# Stocking density effects on broiler welfare: Identifying sensitive ranges for different indicators

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**ABSTRACT** Although stocking density is perceived as a topic of major importance, no consensus has been reached on what density would allow for good welfare. In the present study, the welfare of 4 replicates of birds stocked at 8, 19, 29, 40, 45, 51, 61, and 72 broilers per pen (or 6, 15, 23, 33, 35, 41, 47, and 56 kg actually achieved BW/m$^2$) was studied using 6 welfare indicators. Density did not affect bursa weight, mortality, or concentrations of corticosterone metabolites in droppings but did influence leg health ($P = 0.015$) and footpad and hock dermatitis ($P < 0.001$) and tended to influence fearfulness ($P = 0.078$). However, not every increase in density or group size, or both, led to poorer welfare for the affected indicators: leg health and fearfulness showed unexpected peaks at intermediate densities. Furthermore, the indicators were influenced at different densities: leg strength showed a steep decrease from 6 to 23 kg/m$^2$, hock dermatitis rose from 35 to 56 kg/m$^2$, and footpad dermatitis and fearfulness were only significantly higher at the highest density of 56 kg/m$^2$. No threshold stocking density above which all aspects of welfare were suddenly altered was found in this study. Instead, different aspects of welfare were influenced at different densities or group sizes, or both. Thus, evaluating the effects of stocking density on welfare as a whole would require either identification of acceptable levels for each separate indicator or a weighting of the indicators in an integrated welfare score. A tentative attempt to such an integration, made using equal weights for all parameters, showed a decrease in welfare as density increased ($P < 0.001$). The lowest 2 densities (6 and 15 kg/m$^2$) scored better than most middle densities (23, 33, 35, and 47 kg/m$^2$), whereas all densities scored better than the highest density (56 kg/m$^2$).

**Key words:** stocking density, group size, welfare, broiler

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## INTRODUCTION

The stocking densities under which broiler chickens are kept vary greatly between countries and husbandry systems (SCAHAW, 2000). Citizens perceive stocking density as a top priority for animal welfare and are concerned with the stocking densities used in commercial livestock production (Vanhonacker et al., 2008). Although the use of high stocking densities can diminish individual growth (Sorensen et al., 2000; Feddes et al., 2002; Al Homidan et al., 2003; Dawkins et al., 2004), this has not always been an incentive for producers to decrease stocking densities because the economic benefit per square meter is often still higher if the chickens are stocked more densely (Cravener et al., 1992; Feddes et al., 2002).

Because the economic effect of reducing stocking density is large, it is very important to determine the relationship between stocking density and welfare as precisely as possible so that decisions can be taken on what stocking density is acceptable from an animal welfare point of view. Unlike the relationship between stocking density and farm profitability, however, the relation between stocking density and welfare is much more complex and so more difficult to determine precisely. Although many authors have studied this relationship, different effects have been reported. Some of these may have been caused by differences in study design (under controlled circumstances vs. on-farm, varying density by changing group size or pen size, and so on) and others by the use of different indicators of welfare. This makes it hard to compare these studies.


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As a consequence, no specific range in which density affects welfare has been identified yet.

In the ideal scenario, in which all relevant aspects of the multidimensional concept of welfare respond simultaneously at the same density increase, the determination of such a critical density for broiler welfare as a whole would be straightforward. It seems unlikely, however, that if a critical density exists at all, that it will be the same for different indicators. For instance, animals may adapt their behavior at a relatively low density, whereas effects on mortality may only become apparent at much higher densities. If this is indeed the case, determining a threshold stocking density that is acceptable for animal welfare becomes even more complicated. In such a situation, one could focus on the indicator that is perceived as being of greatest importance, use the most sensitive indicator (the one that is affected at the lowest density), or opt for an integrated welfare score based upon a subjective weighting of indicators (Botreau et al., 2007; Rodenburg et al., 2008).

