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Stocking density and stress induction affect production and stress parameters in broiler chickens

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Abstract

The objective of this paper is to analyse the effects of three different stocking densities on the production, stress and fear parameters of female broilers during a 46-day production period. Chickens were randomly distributed among nine floor pens in groups of 30 broilers with different space allowances for each treatment; namely eight, 20 and 30 chicks m^{-2}. Chicken growth rate was monitored from day eleven to 46 and indicators of stress, including haematocrit, heterophil/lymphocyte ratio and concentrations of plasma corticosterone, as well as tonic immobility, were measured on days 22 and 46. On day 46, the incidence of foot and skin lesions was assessed, and stress was induced to analyse the response of broilers to each stocking density. High stocking densities cause acute stress in broilers; the effects of low and intermediate stocking densities, however, are not so evident, particularly in relation to tonic immobility and response to acute stress.

Keywords: *animal welfare, broiler, fear, health status, stocking density, stress induction*

Introduction

At present, broiler chickens generally tend to be reared at relatively high stocking densities. This is a major welfare issue in intensive livestock production systems. It is assumed that reducing the stocking density increases animal welfare; as a consequence, attempts are being made to limit stocking density to an acceptable level through the passing of laws and regulations (Bessei 2004). Recently, a new European Directive related to maximum stocking densities for broiler production has been published (Directive 2007/43/EC) but, prior to this, the codes of good practice varied considerably between countries. In this new Directive, acceptable stocking density values range between 33 and 39 kg m^{-2}.

High stocking density also contributes to poor litter quality, high ammonia production and heat stress. These indirect effects are also believed to have an impact on animal welfare (Scientific Committee on Animal Health and Animal Welfare [SCAHAW] 2000). However, authors such as Dawkins *et al* (2004) and Jones *et al* (2005) claim that stocking density *per se* is less important to bird welfare than the control of the birds' environment, in particular, factors such as good ventilation, air control, air quality and litter quality — given the role of air temperature and relative humidity on the health and mortality of broilers.

Several reports have dealt with the negative influence of stocking density on certain production parameters (growth performance, carcase yield and skin scratches) (eg Martrenchar *et al* 2000; Hall 2001; McLean *et al* 2002; Dawkins *et al* 2004; Thomas *et al* 2004). Dozier *et al* (2005) indicated that stocking density influenced bodyweight and feed consumption, but meat yields were not significantly altered. Feddes *et al* (2002), assuming no change in performance, pointed out that increasing stocking density results in higher profitability per kilogram of chicken produced. However, according to these authors, there is a significant effect of stocking density on broiler performance and carcase traits, with maximum growth observed at a stocking density of 14.3 birds m^{-2}, as compared to higher densities. Other authors (eg Hall 2001; Thomas *et al* 2004), however, have reported difficulties in distinguishing the effects of stocking density from those of group size.

Thaxton *et al* (2006) questioned whether higher stocking density causes adaptive responses characteristic of physiological stress. But, while production characteristics have been studied in some detail, less has been done to examine the effect of stocking density on broiler stress and fear responses, as indicated by Sanotra *et al* (2001) and Dozier *et al* (2006). In most of these cited studies, basal levels of corticosterone were measured, with the assumption being

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that low basal levels indicate that the animal is not stressed; however, this does not explain how the individual adapts to a stressful situation. In fact, the animal's adrenocortical response to stress is more relevant when analysing stressful situations than the basal corticosterone level (Silverin 1998).

In this paper, the influence of stocking density on production, corticosterone and haematocrit values, as well as the incidence of skin and foot lesions, other health measurements and fear levels of broiler chickens, has been evaluated. In addition, the relationship between stocking density and bird response to induced acute stress was also assessed.

