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Validation of an accelerometer to quantify inactivity in laying hens with or without keel-bone fractures

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Abstract

Accelerometers are used to remotely monitor activity in various species in studies that quantify pain, document behavioural patterns, and measure individual activity differences. Studies validating accelerometers typically quantify various active states; however, targeting states specific to periods of inactivity, such as sitting, sleeping, and standing, has the potential to more accurately quantify inactive behaviours commonly associated with behavioural changes related to pain, sickness, or injury. Our objectives were two-fold: first, validate a commercially available accelerometer (Actical®) for quantifying inactivity in laying hens and, second, compare inactivity levels between hens with severely fractured keel bones and hens with minimal to no keel damage. Correlation between the inactivity level as measured by the accelerometer compared to live, focal observation of stationary, inactive behaviours was high; therefore, the Actical® accurately quantifies inactive states in laying hens. Following validation, the Actical® accelerometer was used to quantify inactivity level differences between hens with or without keel-bone damage. Severely fractured hens spent less time motionless, than hens with minimal to no keel damage. Further investigation into inactivity differences related to keel status before and after acquisition of keel fractures is warranted. Use of the accelerometer has the potential to improve animal welfare research by quantifying the effect of pain or sickness on activity level, mapping daily activity patterns, and measuring individual differences in general activity.

Keywords: *accelerometer, activity, animal welfare, inactivity, keel bone, laying hen*

Introduction

Quantifying physical activity with accelerometers has been used in a variety of animal species as a method to detect behavioural changes related to sickness (Marais *et al* 2013; Smith *et al* 2014), oestrus (Hunnell *et al* 2007; Madureira *et al* 2015), seasonal or temporal organisation (Mann *et al* 2005; Ware *et al* 2012), lameness (Conte *et al* 2015; Dalton *et al* 2016; Solano *et al* 2016), and chronic pain caused by osteoarthritis (Lascelles *et al* 2008; Brown *et al* 2010; Rialland *et al* 2013). Accelerometers have been used since the early 1900s to detect vibrations associated with movement, and technological advances in the 1980s allowed the accelerometer to be used for objective quantification of activity in various species (John & Freedson 2012). Understanding the activity patterns of an animal provides insight into daily rhythms, individual differences in behaviour, and changes to an individual's activity before and after a specified drug treatment or procedure. Technological advances in commercially available accelerometers now offer the ability to cost effectively monitor long-term activity outputs in a quantifiable manner without frequent disruption of the animal.

Acute and chronic pain have been shown to alter physical activity and mobility in several species, such as cats (*Felis catus*) (Lascelles *et al* 2008), dogs (*Canis lupus familiaris*) (Brown *et al* 2010), cattle (*Bos taurus*) (Newby *et al* 2013), and pigs (*Sus scrofa domesticus*) (Conte *et al* 2015). Veterinarians and physicians frequently use the measurement of physical activity as an outcome measure to quantify the recovery process and the response to treatment in companion animals (Hansen *et al* 2007; Wernham *et al* 2011) and humans (Inoue *et al* 2003; van Hemert *et al* 2009; Collins *et al* 2012). In poultry, changes in activity level (Duncan *et al* 1991) and resting behaviours, such as sitting and standing (Hocking *et al* 1997) are reported as indicators of pain. Although changes in activity level are believed to be affected by pain stimulated by locomotion (Duncan *et al* 1991; Hocking *et al* 1997), a method for direct quantification of activity in relation to pain or injury has yet to be explored in poultry.

The use of remote sensing equipment, such as RFID tags and accelerometers, to monitor activity in poultry has been applied to assessing resource and range use in non-cage systems (Quwaider *et al* 2010; Daigle *et al* 2012; Richards *et al* 2012; Gebhardt-Henrich *et al* 2014; Banerjee *et al*

2012 cited in Siegford *et al* 2016), detecting hyperactivity in high feather-pecking genetic lines (Kjaer 2009), monitoring sickness behaviour (Marais *et al* 2013), and quantifying convulsive activity during euthanasia (Dawson *et al* 2007; Rankin *et al* 2013); however, use of remote sensing for assessment of behavioural changes related to pain in poultry has been largely unexplored.

In laying hens (*Gallus gallus domesticus*), the keel bone is a site of frequent fractures during their production life (Fleming *et al* 2004; Rodenburg *et al* 2008; Wilkins *et al* 2011; Petrik *et al* 2015; Casey-Trott *et al* 2017a). Damage to the keel bone alters mobility and flight behaviours (Nasr *et al* 2012a), standing and perching behaviours (Casey-Trott & Widowski 2016), and hens with keel damage respond positively to treatment with analgesics (Nasr *et al* 2012b), suggesting keel damage is painful and negatively affects daily activities. Using an accelerometer to quantify activity in hens with keel damage has the potential to determine if this type of injury impacts daily activity level or behaviour patterns. If a difference exists, further investigation into how analgesics affect the activity of hens with fractured keel bones can be used in conjunction with accelerometers to offer detailed analyses of whether and how pain due to keel fractures changes activity.

Previous use of accelerometers in poultry showed promising results in the measurement of steps and activity; however, the duration of recording was limited to a maximum of 1 h for HOBO® Pendant® data loggers (Onset Computer Corporation, Pocasset, MA, USA) (Dalton *et al* 2016) and 60 h for a wireless sensor device (Quwaider *et al* 2010). Although observation time was limited, the use of accelerometers in pullets and hens to describe the behavioural repertoire and variety in activity levels has the potential to be an extremely valuable tool in housing design and management strategies (Kozak *et al* 2016; Siegford *et al* 2016).