The current experiment aimed to identify separate sensitive density ranges for a large number of welfare indicators: fearfulness, corticosterone concentrations, bursa weight, leg strength, dermatitis, and mortality. The 6 indicators were chosen to include physiological as well as psychological parameters of well-being. Increased density and group size were expected to increase conflicts between the birds, leading to an increase in stress, which would be expressed psychologically by increased fearfulness and physiologically by higher glucocorticoid levels and decreased bursa weight (Ravindran et al., 2006). Leg weakness and dermatitis were included because these represent 2 major welfare problems for broiler chickens, which can both be painful themselves and can lead to other welfare problems (Bradshaw et al., 2002). Mortality was included as a parameter in this study because it can be seen as an endpoint of welfare and so the ultimate indicator. Final BW were collected because of their possible influence on the other indicators (Sanotra et al., 2003).

Because many on-farm studies have shown a large effect of management and housing on welfare (Dawkins et al., 2004; Knowles et al., 2008), we opted to work in a smaller-scale experimental study in which housing and management were standardized. This allowed us to focus on our main interest, namely to investigate stocking density effects that could not be avoided by optimizing husbandry procedures. Although differences may exist between the results acquired in small-scale experimental studies and those obtained on-farm, which limits the degree to which these results can be directly extrapolated to commercial practice, this smaller scale made it possible to investigate a wider range of densities than those found on commercial farms. It should be noted that the experiment, in which gradations of stocking density were created by varying the number of broilers in pens of a constant size, did not allow us to disentangle effects of stocking density from those of group size. This decision was taken because the other alternative, keeping group size constant and varying pen size as a way to create the gradations in stocking density, was thought to be even less applicable to commercial farms where pen edge effects are minimal.

## MATERIALS AND METHODS

### *Birds and Housing Conditions*

Day-old broiler chicks (Ross 308) were placed into 3.3-$m^2$ pens, in groups of 8, 19, 29, 40, 45, 51, 61, and 72 birds. Densities were systematically distributed throughout the room to balance for any pen effects. Males and females were mixed at a ratio of 1:1. In each pen, 8 randomly chosen focal birds (4 males, 4 females) were color-marked to allow individual recognition using nontoxic spray paint, which was renewed weekly. All tests were carried out on these 8 birds per pen, but additional birds were used for the postmortem measurements (see below) and mortality was scored for all chickens involved in the experiment.

Ten water cups and 14 feeders were distributed over the sides of each pen. Food and water were available ad libitum. A 3-phase feeding schedule (0- to 13-d starter diet, 14- to 26-d grower diet, and 27- to 39-d finisher diet) was applied with nutrient content in line with NRC recommendations (NRC, 1994) with the exception of a somewhat lower CP content in the starter (21%) and an $ME_n$ in the different phases of 12.3, 12.5, and 12.7 MJ/kg, respectively. No preventive antibiotics were supplied. Ambient temperature was 31°C on the day of arrival and was subsequently lowered by 1°C daily until a temperature of 21°C was reached (achieved by whole-house brooding, without the use of lamp brooders). Temperature was measured continuously in each pen at chick height. A 21L:3D schedule was used. The litter was changed at the start of wk 3, 5, and 6. The experiment was carried out between March and December 2007 and consisted of 4 replicates, that is to say a total of 1,300 birds.

### *Measurements on Living Birds*

Measurements on living birds were carried out on all focal animals (8 per pen, 248 in total). Levels of fearfulness and corticosteroid metabolites and leg health were determined using these 248 birds.

***Fearfulness.*** Fear was assessed by the duration of tonic immobility (**TI**; Jones and Faure, 1981b), a well-validated fear test (Forkman et al., 2007) that does not require locomotor activity and is thus free from interaction with walking ability. The focal birds were tested in the sixth week of life. If the bird righted itself within 15 s, the attempt was seen as unsuccessful and TI was induced again, to a maximum of 3 times. An upper limit of 600 s per induction was used.

