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Behavioural assessment of pain in commercial turkeys (*Meleagris gallopavo*) with foot pad dermatitis

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Abstract 1. Two experiments were conducted to investigate the differences in susceptibility to foot pad dermatitis (FPD) of two medium-heavy lines of turkeys, and whether FPD is painful, by detailed analysis of behaviour in birds with and without analgesic treatment (betamethasone).

2. Turkeys housed on dry litter in the first experiment generally had more frequent bouts of different behaviours that were of shorter duration than birds on wet litter. T-patterns (behavioural sequences) were more frequent, varied and complex on dry than on wet litter. Betamethasone-injected birds of line B, but not breed A, had shorter resting and longer standing durations on wet litter than saline-injected birds.

3. In the second experiment, turkeys on wet litter given saline stood less and rested more than all other treatment groups, suggesting that they experienced pain that was alleviated in birds receiving betamethasone. Turkeys on dry litter had more frequent, varied and complex patterns of behaviour than turkeys on wet litter and birds kept on intermediate litter wetness. Betamethasone provision increased pattern variety regardless of litter treatment.

4. Turkeys with low FPD scores transferred to wet litter and given saline injections had a longer total duration of resting and shorter duration of standing compared to betamethasone-treated birds. Low FPD birds transferred to wet litter had a similar number of patterns and total pattern occurrence as high FPD birds transferred to dry litter. Betamethasone increased pattern variety and frequency compared to saline injections whereas overall pattern complexity was similar.

5. It was concluded that wet litter affects the behaviour of turkey poults independently of FPD and that betamethasone may also change the behaviour of turkeys. There was some evidence from analgesic treatment and T-pattern analyses that FPD was painful. However, there was no evidence of differences in susceptibility to FPD of the two commercial hybrids.

INTRODUCTION

Foot pad dermatitis (FPD) is a highly prevalent and potentially painful contact dermatitis of commercially reared turkeys in which the metatarsal and digital pads become swollen, discoloured and hard. Hyperkeratosis and necrotic lesions form, increasing in size as the condition progresses (Mayne *et al.*, 2007*a*). These external signs are preceded by histopathological evidence of an inflammatory immune response (Mayne *et al.*, 2007*b*).

In addition to the potential pain and reduced mobility caused by inflammation and mechanical damage, secondary infections may further reduce welfare in affected turkeys through conditions such as synovitis and subsequent lameness (Clark *et al.*, 2002). Buda *et al.* (2002) reported the existence of pain receptors and mechanoreceptor sensory nerve endings in the foot pads of turkeys. Additionally, reduced activity and disrupted behavioural sequences are associated with high FPD scores in turkeys kept on wet litter (Hocking and Wu, 2013). However, affected turkeys should be observed both on dry litter and under analgesic intervention to ascertain whether such behavioural alterations are indicative of pain and a

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major aim of the current research was to critically investigate the hypothesis that FPD in turkeys is painful.

High litter moisture content (LMC) is the main causative factor predisposing poultry to FPD (Mayne *et al.*, 2007*a*) and an inflammatory response may develop in as little as 48 h (Mayne *et al.*, 2007*c*). Similarly, fully developed lesions can form under the same conditions over 2–4 d, and heal within 15 d following transfer to dry litter (Mayne *et al.*, 2007*a*). The relationship with FPD severity is linear when LMC is above a certain proportion depending on the experiment (Wu and Hocking, 2011; Wyneken *et al.*, 2015) consistent with the hypothesis that LMC is the major cause of FPD in turkeys. FPD, however, is multifactorial (Mayne, 2005), and some studies suggest the possibility of genetic factors (Mayne, 2005; Wu and Hocking, 2011). It has been demonstrated that Large White turkey poults are more susceptible to FPD than Broad Breasted Bronze poults reared under the same conditions (Chavez and Kratzer, 1972), and it has been suggested that widely used commercial strains differ in their susceptibility to FPD.

Two questions were therefore addressed in this study: are there differences between two major medium-heavy lines in their susceptibility to FPD; and do turkeys with FPD exhibit behaviours indicative of pain confirmed by analgesic intervention?

MATERIALS AND METHODS

A detailed description of the experimental design of the experiments is presented in the accompanying paper (Wyneken *et al.*, 2015). The research was part of a series of three experiments of which Experiments 1 and 3 were used to assess pain from FPD using analyses of behaviour in floor pens. The results are reported in this paper and the experiment numbers 1 and 3 are retained for ease of comparison with the paper of Wyneken *et al.* (2015). Experiment 3 consisted of two phases (3.1 and 3.2) in a partial crossover experiment.