The lightweight and durable Actical® (Philips Respironics, Bend, OR, USA) accelerometer offers the ability to continuously monitor activity for more than 250 days and it has been used in a variety of species to quantify activity. The Actical® is an omnidirectional accelerometer designed to detect vibrations from movement in all directions, and is quantified by a unit-less, arbitrary activity count that is based on the duration and magnitude of the acceleration. When the device is stationary, with no acceleration detected in any direction, a zero is recorded for the designated period (Lascelles *et al* 2008; John & Freedson 2012). To date, Actical® accelerometers have not been validated for use in poultry. In order to quantify activity levels of hens with keel-bone damage, validation of the accelerometer for use in laying hens is essential.

Previous validation studies using cats and dogs demonstrate a strong correlation between the activity count of the Actical® accelerometer and the distance travelled or time spent mobile; however, discrepancies repeatedly arose during non-locomotor, active behaviours, such as grooming (Lascelles *et al* 2008; Andrews *et al* 2015), shaking, scratching (Andrews *et al* 2015), and tail-wagging (Hansen *et al* 2007). Likewise, birds perform comparable non-locomotor, active behaviours that also have the potential to influence the accelerometer output. Long durations or frequent bouts of preening pose a risk of registering high activity counts during periods of relatively low locomotor behaviour, as do other avian behaviours, such as dustbathing, wing-flapping and body-shaking (Dalton *et al* 2016) which, although short in duration and frequency, have the potential to skew the activity count results due to the relatively high acceleration associated with each of these behaviours.

Previous studies validating the Actical® in other species focused on active behaviours; however, frequently, their results showed that the strongest overlap between the Actical® and specific behaviours occurred during periods of stationary, inactive behaviour classified as resting (Hansen *et al* 2007) or immobile (Lascelles *et al* 2008) behaviour. Behavioural changes in relation to pain or injury in poultry affect levels of inactivity, such as lying time (Duncan *et al* 1991), rather than causing dramatic changes in the intensity or duration of active behaviour. The purpose of this study was to quantify the amount of time hens spend inactive in order to capitalise on the overlap between zero activity (no acceleration detected) as measured by the Actical® and the observation of stationary, inactive behaviours: sitting, standing and sleeping. Following validation, the Actical® was used to quantify the level of inactivity of hens with keel-bone fractures and hens without keel-bone fractures as a secondary objective to determine if keel damage affected daily inactivity level. The hypothesis was that hens with keel-bone damage would have a greater duration of inactive behaviour than hens without keel-bone damage.

Materials and methods

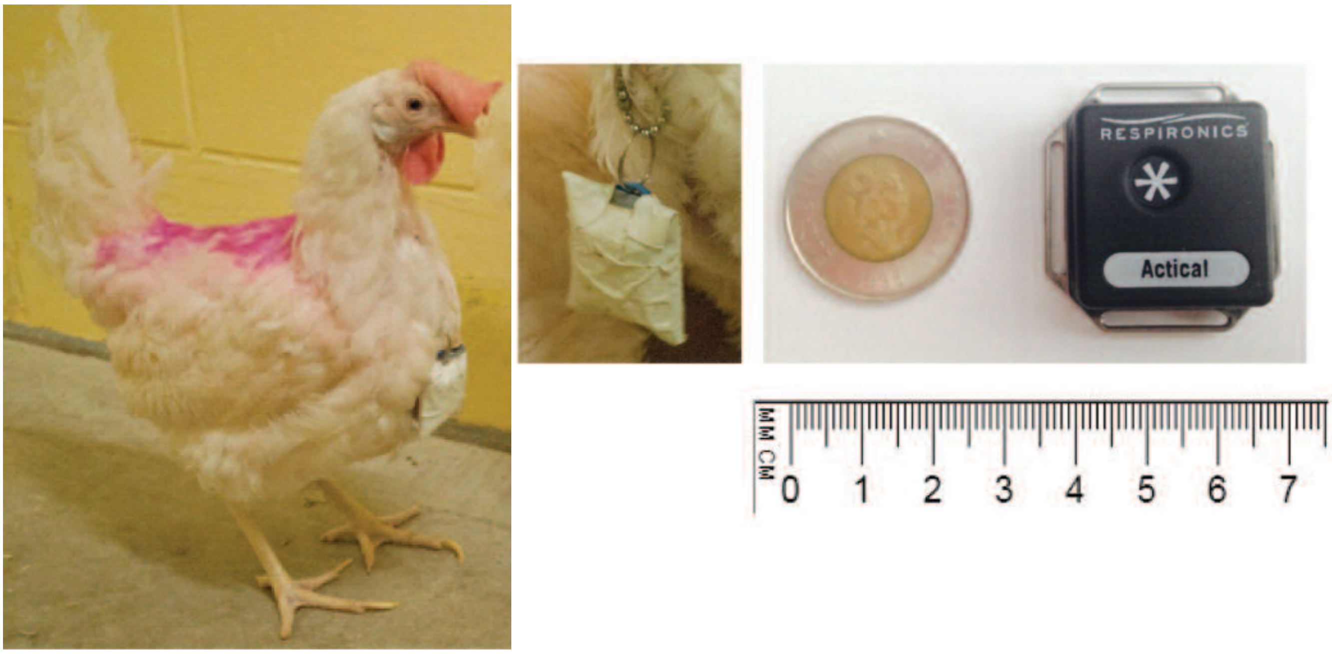
Animal housing and management

Animal use was approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #1947). Two consecutive flocks of 588 Lohmann-selected Leghorn-Lite (LSL-Lite) laying hens were housed from one day of age to 73 weeks of age at the University of Guelph Arkell Poultry Research Station.

