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Associations between behaviour and health outcomes in conventional and slow-growing breeds of broiler chicken

S.M. Abeyesinghe *, N.M. Chancellor, D. Hernandez Moore, Y.-M. Chang, J. Pearce, T. Demmers, C.J. Nicol

The Royal Veterinary College, Hawkshead Lane, Brookmans Park, Hatfield AL9 7TA, United Kingdom

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ABSTRACT

Broiler chickens are prone to a range of complex health and welfare issues. To support informed selection of welfare traits whilst minimising impact on production efficiency and to address a major gap in understanding, we systematically explored associations between health and behavioural indicators of broiler welfare. One conventional (**CNV**, n = 350) and two slow-growing broiler breeds (**SGH** and **SGN**, respectively n = 400) were reared from hatch in pens of 50 birds. Birds were assessed for health (gait, plumage cover and dirtiness, pododermatitis, hockburn, and leg deviations) at 2.2 kg liveweight according to the Royal Society for the Prevention of Cruelty to Animals Broiler Breed Welfare Assessment Protocol. Behaviour and resource-use of 10% of birds per pen, on days 29 (all breeds) and 43 (SGH and SGN), was (i) scan sampled every 60 min between three to six and between twelve to fifteen hours after photoperiod onset; and (ii) continuously sampled sequentially from focal birds for 3 min each in a random order, during 15 min observation periods at three and twelve hours after photoperiod onset. Binary logistic generalized linear models were used, to assess respective associations between pen prevalence of each health outcome and (i) pen mean percentage scans of behaviour, and (ii) pen mean frequency and duration per 3 min focal observation of behaviour. Better growth rate and feed conversion but poorer health outcomes (mortality, gait, pododermatitis, feather cover) were more prevalent in CNV. Strong associations between behaviour and several heath indicators revealed, (i) increases in side-lying inactive, sitting inactive, and use of the litter relative to other resources, as primary and general indicators of poorer health, and (ii) increases in standing inactive, perch use, walking, Comfort, High Energy and Exploratory behaviour as primary and general indicators of better health. Of these, changes in side-lying, standing inactive, walking, Comfort and High Energy behaviour were particularly sensitive to small differences in health outcomes important for breed acceptance in high-welfare schemes. Crucially these behavioural measures additionally represent motivational and affective aspects of welfare not captured by health measures and allow opportunity for earlier intervention. Thus, to provide a comprehensive assessment of broiler experience, behaviour should be incorporated into broiler welfare assessments.

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Implications

There is a growing demand for "better chicken" derived from healthier breeds reared within improved living environments. To ensure transparency in breed approval for such schemes, candidate breeds are tested using defined protocols. Broiler welfare is also assessed on farm. All assessment and auditing protocols involve handling birds to assess health outcomes such as gait score, dermatitis and plumage condition. Certain chicken behaviours are associated with positive welfare states. Our finding that behavioural profile is additionally strongly associated with assessed health outcomes, means that focused observations could potentially replace tests that themselves disturb vulnerable birds, whilst more comprehensively assessing welfare.

Introduction

Broiler chickens are prone to a range of health problems including leg disorders (Dawkins et al., 2004; Knowles et al., 2008; Caplen et al., 2014), contact dermatitis (Haslam et al., 2007; Michel et al., 2012) and circulatory and metabolic disorders (Part et al., 2016). Each condition has a complex and multi-factorial aetiology. Management practices such as thinning (de Jong et al., 2012), house ventilation, temperature and humidity (Jones et al., 2005), light regime (Schwean-Lardner et al., 2013) and stocking density (Sun

* Corresponding author.
E-mail address: sabeyesinghe@rvc.ac.uk (S.M. Abeyesinghe).

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et al., 2013) influence the incidence of these conditions, but there is also a strong genetic component to each. Genetic selection for rapid growth and increased breast muscle has produced birds with high body mass, abnormal morphology (e.g., wide leg spacing and hip and foot rotations) and gait (Caplen et al., 2012) and an increased risk of pathology and susceptibility to infection (Corr et al., 2003). When reared under similar conditions, slower growing genotypes are less susceptible to leg health problems (e.g. Shim et al., 2012; Dixon, 2020) and contact dermatitis (Allain et al., 2009; Yamak et al., 2016). The incidence of footpad dermatitis and hockburn also differs between standard (fast-growing) breeds (Haslam et al., 2007; Skrbic et al., 2015; Yamak et al., 2016).

For many decades commercial breeding goals have largely favoured production characteristics over health and welfare traits. This has prompted campaigns by animal welfare organisations to persuade breeding companies to shift the emphasis of their breeding programmes. In parallel, assurance schemes designed to influence retailers and consumers, promote higher welfare breeds. Specifically, in the United Kingdom the Royal Society for the Prevention of Cruelty to Animals (**RSPCA**) Assured scheme accepts only those breeds that have passed the RSPCA Broiler Breed Welfare Assessment Protocol (**BBWAP**) (RSPCA, 2017; RSPCA Assured, 2020)

Balancing selection for bird welfare traits against economic and environmental considerations poses a conundrum for breeding companies and retailers. The reduced resource efficiency of slower growing birds could potentially increase production costs and environmental footprint. In addition, the assumption that faster growth rate will inevitably result in poorer welfare has been challenged (Dawkins and Layton, 2012). It is therefore important that selection effort is focussed where it has most effect in improving bird welfare alongside the smallest possible negative impact on production efficiency. Meeting this challenge requires additional evidence about bird behaviour and its relations with measures of bird health. Changes in bird behaviour may provide an early warning of a developing pathology, or could provide evidence that some pathologies are more painful than others. Relevant behaviours of this kind could become important targets for selection. When approving breeds for high-welfare production systems, it is also critical to establish whether individual birds perform priority behaviours such as foraging, comfort behaviours and dustbathing (Weeks and Nicol, 2006). All broilers spend a considerable amount of time lying, but lying time increases with gait score (Weeks et al., 2000). Lameness, enforced lying and other aspects of poor health are likely to interfere with birds' ability to perform high priority behaviours, but there is surprisingly little research on the associations between broiler health and behaviour. Although other studies have examined behaviour and health indicators independently, our study explored relationships between these outcomes and assessed whether similar associations hold true across a range of breeds. We predicted that low-resilience (McFarland, 2014), high energy and priority behaviours would decrease, and resting postures would increase with poorer health outcomes. A further aim of our study was to provide information that could be used to inform or refine the RSPCA BBWAP protocol (RSPCA, 2017) which requires that general behaviour and activity should be assessed. Currently, the protocol does not specify how this should be done, so we aimed to identify sensitive and cost-effective behaviour sampling protocols that could potentially be incorporated into future breed assessment trials.

Material and methods

Animals and housing

Fertilized eggs from one conventional (**CNV**) and two slow-growing breeds (**SGH** and **SGN**) were incubated, hatched and allocated to same-breed groups of 50. Batch 1 comprised four groups of CNV, three groups of SGH, five groups of SGN; Batch 2 comprised three groups of CNV, five groups of SGH and three groups of SGN. Each group was housed in an identical pen (6.5 m^2) situated within identical environment-controlled rooms. The pen size was larger than specified in the standard RSPCA BBWAP, as agreed with RSPCA to meet required Home Office specifications for conduct of the research at a Licensed Establishment (certified under United Kingdom Animal (Scientific Procedures) Act 1986). Pens contained wood-shavings bedding, a 1.3 m length wooden perch, a bell drinker with 22 mm trough space per bird, and a bell feeder with 150 mm trough space per bird. Non-limiting feed was provided according to the RSPCA BBWAP (RSPCA, 2017). Birds were reared with a 22 h Light:2h Dark schedule (daylight strip bulbs, dawn onset at 05:30) to three weeks, then the dark period increased by 1 h per week to a maximum of 18 h Light:6h Dark. Temperature was maintained at approximately 22 °C, humidity and light intensity ranged between 32 and 42% and 43 and 58 lux, respectively.

Pens were marked with tape for virtual division of pen areas to facilitate analysis of bird location. On day 5, five birds were randomly selected as behaviour-focal birds and marked using non-toxic spray and leg tags, checked and replaced weekly. Overhead digital cameras and a closed circuit television system using MilestoneXprotect software captured video recordings on days 29 and 43. Camera and recording software settings corrected for parallax error at the point of origin as far as possible. Fig. 1 shows the timeline for all procedures described below.

Bird health assessments

Number of deaths and culls (with reasons) were collected daily for every pen. Birds were bulk-weighed by pen at placement, 2, 4 and 5 weeks of age, one week before health assessment, and at slaughter weight. Health assessments took place as close as possible to the day when birds reached a weight of 2.2 kg. At health assessment, individual birds were sexed and weighed. All birds were assessed for walking ability within a temporary corridor erected within the home pen; two observers used the BBWAP 6-point Gait Scoring system (Table 1) independently scoring each bird before discussing disagreement and agreeing a final score. Subsequently, a random sample (minimum 25) of birds were caught, placed gently into crates and moved to a separate health assessment area. Any marked bird not already captured was added to this sample. These selected birds were scored for feather cover, breast plumage dirtiness, hockburn, pododermatitis and leg deviations (Table 1).