***Corticosteroid Metabolites.*** To avoid additional disturbance by taking blood samples and problems with simultaneous sampling, corticosterone concentrations were assessed noninvasively via quantification of corticosterone metabolites (**CM**) in droppings. The applied method has previously been validated for broiler chickens (Rettenbacher et al., 2006). Droppings were collected in wk 3, 5, and 6 from the boxes in which the focal birds were held before their weekly weighing (per pen). The weighing procedure never exceeded 1.5 h for all birds together, which is shorter than the expected time lag between elevated glucocorticoid levels in the blood and the appearance of CM in the droppings (Rettenbacher et al., 2004). Each dropping was homogenized and 0.5 g was suspended in 5 mL of 60% (vol/vol) methanol by shaking for 30 min on a multivortex. After centrifugation, aliquots of the supernatant were diluted 1:10 with assay buffer and concentrations of CM were determined with a noncommercial enzyme immunoassay (Rettenbacher et al., 2004).

***Leg Health.*** A latency-to-lie (**LTL**) test was used to study leg health. This test measures the amount of time a chicken can remain standing to avoid sitting down in shallow, lukewarm water and is correlated to the walking ability of the chicken (Berg and Sanotra, 2003). The birds were tested without visual or physical contact with other birds.

## Postmortem Measurements

Spontaneous mortality and the number of birds culled because of obvious gait or other health problems were recorded on a daily basis. At d 39, one hundred nine chickens per replicate were randomly selected from the remaining birds for postmortem measurements. These included all focal birds, but at some densities, additional birds were used, leading to postmortem measurements on 8, 19, 8, 8, 25, 8, 8, and 25 birds per replicate per group of 8, 19, 29, 40, 45, 51, 61, and 72 birds, respectively (due to the amount of birds needed for another experiment, not described here). These birds were culled by injection of an overdosed anesthetic into the wing vein and subsequently weighed.

***Bursa Weight.*** Directly postmortem, the bursa of Fabricius was removed from the birds selected for postmortem measurements and weighed. Raw bursa weight as well as the bursa:BW ratio for each bird was used for analysis.

***Dermatitis.*** Footpad and hock dermatitis were scored on the birds selected for postmortem measurements. The footpads were scored using a 3-point scale as described by Algers and Berg (2004): 0 = no unhealed lesions larger than a pinhead; 1 = moderate, superficial lesions; and 2 = large or deep lesions, ulcers, or scabs. The hocks were scored according to the surface area of the lesion, on a 5-point scale (1 = no lesion; 2 = lesion of 1 to 15 $mm^2$; 3 = lesion of 16 to 50 $mm^2$; 4 = lesion of 51 to 120 $mm^2$; 5 = lesion of >120 $mm^2$). The scores of the left and right footpad were averaged, as were those of the hocks.

## Integrated Welfare Score

The scores of all indicators measured on an individual level (TI, LTL, bursa:BW ratio, and hock and footpad dermatitis) were aggregated using a simple method of integration in which all indicators were given an equal weight. All scores were standardized by subtracting the mean and dividing by the SD, to give a mean of 0 and a SD of 1. The direction of the scores was also standardized; thus, higher scores indicated better welfare (Tuyttens et al., 2008). These standardized scores were then used as repeated measures, within individual, to analyze this integrated welfare score.

## Statistical Analysis

Two different types of analysis were used. First, a linear model treating stocking density as a continuous variable was used because this is a sensitive method for finding relationships over a wide range of densities while minimizing the effect of spurious findings resulting from outliers. However, such a model can by definition only result in a linear relationship and thus no critical points can be identified. Therefore, an additional model, treating density as a class variable, was used to detect critical densities by making direct comparisons between different stocking densities.

Mortality was analyzed by a Friedman test in StatXact 5.0.3 (Cytel Inc., Cambridge, MA), using replicate as block. All other data were analyzed in a mixed model using SAS 9.1.3 (SAS Institute Inc., Cary, NC). For TI, bursa weight, bursa:BW ratio, dermatitis, and the standardized integrated welfare score, the fixed factors density, sex, and their interaction were used. Latency to lie was analyzed with these fixed factors plus BW and its interactions as additional fixed factors. Corticosteroid metabolites were analyzed at the pen level because it was unknown which individual had produced which dropping. Values from the same combination of density, week, and replicate were treated as repeated measurements and density and week were used as fixed factors. Replicate was used as a random factor in all mixed model analyses. Nonsignificant fixed effects or interactions were dropped from the analysis.