As part of a concurrent experiment, half of the hens were reared in conventional cages (Ford Dickinsen, Ontario, Canada) and half were reared in an aviary system (Farmer Automatic Portal Pullet rearing system, Clark Ag Systems, Caledonia, Ontario, Canada). Rearing details can be found in Casey-Trott *et al* (2017b).

At 16 weeks of age, pullets from both rearing systems were placed into two rooms holding 12 furnished cages (Farmer Automatic Enrichable [Furnished] Cages; Clark Ag Systems) each, with rearing treatment segregated by cage. Each room contained six large (41,296 cm^2; 60 hens; 688 cm^2 per hen) and six small (20,880 cm^2; 30 hens; 696 cm^2 per hen) furnished cages. Only hens from large cages were used in this experiment. Each bank of six furnished cages had three tier levels. The same rearing and adult rooms were used for each flock.

Figure 1



Actical ® necklace (left) attached to hen and (right) shown attached to Actical® accelerometer with a size comparison for reference.

Hens were fed a commercial layer crumbled pellet diet (UoG Arkell Poultry Layer Breeder, Floradale Feed Mill Ltd, Floradale, Ontario, Canada) with automatic feed chains running every 3 h commencing at the start of a 14-h light period from 0700–2100h with a 15-min sunrise and sunset. Each furnished cage provided a curtained nest area (94 cm^2 per hen), 10-cm high rounded-edge, square plastic perches (15 cm^2 per hen) running parallel to the cage front throughout the middle area, and a smooth plastic scratch area (42 cm^2 per hen). Nipple drinkers with cups were located above the feed auger down the middle of the cage. The feed troughs (12 cm per hen) were located on both outer sides of the cage.

Accelerometer

The Actical® accelerometer measured 28 × 27 × 10 mm (length × width × height), and weighed 17.5 g. The Actical® is an omnidirectional accelerometer constructed of a rectangular piezoelectric bimorph plate and seismic mass designed to detect movement in all directions; however, the device is most sensitive in a position parallel to the longest direction of the case (John & Freedson 2012). To account for this positioning on a laying hen, necklace harnesses were created to hold the Actical® in a vertical plane with the hen. Necklaces were comprised of a #10 brass nickel-coated beaded chain, with a keyring used to attach the accelerometer. The device was protected by white duct tape, for a total weight of < 30 g (Figure 1). Necklaces were designed to break away if caught within the cage to prevent strangulation.

As determined by small pilot studies (data not reported), to achieve maximum sensitivity, all Actical® accelerometers were programmed to record 1-s epochs and set for subjects with the following settings: height 10.0 cm, weight 0.5 kg, gender female, and age 2. These settings were adjusted based on subject size and weight to appropriately calibrate the Actical®. A full description of the Actical® accelerometer mechanism of operation can be found in Lascelles *et al* (2008) and John and Freedson (2012).

Part 1 Accelerometer validation

Seven LSL-Lite laying hens, each housed in a separate group in a furnished cage (60 hens per cage), were selected from various locations throughout the cage, marked with livestock paint, and fitted with Actical® accelerometer necklaces at 70 weeks of age.

After acclimatisation (> 24 h), hens with Actical® necklaces were focally observed for 1 h on each of two consecutive days. Observation periods were equally allocated throughout the hours of 0900–1600h to allow for one morning and one afternoon of observation per hen. Trained observers followed a detailed ethogram (Table 1) to record behaviour during live observation. Reliability among all four observers was acceptable (W = 0.733; χ^2 = 8.8; P = 0.030). All behaviours were recorded using a hand-held computer (Psion Workabout Pro, Schmauburg, IL, USA) with Noldus Pocket Observer 3.1 software (Noldus Information Technology, Wageningen, The Netherlands). All behaviours, except perching, were recorded as mutually exclusive state behaviours in that initiation of one behaviour terminated the recording of the previously occurring behaviour. Occurrence of perching was recorded in conjunction with any behaviours observed while the hen was located on the perch, allowing for a description of specific activities that occurred on the perches.

Table 1 Ethogram used for focal behaviour observations.

Behaviour	Description
Forage	Pecking or scratching at the floor of the cage with head below rump (adapted from Klein *et al* 2000)
Eat	Head in the feed trough or completely through the cage over the feeder. Can include standing breaks of ≤ 5 s followed by resumption of behaviour
Drink	Repeated pecks at nipple drinker followed by swallowing. Can include standing breaks of ≤ 5 s, with beak still within the plane of the drinker, followed by resumption of drinking behaviour
Preen	A hen uses her beak to clean wing and body feathers. Related behaviours include head scratching, wing stretching, feather ruffling and/or feather erection
Walk	Moving more than three paces in one direction, head erect
Stand	Hen standing on feet, legs extended, no movement of the body but with eyes open (adapted from Webster & Hurnik 1990). Head in either erect or relaxed posture
Sit	Hen's body is flush with the bottom of the cage, wings tucked, and head either erect or in relaxed posture. Eyes are open
Sleep	Hen in a relaxed posture, either sitting or standing, with eyes closed. Head may be tucked (adapted from Blokhuis 1984)
Dustbathe	A hen performs vertical wing shakes on the wire, bill raking, circular foot motions. Includes sham dustbathing. Hen may pull feed from feeder to use as substrate. Can be social or individual (adapted from Scholz *et al* 2011)
Perch	A hen has two feet on a perch (or feed auger) for more than 3 s (ie not stepping over the perch)
Accelerometer-directed	Pecking, pulling, or shaking of the accelerometer device by the individual wearing the accelerometer device or by a cage-mate