Bird behaviour assessments

Behavioural data collection occurred as close to health assessment days as possible. The behaviour of all breeds was therefore observed on day 29, based on the prediction that CNV birds would reach an average weight of 2.2 kg by day 33. Breed CNV birds left the trial after this point. The behaviour of breeds SGH and SGN was recorded for a second time on day 43 based on the prediction that they would reach an average weight of 2.2 kg by day 46 (SGH) or day 52 (SGN). Stills and video clips were coded so that observers would be blind to pen, batch and day. It was not possible to blind observers fully to breed due to obvious differences in bird plumage colour. Intra-observer reliability was checked by insertion of a randomly selected 10% of stills or video clips prior to observer review. Behaviour data were collected from marked birds, but where a marked bird could not be identified, the behaviour of an alternative, randomly selected (unmarked) focal bird was recorded instead.

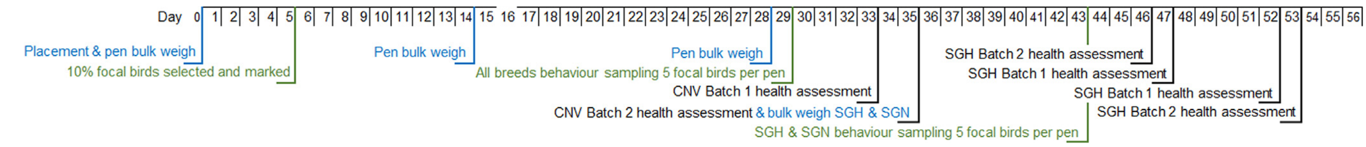

Fig. 1. Time line of procedures showing data collection for each broiler chicken breed over days. Where SGH = Slow-growing breed H, SGN = Slow-growing breed N and CNV = the conventional breed.

Table 1
Health parameters recorded for each pen of broiler chickens (*Gallus gallus domesticus*) at health assessment which took place for each of the three breeds (Slow-growing breed H (SGH) n = 8, Slow-growing breed N (SGN) n = 8, and the conventional breed (CNV) n = 7 pens) as close as possible to the day when birds reached a weight of 2.2 kg.

Health Parameter	Scale
Walking ability[1]	6-Point Gait Scoring system modified from Kestin et al. (1992) from score 0 = smooth, fluid locomotion to score 5 = incapable of sustained movement)
Feather cover[2]	5-Point scale with 0.5 demarcations from score 0 = full feather cover to score 2.0 = body is bare of feathers and wings are patchy of feathers (Dawkins et al., 2004)
Breast Plumage Dirtiness[2]	3-Point scale with 1.0 demarcations, score 0 = clean plumage to 2 = large patches of dirty plumage on breast/breast is completely covered in dirty plumage (Welfare Quality®, 2009)
Hockburn[2,3]	4-Point scale. Score 0: no discolouration or lesions on hocks; score 0.25: very small superficial (<1 mm), slight discolouration in limited area, mild hyperkeratosis/no discolouration or lesions on hocks but hock is pink (P) or swollen (S); score 1: area affected does not extend over the hock, substantial discolouration, dark papillae, superficial lesions, no ulceration; score 2: greater surface of the hock affected, deeper lesions with ulceration, sometimes haemorrhage, scabs of significant size, severely swollen area
Pododermatitis (footpad dermatitis)[2,3]	4-Point scale. Score 0: no lesions present; 0.25: very small superficial lesions (1–2 mm), slight discolouration in a limited area, mild hyperkeratosis/or, no lesions present on the pads, but the pad is pink (P), swollen (S) or recently healed (H); score 1: area affected does not extend over the entire plantar pad, substantial discolouration, dark papillae, superficial lesion and no ulceration; score 2: greater surface of the plantar pad affected, sometimes lesions on toes, deeper lesions, haemorrhage, scabs of significant size or severely swollen foot pad
Leg straightness (leg deviation)[2]	Identified Varus or Valgus (Julian, 1984) deviation for each leg using a novel 2-point scale. Score 0: Leg straight; score 1: Mild varus stance in medial deviation (mildly bowlegged) OR Mild valgus stance in the lateral deviation (mildly "knock kneed"); score 2: Severe varus or valgus stance

[1] Scored for all birds per pen.
[2] Scored for a random minimum sample of 25 birds per pen and all marked birds.
[3] Score 0.25 here numerically represents the 0(P/S) or 0(P/S/H) scores respectively used for hockburn and pododermatitis (RSPCA, 2017).

Scan sampling for focal behaviour and resource-use

On days 29 and 43, instantaneous scan sampling of behaviour and resource-use of marked birds was conducted using 10 s video samples and corresponding stills collected on four occasions at 60 min intervals starting at 0900 h, and on four occasions at 60 min intervals starting at 1800 h (respectively, three to six hours after light onset and six to three hours before the dark period to capture an equivalent morning and afternoon period). Behaviour and resource-use were classified into mutually exclusive activities according to the ethogram in Table 2.

Continuous focal sampling

Continuous video recordings were collected between 0900 and 0915 h; and 1800 and 1815 h (corresponding with the start of the scan sampling periods) and exported into BORIS behaviour coding software (Friard and Gamba, 2016) for analysis. Each marked bird was observed for 3 min focal continuous sampling in turn, in a random order; recording frequency and duration of behaviour according to the ethogram shown in Table 2.

Data analysis

Since standard outcomes from the RSPCA BBWAP are based on mean pen prevalence values, and in view of variation in capture of behaviour of marked birds across samples, we analysed all data at pen level. All analyses involving behaviour therefore assumed that the behaviour of the birds recorded was a representative sample of the pen (≥10% of birds) irrespective of whether marked or randomly selected.

Associations between health outcomes

Pen mean scores were calculated for all health outcomes. Additionally, health score frequencies (gait scores n = 1 074; other variables n = 621) were examined and recoded into binary variables (presence or absence of a moderate or severe health issue); indicated by a gait score of ≤2; feather score of ≤0.5; dirtiness score of ≤1.0; hockburn and pododermatitis scores of ≤0.25; leg deviation ≤1 (representing a deviation score of one or more in one or both legs, irrespective of form). Data were then aggregated into frequencies of cases per pen (prevalence per pen) and calculated as percentage frequencies. Percentage frequencies of mortalities per pen excluding week one (mortality$^{(-wk1)}$) were also calculated. Pen mean scores and pen percentage frequency data were imported into Graphpad Prism [Version 8.0.0 (224)]. Spearman's rank correlations were conducted to test for associations between health outcomes (within data type).

Breed and batch differences in health outcomes

Breed differences in pen (CNV n = 7, SGH n = 8, SGN n = 8) mean health outcome scores and percentage score frequency data were analysed in Graphpad Prism [Version 8.0.0 (224)], using Kruskal–Wallis tests, with a Post Hoc Dunn's test adjusting for multiple comparisons where appropriate. Mann–Whitney tests were used to check for differences between batches.

Associations between behaviours

Some behaviours were collated into larger categories (All Feeding, All Drinking, Comfort, High Energy and Exploratory behaviours, see Table 2). Event behaviours were assigned a duration of 1 s for inclusion in collated category durations. Behaviour and resource-use data were tested for intra-observer reliability and variables with low intra-rater agreement (Cohen's Kappa <0.7, or r < 0.7; Wilcoxon signed rank tests P < 0.05) were excluded, as were behaviours that were too infrequent for further analysis (<4% for scans, <8% for continuous data). For all compliant data, pen mean percentage scans were calculated for scan-sampled behaviour and resource-use, and pen mean frequencies and durations per

Table 2
Ethogram used for scan and continuous observations of focal broiler chickens (*Gallus gallus domesticus*) of the three breeds (Slow-growing breed H (SGH), Slow-growing breed N (SGN), and the conventional breed (CNV).

