frequency while lying down.

Birds with greatest breast muscle yield had the largest number of lying bouts; there was an increase of 0.3 bouts/% (95% Confidence Interval 0.1 to 0.5) for an increase of 1% in breast muscle yield ( $F_{1,20} = 8$ ;  $P = 0.012$ ). Breast muscle yield was not related with total lying time, mean lying bout length, movement frequency while lying down or walking bout frequency. Macroscopic foot lesions were observed in 21 birds out of 330 at the post mortem examination. In most birds (19), the lesions consisted of mild erosions or ulcers in the skin of both hock joints (hock dermatitis), without foot pad dermatitis.

## **Discussion**

Wooden breast myopathy detected by macroscopic inspection of post mortem muscle was associated with a decreased ability to walk. Results on resting behavior, gait and latency to lie showed a detrimental effect of age on mobility of broilers. Earlier studies already indicated that walking ability degenerates as birds grow older (Weeks *et al.*, 2000; Bailie *et al.*, 2013; Kaukonen *et al.*, 2017), in addition, their behavioural activity dwindles and the latency to lie gets shorter (Norrington *et al.*, 2016; Bailie *et al.*, 2013). This study further confirmed these findings of progressive immobility when birds became older as they were lying for longer episodes and had fewer bouts of walking. Lamé birds use longer time resting sternally (Weeks *et al.*, 2000) but it is yet unknown if prolonged resting is caused by muscle weakness or pain or if prolonged resting affects breast muscle development negatively.

Results revealed that wooden breast affected birds had fewer movements while lying down compared with unaffected counterparts. It might be speculated that developing wooden breast myopathy may cause discomfort, manifested as motionless resting behaviour. This observation calls for further studies about possible pain or discomfort involved in wooden breast myopathy that perhaps causes heightened sensitivity of breast area. The effects of wooden breast on other measures of resting behavior and activity are less clear however. Moreover, behavior of birds with high breast muscle yield was characterized by shorter periods of lying, perhaps indicating fragmentation



of resting behaviour. There is need for further investigation of the behavioral effects while wooden breast myopathy is developing.

Results demonstrate that wooden breast myopathy appeared especially in heavier birds with high breast muscle yield. In agreement to this, higher breast muscle yield, weight and faster growth has been correlated with increased white striping and wooden breast (Kuttappan *et al.*, 2012; Kuttappan *et al.*, 2013a, Kuttappan *et al.*, 2017). Myofiber lysis, necrosis and myodegeneration have been described in association with both white striping and wooden breast myopathy (Kuttappan *et al.*, 2013b; Sihvo *et al.*, 2014, Mazzoni *et al.*, 2015). Plasma creatine kinase is a marker of muscle damage and high values have been detected in meat type poultry (Branciarri *et al.*, 2009; Wichman *et al.*, 2010), but the reason for the indicated muscle damage have not been clarified. Yet higher creatine kinase levels have been associated with white striping of breast muscle indicating muscle damage (Kuttappan *et al.*, 2013a). Current results together with histological observations by Sihvo *et al.* (2014; 2017) suggest that damaged fibers exist in breast muscles of broilers who show no clinical signs of illness but have an overall poorer walking ability. The association of this sort of muscle damage with pain still remains to be studied.

Rather unsurprisingly, birds that had poorer gait also had longer resting times and fewer walking bouts, as already shown by Weeks *et al.* (2000). This study is also in line with Caplen *et al.*, (2014) who showed that gait score predicted moving ability. Myodegenerative muscle disease, as described by Sihvo *et al.* (2014) in *pectoralis major* could be hypothesized also to affect the leg muscles thus weakening the walking ability. Yet, it seems implausible as Sihvo *et al.* (2017) found a muscle lesion comparable to wooden breast myopathy from a leg muscle of one bird only. In another scenario, leg muscles may remain unaffected while gait of clinically normal looking birds is affected by subtle sickness behavior, mediated by for example pain in breast muscles or elsewhere. It has previously been suggested that lameness in broiler associates with pain (Hothersall *et al.*, 2016). Wooden breast myopathy manifests in larger breast muscles (Kuttappan *et al.*, 2017) making it difficult to tease apart the effects of body weight, age and wooden breast myopathy. Thus heavy weight or fast growth rate could be a common underlying factor for both wooden

breast myopathy and poor gait. However, there was no statistical interaction between age and wooden breast status indicating that the wooden breast affected birds had poorer gait in all age groups. Thus, even the youngest birds, still agile, demonstrated slightly poorer gait while affected with wooden breast myopathy than healthy birds did.