Following two days of observations, the Actical® necklaces were removed. All data were recorded in 1-s epochs which were then uploaded in 15-s intervals (pre-set by the software for uploading purposes) and the total number of zero counts from each hour that the hen was concurrently focally observed in live observation was summed to create the variable Actical® inactivity (AI). A 'zero count' was any 15-s interval that recorded a '0' for acceleration, meaning that no movement in any direction was detected by the Actical® over the entire 15-s period. The total duration of AI was then converted to total seconds of inactivity for each individual hen on each of the two days.

A variable of the total duration of inactivity, as recorded by live observations, was also created by summing the total duration of stationary, inactive behaviours (sit, sleep and stand) for each hen during each hour of live, focal behaviour observation. This variable was subsequently termed stationary inactivity (SI). A variable of total duration of activity was also created for each hen by summing the total duration of all active behaviours (walk, preen, eat, drink, forage and dustbathe) recorded during live observation.

The data were analysed in SAS statistical software version 9.4 (SAS Institute Inc, Cary, NC, USA) using a Pearson's correlation analysis (PROC CORR command). The duration of AI and SI for each hen on each day were plotted against each other to determine the Pearson correlation (R^2 value). The Pearson correlation coefficients were determined for the duration of SI, active behaviours, perching, and accelerometer-directed behaviour for each individual hen as compared to the amount of AI detected for each individual hen. The level for assessment of statistical significance was set at $P < 0.05$.

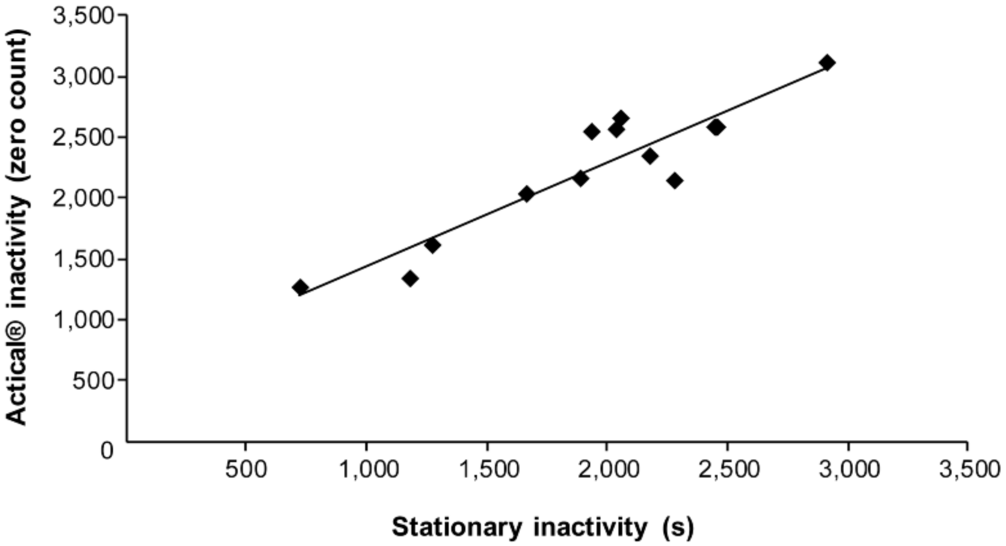
Part 2 Effect of keel status on hen Actical® inactivity

Two hens with fractured keels and two hens without were selected from each of the 24 large, furnished cages when the hens were 71–72 weeks of age (n = 96). Selection for the study was based on the keel-bone status of each individual bird determined by palpation, with a focus on selection for hens with severe keel damage and hens with minimal to no keel damage. Lights were dimmed for ease of handling and hens were caught from various locations from within each cage until two hens with a non-fractured keel and two hens with a severely fractured keel were found. A keel was considered non-fractured if it followed a normal, straight, 180° line without the presence of any sharp bends or periosteal scars or calluses indicative of a healing fracture. A keel was classified as severely fractured if there was the presence of a sharp bend or deviation from the 180° line accompanied by one or more periosteal scars or calluses (Casey-Trott *et al* 2015). All palpation scoring was completed by the lead investigator (TMC-T) who was trained in palpation as described in Casey-Trott *et al* (2017a). Immediately following palpation, selected hens were fitted with an Actical® necklace and marked dorsally with livestock paint to allow for monitoring and retrieval of the devices, and returned to their home cages.

Actical® necklaces were placed on the hens for a total of seven days, with the first day of data collected from the Actical® excluded from analyses to allow for acclimatisation of the hen to the device. Since the necklaces were designed to detach from the hen if caught within the cage, data were only included in the analysis if the necklace remained on the hen for ≥ 4 complete days of data collection. Necklaces were not re-attached if they fell off to ensure that birds were not disturbed beyond daily management routines throughout the entire data collection period.

After seven days of data collection for each group of 24 hens, hens were caught and the remaining necklaces removed. All data were uploaded in 15-s intervals and the total number of zero counts from the lights-on period, 0700 to 2100h, was summed to determine the total time inactive for each hen during daylight hours. The daily duration of daytime inactivity was then averaged to create a mean duration of AI for each individual hen.