Behaviour	Description	Sampling and recording method[1]	State or Event[2]	Mutually exclusive collations
Sitting inactive	Immobile, entire breast touching the ground	S and CF	State	
Side-lying inactive	Similar to sitting but bird is lying on its side. Usually leg is stretched	S and CF	State	
Standing inactive	Immobile on two legs, body not touching the ground	S and CF	State	
Walking	Slow forward movement, breast above the ground, using legs	S and CF	State	
Crawling	Slow forward movement, breast touching the ground, using legs	S and CF	State	
Feed standing	Downward pecking in feeder while standing	S and CF	State	All Feeding
Feed sitting	Downward pecking in feeder while sitting	S and CF	State	All Feeding
Drink standing	Beak dipped into water in drinker followed by tilt head back and swallow while standing	S and CF	State	All Drinking
Drinking sitting	Beak dipped into water in drinker followed by tilt head back and swallow while sitting	S and CF	State	All Drinking
Preen standing	Moving the beak through the feathers while standing	S and CF	State	Comfort
Preen side-lying	Moving the beak through the feathers while side-lying	S and CF	State	Comfort
Preen sitting	Moving the beak through the feathers while sitting	S and CF	State	Comfort
Dust bathing	Vertical wing shakes in a lying position. Or lying on side, scratching at pen floor, rubbing head and neck on floor, opening wings (Nicol et al., 2009)	S and CF	State	Comfort
Foraging	Ground scratching using both legs accompanied by pecking on the ground	S and CF	State	Exploratory
Running	Faster forward movement, breast above the ground, using legs; wings not involved	S and CF	State	High Energy
Wing assisted run	Faster forward movement, breast above the ground, using legs and wings	S and CF	State	High Energy
Play fight	Two birds, hopping and "chest bumping"— no touching necessary; while facing on another. No forceful pecking	S and CF	State	High Energy
Hop/Jump	Single bird (not directed towards another bird) launches from the ground/ pen furniture with both feet – wings may be outstretched but not flapping and elevation is not sustained	S and CF	Event	High Energy
Fly	Single bird (not directed towards another bird) launches from the ground/ pen furniture with both feet – wings flapping and elevation is sustained for at least three consecutive wing beats	S and CF	State	High Energy
Wing assisted jumping	Push off a surface and into the air by using the muscles in legs while using wings	CF only	Event	High Energy
Wing flapping	Bilateral movement of the wings including wing raising (Nicol et al., 2009) while standing	CF only	Event	High Energy
Peck pen wall	Peck at any part of the pen wall or mesh	CF only	Event	Exploratory
Peck at feeder	Peck the feeder wall without touching feed	CF only	Event	Exploratory
Peck at drinker	Peck the drinker wall without touching water	CF only	Event	Exploratory
Peck at perch	Peck the perch frame	CF only	Event	Exploratory
Peck at litter	Peck at the litter. No ground scratching involved	CF only	Event	Exploratory
Peck at accelerometer	Peck at accelerometer attached to another bird	CF only	Event	Exploratory
Peck at mark	Peck at mark on another bird's plumage or the leg tag of another bird (specify in notes)	CF only	Event	
Feather peck/Peck at plumage	The focal bird pecks another bird's feathers or plumage anywhere except a spray-marked area (note if particles of litter on other bird's plumage)	CF only	Event	
Peck at beak	Peck at the beak of another bird (note if feed or water on other bird's beak)	CF only	Event	
Self-Peck mark	Peck at mark on own plumage or own leg tag (specify in notes)	CF only	Event	
Feather ruffle	Feather erection and body shaking	CF only	Event	Comfort
Leg stretch	Stretching one of the legs while standing, usually to the rear of the bird	CF only	Event	Comfort
Wing Stretch	Wing is extended fully in a downwards direction towards the floor, stretching down across the leg	CF only	Event	Comfort
Leg & wing stretch	Stretching one of the legs while standing, usually to the rear of the bird, accompanied by wing extended fully in a downwards direction towards the floor, stretching down across the leg	CF only	Event	Comfort
Head scratch	Scratch head using one foot	CF only	Event	Comfort
Headshake	Rapid rotatory movement of the head accompanied by slight raising of the head and neck feathers – less pronounced side to side flick is occasionally observed (modified from Nicol et al., 2009)	CF only	Event	
Resource-use				
On perch	Both feet on the upper bar of the perch	S only	State	
At feeder	Head perpendicular, or closer to perpendicular than parallel, to the feeder trough, above the trough or with beak in the trough	S only	State	
At drinker	Head perpendicular, or closer to perpendicular than parallel, to the drinker trough, above the trough or with beak in the trough	S only	State	
On litter	Both feet on the litter of the pen (in any posture), but not at the feeder or drinker	S only	State	
Missing observation				
Out of view	Where a bird could not be seen it was recorded as out of view.	S and CF	State	

Abbreviations: S = scan sampling; CF = continuous focal sampling.
[1] When using continuous focal sampling, frequency only was recorded for event behaviours whilst both frequency and duration (s) were recorded for state behaviours.
[2] Combined categories of behaviour used for statistical analysis are indicated in the mutually exclusive collations column. Event behaviours combined with state behaviours within these collations were assigned a duration of 1 s.

3 min focal observation were calculated for continuously recorded focal behaviour. Data were imported into Graphpad Prism (Version 8.0.0(224)), and Spearman's rank correlations were conducted to test for associations between behaviours (within data type).

Breed and batch differences in behaviour

Differences between breeds (CNV n = 7, SGH n = 8, SGN n = 8 pens) and batches in pen mean percentage scans of behaviour and resource-use, and pen mean frequency and duration per 3 min focal observation of behaviour were analysed in Graphpad Prism (Version 8.0.0(224)) in the same way as for the health outcomes.

Associations between behaviour and health outcomes

Binary logistic generalized linear models were used to assess associations between pen prevalence of each health outcome (response variable) and each behaviour variable. Each model was run with only behaviour as an explanatory variable and then additionally with behaviour, breed and behaviour by breed interaction terms to check for any breed by behaviour interactions. For all analyses of day 29 data, breed CNV was set as the reference comparator. For day 43 data, breed SGH was the reference. Significant odds of association referred to the likelihood that a 10% increase in pen mean percentage scans of behaviour or relative resource-use (scan data) or a one unit increase in pen mean frequency or duration (s) of behaviour (continuous data) that focal birds in any pen were observed to perform, was associated with any bird in that pen having a poorer health outcome. There was insufficient variation in pododermatitis score on day 29 or 43, and insufficient variation in feather cover score on day 43 to examine associations with behaviour. To account for the large numbers of statistical tests only results where $P < 0.01$ are considered significant, but complete tables are presented in Supplementary material.

Results

Mortality

Mean ± SD total mortality (including week one) per batch was 3.40 ± 1.53% for breed SGH, 7.47 ± 2.53% for breed SGN and 15.9 8 ± 13.40% for breed CNV. The pattern of cumulative total mortality is shown in Fig. 2. Mortality increased rapidly after day 21 for CNV birds and was due to heart attacks, leg culls (gait score $\geq$4), ascites and other unascertained causes. For SGN early-life mortality was higher in Batch 2 than in Batch 1, but thereafter showed a similar slow rise. Leg culls contributed 0% total mortality for SGH birds, 2.25% total mortality for SCN birds and 2.29% total mortality for CNV birds.

Associations between health outcomes

The prevalence of leg deviations ($\geq$1) was positively correlated with all poor health outcomes except mortality$^{(-\mathrm{wk1})}$. There were also positive associations between prevalence of hockburn and prevalence of poor gait and breast dirtiness; and between prevalence of poor feather cover and poor gait, pododermatitis and mortality$^{(-\mathrm{wk1})}$. Mortality$^{(-\mathrm{wk1})}$ was not associated with any other poor health outcomes. Positive correlations were also seen between mean pen scores for gait and respectively hockburn and feather cover. No other correlations were found between pen mean scores for health outcomes. See supplementary material for all health outcome associations tested (Tables S1a and S1b).

Breed and batch differences in health outcomes

Descriptive data relating to health outcomes for each breed are reported in Table 3, alongside contextual information about hatching rates and bird characteristics at the time of health assessment. Pen mean percentages of mortality$^{(-\mathrm{wk1})}$, pododermatitis and poor plumage, as well as pen mean plumage score were all significantly greater for CNV than both SGH and SGN. Pen mean percentages of poor gait, leg deviations, as well as pen mean growth rate per bird, gait score and hockburn score were all significantly greater for CNV than SGN. Pen mean percentage of hockburn and pen mean percentage and mean score of dirty breast plumage were significantly lower for SGN than both SGH and CNV. There were no significant differences between batches for any health outcome parameter.

Associations between behaviour outcomes

Considering pen mean percentage scans: Standing inactive was negatively correlated with sitting and side-lying inactive on day 29 but with only sitting inactive on day 43. Side-lying inactive was negatively correlated with walking on day 29 but not day 43. Located on the litter was positively associated with sitting inactive and negatively associated with on the perch on day 29 but only negatively associated with All Drinking on day 43. At the feeder was positively associated with All Feeding behaviour only on day 43. See supplementary material for all tested relationships (Tables S2a and S2b).

Considering mean frequencies derived from continuous focal observations, on day 29 positive correlations were seen between standing inactive, walking and High Energy behaviour. Walking was also positively correlated with All Feeding behaviour. No associations were detected on day 43. See supplementary material (Table S3).

Considering pen mean durations derived from continuous focal observations, on day 29 there were negative correlations between sitting inactive and both standing inactive and walking, and between side-lying inactive and walking. Walking was positively correlated with standing inactive and All Feeding behaviour. On day 43 the negative correlation between sitting inactive and walking persisted and All Drinking behaviour was positively correlated with both walking and High Energy behaviour. See supplementary material (Tables S4a and S4b).

Breed and batch differences in behaviour

Table 4 shows breed differences in scan-sampled behaviour and Table 5 shows breed differences in continuously focal-sampled behaviour. The two methods provided similar but not identical profiles of breed behaviour. The most common behaviour identified by both methods was sitting inactive, but there was slight divergence in relative rankings of behaviours beyond this point. For example, scan sampling identified the second most common activity for SGN to be standing inactive while continuous sampling (duration) identified this to be Comfort behaviour (Tables 4 and 5).