Materials and methods

Experimental design

Two hundred and seventy female broiler chicks (Ross®, Aviagen, Alabama, USA) were used in this study, which was carried out in an experimental poultry house in Segorbe, Castellón, Spain. One-day old chicks were reared together until eleven days old, at which time they were randomly assigned to nine experimental pens in groups of 30 animals, until they were 46-days old. Three different densities were obtained by varying the available floor space so as to maintain group size and to avoid confounding the effects of stocking density with those of group size. However, pen size and stocking density were confounded, although, as Leone and Estevez (2007) note, this is inadvertent confounding. The dimensions of the pens were 1.14 × 1.1 m, 1.4 × 1.25 m and 2.0 × 2.0 m (length × breadth) and stocking densities were 30 chickens m^{-2} (high density), 20 chickens m^{-2} (intermediate density) and eight chickens m^{-2} (low density), respectively, corresponding to 70, 50 and 20 kg of bodyweight m^{-2} of floor space at the end of the experiment. A stocking density of 50 chicks m^{-2} was similarly evaluated by Shanawany (1988) (see also Dozier *et al* 2006; Ravindran 2006; Thaxton *et al* 2006). Each treatment was replicated with three pens. Each pen was equipped with new wood shavings (originally 10 cm in depth and when birds reached 35 days of age, 3 cm more were added to each pen), a trough feeder (32 cm in diameter) and one 15-litre manual drinker (35 cm in diameter) in the middle of the pen. The stocking densities were calculated by subtracting 0.25 m^2 of unusable space corresponding to the feeder and drinker areas. Divisions among pens permitted visual and olfactory contact between birds. All of the pens were in the same room, with temperature maintained between 20 and 31ºC and relative humidity between 60 and 70% (in accordance with commercial recommendations), and a continuous 24-h lighting regime, with an average light intensity inside the room of 25 lux.

Three commercial diets (Piensos Ponsa SA, Calders, Spain) were fed during the experimental period: starter between one and eleven-days old (2,741 kcal kg^{-1} of metabolisable energy and 19.2% of crude protein); grower1 between eleven and 22 days of age (3,047 kcal kg^{-1} of metabolisable energy and 20.5% of crude protein); grower2 between 23 and 40 days of age (3,104 kcal kg^{-1} of metabolisable energy and 19.3% of crude protein); and finisher from 41 to 46 days of age (3,100 kcal kg^{-1} of metabolisable energy and 18.5% of crude protein).

Measured parameters

Feed was provided *ad libitum* and measured on alternate days, and water was changed and measured daily. Mortality rates were recorded every day and dead chickens were weighed and examined to determine the cause of death. Unfit broilers, susceptible to death or with leg problems (extracted from Dawkins *et al* 2004), were culled and also examined. When a chicken died, the available pen space was reduced, proportionally, to the theoretical space occupied by a bird. Conversion rates were calculated by dividing total feed intake by the weight of living and dead birds.

All birds were weighed individually on day eleven and randomly assigned to a pen. On this day, ten animals in each pen were also tagged with coloured rings on their legs for individual identification for subsequent blood sampling and tonic immobility tests. Subsequently, all birds in each pen were individually weighed at 18, 22, 32 and 46 days of age.

On day 22, five tagged animals were randomly chosen from each pen, and blood samples were taken by venipuncture from the brachial vein. Approximately 3 ml of blood per bird was collected and placed in tubes containing EDTA as the anticoagulant. The tubes were kept in ice until taken to the laboratory, where they were centrifuged at 1,500 g for ten minutes before the plasma was removed and passed to Eppendorf tubes (250 ml) for storage at –20ºC until the corticosterone analysis. All samples were taken within 3 min of the bird's capture to minimise the effects of sampling on plasma corticosterone levels (Littin & Cockrem 2001). Plasma corticosterone concentrations were determined by radio-immunoassay (Immunochem TM Double antibody, corticosterone 125 RIA kits, MP Biomedicals Inc, Irvine, CA, USA). The mean intra- and inter-assay coefficients of variation were 4.4–10.3% and 6.5–7.2%, respectively, and all the samples were run in duplicate.

To determine the heterophil/lymphocyte (H/L) ratio, one drop of whole blood from each chicken sampled was smeared on a glass slide. The smears were stained with May-Grünwald-Giemsa (Lucas & Jamroz 1961) after immediate 3-min fixation in methanol. One hundred leukocytes were counted on each slide, and the H/L ratio was calculated by dividing the number of heterophils by the number of lymphocytes.

Once in the laboratory, the blood samples were used for haematocrit determination. They were taken up in capillary tubes and centrifuged in a microhaematocrit centrifuge for 5 min at 8,385 g.