# RESULTS

## Fearfulness

In the linear model, there was a significant density × sex interaction ($F_{1,240} = 5.3$, $P = 0.021$) on the duration of TI. Although the modeled response for females was 124 s at all densities, male TI increased with density [regression equation: TI duration = 43.0 + 2.11 × density (in birds/pen)]. The class model showed a slightly different picture. With this analysis, there was only a weak tendency to a density × sex interaction ($P = 0.097$). When this interaction was removed from the model because of this weakness, a somewhat stronger tendency toward a main density effect was found (Table 1). The groups of 72 birds had a longer TI duration than those of 8, 19, 29, 45, and 51 and tended to differ from 61. However, the groups of 72 and 40 birds per pen did not differ from each other.

## Corticosteroid Metabolites

Concentrations of CM were not influenced by density in either the linear ($F_{1,73.4} = 0.3$, $P = 0.620$) or the class model (Table 1) but did show a week effect ($F_{2,74} = 31.2$, $P < 0.001$). The average concentration in wk 3 (68.6 ± 4.2 ng/g) was significantly ($P < 0.001$) higher than in either wk 5 (48.1 ± 43.2 ng/g) or wk 6 (45.1 ± 4.2 ng/g).

## Leg Health

The LTL test showed a decrease of leg strength with increasing density in the linear model [$F_{1,228} = 10.3$, $P = 0.002$, regression equation: LTL = 896 − 2.03 × density − 0.26 × BW d 36 (with density in birds per pen and BW in grams)]. The class model (Table 1) showed that this decrease was caused by a steep drop in LTL duration over the 4 lowest densities, leading to a significant difference between the groups of 8 and all groups ≥40 birds per pen, with the exception of the groups of 61, which tended to show shorter durations ($P = 0.063$).

## Bursa Weight

No significant density effects were found on either the absolute bursa weight or the bursa:BW ratio ($F_{1,230} = 1.4$, $P = 0.237$ and $F_{1,230} = 1.6$, $P = 0.202$, respectively, in the linear model). Sex, however, did influence the absolute bursa weight ($F_{1,226} = 8.26$, $P = 0.004$).

Females had lighter bursas (4.0 ± 0.13 g) than males (5.4 ± 0.13 g). Similarly, the bursa:BW ratio ($F_{1,230} = 10.1$, $P = 0.002$) was lower for females (0.167% ± 0.005) than for males (0.189% ± 0.005).

## Dermatitis

Both hock and footpad dermatitis were significantly worse with increasing density when modeled linearly. Footpad dermatitis showed the smaller effect of density [$F_{1,411} = 47.1$, $P < 0.001$, regression equation: footpad score = −0.11 + 0.004 × density (in birds/pen)] and pairwise comparisons showed that this was entirely due to the birds in groups of 72, which scored significantly higher than those in any other group (Table 1).

Hock dermatitis [$F_{1,411} = 305.3$, $P < 0.001$, regression equation: hock score = 0.14 + 0.036 × density (in birds/pen)] showed a more gradual increase with density, with pairwise comparisons showing significantly elevated levels from 51 birds/pen and onward (Table 1).