Current results from the modified latency to lie test, where no water was used, are on a comparable level with the latencies found by Bailie *et al.* (2013), employing a similar technique with no water. However, compared with Berg and Sanotra (2003), who used a 3 cm water bath this test resulted in much shorter latencies and was very fast to deliver. In the test most birds lied down in less than a half minute compared to the tests used by Weeks *et al.* (2002), Berg and Sanotra (2003) and Caplen *et al.* (2014) where birds could stand for as long as 10 to 15 minutes. Results indicate that broilers lie down faster in their pen than when tested with water and thus find water aversive and avoid lying down.

Very low rates of foot pad dermatitis reflect positive effects of very clean and dry bedding that could be achieved with regular renewal of litter. However, the presence of few cases of hock dermatitis suggests that hock skin might be very vulnerable or posture of some birds is altered. In conclusion, the majority of slaughter aged broilers were not walking well. Wooden breast myopathy was associated with an additional impairment of gait scores, and might thus be linked to the common walking abnormalities in broilers. Findings of this study call for further investigation on effects of wooden breast myopathy on broiler welfare.

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### **Declaration of interest**

None.

### **Ethics statement**

None.

### **Software and data repository resources**

None.

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**Table 1.** Differences between wooden breast affected and non-affected birds in behavior and weight (*Mean ± SE, N = 266*)

	Wooden breast	Non wooden breast	P-value
Gait score	2.9 ±0.1	2.6 ±0.1	0.001
Latency to lie s	16 ±1	17 ±1	ns
Body weight g	2774 ±91	2620 ±91	0.001
Breast muscle yield %	20.5 ±0.5	19.4 ±0.5	0.001
Number of movements per 10 minutes while lying down	2.0 ±0.4	3.2 ±0.4	0.046

**Table 2.** Body weight and breast muscle yield of broilers at different ages (*Mean ±SE, N = 266*)

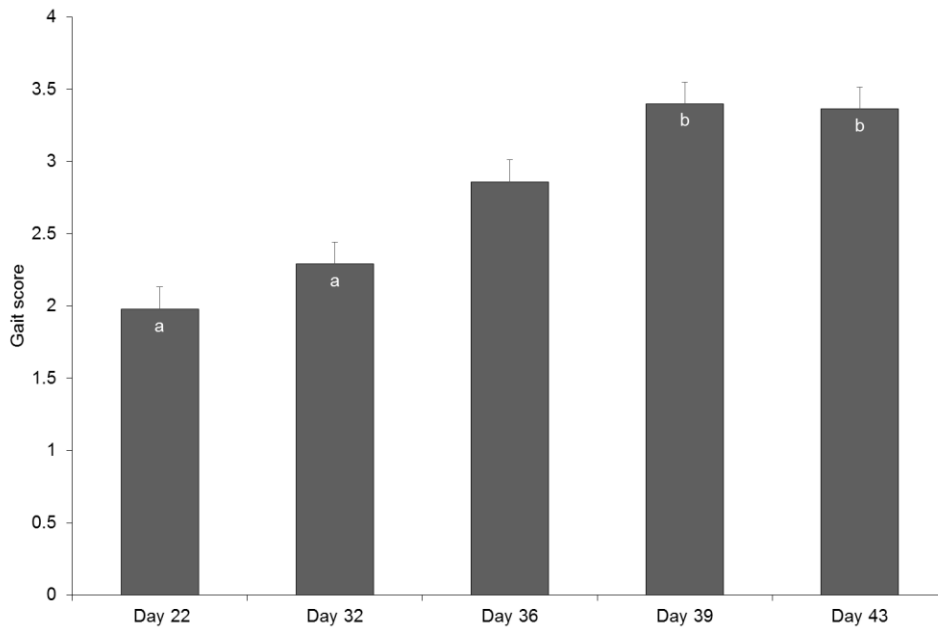
	Day 22	Day 32	Day 36	Day 39	Day 43
Body weight, g	1325 ±94 <sup>a</sup>	2411 ±94 <sup>b</sup>	2797 ±94 <sup>c</sup>	3233 ±93 <sup>d</sup>	3719 ±93 <sup>e</sup>
Breast muscle yield %	17 ±0.5 <sup>a</sup>	19 ±0.5 <sup>b</sup>	20 ±0.5 <sup>c</sup>	21 ±0.5 <sup>c</sup>	22 ±0.5 <sup>d</sup>