Tonic immobility (TI) is defined as a state of motor inhibition and reduced responsiveness to external stimuli induced by a brief period of physical restraint (Gallup 1977; Jones 1990), and it is a widely-used measurement of fear (eg Bilcik *et al* 1998; Campo *et al* 2001; Bizeray *et al* 2002; Hocking *et al* 2005). TI was assessed in the ten birds tagged from each pen on day 25–26 and on day 44. As soon as each

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broiler was caught, TI was induced in a nearby room by inverting the bird onto its back with its head hanging over the edge in a U-shaped wooden cradle covered with a thick layer of cloth. The bird was restrained for 15 s by placing one hand on the sternum while covering the head with the other hand, in accordance with the procedure described by Jones and Faure (1981). The observer sat in full view of the chicken and at a distance of about 2 m from the bird. If the bird remained immobile for 10 s after the experimenter removed his/her hands, the time until the bird showed a righting response was recorded. If the bird showed no righting response over a 15-min period, the session was ended and a maximum score of 15 min (900 s) was assigned (Stub & Vestergaard 2001). Conversely, if the bird righted itself in fewer than 10 s, it was then considered that tonic immobility had not been induced and the restraint procedure was repeated. The number of inductions necessary to induce TI for at least 10 s was recorded and if TI was not induced after five attempts, the bird was deemed not to be susceptible and its TI duration score was 0 s (Bizeray *et al* 2002).

On day 46, before slaughter, the following health measurements were taken. Foot-pad dermatitis and hock burns were evaluated according to the scoring system developed by Ekstrand *et al* (1998). Lesions were classified from 0–3 as follows: 0 = No lesions (only mild hyperkeratosis, no discoloration or scars); 1 = Mild lesions (superficial sores, erosions, papillae and discolouration); 2 = Severe lesions (deep lesions, ulcers, and scabs). The presence of ascites and ocular lesions were assessed as 0 when no visual signs were observed and as 1 when there was a degree of inflammation of the abdominal zone or secretion of the eyes. Finally, a bird's respiratory noise level was recorded as follows: 0 = no noise was heard; 1 = low noise; 2 = noise was clearly audible.

On days 41, 42 and 43, another bleeding was carried out as described above, but in this case, two samples were collected: after collecting the first sample, stress was induced in the bird following a modification of the protocol proposed by Silverin (1998). Birds were isolated inside a 50-cm diameter dark box and left undisturbed for 30 min. After this period, a second blood sample was taken to study the corticosterone response of each bird to acute stress.

Statistical analyses

Performance data, post-induction levels of corticosterone and proportionate levels of increase were subjected to an analysis of variance, according to the following model:

$$Y_{ij} = D_i + P_j + \varepsilon_{ijk}$$

Where Y_{ij} is the response variable (bodyweight, feed consumption, water consumption or conversion rate) at a certain moment; D_i is the effect of stocking density ($_i$ = HD, ID, LD); P_j is the effect of the pen ($_j$ = 1, …, 9) and ε_{ijk} is the error (being $_k$ the number of individuals). Stocking density was considered to be a fixed effect and pen was assumed to be a random effect, as well as the experimental unit. Initial bodyweight (at eleven-days old) was used as a covariate when analysing bodyweight. Analyses were performed by Mixed Linear Models Procedure (Proc Mixed) using the SAS System® (SAS Institute 2001). Tukey-Kramer adjustments were used for *post hoc* comparisons. Logarithmic transformations for corticosterone levels were used for analysis of variance but, in the least square means table, the back-transformed data are reported. Corticosterone values, H/L ratio, haematocrit and duration of tonic immobility were analysed using Proc Mixed of the SAS System®, but with stocking density and time as fixed effects and pen as a random effect, so the model tested was:

$$Y_{ijkl} = D_i + P_j + T_k + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the response variable; D_i is the effect of stocking density ($_i$ = HD, ID, LD); P_j is the random effect of the pen ($_j$ = 1, …, 9); T_k is the effect of time ($_k$ = 1, 2) and ε_{ijkl} is the error (l being the number of individuals). Logarithmic transformations for the duration of TI (according to Campo & Redondo 1996), corticosterone and leukocyte components were used for analysis of variance, but the back-transformed data are presented in the least square means table.