Animals and husbandry

In Experiment 1, a total of 240 one-day-old male poults of two medium-heavy commercial lines, 120 of line A and 120 of line B, from breeder flocks that were 33 weeks old were obtained from a commercial hatchery. The poults were beak-trimmed by an infrared method in the hatchery in Experiment 3 but not in Experiment 1. Each pen was sealed with mastic and measured 1.25 × 1.77 m. A brooding lamp was provided until 26 d (Experiment 1) or 28 d (Experiment 3). Each pen was littered with 4.6 kg white wood shaving to a depth of about 50 mm. Feed and water in Experiment 1 were provided by a wall-mounted, gridded feeder and a graduated water tank connected to a suspended bell drinker. For Experiment 3, water was supplied in suspended nipple drinkers (10 nipples per drinker). At 64 d in Experiment 3, 72 birds from the larger flock were rehoused in 12 pens (2.5 × 1.77 m) of 6 birds from the same rearing pen and were given feed and water in suspended feeders and bell drinkers. Pens were cleaned and fresh litter provided on d 1 of treatments in Experiment 1 only.

The poults were given 3 h of light (100 lux) followed by 3 h dark alternately until 23:30 h. Thereafter, the photoperiod was 16 h light (07:30–23:30 h), and 8 h dark. Light intensity was 50 lux for d 2 and d 3, and 12 lux from d 4 onward. Ambient temperatures decreased from 28°C at hatch to 16°C at 10 weeks. Feed (Target Feeds Ltd, Whitchurch, UK) was a standard wheat-soya-based commercial turkey starter ration supplied as crumbs in Experiment 1. Birds in Experiment 3 were fed on 4 diets based on maize or wheat and soya or non-soya ingredients as crumbs from 0 to 4 weeks and 3 mm pellets thereafter (5–8 weeks and 9–12 weeks).

Experimental treatments

Experiment 1

The poults were initially housed by line at 10 poults per pen for 26 d. On d 27, litter treatments and birds were randomly allocated across 48 pens (4 poults per pen). Unfamiliar poults were not mixed and excess poults were humanely culled. No water was added to the control (dry, 1D1) pens and in the "wet" treatments water was added to the litter to achieve an initial moisture content of 30%, 40% or 50% (1W1, 1W2, 1W3). The added water was estimated on the basis of the weight of litter in the pen and the previously determined moisture content of dry wood shavings. Water was added daily thereafter for 7 d to maintain the required condition of the litter and induce foot pad lesions as described by Mayne *et al.* (2007*a*) except that excreta were not removed.

Between 09:30 h and 10:30 h on d 3 of litter treatment, two randomly selected birds from each pen received a 0.4 ml injection into the breast tissue of either saline (control) or a solution of betamethasone. The concentration of analgesic administered was 0.04 mg/kg of betamethasone sodium phosphate (Betnesol Injection, RPH Pharmaceuticals AB, Haninge, Sweden) as calculated from the average bird weight recorded on d 1 of litter treatments (1162 g). Birds were marked for identification and injected as described for a further 2 d to ensure that an effective concentration of betamethasone was present before filming began. On the third day of injections, aerial-view home-pen recordings were made using 12 monochrome cameras (WV-BP330 Panasonic CCTV, Suzhou Co. Ltd, Suzhou, China) and three 4-channel digital recorders (INS-DVR04V2-5 DVRs, Inspired Security Solutions Ltd, Hastings, UK.) suspended above the centre of each pen. The one hour films were taken twice for each pen at either (i) 11:00–12:00 h and 15:00–16:00 h or (ii) 13:00–14:00 h and 17:00–18:00 h. Half the pens were filmed on d 3 and half on d 4 starting at 11:00 or 13:00 in alternate pens in each of three blocks.

Experiment 3.1

Between 15:00 and 15:30 h on d 64, water was added to 8 pens to achieve litter scores for treatments 3W1 and 3W2 of 3 and 5 respectively on the Tucker and Walker (1999) scale and no water was added to the 4 dry control pens (3D). Litter scores were maintained as previously described. Daily injections in the morning were conducted on d 10–17 of litter treatments. For birds receiving betamethasone (0.04 mg/kg), doses were calculated using individual bird weights on d 1 of injections and recalculated after 5 d to account for weight gain. In order to minimise potential discomfort from the volume of injected solution, half the dose was injected into each breast. Filming was conducted over one day (injection d 4, litter treatments d 14), with all pens being filmed from 11:00 to 12:00 h and again from 15:00 to 16:00 h.

Experiment 3.2

At 15:00 h on d 16 of litter treatments, the birds were switched from 3D to 3W2 pens and vice versa. Birds from 3W1 pens were not included. Litter and analgesic treatment times were maintained and birds were left to acclimatise overnight (20 h) on the same diets as before. Filming occurred as described for Experiment 3.1 on injection d 7 (litter treatment d 17).

Observations

Individual weights were recorded at the start and end of the experimental periods. LMC in each pen was determined as previously described (Wyneken *et al.*, 2015). Foot pad scores were obtained for every bird at the end of each experiment on an 8-point scale (Mayne *et al.*, 2007*a*).