**Table 1.** The effects of density on the welfare indicators as calculated by the class model[1]

| Indicator | Test statistic | *P*-value | 8 birds/pen, 6 kg/m$^2$ | 19 birds/pen, 15 kg/m$^2$ | 29 birds/pen, 23 kg/m$^2$ | 40 birds/pen, 33 kg/m$^2$ | 45 birds/pen, 35 kg/m$^2$ | 51 birds/pen, 41 kg/m$^2$ | 61 birds/pen, 47 kg/m$^2$ | 72 birds/pen, 56 kg/m$^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| TI duration[2] (s) | $F_{7,236} = 1.9$ | $P = 0.078$ | $112^{a}$ (28) | $101^{a}$ (27) | $105^{a}$ (27) | $146^{ab}$ (27) | $126^{a}$ (27) | $80^{a}$ (31) | $131^{ab\#}$ (27) | $198^{b}$ (27) |
| CM[3] (ng/g) | $F_{7,67.3} = 1.6$ | $P = 0.154$ | 49.4 (4.6) | 56.7 (5.4) | 66.0 (5.4) | 55.8 (4.6) | 56.2 (5.4) | 57.1 (6.5) | 57.3 (5.4) | 54.4 (4.6) |
| Bursa weight (g) | $F_{7,224} = 0.6$ | $P = 0.733$ | 4.8 (0.3) | 4.7 (0.3) | 4.8 (0.3) | 4.8 (0.3) | 5.0 (0.3) | 4.8 (0.3) | 4.6 (0.3) | 4.3 (0.3) |
| Bursa:BW (%) | $F_{7,224} = 0.8$ | $P = 0.562$ | 0.18 (0.01) | 0.18 (0.01) | 0.18 (0.01) | 0.18 (0.01) | 0.19 (0.01) | 0.18 (0.01) | 0.17 (0.01) | 0.16 (0.01) |
| Latency to lie (s) | $F_{7,222} = 2.6$ | $P = 0.022$ | $336^{a}$ (36) | $288^{ac}$ (36) | $214^{ac}$ (36) | $198^{c}$ (36) | $189^{b}$ (36) | $203^{c}$ (41) | $239^{a\#bc}$ (38) | $179^{b}$ (36) |
| Hock dermatitis | $F_{7,405} = 70.6$ | $P < 0.001$ | $1.0^{a}$ (0.3) | $1.0^{a}$ (0.3) | $1.1^{a}$ (0.3) | $1.3^{ab}$ (0.3) | $1.2^{a}$ (0.3) | $1.9^{bc}$ (0.3) | $2.0^{c}$ (0.3) | $3.1^{d}$ (0.3) |
| Footpad dermatitis | $F_{7,405} = 12.3$ | $P < 0.001$ | $0.0^{a}$ (0.1) | $0.0^{a}$ (0.1) | $0.0^{a}$ (0.1) | $0.0^{a}$ (0.1) | $0.0^{a}$ (0.1) | $0.0^{a}$ (0.1) | $0.0^{a}$ (0.1) | $0.26^{b}$ (0.1) |
| Mortality (%) | FR (x) = 5.9 | $P = 0.553$ | 3.1 (3.1) | 6.6 (2.9) | 5.2 (2.1) | 5.6 (1.8) | 8.3 (2.1) | 1.3 (0.9) | 5.7 (1.5) | 3.5 (1.1) |
| Mortality excluding replicate 2 (%) | FR (x) = 4.5 | $P = 0.726$ | 4.2 (4.2) | 1.8 (1.8) | 2.3 (1.6) | 2.5 (1.4) | 4.4 (1.8) | 1.3 (0.9) | 3.8 (1.4) | 2.8 (1.1) |
| Day 39 BW (kg) | $F_{7,234} = 0.4$ | $P = 0.891$ | 2.65 (0.04) | 2.65 (0.04) | 2.64 (0.04) | 2.70 (0.04) | 2.62 (0.04) | 2.63 (0.05) | 2.67 (0.04) | 2.61 (0.04) |
| Integrated welfare score | $F_{7,1185} = 11.0$ | $P < 0.001$ | $0.24^{a}$ (0.10) | $0.24^{a}$ (0.10) | $0.14^{b}$ (0.10) | $-0.01^{b}$ (0.10) | $0.00^{b}$ (0.10) | $0.05^{ab}$ (0.11) | $-0.02^{b}$ (0.10) | $-0.58^{c}$ (0.10) |

[a–d]Values within a row lacking a common superscript differ ($P < 0.05$). When common superscripts are followed by a #, means tend to differ ($P < 0.10$).