The Chi-squared test was used to assess the effect of stocking density on mortality and the number of attempts to induce tonic immobility. The health measurements' analysis was performed by Proc Genmod of the SAS System® (generalised linear models), according to a Poisson distribution.

Results

Mortality

Mortality rates did not differ due to density or age, and only eight animals died during the entire experiment. Post-mortem examinations of dead animals revealed that the main causes of death were firstly ascites, and subsequently dilated cardiomyopathy and respiratory diseases, which affected all treatments equally.

Bodyweight and feed and water intake

Mean bodyweights (BW) are indicated in Table 1. Significant differences were found among the three studied stocking densities. Weight gain in low density (LD) from the first to the last weighing day was greater than in the other treatments, but there were no significant differences between intermediate density (ID) and high density (HD) values, not even at the end of the production cycle.

Regarding feed consumption, food intake during the experimental period is presented in Figure 1. As observed in this Figure, during the last week at LD, feed consumption underwent a noticeable increase in comparison to ID and HD. From day 33, the differences among all three densities became significant (164.7, 171.97 and 185.94 g per bird per day at HD, ID and LD, respectively for the last measurement). The conversion rate, defined as kg feed per kg meat, did not differ statistically among the treatments.

In Figure 1, water consumption is also displayed. Statistically-significant differences were only seen when the birds were 31-days old.

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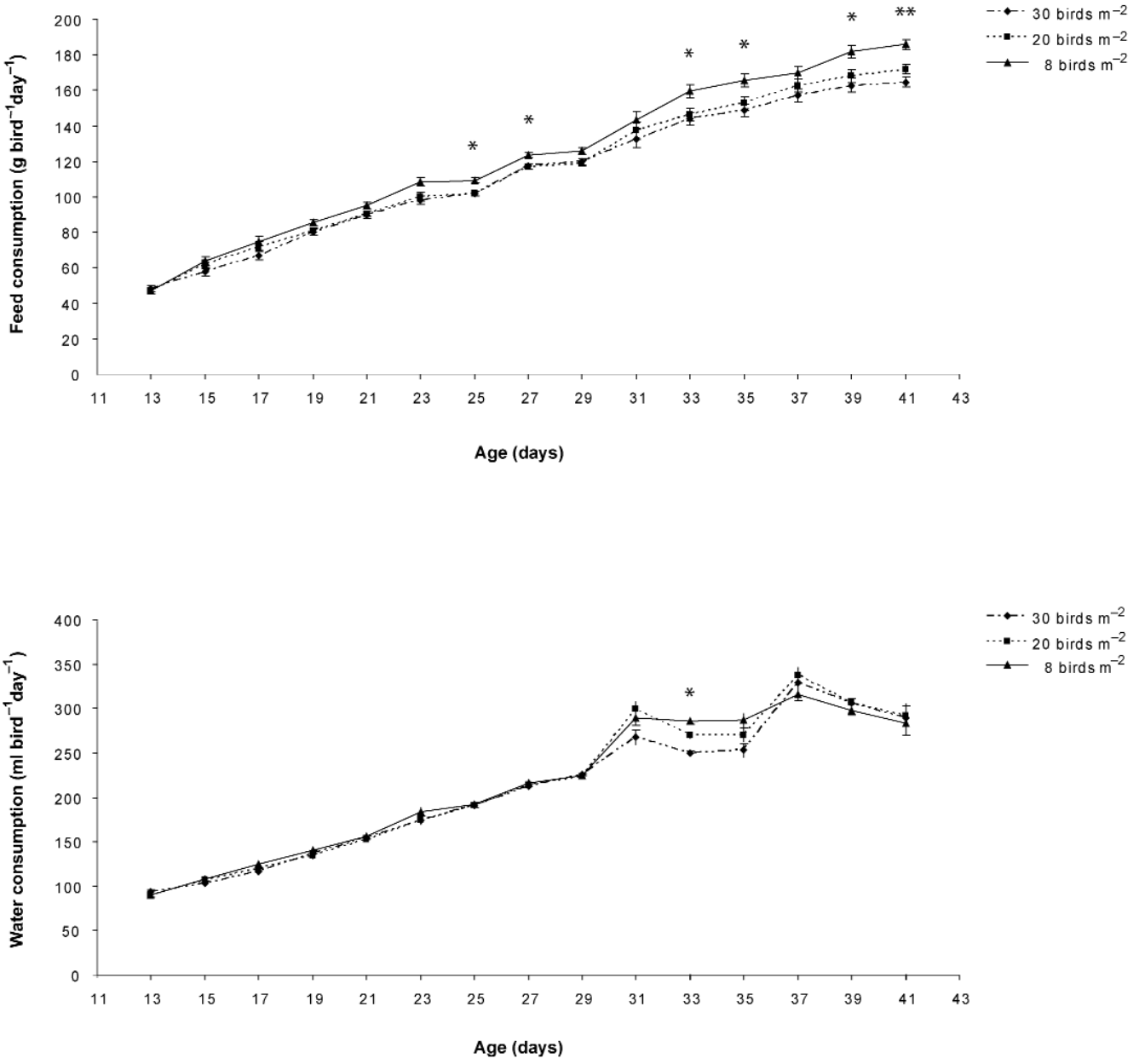
Table 1 Individual bodyweight (kg) of female broilers at three different densities on different days.