For 1D and 1W3 treatments in Experiment 1 and all treatments in Experiments 3.1 and 3.2, home pen recordings were imported into The Observer XT version 11 (Noldus Information Technology, Wageningen, NL) and 25-min clips from each file were randomly selected for analysis. The behaviour of the two injected birds in each clip was scored using focal sampling and the ethogram shown in Table 1. Within each behavioural group (point events, postures and activities), behaviours were mutually exclusive. By definition, point events in Observer have no timescale; therefore, end points are not defined in the ethogram. Inclusion of “reduced activity” was necessary as sets of state events must add up to 100%. Each clip was watched twice as birds were scored individually. Prior to this, 20 h of practice scoring was conducted on non-selected clips to minimise the risk of order effects and to determine optimal software settings. Data were extracted from Observer and processed for THEME version 5 (Noldus Information Technology, Wageningen, NL) using Noldus PatternVision DEP software (PatternVision Ltd, Reykjavik, ISL) for detection and analysis of hidden behavioural patterns (T-patterns). The optimal parameters for pattern detection were identified as a *P*-value of 0.0001 and a minimum pattern occurrence of 3 (Magnusson, 2000). The simulation filter and randomisation testing options were applied (parameters as for real data) to further reduce the chance of type 1 errors.

Bird welfare

All experiments and procedures were conducted after ethical approval under project licence number PPL60/45067. The health of the turkeys was inspected on a daily basis and severely affected birds were humanely killed. All turkeys were killed at the end of each experiment with an intravenous sodium pentobarbital injection (Euthatal, Merial, Toulouse, France).

Statistical analysis

Statistical analyses were conducted in Genstat version 13 (VSN International, Hemel Hempsted, UK). Residual plots were inspected for normality and homogeneity of variance and transformations were used where appropriate (see below). Where residuals violated model assumptions due to outliers (i.e. were 4 or more standard deviations from the mean), the related data points were removed and the analysis repeated; where no notable difference to the output resulted, the original analyses are presented. *Post hoc* analyses (*t*-tests) were conducted as necessary to evaluate prior contrasts for significant effects of factors with more than two levels.

Data transformations to achieve normality and homogeneity of variance for Experiment 1 were $\log_e$ for mean duration of rest and reduced activity; $\log_e + 1$ for frequency/h of feeding, drinking, other, peck feeder, peck ground, peck wall,

Table 1. *Ethogram for behavioural observations*

Behaviour	Description
Point event behaviours	
Rest together	Bird transitions to rest within one body length's distance of nearest bird
Rest alone	Bird transitions to rest over one body length's distance from nearest bird
Peck and modifiers	Bird's head is thrust forward then retracted quickly, causing the beak to make/almost make contact with the (i) ground (peck ground), (ii) walls (peck wall), (iii) other birds (peck others), (iv) bird itself (allo-peck), (v) drinker or (vi) top/side of feeder (peck feeder). Peck total is the sum of all pecks
State event "posture" behaviours	
Stand	Bird is stationary; body is elevated from the ground/feeder. Behaviour ends when two or more steps are taken in one direction, or body touches the ground/top of feeder for one or more seconds
Rest	Bird is stationary; body is touching the ground/top of feeder. Behaviour ends when body is elevated from the ground/feeder for one or more seconds
Walk	Standing bird takes two or more consecutive steps in one direction. Behaviour ends when bird is stationary for two or more seconds
State event "activities" behaviours	
Feed	Bird pecks inside feeder. Behaviour ends when bird has not pecked into feeder for five or more seconds or takes two consecutive steps away from the feeder
Drink	Bird dips beak into the moat/pecks at the nipple of the drinker. Behaviour ends when bird has removed beak from the moat/has not pecked at the nipple for five or more seconds, or takes two consecutive steps away from drinker
Preen	Bird rubs beak through plumage. Behaviour ends when head is not in contact with feathers for two or more seconds or head remains stationary for five or more seconds
Other	Bird carries out a low-frequency behaviour (leg/wing stretch, fly, flap, dust bathe, fighting/aggression, display)
Reduced activity (RA)	Bird exhibits no behaviours in the "activities" category. Behaviour ends when any behaviour from this category begins
State event behaviours comprising "other"	
Leg/wing stretch	Bird extends one or both leg(s) or wing(s) in a slow, prolonged manner. Behaviour ends when limb returns to original position or is left extended for five or more seconds
Fly	Bird raises and lowers wings repeatedly until no longer in contact with the ground/top of feeder. Behaviour ends when bird is in contact with the ground/top of feeder again
Flap	Bird extends wings, raising and lowering them one or more times but does not take flight. Behaviour ends when wings return to a stationary
Dust bathe	Bird rests on the ground and flicks wood shavings into its plumage. Behaviour ends when bird is motionless for two or more seconds or transitions to standing
Fighting/aggression	Bird displays one or more of the following behaviours: (i) repeatedly presses body/neck against conspecific in a forceful, jerking manner, (ii) runs in tight circles leaning onto conspecific and (iii) faces and pecks at conspecific forcefully possibly retaining hold of flaps of skin/feathers for several seconds. Behaviour ends when birds are no longer in contact for 5 or more seconds
Display	Bird alters appearance and increases size by means of piloerection, lifting its tail and stretching its wings downwards. Behaviour ends when bird's appearance returns to normal.

peck others, peck total and also for T-pattern occurrences and original T-patterns. Arc-sine√ transformations were required in Experiment 3.1 for percentage duration of rest, stand and preen standing and in Experiment 3.2 for percentage duration of walk and preen resting. T-pattern occurrences and original T-patterns in Experiment 3.2 were transformed to $\log_e + 1$. Where residuals of untransformed data appeared normal and *P*-values were not notably altered by the transformation, original data are presented; otherwise, transformed data are presented with backtransformed means in parentheses.