On day 29 SGN spent significantly fewer mean percentage scans per pen side-lying inactive compared to both SGH and CNV, as well as greater standing inactive and walking compared to CNV (Table 4). Both SGH and SGN spent a greater pen mean percentage scans on the perch than CNV on day 29 (Table 4). On day 43 SGN spent fewer scans side-lying inactive and greater standing inactive than SGH (Table 4).

On day 29, on average within a 3 min focal observation, CNV pens showed more frequent side-lying and less frequent and shorter duration (s) standing inactive than SGH and SGN (which did not differ) (Table 5). No breed differences were identified for

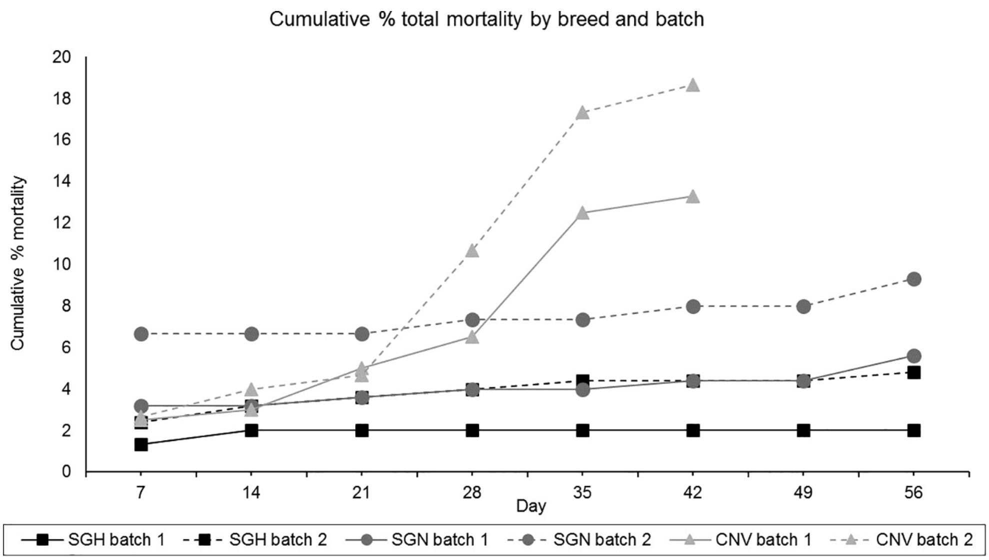

Fig. 2. Weekly cumulative percentage pen mortality up to abattoir date by broiler chicken breed and batch. Where SGH = slow-growing breed H, SGN = slow-growing breed N and CNV = the conventional breed.

Table 3
Pen health and performance outcomes from two slow-growing and one conventional breed of broiler chicken (*Gallus gallus domesticus*).

Health/performance outcome	Breed[1]			H[2,3]	*P*-value[3]
	SGH	SGN	CNV		
Parent flock age (across batches)	44–52	40–44	40–44	–	–
Hatching rate (across batches)	46–60%	63–67%	47–51%	–	–
Age range (d) at health assessment (target 2.2 kg)	46–47	52–53	33–35	–	–
Male:female ratio at health assessment	1.06	0.84	0.24	–	–
Mean ± SD weight (kg) at health assessment (birds weighed individually)	2.31 ± 0.80	2.39 ± 0.54	2.20 ± 0.75	–	–
Median (25–75% quartiles) mean pen daily growth rate per bird (g) (birds bulk weighed)	**49.00**[ab] (48.25–50.75)	**45.50**[a] (44.25–46.75)	**66.00**[b] (64.00–68.00)	**19.65**	**0.0004**
Mean (25–75% quartiles) Feed Conversion Ratio (birds bulk weighed)	**1.71**[a] (1.47–1.92)	**2.04**[b] (2.02–2.13)	**1.40**[a] (1.36–1.41)	**15.73**	**<0.0001**
Median (25–75% quartiles) mean pen % total mortality (excluding week 1)	**2.00**[a] (2.00–5.50)	**3.00**[a] (2.00–6.00)	**12.0**[b] (8.00–20.00)	**11.89**	**0.0026**
Median (25–75% quartiles) mean pen gait score	**1.46**[ab] (1.43–1.55)	**1.25**[a] (1.13–1.34)	**2.22**[b] (2.14–2.29)	**19.57**	**<0.0001**
Median (25–75% quartiles) mean pen % poor gait (score ≥2)	**43.17**[ab] (41.89–51.80)	**31.50**[a] (23.57–35.33)	**92.50**[b] (89.13–97.83)	**19.57**	**<0.0001**
Median (25–75% quartiles) mean pen hockburn score	**0.20**[ab] (0.17–0.23)	**0.10**[a] (0.04–0.18)	**0.22**[b] (0.19–0.27)	**11.60**	**0.0030**
Median (25–75% quartiles) mean pen % hockburn (score ≥0.25)	**78.85**[a] (69.00–91.35)	**30.50**[b] (14.71–71.65)	**88.46**[a] (78.13–88.89)	**10.92**	**0.0043**
Median (25–75% quartiles) mean pen pododermatitis score	0.00 (0.00–0.03)	0.00 (0.00–0.00)	0.01 (0.00–0.03)	5.178	0.0751
Median (25–75% quartiles) mean pen % pododermatitis (score ≥0.25)	**0.00**[a] (0.00–19.39)	**0.00**[a] (0.00–0.00)	**100.00**[b] (100.00)	**18.14**	**<0.0001**
Median (25–75% quartiles) mean pen breast plumage dirtiness score	**0.862**[a] (0.545–0.961)	**0.197**[b] (0.158–0.267)	**0.630**[a] (0.370–0.731)	**15.26**	**0.0005**
Median (25–75% quartiles) mean pen % dirty breast plumage (score ≥1.0)	**74.46**[a] (51.62–88.94)	**19.68**[b] (15.82–25.75)	**62.96**[a] (37.04–73.08)	**15.18**	**0.0005**
Median (25–75% quartiles) mean pen % presence of leg deviations (≥1)	**71.15**[ab] (64.35–84.11)	**59.63**[a] (50.29–64.56)	**92.31**[b] (77.78–93.55)	**13.47**	**0.0012**
Median (25–75% quartiles) mean pen % varus leg deviations	0.00[a] (0.00–0.00)	5.23[b] (0.00–7.85)	0.00[a] (0.00–0.00)	9.220	0.0100
Median (25–75% quartiles) mean pen % valgus leg deviations	**71.15**[ab] (64.35–84.11)	**51.93**[a] (35.48–65.38)	**92.31**[b] (77.78–93.55)	**13.47**	**0.0012**
Median (25–75% quartiles) mean pen plumage score	**0.00**[a] (0.00–0.00)	**0.00**[a] (0.00–0.00)	**0.26**[b] (0.16–0.61)	**18.07**	**0.0001**
Median (25–75% quartiles) mean pen % poor plumage (score ≥0.5)	**0.00**[a] (0.00–0.00)	**0.00**[a] (0.00–0.00)	**48.15**[b] (31.25–76.92)	**18.06**	**0.0001**

Abbreviations: SGH =slow-growing breed H (*n* = 8 pens), SGN = slow-growing breed N (*n* = 8 pens), CNV = the conventional breed (*n* = 7 pens),
[1] Results considered significant are emboldened.
[2] H = test statistic from the Kruskal–Wallis test of differences between the breeds.
[3] '–' represents untested relationships due to low variation, reliability or frequency.
[a–b] Values within a row with different superscripts differ significantly at $P < 0.05$ as identified by post hoc Dunn's method tests corrected for multiple testing using [Graphpad prism 8.0.0 (224)].

Table 4
Pen scan-sampled behaviour and resource-use data from two slow-growing and one conventional breed of broiler chicken (*Gallus gallus domesticus*): Median (25–75% quartiles) mean % of scans per pen.