Day number	30 birds m^{-2}	20 birds m^{-2}	8 birds m^{-2}	SEM	df	Significance level
11	0.185	0.193	0.203	0.007	266	ns
18	0.483[a]	0.512[ab]	0.550[b]	0.017	266	0.0391
22	0.733	0.765	0.805	0.024	266	ns
32	1.437[a]	1.477[ab]	1.551[b]	0.039	266	ns
46	2.435[a]	2.536[a]	2.689[b]	0.052	266	0.0055

[abc:] Means within the same row with no common superscript ($P < 0.05$).

Figure 1



Feed (g bird^{-1} day^{-1}) and water (ml bird^{-1} day^{-1}) consumption of female broilers from days 11 to 41 at three different stocking densities. * $P < 0.05$, ** $P < 0.01$.

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Table 2 Mean (± SEM) heterophil/lymphocyte ratio of female broilers at 22 and 41, 42 and 43 days of age at three different stocking densities.

Day number	30 birds m^{-2}	20 birds m^{-2}	8 birds m^{-2}	Significance level
22	0.346 (± 0.107)[a]	0.392 (± 0.120)[a]	0.538 (± 0.108)[a]	
41, 42 and 43	0.991 (± 0.991)[c]	0.804 (± 0.108)[bc]	0.553 (± 0.105)[ab]	0.0007

[abc:] Means with no common superscript differ significantly ($P < 0.0001$).

Table 3 Mean (± SEM) health measurements of 46-day old female broilers at three different stocking densities.

Category of health measurement	30 birds m^{-2}	20 birds m^{-2}	8 birds m^{-2}	Significance level
Respiratory noises	0.889 (± 0.161)[ab]	1.174 (± 0.158)[a]	0.767 (± 0.153)[b]	0.0201
Foot-pad dermatitis	1.889 (± 0.095)	1.522 (± 0.091)[b]	0.367 (± 0.089)[c]	< 0.001
Hock burn	1.741 (± 0.092)[a]	1.519 (0.089)[a]	0.967 (± 0.087)[b]	< 0.001
Ascites	0.963 (± 0.061)	0.796 (0.059)	0.800 (± 0.0578)	ns
Ocular secretion	0.148 (± 0.069)[ab]	0.311 (± 0.066)[a]	0.033 (0.065)[b]	0.0141

[abc:] Means within a row with no common superscript differ significantly ($P < 0.05$).

Only data until day 41 are presented, because on that day blood sampling began and consumption could have been affected by the stress of handling.

Haematology

There were no significant differences seen in haematocrit values ($P = 0.6845$) among densities; the mean value was 30.21 (± 0.83). Table 2 also shows the results of the H/L ratio. On day 22, density did not affect the H/L ratio, but this pattern changed over time, as shown by the statistically-significant interaction between age and stocking density. On days 41, 42 and 43, the highest H/L ratio was seen in HD, which differed significantly from LD, although there were no significant differences found between HD and ID, or between ID and LD.