Experiment 1 was a randomised block design with 48 pens in 6 blocks (replicates) filmed twice. Behaviours were analysed for injected birds in dry and 1W3 litter treatments to give 96 observations (24 pens × 2 birds/pen × 2 times). The mean frequencies (F, converted into frequency/h) and, where applicable, mean bout durations (BD, s) and mean total duration (TD, %) of different behaviours were obtained from the Observer output. General analysis of variances (ANOVAs) were conducted on these variables for all behaviours. Model effects were line, litter (which was confounded with FPD score), provision of analgesia (analgesia vs. saline), time of day (11:00–14:00 h and 15:00–18:00 h) and their interactions. Blocking factors were bird ID nested within pen, nested within block. Analysis of the T-patterns identified by THEME were conducted for the number of unique behavioural patterns (variety), the number of occurrences of all patterns (frequency) and the mean number of behaviours within a pattern (complexity). Effects and blocking factors were the same as described previously for the Observer output analyses.

Experiment 3.1 was a completely randomised design with 12 pens (three litter treatments × 4 replicates) filmed twice, giving 24 observations. Each treatment was replicated within each of the 4 diets. Behaviours were analysed for injected birds in all litter treatments (n = 4 for each injection and litter treatment). Behavioural data (as described for Experiment 1) were obtained from Observer and THEME and analysed using general ANOVAs. Effects included for both outputs were litter, analgesia, time of day (11:00–12:00 h and 15:00–16:00 h) and their interactions. Blocking factors were bird ID nested within pen.

Experiment 3.2 was a completely randomised design with 8 pens (two litter treatments ("wet moved to dry" and "dry moved to wet") × 4 replicates) filmed twice giving 16 observations. Behavioural data (as described for Experiment 1) were obtained from Observer and THEME, combined with the data from the same birds in Experiment 3.1, and analysed using general ANOVAs. Effects for the Observer and THEME ANOVAs were original litter (confounded with FPD score and referred to as FPD hereafter), analgesia, stage (1 and 2, i.e. pre- and post-relocation), time (11:00–12:00 h and 15:00–16:00 h) and their interactions. Blocking factors were bird ID nested within pen.

RESULTS

Litter scores and production variables are discussed in the companion paper (Wyneken *et al.*, 2015). Mean FPD scores for 1D and 1W3 treatments respectively in Experiment 1 were 0.8 and 6.2 and for 3D and 3W2 in Experiment 3 they were 0.5 and 6.3.

Standing and resting behaviours were scored as occurring on either the ground or near the feeder in Experiment 1; however, as the latter were rarely observed this distinction was removed from the coding scheme for Experiment 3. "Peck ground" and "peck wall" were combined into "peck environment" for Experiment 3. "Peck drinker" in Experiments 1 and 3 and "peck feeder" in Experiment 3 were not analysed because they were rarely observed.

Time was included in the model for all behavioural analyses, but as it was accounted for in the analysis and is not relevant to this study, these results have been omitted. Main and interaction effects have also been omitted when they are superseded by higher-order interaction effects. Finally, effects that were too small to be biologically important are omitted in the presentation of results. For completeness and economy of space, means, SEDs (standard error of a difference) and *P*-values of main effects for all behavioural parameters are presented in Supplementary Tables 1–14.

Experiment 1

The mean frequency and duration of different behaviours, SEDs and significance are presented in Table 2. Compared to birds housed on 1W3 litter (high FPD scores), those on dry litter (1D) with lower FPD scores had more frequent bouts of standing, walking, preening, ground-pecking and total pecking, whereas the total duration of bouts of feeding, walking and standing was shorter, and the total duration of preening was higher.

Table 2. *Effect of dry (1D) and wet (1W3) litter treatments on the number of bouts and total duration of different behaviours in Experiment 1*

Behaviour	1D	1W3	SED	*P*-value
Mean bout frequency, n/h				
Stand	57.2	30.7	6.90	0.002
Walk	45.2	23.6	6.15	0.003
Preen	22.8	16.1	2.01	0.005
Peck ground	4.15 (62.4)[a]	1.62 (4.1)	0.274	<0.001
Peck total	5.20 (180.3)	4.47 (86.4)	0.305	0.031
Mean bout length, s				
Feed	40.7	72.1	13.91	0.039
Stand	23.4	52.7	9.66	0.008
Walk	4.87	6.19	0.589	0.041
Total bout duration, %				
Preen	15.79	9.64	2.102	0.010

[a]Backtransformed mean in parenthesis.