Behaviour	Breed Day 29 (*n* = 23)[1]					Breed Day 43 (*n* = 16)[1,4]			
	SGH	SGN	CNV	H[2]	*P*-value	SGH	SGN	U[3]	*P*-value
Sitting inactive	65.00 (57.50–68.75)	55.00 (48.13–69.38)	65.00 (57.50–75.00)	2.88	0.2369	66.25 (62.50–74.38)	58.75 (51.25–64.38)	13.50	0.0552
Side-lying inactive	**6.25**[a] (3.13–10.00)	**0.00**[b] (0.00–0.00)	**17.50**[a] (12.50–20.00)	**17.26**	**0.0002**	**7.50** (3.13–10.00)	**0.00** (0.00–2.50)	**7.00**	**0.0065**
Stand-inactive	**7.50**[ab] (3.75–11.88)	**21.25**[a] (6.25–24.38)	**2.50**[b] (0.00–5.00)	**11.43**	**0.0033**	**8.75** (7.50–12.50)	**17.50** (13.13–22.50)	**5.00**	**0.0034**
Walking	**1.25**[ab] (0.00–4.38)	**6.25**[a] (3.13–9.38)	**0.00**[b] (0.00–0.00)	**10.61**	**0.0050**	22.50 (18.13–37.50)	28.75 (18.13–41.25)	27.00	0.6267
All Feeding	3.75 (0.63–6.88)	5.00 (3.13–6.88)	7.50 (2.50–10.00)	1.07	0.5870	2.50 (2.50–5.00)	3.75 (2.50–7.50)	27.00	0.6291
All Drinking	1.25 (0.00–2.50)	3.75 (0.00–5.00)	0.00 (0.00–2.50)	2.25	0.3255	1.25 (0.00–2.50)	2.50 (0.00–9.38)	23.00	0.3343
Comfort	8.75 (5.63–10.00)	6.25 (5.00–9.38)	5.00 (2.50–7.50)	3.51	0.1729	7.50 (2.50–11.88)	7.50 (7.50–10.00)	27.00	0.6081
On perch[3]	**12.50**[a] (6.25–17.50)	**7.50**[a] (5.00–10.00)	**0.00**[b] (0.00–0.00)	**13.62**	**0.0011**	-	-	-	-
At feeder	5.00 (0.63–6.88)	5.00 (3.13–7.50)	7.50 (2.50–10.00)	1.07	0.5870	2.50 (2.50–5.00)	3.75 (2.50–7.50)	27.00	0.6291
On litter	81.25 (76.25–89.38)	82.50 (78.13–87.50)	90.00 (87.50–97.50)	6.40	0.0408	92.50 (90.00–97.50)	87.50 (81.88–91.25)	15.50	0.0918

Abbreviations*:* SGH = slow-growing breed H (*n* = 8 pens), SGN = slow-growing breed N (*n* = 8 pens), CNV = the conventional breed (*n* = 7 pens),
[1] Results considered significant are emboldened.
[2] H = test statistic from the Kruskal–Wallis test of differences between the breeds.
[3] U = test statistic from the Mann–Whitney test of differences between the breeds.
[4] '–' represents untested relationships due to low variation, reliability or frequency.
[a–b] Values within a row with different superscripts differ significantly at $P < 0.05$ as identified by post hoc Dunn's method tests corrected for multiple testing using [Graphpad prism 8.0.0 (224)].

day 43. There were no differences between batches for any scan or continuously sampled behaviours for either day.

Associations between health and behaviour

Associations between behaviour and health outcomes

The significant test statistics for associations between pen level health outcomes and, respectively, scan observations of focal bird behaviour and resource-use, are shown in Table 6 and continuous observations of focal bird behaviour are shown in Table 7.

Positive associations refer to greater likelihood of poorer pen health (indicated with ↓), negative associations refer to a reduced likelihood of poorer pen health (indicated with ↑). Full tables of all significant and non-significant results are provided in the Supplementary material (Tables S5 and S6).

Consistency of behaviour and health associations across ages

Overall, more significant results were obtained at day 29, when three breeds were included in the analysis, providing greater study power. No significant associations between behaviour and mortality$^{(-wk1)}$ were noted at day 43. Standing inactive (scan; negative), side-lying inactive (continuous; positive) and walking (continuous, negative) were associated consistently with gait at both 29 and 43 days of age. Most associations were consistent across ages for hockburn: sitting inactive (scan; positive), side-lying inactive (continuous; positive) and walking (continuous, negative), were associated consistently at both 29 and 43 days, High Energy behaviour (continuous) was associated with better (lower) hockburn scores at 29 days but this effect reversed at 43 days. For breast plumage dirtiness, consistency across ages was found for sitting inactive (scan, positive), standing inactive (scan and continuous, negative), walking (continuous, negative), and between frequency of High Energy behaviour at 29 days and duration of High Energy behaviour at 43 days (continuous, negative). Some associations were apparent only at 43 days, for example, the positive associations between dirty breast plumage and both location on the litter (scan) and All Feeding behaviour (continuous), and the negative associations between Comfort behaviour (scan and continuous) and hockburn, and between Comfort behaviour (scan) and pen prevalence of leg deviations.

Day 29 only – Associations of behaviour with many different health outcomes

Considering further the full dataset obtained at 29 days of age, some behaviours (measured either by scan or continuous observation) were associated with many poor health outcomes. The most informative in this regard were (scans) side-lying inactive (positively associated with *all* health outcomes) and both relative location on the perch (scans) and standing inactive (scans and continuous) (negatively associated with *all* health outcomes, with the exception of perch location and dirty breast plumage which were positively associated) detected in the observations. Walking and High Energy behaviour were negatively associated, and sitting inactive was positively associated with almost all poor health outcomes (excluding leg deviations, mortality$^{(-wk1)}$ and feather cover respectively). Other informative categories were the collated categories of Comfort behaviour (scans), which was negatively associated with poor gait, feather cover and mortality; and Exploratory behaviour (continuous), which was negatively associated with poor gait, dirty breast plumage and leg deviations. Finally scan observations revealed positive associations between relative location on the litter with poor gait, poor feather cover and mortality$^{(-wk1)}$, relative location at the feeder with poor gait and poor feather cover, and All Feeding behaviour with poor feather cover.

Day 29 only – Consistency in associations across methods

The two methods allowed us to obtain different information: resource-use data were obtained only during scans, whilst some behavioural events were recorded only during continuous observations (see Table 2). However, a set of behaviours was recorded using both methods. All the health outcome associations with side-lying inactive and standing inactive described above were

Table 5
Pen continuously sampled behaviour from two slow-growing and one conventional breed of broiler chicken (*Gallus gallus domesticus*): Median (25–75% quartiles) pen mean frequency [F] and total duration (s) [D] per bird during 3 min observation.

		Breed Day 29 (n = 23)[1,2]					Breed Day 43 (n = 16)[1,2]			
Behaviour	Measure	SGH	SGN	CNV	H[3]	*P*-value	SGH	SGN	U[4]	*P*-value
Sitting inactive	F	–	–	–	–	–	–	–	–	–
	D	114.5 (81.3–131.7)	99.9 (89.5–144.6)	126.6 (107.3–139.3)	1.36	0.5074	117.7 (107.5–124.3)	111.6 (107.4–119.2)	28.00	0.7209
Side-lying inactive	**F**	**0.10**[a] (0.00–0.30)	**0.05**[a] (0.00–0.18)	**0.40**[b] (0.30–0.80)	**10.89**	**0.0043**	0.15 (0.10–0.28)	0.10 (0.00–0.10)	13.00	0.0544
	D	2.2[ab] (0.0–8.1)	0.2[a] (0.0–2.7)	9.4[b] (1.6–28.7)	6.79	0.0335	6.6 (2.2–15.2)	0.5 (0.0–9.1)	15.00	0.0822
Stand-inactive	**F**	**1.20**[a] (1.03–2.00)	**1.25**[a] (0.95–2.13)	**0.50**[b] (0.50–0.60)	**10.02**	**0.0067**	1.05 (0.85–1.60)	1.35 (0.88–2.00)	23.50	0.3949
	D	**13.5**[a] (8.2–22.2)	**12.1**[a] (7.8–18.1)	**3.2**[b] (2.1–7.4)	**9.45**	**0.0089**	8.2 (5.0–14.1)	12.3 (5.4–15.1)	26.00	0.5737
Walking	F	1.15[a] (0.70–1.98)	1.60[a] (0.88–2.30)	0.60[b] (0.20–1.00)	6.04	0.0488	0.90 (0.73–1.15)	1.40 (0.65–1.85)	23.00	0.3661
	D	9.2 (3.7–12.5)	11.2 (6.3–14.4)	3.3 (1.1–4.1)	6.85	0.0325	4.7 (3.2–5.6)	7.5 (3.7–11.3)	20.00	0.2345
All Feeding[c]	F	0.30 (0.03–0.58)	0.25 (0.10–0.30)	0.30 (0.00–0.40)	0.06	0.9706	0.25 (0.20–0.48))	0.30 (0.05–0.53)	30.00	0.8622
	D	7.2 (0.1–27.2)	6.8 (1.9–13.3)	11.0 (0.0–19.8)	0.16	0.9224	7.6 (3.0–10.9)	2.2 (0.3–8.7)	19.00	0.1866
All Drinking[c]	F	0.10 (0.00–0.18)	0.10 (0.00–0.28)	0.20 (0.00–0.20)	0.54	0.7632	0.15 (0.00–0.28)	0.10 (0.03–0.30)	28.50	0.7692
	D	2.8 (0.0–8.0)	2.1 (0.0–8.3)	3.9 (0.0–7.1)	0.07	0.9628	1.7 (0.0–6.7)	1.1 (0.1–9.8)	28.00	0.7156
Comfort[c]	F	–	–	–	–	–	–	–	–	–
	D	16.5 (15.2–22.3)	13.2 (8.0–25.5)	9.3 (7.7–20.7)	1.71	0.4247	14.1 (9.8–25.0)	24.3 (13.8–29.5)	21.00	0.2786
Exploratory[c]	F	–	–	–	–	–	–	–	–	–
	D	0.0 (0.0–0.0	0.0 (0.0–1.3)	0.0 (0.0–0.0)	3.92	0.1408	0.0 (0.0–0.0)	0.0 (0.0–0.0)	28.00	>0.999
High Energy[c]	F	0.15 (0.10–0.30)	0.30 (0.05–0.90)	0.10 (0.00–0.30)	2.15	0.3413	0.10 (0.00–0.35)	0.10 (0.00–0.40)	31.50	0.9977
	D	0.7 (0.2–1.1)	0.4 (0.0–1.4)	0.7 (0.0–0.9)	0.28	0.8703	0.0 (0.0–0.3)	0.0 (0.0–1.0)	24.00	0.4126