Fear

Density itself did not influence the number of attempts to induce TI ($P = 0.1238$). This parameter was much more strongly affected by the age of the birds ($P < 0.0001$): from the first time the test was carried out (at 25–26 days old) until the second (at 44 days), the number of inductions required, decreased from 2.43 to 1.59. In addition, Chi-squared results revealed that when birds were 44 days old, TI was induced in all the chickens. At this age, five attempts were unnecessary for induction and most birds were induced during the first attempt (25%). Nonetheless, even when the birds were 25–26 days old, TI was induced in most of the chickens during the second attempt (22.26%), while induction was not achieved in 6.25% of the chickens.

Conversely, the duration of TI did not differ due to treatment, age, or the interaction of treatment and age, and the average duration was 226 s. While age seems to increase TI duration numerically that difference was not found to be significant.

Health measurements

Table 3 summarises the results for this analysis. Density was found to significantly affect the aspects of health studied, with the exception of ascites.

In short, LD had the lowest scores for all of the health measurements assessed, and values were significantly lower than those of ID and HD. However, HD and ID did not significantly differ from one another, except for foot-pad dermatitis which was significantly higher for HD. In addition, HD and ID had the highest scores for foot-pad dermatitis.

Concentrations of corticosterone

Density did not affect the concentration of corticosterone ($P = 0.26$), but an interaction was seen between density and age (Table 4). At 22 days old, chickens had the highest corticosterone levels at ID and LD, and the lowest levels at HD, although these differences were not statistically significant. In contrast, at days 41, 42 and 43, HD had the highest level but did not differ significantly from LD, while ID had the lowest level.

Stress induction

With regard to the differences in plasma corticosterone levels after 30 min of stress induction, they differed significantly due to stocking density, as shown in Table 4. The greatest increase, after stress induction, occurred at HD, but there were no significant differences between LD and ID, both of which showed similar increases. However, the proportion of increase from the baseline sample suggests that all the densities seemed to experience a similar proportion of increase.

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Table 4 Plasma corticosterone concentrations (ng ml^{-1}) at 22 and 41, 42 and 43 days of age at three different stocking densities.

	30 birds m^{-2}		20 birds m^{-2}		8 birds m^{-2}		Significance level
	Range	Mean	Range	Mean	Range	Mean	
Day 22	2.49–3.57	2.98^{a}	2.99–4.29	3.58^{a}	3.28–4.70	3.93^{a}	
Days 41, 42 and 43	2.74–3.75	3.13^{a}	1.37–1.88	1.57^{b}	1.83–2.50	2.09^{ab}	0.0178
Post-induction level	10.11–12.81	11.38^{a}	5.74–7.31	6.48^{b}	7.09–8.99	7.98^{b}	0.0042
Proportion of increase	4.22 (± 1.09)		5.69 (± 1.12)		4.76 (± 1.09)		ns

^{ab} Means within the same row with no common superscript ($P < 0.05$).
Ranges: Upper limit = Inv ($\mu_{\ln[\text{corticosterone concentration}]} + SE_{\ln[\text{corticosterone concentration}]}$), Lower limit = Inv($\mu_{\ln[\text{corticosterone concentration}]} - SE_{\ln[\text{corticosterone concentration}]}$)

Discussion

Mortality

In the present study, mortality was not affected by stocking density. This lack of difference between treatments may be due to the small number of birds housed in each pen. For example, some problems in commercial farming that may contribute to increased mortality (such as hysteria) cannot be reproduced in these experimental conditions. Hall (2001), working with group sizes similar to those of commercial groups, detected significant differences between 17 and 20 birds m^{-2}, with lower mortality values in the first group, although, in his experiment, group size and density were confounded. Some early investigations found no effect of stocking density on mortality (eg Shanawany 1988), and other, more recent studies (Puron *et al* 1995; Feddes *et al* 2002; McLean *et al* 2002; Thomas *et al* 2004) reported similar results. However, due to the constraints imposed by the current study, we are unable to state conclusively that mortality is not affected by stocking density.

Bodyweight and feed and water intake

In this study, the performance results varied according to experimental period (Table 1). At 18 days of age, mean bodyweight increased as stocking density decreased, and this pattern was maintained until day 46, when the differences between HD and ID became greater than during the previous measurements. Nevertheless, the weight difference between these treatments was only 200 g.