[1]Values are presented as least squares means (±SE), except for mortality where means (±SE) are presented.

[2]Tonic immobility.

[3]Corticosteroid metabolites.

### Mortality

Overall mortality was 5.0% (±SE 0.6). Unfortunately, this figure was heavily influenced by an outbreak of *Escherichia coli* in the second week of the second replicate, which necessitated the redistribution of birds over groups and the removal of the group of 51 birds from this replicate. When the second replicate was excluded from the analysis, overall mortality was 2.9%. No density effect was found (Table 1). However, it could be argued that our sample size was too small to give a representative view of mortality because we prioritized a wide range of densities, rather than large numbers of birds in each specific density group.

### Final BW

The final BW acquired at 39 d of age were not significantly affected by density in either of the statistical models employed (linear model: $F_{1,240} = 0.2$, $P = 0.640$). However, males were heavier than females [2.87 vs. 2.42 (±0.02) kg, $F_{1,241} = 212.8$, $P < 0.001$].

### Integrated Welfare Score

When the indicators scored on an individual level (TI, LTL, relative bursa weight, and hock and footpad dermatitis) were summarized into an integrated welfare score, increasing density was found to decrease welfare in both the linear ($F_{1,1192} = 54.3$, $P < 0.001$) and the class model (Table 1). The lowest 2 densities (6 and 15 kg/m$^2$) showed better scores than most middle densities (23, 33, 35, and 47 kg/m$^2$), whereas all densities scored better than the highest density (56 kg/m$^2$; Figure 1). In addition, females had a slightly higher welfare score than males (0.09 vs. −0.08 ± 0.07, $F_{1,1187} = 8.9$, $P = 0.003$).

## DISCUSSION

In this experiment, stocking density was varied by housing different numbers of birds in equally sized pens under controlled circumstances. Corticosteroid levels, bursa weight, final BW, and mortality were not significantly affected by stocking density. However, leg strength decreased and dermatitis and fearfulness (in males only) increased with increasing stocking density. Nonetheless, closer inspection of the data revealed that it would be wrong to conclude that every increase in stocking density resulted in a similar or consistent reduction of these welfare aspects. The integrated welfare score showed no difference between densities in the range of 23 to 47 kg/m$^2$ but did show differences at the more extreme densities. In addition, the specific stocking density at which a change in welfare occurred differed for the separate indicators. Latency to lie was affected first, showing a downward slope from 6 to 33 kg/m$^2$ before leveling off at the higher densities. In contrast, dermatitis and fearfulness showed effects at the high end of the density scale only. Hock dermatitis showed stable values from 6 to 35 kg/m$^2$ and subsequently increased to peak at 56 kg/m$^2$, whereas footpad dermatitis and fearfulness were only affected at the highest density (56 kg/m$^2$). This shows the importance of studying a wide range of densities because the differences in leg strength would have gone unnoticed if only commercial stocking densities had been tested, whereas the effects on fearfulness and footpad dermatitis would not have been found if the highest density had been omitted.

Some caution should be taken when extrapolating these results to commercial practice. First, as stated earlier, our experiment was conducted on a small scale to be able to include the lower densities and to control environmental influences. Nevertheless, environmental influences can have a large effect on welfare (Estevez, 2007) and where husbandry procedures, like climate control and litter management, are not optimized in practice, the effect of stocking density on welfare found in this study may be either obscured or enhanced by these environmental influences. Second, we used only 1 (although commercially important) genetic line of fast-growing broilers and our density categories were expressed as final BW per square meter. This is the most common method in both research and legislation (Estevez, 2007). But changes in this kilograms per square meter to number of birds per square meter ratio might be expected to change the results. This would be, for example, if lighter birds than the ones in the present experiment were used, then the same stocking density would encompass more birds per square meter, or vice versa if heavier birds were used. Because group size and stocking density were confounded in this study, these effects could not be disentangled.