Abbreviations: SGH = Slow-growing breed H (n = 8 pens), SGN = Slow-growing breed N (n = 8 pens), CNV = the conventional breed (n = 7 pens).
[1] Results considered significant are emboldened.
[2] '–' represents untested relationships due to low variation, reliability or frequency.
[3] H = test statistic from the Kruskal–Wallis test of differences between the breeds.
[4] U = test statistic from the Mann = Whitney test of differences between the breeds.
[a–b] Values within a row with different superscripts differ significantly at $P < 0.05$ as identified by post hoc Dunn's method tests corrected for multiple testing using [Graphpad prism 8.0.0 (224)].
[c] Indicates a collated category of behaviour.

detected using *both* methods (for the continuous observations this was sometimes detected by frequency, sometimes by duration and sometimes by both measures). Scan observations detected associations with sitting inactive (e.g. for poor gait, dirty breast plumage, hockburn and leg deviations) and with Comfort behaviour (e.g. for gait and mortality$^{(-wk1)}$) that were not detected with the continuous observations. In contrast, continuous observations detected associations for walking and the collated category High Energy behaviour (e.g. for poor gait, feather cover, dirty breast plumage, hockburn, and mortality$^{(-wk1)}$/leg deviation) that were not detected with the scan observations.

Discussion

Our study aimed to generate a greater understanding of the relationships between measures of broiler health and behaviour, and to inform RSPCA BBWAP behavioural monitoring requirements. Our hypotheses were generally supported across a range of welfare outcomes. Consistent with Dixon (2020), who examined health and behaviour of some breeds in common with ours, we found the faster growing conventional breed had higher prevalence of poorer health outcomes (mortality, gait, pododermatitis, feather cover), but better growth and feed conversion than the slower growing breeds. Mortality was high for the CNV compared to the commercial norm, particularly in weeks 4 and 5, with birds primarily found dead or experiencing heart attacks. This is perhaps unsurprising when considering the RSPCA BBWAP use of low stocking density and non-limiting, high energy and high protein feed to maximise genetic potential. Leg deviations were also high and it is possible this was scored sensitively with our novel scale. Good photo-based scales for leg deviations are not available and are sorely needed. Our day 29 scanned behaviour was broadly equivalent to the day 30 scanned behaviour reported by Dixon (2020) but our slower growing breed spent more scans sitting, fewer scans standing and almost none foraging or dustbathing. The absences of dustbathing and foraging may potentially be influenced by our sampling times in comparison to Dixon's (2020) 24 h recording period.

Key behavioural indicators of broiler welfare

Side-lying inactive, sitting inactive, and greater use of the litter relative to other resources, were revealed as primary and general indicators of poorer health, whose increase may also precede more

Table 6
Significant test statistics for the pen level analysis of associations between pen prevalence of broiler chicken (*Gallus gallus domesticus*) health outcomes[1] (up to 2.2 kg liveweight) and pen percentage scans of focal bird behaviour and resource use (breeds pooled).

Health outcome by behaviour/ resource-use association	Day 29 (CNV, SGH, SGN) *n* = 23 pens				Day 43 (SGH, SGN) *n* = 16 pens[3]			
	Odds ratio for a 10% increase in behaviour[2]	95% confidence interval	χ^2	*P*-value	Odds ratio for a 10% increase in behaviour[2]	95% confidence interval	χ^2	*P*-value
Pen prevalence poor gait								
Sitting inactive	1.57↓	1.38–1.79	46.3	<0.001				
Side-lying inactive	4.12↓	3.30–5.13	159.5	<0.001	–	–		–
Standing inactive	0.40↑	0.34–0.46	129.0	<0.001	0.65↑	0.51–0.83	11.5	0.001
Comfort[c]	0.59↑	0.43–0.80	11.6	0.001				
On perch	0.39↑	0.32–0.48	78.2	<0.001	–	–	–	–
At feeder	1.53↓	1.11–2.11	6.9	0.009				
On litter	1.89↓	1.60–2.24	55.3	<0.001				
Pen prevalence poor feather cover								
Side-lying inactive	9.81↓	6.59–14.60	126.5	<0.001	–	–	–	–
Standing inactive	0.04↑	0.02–0.09	64.1	<0.001	–	–	–	–
All Feeding[c]	4.53↓	2.67–7.71	31.2	<0.001	–	–	–	–
Comfort[c]	0.21↑	0.10–0.43	17.8	<0.001	–	–	–	–
On perch	0.01↑	0.00–0.02	62.5	<0.001	–	–		–
At feeder	5.26↓	2.98–9.28	32.7	<0.001	–	–		–
On litter	3.99↓	2.74–5.82	51.6	<0.001	–	–		–
Pen prevalence dirty breast plumage								
Sitting inactive	1.30↓	1.10–1.53	10.0	0.002	2.31↓	1.79–2.98	41.1	<0.001
Side-lying inactive	2.41↓	1.90–3.04	53.3	<0.001	–	–	–	–
Standing inactive	0.54↑	0.44–0.66	37.9	<0.001	0.22↑	0.14–0.33	52.2	<0.001
Comfort[c]					0.29↑	0.17–0.51	18.5	<0.001
On perch	1.59↓	1.23–2.07	12.3	<0.001	–	–	–	–
On litter					1.97↓	1.41–2.77	15.5	<0.001
Pen prevalence hockburn								
Sitting inactive	1.72↓	1.44–2.06	35.0	<0.001	1.57↓	1.24–1.98	14.0	<0.001
Side-lying inactive	3.24↓	2.39–4.39	57.5	<0.001	–	–	–	–
Standing inactive	0.37↑	0.30–0.46	87.0	<0.001	0.34↑	0.24–0.48	37.4	<0.001
Comfort[c]					0.47↑	0.26–0.82	7.1	0.008
On litter					2.95↓	2.07–4.21	35.9	<0.001
Pen prevalence leg deviation								
Sitting inactive	1.41↓	1.18–1.68	13.9	<0.001				
Side-lying inactive	1.99↓	1.51–2.62	23.9	<0.001	–	–	–	–
Standing inactive	0.55↑	0.45–0.67	35.3	<0.001				
Comfort[c]					0.46↑	0.25–0.83	6.7	0.009
On perch	0.62↑	0.47–0.82	11.2	0.001	–	–		–
Pen prevalence mortality$^{(-wk1)}$[1]								
Side-lying inactive	2.13↓	1.58–2.87	24.6	<0.001	–	–		–
Standing inactive	0.49↑	0.34–0.71	14.6	<0.001				
Comfort[c]	0.20↑	0.09–0.46	14.5	<0.001				
On perch	0.39↑	0.24–0.61	16.1	<0.001	–	–		–
On litter	1.74↓	1.24–2.45	10.2	0.001				

Abbreviations: SGH = slow-growing breed H (*n* = 8 pens), SGN =slow-growing breed N (*n* = 8 pens), CNV = the conventional breed (*n* = 7 pens), χ^2 = Wald Chi-square with degrees of freedom of 1.

[1] Health outcomes were pen prevalence of poor gait (scores ≥2), poor feather cover (scores ≥0.5), dirty breast plumage (scores ≥1), leg deviations (birds with one or more leg deviations of any form), hockburn (scores ≥0.25), and mortality$^{(-wk1)}$ (mortality excluding week 1: *n* = 76 comprising leg culls 14.4%, heart attacks 10.5%, ascites 3.9%, runt culls 13.2%, other culls 17.1% and found dead 40.8%).

[2] A positive odds ratio is >1 and a negative odds ratio is <1. Significant positive odds ratios are indicated with ↓ as they indicated behaviour associated with a worse health outcome and significant negative odds ratios are indicated with ↑ as they indicated behaviour associated with a better health outcome.

[3] '–' represents untested relationships due to low reliability, frequency or variation.

[c] Indicates a collated category of behaviour.

clinical or difficult to assess signs of poor welfare outcomes. Standing inactive, greater use of the perch, walking, total Comfort behaviour (preening, dustbathing, leg and wing stretching, head-scratching, feather ruffle), total High Energy behaviour (running, wing-assisted running, wing flapping, playing, fighting, hopping/jumping, wing-assisted hopping/jumping, flying) and, to a lesser extent, total Exploratory behaviour (foraging, litter pecking and pecking at pen furniture) were revealed as primary and general indicators of better health. Their reduction or absence may also precede clinical observation of breast plumage dirtiness, gait score, hockburn and leg deviation. Of these behaviours, side-lying, standing, walking Comfort and High Energy behaviour were most sensitive to small differences in health outcomes important for breed acceptance in high-welfare schemes and may be useful targets for selection efforts.