On the other hand, feed consumption was very similar for HD and ID, but birds consumed less feed than those housed at LD, an effect which was more pronounced at the end of the experimental period. This was similar to the results of Puron *et al* (1995), McLean *et al* (2002), and Ravindran *et al* (2006) although, in the three treatments in this study, there was a slight decline in feed consumption during the last days of the experiment. However, this did not appear to affect bodyweight, and the conversion rate did not differ due to treatment or time, although the access to feeders and drinkers was probably increasingly limited as the birds grew and had difficulty reaching the feeder zone (Shanawany 1988). This supposition is not borne out when access to drinkers is considered since water consumption was not affected by stocking density at the end of the experimental period.

Conversely, it is worth noting that in our study, these data could not be related to feeder or drinker space per bird, because group size was identical for all pens. In general, some negative effects of stocking density on growth performance can be improved by increasing feeder space as broilers approach heavy weights (Dozier *et al* 2005).

Another hypothesis was proposed by Bessei (2006), who noted that the influence of stocking density on broiler growth acted through heat stress rather than through physical restriction of the animals' space for movement. This could have been the case in the current experiment, as growth rate was very similar for all densities.

Nevertheless, Ravindran *et al* (2006) with densities of up to 24 birds m^{-2} and Thomas *et al* (2004) with densities ranging from 5 to 20 birds m^{-2}, suggested that bodyweight increases did not differ significantly at different stocking densities, although there was a clear tendency for increased bodyweight at lower stocking densities. The small but significant differences between densities in the current study might be due to the fact that all the animals were female broilers, while Puron *et al* (2005) indicated that differences between stocking densities were only found in male chickens.

In any event, it seems that ID is similar to either LD or HD in terms of the lack of significance, although the small differences between ID and HD (mainly in bodyweight) are probably related to the fact that both are very high stocking densities.

Haematology

It can be assumed that the H/L ratio is a reliable index for determining stress in poultry (Gross & Siegel 1983). High values indicate chronic stress (de Jong *et al* 2002) and the influence of certain stressful situations on H/L ratios has been studied extensively: Altan *et al* (2003) and Borges *et al* (2004) demonstrated that the H/L ratio increases with heat stress, as well as ACTH administration (Puvaldopirod & Thaxton 2000).

In the current study, the effect of stocking density on this parameter was significant at the end of the experimental

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period, being higher when the broilers were housed at HD. This suggests that birds were more stressed when they were housed at HD than at ID and LD at the end of the rearing period (days 41, 42 and 43).

In addition, the H/L ratio increased linearly with stocking density. This is similar to Thaxton *et al* (2006) who found linear increases in the H/L ratio as stocking density increased from 30 to 45 kg of bodyweight m^{-2} although, in their study, this did not occur when stocking density increased from 20 to 55 kg of BW m^{-2}. However, the intermediate density value in the present study (20 birds m^{-2}) was not high enough to cause significant differences compared to much lower stocking densities (LD), although the numerical differences between the two treatments might be relevant. Furthermore, only the H/L ratios for LD are close to optimum stress levels, according to Gross and Siegel (1993), ie between 0.2 (low level of stress) and 0.8 (high level of stress), suggesting that the stressful situation, even at ID, is considerable as is the H/L ratio value at the higher end of this range.

Fear

The effect of stocking density did significantly affect the number of attempts to induce TI. However, assuming that the number of attempts to induce TI is an indicator of fear in broilers, with a lower number of attempts indicating a higher fear response (Faure *et al* 2003), it may be said that the fear response is higher when birds are at the end of the rearing period as the number of attempts is lower. It would appear that the age of the chickens is important in terms of the fear reaction, with birds becoming more reactive as they mature. In the current study, these differences between ages, which equally affected the three densities, could have been caused by one of two reasons: i) the fear reaction increases with age as a direct consequence of the stress the animals suffer during the rearing period, or ii) in this experiment there was an exogenous stressful factor affecting the broilers' reaction in all three treatments. Similar results were found in male ducks by Faure *et al* (2003), who concluded that differences in the number of inductions disappeared when the ducks were 10-weeks old, when practically all the individuals were in TI after one attempt. Heiblum *et al* (1998) also found that age (from one to seven days old) decreased the number of inductions necessary to cause TI, although these results are not comparable due to the different ages measured. Taking these findings into account, it is possible that age itself affects the birds and increases their fear response.