It is known that certain group sizes may represent a social dilemma for laying hens because hierarchy is established differently in small and large groups (Pagel and Dawkins, 1997) and that at intermediate group sizes choosing between these 2 strategies may become problematic (Keeling et al., 2003). Although there has previously been some debate on whether broilers actually grow to be old enough to develop a hierarchy, its

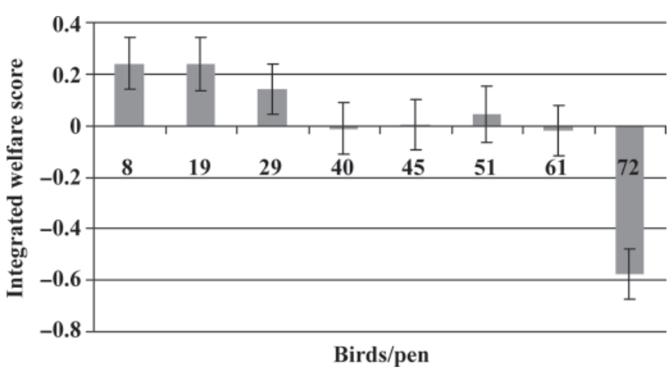


**Figure 1.** Aggregated welfare score integrating relative bursa weight, tonic immobility, latency to lie, hock dermatitis, and footpad dermatitis scores.

formation has been described in broilers from ages as early as 3 to 4 wk (Estevez et al., 1997; Pettit-Riley et al., 2002). This problem with intermediate group sizes, found at around 30 laying hens, may be the underlying cause of the higher-than-expected duration of TI in the groups of 40 broilers. It would account for the lack of a difference in the TI test between groups of 40 and those of 72, despite the fact that groups of 72 birds differed from groups greater than and smaller than 40 birds. However, further research with stable group sizes at different stocking densities or stable stocking densities with increasing group sizes would be needed to assess whether this is actually the case. The linear model identified an interactive effect of density and sex on TI; although males showed a 3-fold increase between 8 and 72 birds per pen (from 60 to 192 s), females showed no reaction at all. This is in line with previously reported differences in the level and ontogeny of fearfulness between sexes (Jones and Faure, 1981a; Campo and Carnicer, 1993; Balazova and Baranyiova, 2008).

The LTL measurements also showed an unexpected peak at 61 birds per pen. No direct explanation could be found for this peak (no extra leg culls or mortality occurred in this group before testing), but analysis of behavioral data (not reported here) showed that the groups of 61 had 20% longer walking bouts than the other groups in the fourth week of life. This extended exercise may have led to the increased leg strength because training is known to increase bone density and decrease bending and twisting (Reiter and Bessei, 1998). Because of its early occurrence, it does seems likely that the activity was the cause, rather than a consequence, of the improved leg strength. In general, leg health decreased with density, as was expected a priori and shown before on-farm as well as under controlled circumstances (Sorensen et al., 2000; Hall, 2001; Dawkins et al., 2004; Thomas et al., 2004; Knowles et al., 2008). However, this parameter has mostly been studied within the range of 24 to 50 kg/m$^2$, whereas the decrease in LTL in our experiment was the greatest between densities below this range. To our knowledge, only Thomas et al. (2004) studied lower densities. In accordance with our study, these authors found their greatest decrease in leg health between densities below those commonly studied, although not at the exact same densities as in the present experiment.

There was no significant difference in the final BW achieved in the various density treatments. A decrease in final BW at high densities has been reported by other authors (Cravener et al., 1992; Elwinger, 1995; Thomas et al., 2004). However, factors that may cause a decrease in production in large groups did not affect our study, in which temperature and air quality were well controlled at all densities, whereas the large number and the placement of the feeders ensured proper accessibility for all birds.