Figure 2



Correlation between stationary inactivity (SI), as determined by focal, live bird observation and Actical® inactivity (AI) zero count (Pearson's correlation coefficient: $R^2 = 0.859$; $P < 0.001$; n = 13 hens).

Following the final data collection, hens were euthanased by cervical dislocation, dissected and classified by keel status at dissection: F0 = No fracture or only minimal damage (single, green-stick fracture at the caudal tip of the keel, potentially accompanied by < 5° deviation from 180°; n = 41); F1 = Severe keel damage (multiple complete fractures, potentially accompanied by > 5° deviation from 180°; n = 20). The decision to compare hens with severely damaged keel bones to a combined category of hens with minimal to no keel-bone damage was based on our previous finding that the behaviour of hens with minimal keel-bone damage closely resembled the behaviour of hens with no keel-bone damage, whereas hens with severe keel-bone damage showed behavioural differences from both groups (Casey-Trott & Widowski 2016). The discrepancy between sample sizes was a result of the loss of necklaces due to their intentional break-away design.

For all statistical analyses, only the true damage status of the keel, as determined by dissection, was used. The level for statistical significance of differences was set at $P < 0.05$.

The mean daily duration of daytime AI zero count for each keel status category was assessed using SAS 9.4. The mean AI zero count was analysed using a general linear mixed model analysis (PROC MIXED command) with keel status (F0 or F1), bodyweight, room, rearing system, and tier as fixed effects. Flock number was included as a random effect to control for variation between flocks. There was no need to include cage as a variable, as only one cage met each of the permutations of the variables Room, Tier and Flock.

All data were tested for normality and normality of residuals (PROC UNIVARIATE command), and the data did not require transformation.

Results

Part 1 Accelerometer validation

From periodic visual observation over the first 24 h, the Actical® accelerometer was well tolerated by the hens, with the most disturbance of behaviour occurring within 2 h of application. Accelerometer-directed behaviours, such as pecking at or shaking the device, were most frequently observed and most vigorous during this time. Walking backwards, a behaviour not commonly expressed by hens, was observed in a few birds within the first 30 min of necklace attachment, but not seen thereafter. On the day following necklace attachment, a full range of behaviours (sit, stand, walk, forage, eat, drink, perch, dustbathe, preen, wing-flap and sleep) were exhibited by each hen involved without impedance by the Actical® necklace.

A total of 14 h of simultaneous focal, live bird observation and Actical® accelerometer data were collected. One observation was considered an outlier, as it was greater than ± 2 standard deviations from the group mean difference, and was subsequently removed. The corresponding behaviour that occurred during the Actical® data collection of this outlier was frequent bouts of preening and continuous standing in a high traffic area within the furnished cage for the entire 1-h observation period.

There was a strong, positive correlation between periods of SI and AI, with an R^2 value of 0.859 ($P < 0.001$; Figure 2). Likewise, the relationship between periods of active behavioural states and AI had an inverse relationship with an R^2 value of 0.590 ($P = 0.002$). The total duration of perching had a positive relationship with AI ($R^2 = 0.493$; $P = 0.024$). The correlation between AI and

Figure 3

Comparison of (upper) the Actical® Actogram graph output and (lower) focal behaviour observation output of a single bird over a concurrent 1-h period.

the total duration of Actical®-directed behaviours was not significant ($R^2 = 0.293$; $P = 0.065$) although it did show an inverse trend with AI.

The mean (± SEM) duration of SI, as recorded by focal behaviour observations, was 37 (± 2.5) min, and 32 (± 2.7) min for AI, per hour-long observation.

A graphical representation of the 1-h focal behaviour observation output, compared to the concurrent Actical® Actogram activity output, is shown in Figure 3.

Part 2 Effect of keel status on hen Actical® inactivity

Keel-bone status had a significant effect on AI; hens with severely damaged keel bones (F1) spent less time inactive (1,280 [± 202] zero counts per day) than hens with minimal or no keel-bone damage (1,461 [± 196] zero counts per day; $F_{1,53} = 4.54$; $P = 0.036$). There was no effect of bodyweight ($F_{1,53} = 0.89$; $P = 0.394$), room ($F_{1,53} = 0.25$; $P = 0.543$), rearing system ($F_{1,53} = 0.03$; $P = 0.930$), or tier ($F_{1,53} = 0.91$; $P = 0.595$) on AI. The distribution of individual F0 and F1 hens by their mean AI zero count can be seen in Figure 4.

With AI converted to a unit of time, the raw mean (± SEM) total duration of daytime inactivity for F0 was 316 (± 20) min and 243 (± 12) min for F1. An example of an Actical® Actogram activity output for an individual hen over a period of nine consecutive days is shown in Figure 5.