Unlike other health outcomes collected after behaviour recordings, mortality was measured daily throughout rearing. Even so, day 29 behaviour (side-lying, standing, Comfort, and relative locations on the perch and litter) predicted ultimate pen prevalence of mortality$^{(-wk1)}$. Of the CNV bird deaths, approximately half died from ascites, heart attacks or other unascertained causes that did not appear to bear any relation with skin or leg health problems. A relatively high proportion of SGN birds were culled for leg problems during the first week of life, especially in Batch 2 (see Fig. 1) but this appeared to reflect a developmental disorder that was not followed by subsequent leg problems in older SGN birds.

Although, to our knowledge, no previous work has examined detailed associations between health outcomes and behaviour, we found points of consistency with other studies. For example, although they did not directly test associative relationships

Table 7
Significant test statistics for the pen level analysis of associations tested between pen prevalence of broiler chicken (*Gallus gallus domesticus*) health outcomes[1] (up to 2.2 kg liveweight) and pen mean frequencies (F) and durations (D [s]) of focal bird behaviour (breeds pooled).

Health outcome by behaviour association[2]	Measure	Day 29 (CNV, SGH, SGN) n = 23 pens				Day 43 (SGH, SGN) n = 16 pens			
		Odds ratio for a one unit increase in behaviour[3]	95% confidence interval	χ^2	*P*-value	Odds ratio for a one unit increase in behaviour[3]	95% confidence interval	χ^2	*P*-value
Pen prevalence poor gait									
Side-lying inactive	F	56.19↓	27.58–114.49	123.1	<0.001	18.47↓	4.55–74.97	16.6	<0.001
	D	1.08↓	1.06–1.11	59.7	<0.001				
Standing inactive	F	0.41↑	0.34–0.50	81.4	<0.001				
	D	0.93↑	0.91–0.95	67.7	<0.001				
Walking	F	0.49↑	0.42–0.58	67.5	<0.001				
	D	0.91↑	0.89–0.93	67.3	<0.001	0.94↑	0.90–0.98	7.2	0.007
All Feeding[c]	F					2.45↓	1.28–4.70	7.3	0.007
	D					1.06↓	1.03–1.10	14.0	<0.001
Exploratory[c]	F	–	–	–	–	–	–	–	–
	D	0.89↑	0.83–0.95	10.7	0.001				
High Energy[c]	F	0.41↑	0.30–0.56	31.7	<0.001				
	D								
Pen prevalence poor feather cover									
Sitting inactive	F	–	–	–	–	–	–	–	–
	D	1.01↓	1.01–1.02	9.3	0.002	–	–	–	–
Side-lying inactive	F	47.52↓	20.92–107.97	85.0	<0.001	–	–	–	–
	D	1.04↓	1.03–1.06	38.3	<0.001	–	–	–	–
Standing inactive	F	0.03↑	0.01–0.07	75.8	<0.001	–	–	–	–
	D	0.66↑	0.61–0.72	92.2	<0.001	–	–	–	–
Walking	F	0.36↑	0.26–0.52	32.4	<0.001	–	–	–	–
	D	0.89↑	0.85–0.93	27.4	<0.001	–	–	–	–
Comfort[c]	F	–	–	–	–	–	–	–	–
	D	0.93↑	0.90–0.96	18.7	<0.001	–	–	–	–
High Energy[c]	F	0.05↑	0.01–0.17	21.7	<0.001[A]	–	–	–	–
	D					–	–	–	–
Pen prevalence breast plumage dirtiness									
Side-lying inactive	F					13 663.80↓	1 128.06–165 504.71	56.0	<0.001
	D					1.08↓	1.04–1.11	23.8	<0.001
Standing inactive	F								
	D	0.97↑	0.95–0.99	7.9	0.005	0.94↑	0.91–0.98	10.5	0.001
Walking	F	0.73↑	0.59–0.90	8.5	0.003	0.56↑	0.38–0.83	8.3	0.004
	D					0.88↑	0.83–0.94	17.3	<0.001
All Feeding[c]	F								
	D					1.06↓	1.02–1.11	8.1	0.004
All Drinking[c]	F					0.10↑	0.03–0.37	11.8	0.001
	D					0.92↑	0.88–0.96	13.7	<0.001
Exploratory[c]	F	–	–	–	–	–	–	–	–
	D	0.83↑	0.75–0.93	10.3	0.001				
High Energy[c]	F	0.53↑	0.35–0.81	8.9	0.003				
	D					0.32↑	0.19–0.54	18.7	<0.001
Pen prevalence hockburn									
Side-lying inactive	F	6.16↓	2.84–13.38	21.1	<0.001	7 815.63↓	549.87–111 088.21	43.8	<0.001
	D	1.03↓	1.01–1.04	9.1	0.003	1.16↓	1.11–1.20	54.0	<0.001
Standing inactive	F	0.52↑	0.41–0.67	26.7	<0.001				
	D	0.93↑	0.91–0.95	35.1	<0.001				
Walking	F	0.53↑	0.42–0.67	30.2	<0.001				
	D	0.93↑	0.91–0.96	23.2	<0.001	0.93↑	0.88–0.98	7.3	0.007
Comfort[c]	F	–	–	–	–	–	–	–	–
	D					0.97↑	0.95–0.99	11.8	0.001
Exploratory[c]	F	–	–	–	–	–		–	–
	D					4.3×10^{-7}↑	4.5×10^{-10}–0.00	17.5	<0.001
High Energy[c]	F	0.25↑	0.17–0.39	39.8	<0.001	4.96↓	1.90–12.96	10.7	0.001
	D								
Pen prevalence leg deviation									
Side-lying inactive	F	3.82↓	1.78–8.23	11.7	0.001				
	D								
Standing inactive	F	0.60↑	0.47–0.78	15.8	<0.001				
	D	0.96↑	0.94–0.98	12.7	<0.001				
Walking	F	0.63↑	0.50–0.79	15.45	<0.001				
	D	0.95↑	0.92–0.97	11.5	0.001				
Exploratory[c]	F	–		–	–	–		–	–
	D	0.85↑	0.78–0.93	13.4	<0.001				
High Energy[c]	F	0.55↑	0.37–0.83	8.4	0.004				
	D								

Table 7 (*continued*)

Health outcome by behaviour association[2]	Measure	Day 29 (CNV, SGH, SGN) n = 23 pens				Day 43 (SGH, SGN) n = 16 pens			
		Odds ratio for a one unit increase in behaviour[3]	95% confidence interval	χ^2	*P*-value	Odds ratio for a one unit increase in behaviour[3]	95% confidence interval	χ^2	*P*-value
Pen prevalence mortality$^{(-wk1)}$									
Side-lying inactive	F	2.74↓	1.32–5.67	7.4	0.007				
	D								
Standing inactive	F	0.402	0.26–0.63	15.5	<0.001				
	D	0.92	0.88–0.96	16.8	<0.001				
Walking	F	0.57	0.41–0.81	10.0	0.002				
	D								

Abbreviations: SGH = slow-growing breed H (n = 8 pens), SGN = slow-growing breed N (n = 8 pens), CNV = the conventional breed (n = 7 pens), χ^2 = Wald Chi-square with degrees of freedom of 1.

[1] Health outcomes were pen prevalence of poor gait (scores ≥2), poor feather cover (scores ≥0.5), dirty breast plumage (scores ≥1), leg deviations (birds with one or more leg deviations of any form), hockburn (scores ≥0.25), and mortality$^{(-wk1)}$ (mortality excluding week 1: n = 76 comprising leg culls 14.4%, heart attacks 10.5%, ascites 3.9%, runt culls 13.2%, other culls 17.1% and found dead 40.8%).

[2] '–' represents untested relationships due to low reliability, frequency or low variation.

[3] A positive odds ratio is >1 and a negative odds ratio is <1. Significant positive odds ratios are indicated with ↓ as they indicated behaviour associated with a worse health outcome and significant negative odds ratios are indicated with ↑ as they indicated behaviour associated with a better health outcome.

[c] Indicates a collated category of behaviour.

[A] Indicates warnings associated with uncertainty in the statistical model due to reaching the maximum number of step halvings with results based on the last iteration, primarily due to large numbers of zeros in the data or limited variation across welfare outcome categories. In all cases data were plotted to check consistency with findings.

Meyer et al. (2020) reported greater concurrent day 30 inactivity with greater lameness and contact dermatitis in conventional US broilers. Greater time (particularly resting) on the litter and correspondingly less frequent visits to resources are consistent with those of previous work on lameness (Weeks et al., 2000). Weeks et al., (2000) also proposed that increased side-lying by lame broilers may be motivated by relief of pain or discomfort in the legs. Although side-lying was infrequent in our study, it was associated with all poorer health outcomes. The wider association of side-lying with other health outcomes, albeit some co-associated, in our study suggests this posture could more subtly reduce discomfort associated with long periods of resting or avoidance of movement. Dawkins et al. (2013 and 2017) previously reported negative correlations between walking (mean percentage of birds) and hockburn, and between high energy behaviour (fast moving birds measured using optical flow) and hockburn, although it is surprising that in our study walking was sensitive to the mild hockburn scores we recorded.