Dermatitis was affected at the high end of the density scale: the average hock score rose from 35 kg/m$^2$ on, whereas an increase in footpad dermatitis occurred at the highest density only (56 kg/m$^2$). Footpad dermatitis occurred almost exclusively in groups stocked at 56 kg/m$^2$ birds per pen. Only 3 birds were affected in all other groups combined, and even within groups stocked at 56 kg/m$^2$, it was rare: only 8.3% of the birds were affected. However, both hock and foodpad dermatitis figures probably represent an underestimation of the effect of stocking density on dermatitis because litter quality was kept high in this experiment by regular refreshing with new litter. Litter quality has a large effect on dermatitis (Haslam et al., 2006), but under commercial circumstances, litter is not replaced within 1 replicate and is only rarely “topped up” in cases of extreme wetness. Surprisingly, hock dermatitis occurred more often and at lower densities than did footpad dermatitis. This is unusual (Berg, 2004) and may have been caused by the fact that footpad dermatitis depends mostly on litter quality, whereas hock dermatitis is also linked to low activity levels (Haslam et al., 2006).

High stocking densities were expected to lead to higher glucocorticoid levels, especially because these were combined with increasing group size in this study, as an expression of increased stress. This was expected to be stronger in the later weeks when conditions in the broiler house became more crowded. However, no increase with density was found, nor was there an increase of CM concentrations throughout the weeks. In fact, the concentrations measured decreased with age, in line with previous research on plasma corticosterone (Hocking et al., 1996; Jong et al., 2002; Thaxton et al., 2005). The absence of a density effect on CM concentrations is supported by previous work (Dawkins et al., 2004; Dozier et al., 2006; Thaxton et al., 2006). However, recent reviews on corticosteroid measurements have indicated that the magnitude of the corticosteroid response to an acute stressor is a more reliable way of assessing chronic stress than simply measuring basal corticosteroid levels (Korte, 2001; Mormede et al., 2007). Therefore, further research is needed to find out if stressors such as stocking density have the potential to alter the hypothalamic-pituitary-adrenal axis or lead to an altered response of the hypothalamic-pituitary-adrenal axis to an acute stressor, or both. There was a similar absence of an effect for both the absolute bursa weight and the bursa:BW ratio. The weight of lymphoid organs is reported to decrease with increased stress (Ravindran et al., 2006) and therefore decreased weights had been expected with increasing density. The lack of an effect in our study contrasts with previous work (Dafwang et al., 1987; Heckert et al., 2002; Ravindran et al., 2006) but finds some support in studies by Heckert et al. (2002) and Thomas et al. (2004), who found no effect of density on the relative weight of the spleen and adrenal glands.

In our experiment, density affected different indicators of welfare at different levels and no single critical stocking density could be identified. This has implications for determining a specific acceptable stocking density. One possible way to remedy this, which was used in this study, is by integrating several indicators into 1 welfare score. Because such methods are not yet fully developed, and for the sake of simplicity because we had no objective criteria for allocating more weight to one indicator than another, we allocated equal weight to all indicators. The disadvantage with our method is that a low score on one indicator, representing a real welfare problem, could be masked by a high score on another indicator. On the other hand, when no integration is practiced, a system could be judged as acceptable when all indicators just reach a minimum acceptable level, whereas for the animal having several moderate welfare problems at once may be just as problematic as having 1 severe problem. Nevertheless, as an exercise, the integration analysis in this study highlights how this set of indicators affected an overall welfare score. This score was mostly influenced by differences in leg strength at the lowest densities and by fearfulness and dermatitis at the high densities, whereas it remained relatively stable at the middle densities.

Recently, new legislation on stocking density was passed in the European Union, setting an upper limit at 42 kg/m$^2$. From our experimental study, we tentatively deduce that this limit would prevent the increase in welfare problems observed at the highest density in this study, namely fearfulness and footpad dermatitis. However, to achieve the additional welfare benefits on leg strength observed at the lowest densities in this study, much more stringent criteria would be needed. Obviously, though, the extent to which such conclusions from this small-scale experimental study with small group sizes can be extrapolated to commercial conditions with much larger group sizes still ought to be investigated.

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