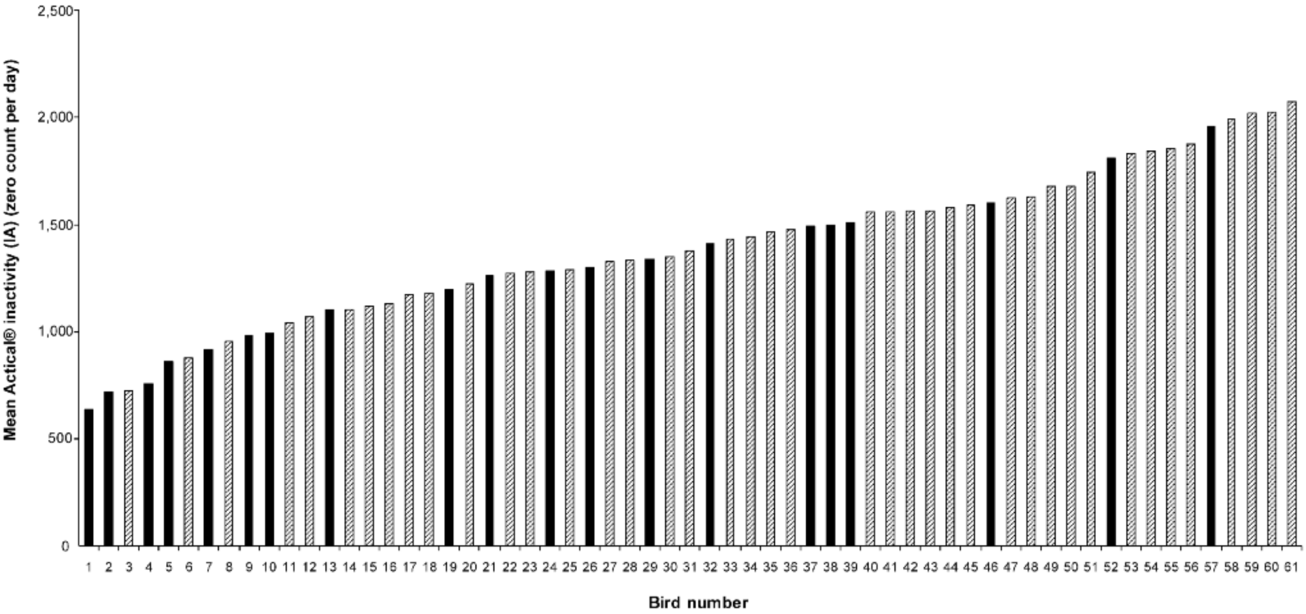
Discussion

Part 1 Accelerometer validation

The Actical® accelerometer output correlates strongly with periods of stationary inactivity corresponding with sitting, sleeping and standing behaviours in laying hens. To our knowledge, this is the first study to assess the validity of the Actical® accelerometer for use as a measure of inactivity in poultry. Previous studies validating the Actical® as a tool for monitoring activity, reported that the relationship between Actical® activity counts and distance travelled produced R^2 values within the range of 0.80–0.90 for cats (Lascelles *et al* 2008) and 0.78 for dogs (Hansen *et al* 2007). The R^2 value reported here falls within that range and demonstrates the validity of using the Actical® to strictly quantify periods of inactive behaviours that correspond with a motionless Actical® with no acceleration detected.

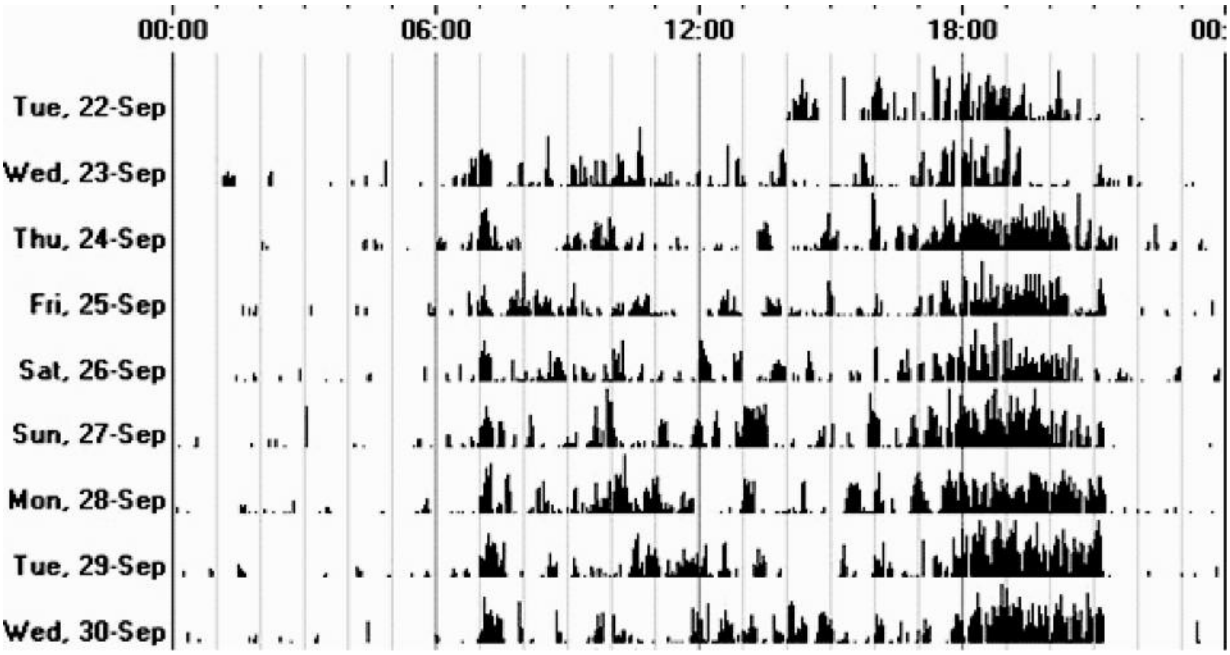
Since the Actical® accelerometer calculates the arbitrary activity count by accounting for both the duration and intensity of a given acceleration, activities such as dustbathing, wing-flapping, or body-shaking produce a relatively high activity count with virtually no locomotor movement. In dogs, periods of vigorous tail-wagging, ground-sniffing, and toy chewing produced exaggerated activity counts in relatively sedentary subjects (Hansen *et al* 2007); the researchers also noted that the strongest overlap in observed behaviours and Actical® activity counts

Figure 4



Distribution of mean Actical® inactivity (AI) zero count per day by keel-bone status for each individual hen. Solid bars represent hens with severe keel damage (F1; n = 20) and striped bars represent hens with minimal to no keel damage (F0; n = 41).