Certain behaviours, (e.g. comfort behaviours) show low-resilience; are among the first to reduce under challenge (Littin et al., 2008; Mandel et al., 2017) and frequently precede clinical signs, providing an early warning (Littin et al., 2008). We propose that effortful behaviours will tax even mildly challenged broiler chickens and thus subtle behavioural changes may occur to conserve energy. This is supported by our finding that mildly poorer feather cover was very strongly positively associated with side-lying and greater time on the litter and negatively associated with Comfort behaviour, walking and perching, despite limited variation in our feather cover scores (day 29). Importantly, although our tested associations are based on the presence or absence of poor health outcomes, there is good independent evidence that comfort behaviours such as preening are associated with positive states (environmental choice; Nicol et al., 2009). Thus recording prevalence of such behaviours contributes more to our understanding of broiler welfare than the prevalence of (negative) health outcomes alone.

How should behavioural indicators be recorded?

Of the two sampling approaches to data collection (scan sampling and continuous sampling, from five focal birds per pen), scan sampling generally resulted in a better balance of measures of positive and negative behavioural indicators that most robustly (across health outcomes and across days) and sensitively (with lower score ranges in welfare outcomes and a smaller sample [SGH and SGN subpopulation at 43 days]) detected changes in welfare. Scan sampling was able to capture side-lying inactive, sitting inactive, standing inactive, Comfort behaviour and resource-use. Walking, Exploratory and High Energy behaviour required continuous sampling, which was also able to capture side-lying inactive (frequencies with generally higher odds), and standing inactive, though not as many associations (dirty breast plumage associations were missed). A caveat is that only continuously recorded side-lying inactive could be tested for associations on day 43 (of which three were identified) because frequency of observations was insufficient for scan data. More frequent scan sampling could address issues of this kind.

Continuous sampling was not without limitations. Not only were continuous recordings of walking, Exploratory and High Energy behaviours less consistent in associations across days, but duration data were limited by our observation period of 3 min per bird. True durations of bouts of some behaviours may have exceeded 3 min. A combination of approaches may be the ideal for the most comprehensive picture, but at minimum scan sampling should be conducted.

Cost effectiveness and timing of behaviour data collection

Welfare assessment protocols will be most widely used if they are valid and sensitive but also not too time-consuming or expensive to conduct (Mullan et al., 2009; Heath et al., 2014). Consistent with Meyer et al (2020) a very positive finding from our study is that data capture of behaviour from five randomly selected 'sentinel' birds (10% of the pen) at each of a limited range of sample points on a single day is sufficient to detect even relatively mild differences in pen prevalence of ultimate welfare outcomes. Our further finding that marking is unnecessary is a significant advantage since it reduces labour and repeated pen disturbance with remarking, and avoids the risk of associated alterations in behaviour towards or by focal birds (Dennis et al., 2008). Our video image processing and data recording took approximately 2.5 h per pen for scan sampling and approximately 1.6 h per pen for continuous sampling. This may still currently be prohibitive compared to other measures used for monitoring or genetic selection, but supports exploration of the potential for automated monitoring techniques to capture key behavioural indicators.

In our study, day 29 behaviour data collection was, respectively, an average of 4, 17 and 23 days in advance of the health assessment for CNV, SGH and SGN. Day 29 incorporation of the CNV data extended the range in scores for health outcomes and the proximity of CNV to health assessment and greater weight of this breed at this age may have more greatly affected their behaviour. It is not currently possible to disentangle age, weight and breed effects. On day 43, behaviour data collection was an average of 3 days in advance of the SGH and 11 days in advance of the SGN birds. Our current data therefore suggest that data collection three days in advance of the health assessment would pick up differences in behaviour associated with health outcomes, although it is plausible that a week in advance may also do so.

Is there possible redundancy between measures of broiler welfare?

The RSPCA BBWAP requires that all birds are gait scored, whilst other health parameters are conducted on 50% of the sample. Previous research indicates that chicken welfare assessments can include redundant (unnecessary) measures (Nicol et al., 2011) or lack sensitivity (e.g. Buijs et al., 2017), unnecessarily increasing labour, cost and bird stress. Simple correlations of our data indicated that the pen prevalence of one or more leg deviations was strongly associated with poor gait and moderately associated with all other health outcomes. De Jong et al (2016) also report moderate correlations between on farm prevalence measures of hockburn and both gait and plumage cleanliness. This suggests that gait scoring, which is particularly disturbing and stressful for lame birds, may not be necessary to assess for all, or perhaps even any, birds if these other parameters are recorded.

It is also important to consider whether undisturbed behaviour data capture could reduce or replace the necessity for intrusive health assessments. Based on strong associations between on farm measurements and assessment post-slaughter, De Jong et al (2016) suggested that abattoir data could replace on farm assessment. However, behaviour measurements can be scheduled to assess changes in bird health over time, and some behaviours can additionally be used as indicators of positive or negative affect (Nicol et al., 2009). Our data suggest capture of strong behavioural predictors of moderately poor gait, hockburn, plumage cleanliness, leg deviations, mortality$^{(-wk1)}$, and potentially feather cover is possible using scan sampling. Clearly this would not substitute good stockmanship nor examination of vulnerable birds, but could predict health status at group level earlier than standard assessments would allow, particularly if automation is feasible. Targeted examination or further assessment could then occur where appropriate, rather than as a unilateral audit for all birds, which may unnecessarily increase stress.

Study limitations

A general assumption of our study was that relationships between behaviour and welfare outcomes would be consistent across breeds. However, there was confound between breed and some health outcome parameters, whereby breeds were not uniformly represented across the score ranges. For example, the majority of higher gait scores and poorer feather cover were recorded for CNV and very few CNV birds contributed to the perfect gait or feather cover categories. Further, CNV birds were over-represented by females, potentially underrepresenting the range in weight and walking ability at a given age in this population, since males tend to be heavier and have poorer gait scores (Sanotra et al., 2001). In addition, darker plumage and skin colouration in SGN may have contributed to underestimation of hockburn, pododermatitis and plumage cleanliness. Where breeds are likely to vary in pigmentation, investigation of alternative ways of recording these parameters is warranted. Some differences in breed by behaviour interactions on health outcomes were identified. Most could be explained by differences in breed weight at the time of recording, proximity to welfare assessment and/or frequencies of behaviour recorded. An informal glance comparing the behavioural data for the CNV on day 29 and the slow-growing breeds on day 43 suggests the slow-growing birds were still more active at comparable weight, so perhaps age may have less of an impact on behaviour than weight, but this was of course additionally associated with differences in health outcomes. It is currently impossible to determine the true relative impacts of the differences in age and weight in our data. Further research systematically investigating the relationships between age, weight, sex, behaviour and health outcomes is crucial to corroborate our findings and confirm the best time to sample behaviour across breeds.

Summary

In summary, we have identified key primary and general behavioural indicators of poor (side-lying inactive, sitting inactive, and location on the litter relative to other resources) and positive (standing, location on the perch, walking, Comfort behaviour, High Energy behaviour, Exploratory behaviour) welfare, which may be useful targets for selection efforts. Non-intrusive scan sampling of behaviour of 10% of the pen, randomly selected at the sample point, appears to capture the majority of strong associations collectively covering all health outcomes measured. Testing these predictions with an independent data set is required to demonstrate external validation. However the consistency and sensitivity of relationships identified suggests substantial promise, and that measurement of at least some health outcomes which involve bird stress can be reduced, or potentially even non-invasively replaced with developments in automated visual analysis, increasing cost effectiveness and improving welfare. Crucially, these behavioural measures of bird welfare may not only precede more clinical or difficult to assess signs of poor health outcomes, but they additionally represent motivational and affective aspects of welfare not currently incorporated into standard welfare assessments for broilers and should be considered in future broiler welfare standards.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100261.

Ethics approval

The work was approved by the Royal Veterinary College Clinical Research Ethical Review Board, reference: URN 2018 1814-3.

Data and model availability statement

None of the data were deposited in an official repository. Data available upon request.

Author ORCIDs

S.M. Abeyesinghe: https://orcid.org/0000-0002-9977-7949.
N.M. Chancellor: N/A.
D. Hernandez Moore: N/A.
Y.-M. Chang: https://orcid.org/0000-0001-6388-9629.

J. Pearce: N/A.
T. Demmers: https://orcid.org/0000-0003-2151-3502.
C.J. Nicol: https://orcid.org/0000-0001-6734-2177.

Author contributions

SA: Investigation, Formal Analysis, Project Administration, Supervision, Writing-Original Draft, Data Curation.
NC: Investigation, Resources, Writing – Review & Editing.
DHM: Investigation, Writing – Review & Editing.
Y-MC: Formal Analysis, Writing – Review & Editing.
TD: Resources, Writing – Review & Editing.
JP: Investigation, Writing – Review & Editing.
CN: Conceptualization, Funding Acquisition, Investigation, Formal Analysis, Writing-Original Draft, Supervision.

Declaration of interest

The authors have no interests to declare.

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