A significant (t = 2.689, P < 0.01, P = 0.027) interaction of litter and analgesic occurred for the total duration of walking that was associated with the longer total duration of walking in saline-injected birds on dry (low FPD) compared to wet litter (high FPD). Means for birds on dry (1D) litter were 5.61% and 6.69% for betamethasone and saline injections respectively compared to 4.77 % and 3.49 % (SED 0.70 %) on wet (1W3) litter.

Significant interactions of line, litter and analgesic occurred for the total duration of resting and the total duration of standing (P < 0.05). Betamethasone-injected birds of line B, but not A, had shorter resting (t = 2.37, P < 0.05) and longer standing (t = 2.37, P < 0.05) durations than saline-injected birds when both were on wet (1W) litter (Table 3).

T-pattern characteristics of birds on dry litter with low FPD scores showed that behavioural sequences were more frequent, varied and

Table 3. *Mean total duration of different behaviours with significant line × litter × analgesic interactions in Experiment 1*

	Line A		Line B			
Analgesia	1D	1W3	1D	1W3	SED	*P*-value
Resting, %						
Betamethasone	50.5	58.6	65.7	51.9	8.45	0.028
Saline	57.2	55.3	60.8	66.9	(6.34)[a]	
Standing, %						
Betamethasone	29.5	36.5	29.2	43.3	7.12	0.018
Saline	35.6	41.3	32.4	29.5	(5.74)[a]	

[a]SED for same level of line × litter.

Table 4. *Effect of dry (1D) and wet (1W3) litter treatments on T-pattern characteristics in Experiment 1*

T-pattern characteristic	1D	1W3	SED	*P*-value
Pattern occurrences, *n*	5.73 (307)[a]	4.15 (62)	0.377	<0.001
Original patterns, *n*	3.62 (36)	2.47 (11)	0.262	<0.001
Behaviours/pattern, *n*	4.04	3.25	0.246	0.006

[a]Backtransformed mean in parenthesis.

complex than in birds on wet litter with high FPD scores (Table 4).

Experiment 3.1

Means for the frequency and duration of different behaviours in Experiment 3.1 are presented in Table 5. Walking, standing, resting, resting together, environment pecking and total pecking were performed more frequently by low FPD (3D) compared to high FPD birds (3W1) and by 3W1 compared to 3W2 ($P < 0.05$). Birds on dry litter also had a larger total duration of walking than 3W1, and 3W1 than 3W2 birds ($P < 0.01$). This pattern was reversed for the duration of resting bouts, with 3D performing the shortest bouts and 3W2 birds performing the longest ($P = 0.011$). Differences between 3D and 3W1 birds for resting bout duration and between 3W1 and 3W2 birds for environment pecking were not significant.

In contrast to the litter treatments, there were relatively few significant differences between birds given betamethasone or saline. Compared to saline-treated birds, betamethasone-injected poults had longer ($P < 0.05$) bouts of preen-standing, walking and standing regardless of litter treatment (Table 6). Significant interaction ($P < 0.05$) effects of litter and analgesic were present for the total duration of standing and resting (Table 6). Turkeys on wet (3W2) litter given saline had a shorter total standing duration compared to birds given betamethasone ($P < 0.001$) and birds on both 3W1 and 3D litter given saline

Table 5. *Mean effect of dry (3D) and wet (3W1 and 3W2) litter treatments on the number of bouts, bout duration and total bout duration of behaviours in Experiment 3.1*

Variable	3D	3W1	3W2	SED	*P*-value
Bout frequency, n/h					
Walk	30.9	15.8	5.3	5.32	0.003
Stand	40.2	23.0	9.0	5.85	0.002
Rest	11.1	8.6	4.5	1.05	<0.001
Rest together	7.7	5.1	2.4	1.00	0.002
Peck environment	365	119	49	66.8	0.003
Peck total	413	259	96	71.6	0.005
Mean bout length, s					
Rest	248	336	788	147.8	0.011
Mean total duration, %					
Walk	5.51	3.08	1.06	0.961	0.004

Table 6. *Mean effect of analgesia on bout duration of walking, standing and preen standing and analgesia × litter on total duration of standing and resting in Experiment 3.1*

Variable		Betamethasone	Saline	SED	*P*-value
Mean bout length, s					
Walk		6.97	4.98	0.715	0.021
Stand		85.5	42.3	17.45	0.035
Preen standing		16.4	9.4	3.1	0.050
Total bout duration, %					
Stand	3D	34.3 (32)[a]	30.9 (26)	7.96	0.016[c]
	3W1	36.7 (36)	31.0 (27)	(4.04)[b]	
	3W2	36.0 (35)	13.4 (5)		
Rest	3D	51.3 (61)	56.5 (70)	8.38	0.024[c]
	3W1	51.0 (60)	57.3 (71)	(4.36)[a]	
	3W2	51.9 (62)	75.8 (94)		

[a]Backtransformed mean in parenthesis.
[b]SED for same level of litter.
[c]Significance of the interaction of analgesia and litter treatment.