<sup>abcde</sup> Means without common letter within row differ  $P < 0.05$

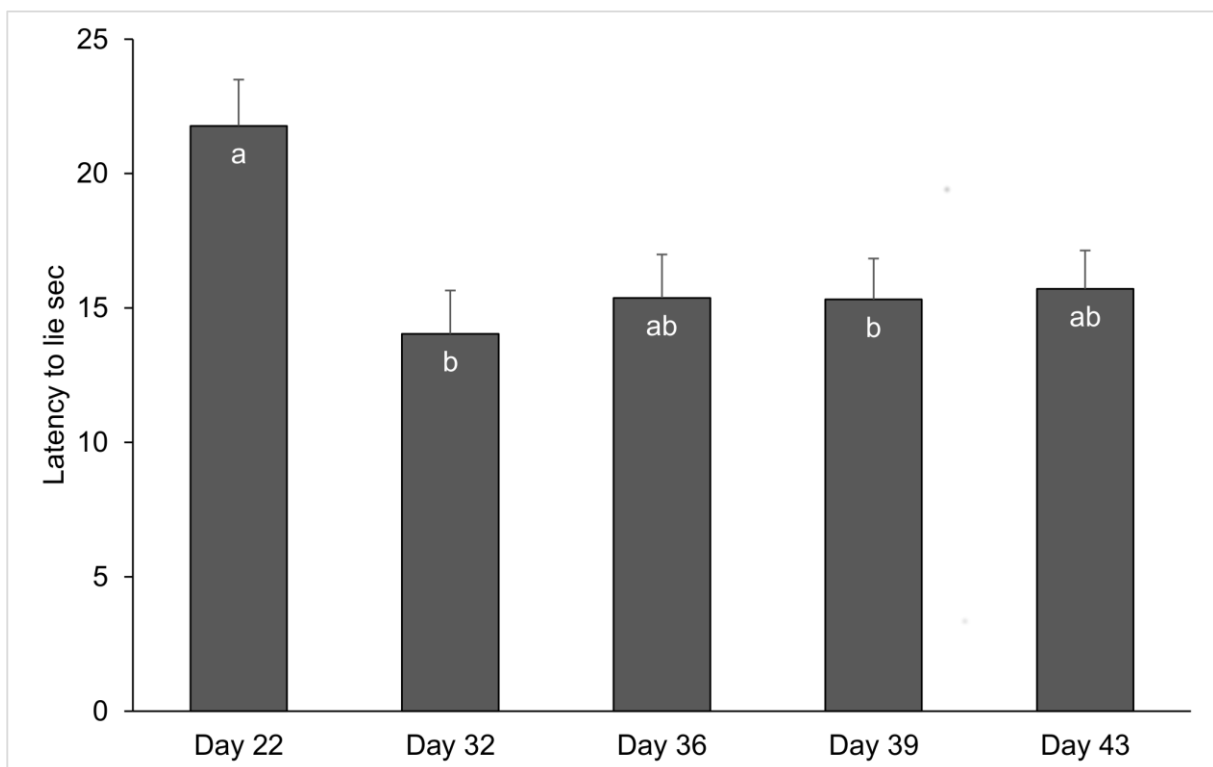
**Table 3.** Lying and activity of broilers at different ages during 10 min observation period (*Mean ±SE, N = 24*)

	Day 12	Day 17	Day 25	Day 34	Day 42
Total lying time s	462 ±12 <sup>a</sup>	485 ±12 <sup>ab</sup>	517 ±12 <sup>bc</sup>	495 ±12 <sup>abc</sup>	535 ±13 <sup>c</sup>
Mean lying bout length s	122 ±20 <sup>a</sup>	179 ±20 <sup>ab</sup>	177 ±20 <sup>ab</sup>	168 ±20 <sup>ab</sup>	251 ±22 <sup>b</sup>
Number of lying bouts	5.3 ±0.4 <sup>a</sup>	4.6 ±0.4 <sup>ab</sup>	3.9 ±0.4 <sup>b</sup>	4.4 ±0.4 <sup>ab</sup>	3.2 ±0.4 <sup>b</sup>
Number of walking bouts	12.0 ±0.9 <sup>a</sup>	9.4 ±0.9 <sup>a</sup>	5.6 ±0.9 <sup>b</sup>	5.3 ±0.9 <sup>b</sup>	2.3 ±1.0 <sup>b</sup>

<sup>abc</sup> Means without common letter within row differ  $P < 0.05$



**Figure 1. Gait score increased as birds aged (mean, SE). ab) Means without common letter differ ( $P < 0.05$ ;  $N = 266$ )**



**Figure 2. Latency to lie increased as birds aged (mean, SE). ab) Means without common letter differ ( $P < 0.05$ ;  $N = 266$ )**