Figure 5



Example of Actical® Actogram activity graph for a single hen over nine days. Lights on at 0700h and off at 2100h.

occurred during periods of quiet rest. By focusing only on periods of inactivity, the effects of behaviours that skew the Actical® activity count can be reduced. For future studies, if the intention is to quantify behavioural patterns or the frequency of occurrence of specific behaviours, the approach of classifying activities by threshold, as described by Kozak *et al* (2016) and Banerjee *et al* (2012) cited in Siegford *et al* (2016), should be used.

For laying hens in the relatively confined environment of a furnished cage, correlating Actical® activity with distance travelled might not be biologically relevant, as the degree of locomotion and behavioural expression varies within different housing systems (Hansen 1994). Laying hens spend approximately 90% of their active time performing feeding behaviour (Dawkins 1989) and have reduced the frequency of behaviours that are energetically costly as a result of genetic selection for feed efficiency (Schutz *et al* 2001; Schutz & Jensen 2001). Especially in an enclosed environment, such as a furnished cage, many active behaviours such as preening, eating, dustbathing and spot-pecking take place in a single location with virtually no distance locomotor movement. Even foraging, arguably one of the more active locomotor behaviours in hens, does not involve a large distance travelled as it does in mammals; rather, foraging behaviour in hens is focused on repeated ground-scratching movements and ground-pecking in a relatively small area (Lindqvist 2008) often in close association with the feed trough in caged birds (Mench 2009). Focusing only on periods of stationary inactivity serves to highlight the diversity of active behaviours expressed in laying hens and offers a conservative approach to quantifying true periods of inactivity.

The decision to include standing, in addition to sitting and sleeping postures, in the definition of SI behaviours was based on previous focal behaviour observations by the researcher where dozing behaviour was frequently observed. Dozing is thoroughly described by Blokhuis (1984) as a stationary behaviour that is part of the normal rest repertoire of poultry. It can take place in either the sitting or standing position, and while it is believed to offer a form of rest in and of itself, it was also believed to transition into more explicit sleeping positions in which the head is tucked beneath the wing. Blokhuis (1984) also noted that the commercial hybrid strain spent a significantly larger proportion of their resting time in a standing position, 20.4% compared to only 4.9% in Red Jungle Fowl. Since we observed a similar, high percentage of standing behaviour in a previous study (Casey-Trott & Widowski 2016), we decided that stationary standing behaviour should be included in the description of SI behaviours, as it represents a substantial portion of resting strategy of commercial hens.

Similar to previous accelerometer validation studies, in which 4–6% of observations were considered to be outliers and excluded from the analysis, < 7% of the observations in our study showed a discrepancy between AI and SI greater than two standard deviations from the mean. The one observation that was considered to be an outlier and subsequently excluded resulted from a hen that spent 40 min standing in the middle of the cage, with more than 10 min spent preening. The large discrepancy between the low value of recorded AI and the high value of SI recorded by visual observation is likely a combination of two factors. First, the standing position was seen in the middle of the cage, in a relatively high traffic area located in a narrow pathway between the nest-box curtains and end of the perches. It is possible that even though the hen was standing in a stationary position, the loosely attached Actical® necklace may have been stimulated by contact with passing cage-mates. The placement of the Actical® on a loosely attached necklace was intentional and allowed for detection of even slight movement as well as a break-away mechanism if the equipment became caught within the cage. While this is an advantage for detecting motion in a relatively small, lightweight species such as a laying hen and been used in marmosets (*Callithrix jacchus*) (Mann *et al* 2005), the disadvantage of a necklace attachment is that the Actical® likely underestimated the amount of inactivity due to a degree of extraneous movement of the Actical® caused by contact with nearby chickens and the ability of the hen or cage-mates to peck or shake the device. Although there was a trend highlighting an inverse relationship between Actical®-directed behaviours (pecking or shaking the device) and inactivity, the lack of significance suggests that Actical®-directed behaviours did not dramatically influence inactivity output. Perhaps a more secure location of Actical® attachment may reduce this extraneous stimulation; however, this problem was only a concern for one observation out of 14.

The second likely reason for the low AI and high SI discrepancy of the outlier hen was the high level of preening expressed by this hen, which was greater than any other hen by more than 1 min and greater than the average preening time of the group by 6.5 min. Lascelles *et al* (2008) and Andrews *et al* (2015) reported a similar problem with cats that spent a large amount of time grooming. Although we initially hoped that the skewed activity count triggered by immobile grooming behaviours would be reduced by focusing on only the AI periods, this appeared not to be the case. Frequent, short bouts of preening triggered enough movement of the Actical® to record a value other than zero acceleration. Since the strategy of this study was to take a conservative approach and include only complete periods of 15 s with entirely zero acceleration as recorded by the Actical®, slight preening motions might have triggered very brief, low threshold bouts of activity reducing the overall total zero count for this particular hen. Again, this only occurred in one individual, which is consistent with other studies (Lascelles *et al* 2008; Andrews *et al* 2015). Although this may be initially perceived as a problem, the ability of the Actical® to detect such subtle movements as preening does provide the opportunity for other applications, for example, to study individual differences in levels of excessive preening or hyperactivity, which have been shown to be related to feather-pecking and genetic selection (Kjaer 2009).