Table 7. *Effect of dry (3D) and wet (3W1 and 3W2) litter treatments on T-pattern characteristics in Experiment 3.1*

T-pattern characteristic	3D	3W1	3W2	SED	*P*-value
Pattern occurrences, *n*	108.2	41.1	16.4	15.77	<0.001
Original patterns, *n*	19.2	7.3	3.6	2.18	<0.001
Behaviours/pattern, *n*	3.82	3.16	1.11	0.306	<0.001

injections ($P < 0.05$). The 3W2 poults given saline had a longer total duration of resting compared to 3W2 birds given betamethasone ($t = 5.48$, $P < 0.001$), 3W1 given saline ($t = 2.21$, $P < 0.05$) and 3D birds ($t = 2.30$, $P < 0.05$).

Results of the THEME analysis are presented in Table 7. There was a significant ($P < 0.001$) effect of litter on pattern frequency, variety and complexity. Birds with low FPD scores (3D) had more frequent ($P < 0.001$), varied ($P < 0.001$) and complex ($P < 0.05$) behaviour patterns compared to 3W1 birds, and also when compared to 3W2 birds ($P < 0.001$) with high scores for FPD. Behavioural patterns in 3W1 birds were more complex than those of 3W2 ($P < 0.001$) and betamethasone provision increased pattern variety regardless of litter treatment (12.8 vs. 7.3, SED 1.81, $P = 0.015$).

Experiment 3.2

Significant ($P < 0.05$) interaction effects of FPD × analgesia × stage were identified for the total duration of resting and standing (Table 8). In low FPD (dry) birds moved to wet litter, those receiving betamethasone had a shorter total resting duration than those receiving saline ($P < 0.01$). The group mean for saline-treated-birds was virtually unaltered between Experiments 3.1 and 3.2. In Experiment 3.1, high FPD saline birds rested more on wet litter

Table 8. *Mean total bout duration of behaviours of turkeys with low (dry litter, 3D) or high (wet litter, 3W2) FPD scores transferred respectively to wet or dry litter with significant interactions of FPD score (wet or dry litter), analgesia and stage (Experiment 3.1 or 3.2)*

	Treatment					
Analgesia	3D	3D/3W2	3W2	3W2/3D	SED	*P*-value
Resting, %						
Betamethasone	60.7	47.3	62.9	71.4	11.19[a]	0.036
Saline	68.8	68.4	90.1	74.9		
Standing, %						
Betamethasone	32.1	43.7	35.1	25.3	10.38[b]	0.041
Saline	27.1	28.5	9.6	24.2		

[a]SED except for the same level of original litter and original litter × stage = 7.98; original litter × analgesic = 8.39. [b]SED except for the same level of original litter and original litter × stage = 7.68; original litter × analgesic = 8.15.

than their betamethasone-injected pen mates, whereas when they were moved to dry litter the treatment means were not significantly different. In low FPD turkeys on wet litter, betamethasone-treated birds had a longer total standing duration than saline birds ($t = 1.98$, $P < 0.05$). In high FPD birds on dry litter, there was no significant difference between the betamethasone and saline treatments.

T-pattern characteristics are presented in Table 9 and show significant ($P < 0.01$) interactions between FPD and LMC for the number of patterns and pattern frequency. Low FPD birds transferred from dry to wet litter had a similar number of patterns, pattern frequency and behaviours/pattern as on dry litter. The T-patterns of high FPD birds transferred from wet to dry litter were similar to those of low FPD birds on wet litter. Compared to saline injection, betamethasone increased pattern frequency (4.06 (57) vs. 3.20 (24), SED 0.230, $P < 0.01$) and number of patterns (i.e. variety) (2.29 (9) vs. 1.88 (6), SED 0.155, $P < 0.05$). A significant ($P = 0.013$) interaction of analgesia and LMC was associated with a single high value for saline-injected turkeys on dry litter and is likely anomalous (data not shown and overall pattern complexity for betamethasone and saline injections respectively were similar (3.34 (28) and 3.38 (28), SED 0.107), not significant).

Table 9. *T-pattern characteristics in Experiment 3.2 for turkeys with low (dry litter) or high (wet litter) foot pad scores in Experiment 3.1 (3D and 3W) transferred from dry to wet litter (3D to 2W) or wet to dry litter (3W to 3D)*

FPD	Treatment	Occurrence, *n*	Patterns, *n*	Behaviours/pattern, *n*
Low	3D	4.69 (108)	2.84 (16)[c]	3.82
	3D to 3W2	4.26 (70)	2.39 (10)	3.62
High	3W2	1.93 (6)	1.08 (2)	2.83
	3W2 to 3D	3.62 (36)	2.04 (7)	3.16
SED[a]		0.554	0.293	0.187
SED[b]		0.476	0.301	0.224
Interaction[d], *P* =		0.003	0.002	0.101

[a]SED for different levels of FPD (low, high). [b]SED for the same level of FPD (low, high). [c]Backtransformed mean in parenthesis. [d]Interaction of FPD score (low, high) and litter condition (dry, wet).