Health measurements

It seems that the severity and incidence of these measures was lower at LD and thus LD birds would have a better welfare status in terms of this parameter. It is worth emphasising that ascites was present in the majority of the chickens at all densities, which indicates that this was a factor that affected all pens equally and all treatments, probably as a result of the wet litter. Moreover, post-mortem analysis indicated ascites and dilated cardiomyopathy as being the main causes of death.

In addition, the incidence of foot-pad dermatitis was quite high in all pens which could, again, be due to the deterioration in litter quality, although it was not assessed in the current experiment. However, Appleby *et al* (2004) stated that wet litter is a particular problem for densely-stocked broiler chickens close to the end of their housing period as they produce large amounts of droppings and spend much time sitting or lying. Moreover, this experiment was carried out during winter (when the outside relative humidity was high) and Dawkins *et al* (2004) claimed that the incidence of lesions may be influenced by season because relative humidity changes and may influence indoor humidity. Thus, it is possible that outside conditions increased indoor relative humidity which, then, affected the litter status, in addition to the stocking density itself.

However, certain studies (McLean *et al* 2002; Thomas *et al* 2004) concluded that there are no differences in certain lesions between different stocking densities, despite the increase in the moisture content of the litter with density, although in these cited studies, no lesions appeared in any treatment and the birds were housed at lower densities than those in this study.

Plasma concentrations of corticosterone

Plasma concentrations of corticosterone are in accordance with the basal levels reported by de Jong *et al* (2001), and were lower for birds at days 41, 42 and 43 when they were housed at ID and LD, although no differences were found between the rest of the measurements. According to this, when chickens grew, those housed at ID became less stressed as their level of corticosterone was lower than at the other two stocking densities. These differences may indicate that at HD (and probably at LD, since differences were not significant), birds undergo a seriously stressful situation and are unable to adapt to the housing conditions. Furthermore, ID leads to the lowest corticosterone levels and could result in a lower level of stress than LD or HD, and the level of adaptation of the animals could therefore improve.

Stress induction

Regarding stress induction, post-induction levels indicated that the response to stress induction is significantly greater when broilers are housed at HD than at ID or LD (Table 4). At 22 days old and before induction, the lowest values were obtained at ID, at which the chickens appeared to have the best level of adaptation, but there were no differences from LD. In this regard, de Jong *et al* (2002) found similar results in feed-restricted birds (a potentially stressful situation which can be compared to density), which had a higher stress response to management. One hypothesis to consider is that the greater response to an isolation test in HD birds is seen because they are more familiar with the close proximity of other birds but, even at LD, birds are in close contact. These findings might confirm that the corticosterone response to an acutely stressful event could become more pronounced in chronic stress situations, such as when birds are housed at excessive density. Nevertheless, this conclusion may be incorrect since, when the proportion of

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increase is studied (Table 3), significant differences disappear and the proportional increase of the level of corticosterone before and after the stress induction is the same in all the three stocking densities. Therefore, results of stress induction have to be very carefully evaluated in order to glean as much information as possible to determine whether it is an appropriate method of detecting welfare problems.

Animal welfare implications

In conclusion, these data indicate that reducing stocking density from 30 to 8 chickens m^{-2} improved final bodyweight, although the increase was modest; it did not have a clear effect on feed consumption. The possible positive effects of this reduction in density on welfare were evident when the results related to the H/L ratio and some health measurements were studied. Nevertheless, other measurements such as haematocrit, tonic immobility or corticosterone seem to be insufficient to reveal these findings. In addition, most of the cited parameters did not differentiate between the effects of 20 birds m^{-2} and 30 birds m^{-2} on welfare aspects. Response to acute stress or management (eg the studied stress induction or the tonic immobility test) may be sensitive parameters to detect possible welfare problems, even more so than parameters such as plasma corticosterone concentration, when the animals are housed under potentially stressful conditions (eg high stocking densities). But this methodology of stress induction has to be improved and tested and further research should be carried out before it can be proposed as an adequate measure of stress. Finally, all of these results have to be studied carefully, due to the large amount of factors that can influence them.

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