Part 2 Effect of keel status on hen Actical® inactivity

Across a variety of species, sickness behaviour manifests as several non-specific behavioural characteristics, such as malaise, anorexia, lethargy, and withdrawal from social activities (Dantzer & Kelley 2007). More specifically, chickens express sickness behaviour by increasing the time spent sitting while reducing other behaviours such as eating, drinking, standing and moving (Cheng *et al* 2004). Likewise, acute or chronic pain and inflammation in turkeys (*Meleagris gallopavo*) with untreated hip disorders (Duncan *et al* 1991), laying hens in response to a sodium urate injection (Hocking *et al* 1997), and broilers with untreated articular inflammation (Hocking *et al* 2001) indicate painful stimuli induce higher levels of inactivity. Evidence of greater levels of inactivity in animals experiencing pain or sickness led to the original hypothesis of this study in that hens with keel-bone damage would demonstrate a greater level of inactive behaviour in comparison to hens without keel-bone damage; however, in this study the opposite was found to be true. Hens without keel-bone damage spent more time inactive than hens with keel-bone damage. Perhaps this indicates that pain related to keel-bone damage is experienced in a different manner, such as, for example, visceral pain in dairy cattle being shown to increase overall activity, inducing a state of restlessness (Rialland *et al* 2014). All of the conditions listed above use changes in inactivity as an outcome measure of assessment, a value that appears to be easily quantifiable by measuring AI as demonstrated by this validation study.

The results of the second experiment demonstrate an interesting contradiction with current literature in that although keel fractures have been demonstrated to be painful (Nasr *et al* 2012b), hens with fractures spent less time stationary and inactive than hens without keel fractures; however, this lower level of stationary inactivity may be related to lower levels of standing behaviour seen in hens with keel fractures, as described in Casey-Trott and Widowski (2016). While these results may lead to the premature assumption that keel fractures are not painful since they do not increase levels of inactivity, it is important to realise that if that were the case, the keel-fractured hens would show no differences in inactivity level from the non-fractured hens. In order to truly understand the dynamics of pain related to keel damage, a traditional pain study with subjects as their own controls and monitoring Actical® inactivity before and after treatment with analgesics needs to be carried out.

The difference in Actical® inactivity between severely fractured hens and hens with minimal to no keel damage does indicate that hens with keel damage are behaviourally different; however, it is important to note that this difference could manifest differently depending on the housing situation (cage vs non-cage) of the bird. One possibility suggests a causal relationship, in which hens that spend less time stationary are subsequently exposed to greater risk of injury to the keel. Tracking the activity of individual hens before and after the development of keel-bone damage could more directly assess whether 'hyper-activity' puts hens at a greater risk of keel damage; however, this was not the focus or design of the current study. Alternatively, perhaps the coping strategy of hens with keel damage does not follow the traditional description of coping with pain related to lower limb injuries which describes the majority of pain research in poultry. Keel-bone damage may be more closely related to the visceral pain. Rialland *et al* (2014) reported that cattle with higher levels of visceral pain exhibited higher levels of activity due to a state of restlessness. Our previous research indicated that hens with keel-bone fractures spent less time standing and had shorter bouts of standing compared to hens with no keel-bone damage (Casey-Trott & Widowski 2016). Since stationary standing was one component within our definition of SI, and SI correlated strongly with AI, it is possible that the lower AI exhibited by hens with keel fractures compared to hens without keel damage is related to a decrease in stationary standing behaviour.

Although the keel bone serves as the anchor for flight muscles and is subjected to the gravitational weight of the visceral cavity, it is not a load-bearing bone in the traditional sense of standing and walking activity. Discomfort related to a keel-bone injury may not be alleviated by increasing the duration of sitting behaviour as it has been shown to in previous studies investigating pain in the lower limbs. Instead, hens with severe keel damage may be spending less time inactive because it is uncomfortable to remain in a sitting or standing position with pressure or strain on the keel for long periods of time resulting in more frequent shifting of position or changes in behavioural state. Restless behaviour and disturbed sleep patterns are commonly reported in human patients experiencing musculoskeletal pain (Moldofsky 2001; Smith & Haythronthwaite 2004), and restlessness is used as an outcome measure for pain assessment in non-verbal infants (Patel *et al* 2001). Similarly, we found shorter bout length for standing behaviour and a trend for more frequent sitting bouts in hens with keel fractures compared to hens with no keel damage (Casey-Trott & Widowski 2016).

Animal welfare implications and conclusion

The Actical® accelerometer effectively measures inactive states in laying hens, and the device has the potential to be used as a tool in animal welfare research by quantifying various daily behaviours in poultry, describing individual differences in behavioural patterns, and measuring changes in behaviour due to pain or injury status. Within the confines of the current study it is impossible to state, definitively, the cause of the decreased AI exhibited by the hens with fractured keel bones; however, it is clear that a difference in inactivity level does exist between hens with minimal to no keel-bone damage and hens with severely damaged keels. Further investigation into understanding the level of inactivity expressed by individual laying hens serving as their own control may permit us to quantify changes in inactivity level due to administration of analgesics in pain trials, age, injury, environmental or husbandry conditions, or disease status.

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