DISCUSSION

Birds and other animals in chronic pain may exhibit several behavioural indicators of pain, including self-imposed isolation, changes in appetite and reductions in activity, preening, interest in surroundings and the use of injured body parts (Morton and Griffiths, 1985; Gentle and Corr, 1995; Weary *et al.*, 2006; Lierz and Korbel, 2012). Analgesic intervention can provide additional evidence that these changes are caused by pain. Standing and activity levels were shown to increase in turkeys with severe degenerative hip joint lesions following provision of betamethasone (Duncan *et al.*, 1991); in birds with less extensive joint damage, activity levels were similar to those of unaffected birds and were not increased by provision of betamethasone (Hocking *et al.*, 1999), suggesting that such behavioural changes in turkeys are pain-induced.

Behavioural pattern sequencing analysis potentially reveals more subtle behavioural changes than gross behavioural changes. Reductions in pattern frequency, variety and complexity indicate that either performance of certain behaviours (or activity in general) has decreased, or that durations between behaviours in some patterns have become more variable. Such disruptions have been shown to be associated with stressors such as disease and hunger (Alados *et al.*, 1996; María *et al.*, 2004; MacIntosh *et al.*, 2011).

Experiment 1

Longer feeding bouts without an increase in total feeding duration were observed on wet litter and are reflected in the lower frequency and longer bout duration of standing (Table 2). These changes in feeding behaviour have also been reported in lame broiler chickens (Weeks *et al.*, 2000); however, in the absence of altered feed intake and an effect of analgesia, pain is an unlikely cause of these differences. Birds on wet litter also had lower frequencies but higher durations of walking, possibly due to differences in walking pace and/or reduced interest in stopping to engage with the environment or conspecifics. In saline treatments, birds on wet litter had a shorter total walking duration than those on dry litter, whereas when analgesia was provided, no difference was apparent, suggesting that pain due to the more severe lesions seen on wet litter was the cause.

In line B birds housed on wet litter, shorter resting and longer standing durations were observed when betamethasone was provided, suggesting that these changes were due to pain relief; line A birds showed no such change (Table 3). This may indicate subtle differences in FPD severity missed during scoring, or breed variation in pain perception or responses to pain (Short, 1998). If a variation in pain perception is responsible, in spite of similar breed susceptibility, the negative welfare impact of FPD could differ between breeds.

Frequency, variety and complexity of T-patterns were all lower on wet litter (Table 4), again suggesting that high litter moisture had a negative impact on welfare.

Experiment 3.1

A linear decrease in activity occurred in response to increasing litter moisture as measured by reductions in the frequency of walking, standing, resting, environmental pecking and total pecking as well as reductions in total walking duration and an increase in resting bout duration (Table 5). Lower pecking frequencies in birds kept on wet litter in Experiments 1 and 3.1 compared to birds kept on dry litter are likely to reflect differences in litter conditions such as friability.

The increases in the frequency of stand-preening and bout duration of walking, standing and stand-preening observed in betamethasone-injected birds occurred regardless of litter condition or FPD score (Table 6). These differences may therefore have been due to the presence of other, unknown sources of leg pain that were alleviated with betamethasone injection. Alternatively, this result may reflect a side effect of betamethasone. Betamethasone is a potent glucocorticoid analgesic with anti-inflammatory activity that may also affect behaviour such as increased feeding or foraging (Berthon *et al.*, 2014; Liu *et al.*, 2014). Side effects were not observed in a previous study in a test situation in turkeys (Duncan *et al.*, 1991) or in the companion study on gait analysis in a walkway test (Wyneken *et al.*, 2015). Increased scratching and pecking at the litter, decreased standing and lower mating activity were observed in adult broiler breeders with or without musculoskeletal disease (Hocking, 1994), whereas betamethasone had no effect on activity in adult male turkeys with or without relatively mild musculoskeletal lesions (Hocking *et al.*, 1999). In the current series of experiments, any side effect of betamethasone was controlled for by the use of birds with and without FPD that enabled the effect of the drug on pain perception to be distinguished from that arising from any side effects of the drug.

Birds with high FPD (3W2) receiving saline stood less and rested more than all other treatment groups, suggesting that these were the only birds in pain, which was alleviated in the 3W2 birds receiving betamethasone. Although FPD scores for 3W1 and 3W2 birds were similar, due to the need of the related study their feet were scored 4 d after filming. During this period, 3W1 birds were kept on wet litter for an additional 2 d during which foot pad quality is likely to have deteriorated notably, whereas little, if any, healing would have occurred in the 3W2 birds (Mayne *et al.*, 2007*a*). The difference between 3W1 and 3W2 lesion scores is therefore likely to have decreased during this period and may, in part, explain discrepancies between FPD scores and the effects of litter × analgesic interactions between 3W1 and 3W2 and this experiment.

Regardless of analgesic or stage, increasing LMC across litter treatments resulted in a gradual reduction in T-pattern frequency, variety and complexity (Table 7). These results are generally consistent with the results of Experiment 1 and support the suggestion that an LMC-associated stressor other than pain (possibly the presence of cold, wet, non-friable litter) was responsible for these behavioural differences.

Experiment 3.2

In birds with high FPD scores housed on dry litter, significant differences between saline and betamethasone treatments were not present for total duration of standing or resting (Table 8). In birds with low FPD scores housed on wet litter, saline birds had a longer total duration of resting and shorter total duration of standing than betamethasone birds. These results appear to suggest that the behavioural differences on wet litter (and lack thereof on dry litter) in Experiments 3.1 and 3.2 were due to LMC rather than pain. However, on wet litter, a motivation to avoid resting on cold, soggy litter may be present. There was a tendency towards standing more and resting less in betamethasone birds transferred from dry to wet litter that is consistent with such an explanation. The means of both behaviours were virtually unchanged by transfer to wet litter in saline birds, suggesting that this motivation was possibly overridden by pain in these birds.

When moved to dry litter, high FPD saline-injected birds showed a tendency to behave more like their betamethasone-injected pen mates (Tables 8 and 9). This could indicate pain suppression caused by an increased interest in their new environment and/or a positive affective state. Conversely, the lack of such an effect in saline birds moved to wet litter may have been caused by an overriding negative affective state due to the introduction of cold, wet, non-friable litter. Cognitive biases are considered good indicators of affective state in both humans and animals (Mendl *et al.*, 2009). Positive cognitive biases (suggestive of positive affective states) occur in animals when environmental improvements are introduced (Matheson *et al.*, 2008; Brydges *et al.*, 2011; Douglas *et al.*, 2012), while animals kept in poor conditions (and those for which good conditions are negatively altered) exhibit negative cognitive biases, suggesting the presence of negative affective states (Bateson and Matheson, 2007; Destrez *et al.*, 2013). It is therefore possible that turkeys moved from dry to wet litter experienced a negative affective state while those moved from wet to dry litter experienced a positive affective state.

In humans, pain perception can be increased or decreased respectively by negative or positive affective states (Villemure and Schweinhardt, 2010). Rats show heightened responses to pain in stressful situations (Andre *et al.*, 2005). Turkeys may experience a similar increase in pain perception due to a negative affective state induced by wet litter. Pain suppression also occurs in humans and chickens when attention is directed away from pain towards other stimuli (Gentle and Corr, 1995; Villemure and Schweinhardt, 2010) and pain perception in turkeys could be subject to similar alterations. If this is indeed the case, turkeys in the earliest stages of FPD onset may experience pain when housed in poor conditions including (though not exclusively) when kept on wet litter. The study of turkey behaviour in commercial settings could therefore be informative and yield more pronounced behavioural changes at lower FPD scores than in this experiment. An alternative explanation for the results of Experiments 3.1 and 3.2 is that none of the turkeys were experiencing pain and that differences were due to environmental stress per se. However, this would not explain the role of betamethasone in the litter × analgesia effect observed in Experiment 3.1 and in line B in Experiment 1.

Synthesis and critique

Wet bedding is regularly used to induce depression-like states in rats (Kompagne *et al.*, 2008). Similar states may have been induced by wet litter in the current experiments and would explain the lower activity levels observed on wet litter. Reduced ground (and total) pecking on wet litter was probably influenced by low litter friability. However, the frequency and total duration of preening, a possible indication of frustration (Duncan and Wood-Gush, 1972), were also lower on wet litter which implies that reduced welfare was the main cause of activity reductions in wet litter birds.

Pain intensity in humans is known to increase with injury depth (Fruhstorfer *et al.*, 1995). This is also known to be true in rats, where skin-deep incisions to the paw fail to elicit the guarding behaviours that follow deeper incisions (Xu and Brennan, 2009). The scoring system used in this study does not separate superficial tissue damage from deeper lesions in which the dermis is exposed and has previously produced external scores which correlated poorly with histopathological findings (Mayne *et al.*, 2007*b*). A histologically validated scoring system has recently been formulated for use in both chickens (Michel *et al.*, 2012) and turkeys (Allain *et al.*, 2013) which incorporates lesion depth and may be more suitable for future studies.

Attentional biases and affective states are likely to affect pain perception and, even in the earliest stages of FPD, turkeys may be experiencing pain when in negative affective states and barren and/or stressful environments.

CONCLUSIONS

It is clear from these results that wet litter is associated with behavioural changes that suggest a compromised state of welfare in growing turkeys regardless of FPD. T-pattern analysis supplemented the standard analyses of frequency and duration of behaviour and differences between treatments were large and consistent with the conclusion that wet litter adversely affected the welfare of the birds. These results highlight the importance of the environment (wet vs. dry litter) on behaviour and the potential confounding effects of foot pad lesions and litter conditions that will need to be accounted for in future experiments to evaluate pain from FPD.

Betamethasone used as an analgesic also changed behaviour and may indicate that this steroid drug stimulated general activity. There was evidence that FPD was painful in the comparison of analgesia and saline in line B of Experiment 1 and the results of Experiments 3.1 and 3.2. Taken together, these indicate that the welfare of birds with high FPD scores is associated with pain that may be attenuated by attentional shifts.

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SUPPLEMENTAL DATA

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