Effect of a Simulated Heat Wave on Stress Parameters of Broiler Chickens Housed at Two Different Stocking Densities

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ABSTRACT

High temperatures and relative humidities are common in certain areas in summer (for example in the Mediterranean area), and can raise the indoor temperature of the farms. The aim of this paper was to assess the possible differences in the response to this situation of broilers housed at two different stocking densities (15 and 20 birds/m^2). On day 29 until day 36, the indoor temperature was increased to 32ºC from 10:00 to 14:00 and maintained at 28ºC for the rest of the day and relative humidity was maintained at 75%. Measured variables before and after this treatment were weight, plasma corticosterone, creatine kinase (CK), aspartate transaminase (AST), alanine transferase (ALT), glucose (GLU), heterophil lymphocyte ratio and rectal temperature. Results showed that no differences were found between the animals housed at 15 birds/m^2 and those at 20 birds/m^2, so stocking density had no significant effect on the measured parameters. In addition, corticosterone concentration and alanine transferase appeared as not useful parameters to study this situation.

Key words: broiler, stocking density, enzymes activity, heat wave, hot conditions

1. INTRODUCTION

Considering the last decades, it is possible to admit that there has been an increase in the frequency of occurrence of hot climate conditions in Portugal, Spain and part of the Mediterranean area of France (outdoor temperatures may rise to more than 40ºC and relative humidity to more than 70%) and these areas could experience even more intense heat waves (considering that a heat wave is a period of abnormally and uncomfortably hot and usually humid weather according to the American Society of Meteorology, 2000) in the future (Blanes-Vidal *et al.*, 2007). These frequently hot climate conditions (high temperatures, warm nights and heat waves) can cause problems in animal housing production, mainly when this occurs at the end of the productive cycle (for example, thousands of broilers can die, as occurred, among others, in the east of Spain in 2003 or in the southwest of France in 2006). It is recognized that heat stress produced by adverse hot climate conditions has several negative effects on animal

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behavior and production. Animals can be adapted to a wide range of climatic conditions, but can be severely challenged by sudden heat waves (Blanes-Vidal *et al*., 2007). Thus, maintaining the proper temperature and relative humidity inside the farm buildings becomes crucial, although it is not an easy task. Therefore, the impact on the animals and their resistance has to be assessed as acclimatization requires time and sudden heat waves do not provide this time for adaptation to the new heat conditions. Consequently, there is a need to identify practical solutions that could help and prepare breeders to deal with hot climate conditions and heat waves, to minimize its impact in livestock production.

On the other hand, one of the major issues in the debate on broiler welfare is the stocking density. Very high densities may impair the birds' welfare directly through physical restriction of movement and indirect effects through poor litter quality, high ammonia level and heat (SCAHAW, 2000).

This possible welfare reduction, translated into additional stress, can be measured with some widely used plasma enzymes, such as creatine kinase (CK), aspartate transaminase (AST), lactate deshydrogenase (LDH) and alkaline phosphatase (ALP). Elevation of their plasma concentrations reflects alterations in tissue function or indicates cell damage or necrosis (SCAHAW, 2000). In addition, plasma activities of AST and CK are signs of stress-induced tissue damage (Hocking *et al*., 1993).

Due to the pressures currently being exerted in the European Union relating to stocking density, its relationship with temperature and the cited problems in certain countries with heat waves, the aim of this work was to evaluate the possible effect of varying stocking density when the broilers have to cope with high temperatures at the end of the rearing period.

2. MATERIAL AND METHODS

A total of 2898 male and female chickens (50% each) Ross®xRoss® 308 were used in this study. The experiment was carried out in three identical experimental rooms (13.2x5.9 m) and each of them was considered as a replicate of the rest and they were provided with environmental control systems based on heating and cooling systems as well as mechanical ventilation.

Each room was divided lengthways into two different parts (pens), with different sizes, and 483 one day old chickens were located in each pen. Studied stocking densities were 15 and 20 chickens/m^2.

Each pen was equipped with 10 cm deep of new rice hulls, and the same number of automatic feeders (45 birds/feeder) and nipple drinkers (12 birds/drinker). Divisions among pens permitted visual and olfactive contact among birds. Temperature and relative humidity were established according to commercial recommendations, and lighting regime varied from 23:1 the first three days to 16:8 on the last day of the rearing period.

Feed and water were provided *ad libitum* throughout the productive cycle. Three commercial diets were used during the experimental period (starter: between 0 and 21 days; grower: 21 to 42 days; and finisher: 42 to 45).

On day 29 and until day 36, the temperature of the three rooms was altered to simulate a heat wave. According to its duration, this heat wave can be considered as increased persistence (Hahn and Mader, 1997). Temperature was maintained at 28ºC throughout the day and increased to 32ºC for four hours (from 10 h to 14 h). During this four hour period, the cooling system was forced to work, so that indoor relative humidity was increased to 75%. In this way, indoor conditions were very similar to the real situation found in commercial farms in the Mediterranean area in summer.

All the one-day old chickens were weighed and then randomly distributed among pens. On day 7, thirty animals from each pen were randomly selected and individually weighed. After weighing, they were individually tagged for easy recognition or identification. Afterwards, these tagged birds were individually weighed again on days 14, 21, 28, 37, 42 and 46, in order to monitor the fattening period.

The mortality rate was recorded daily and broilers which appeared ill and susceptible to death were culled with sodium penthobarbital.

On days 27 and 28 and on days 38 and 39, the thirty tagged chickens from each pen (180 birds) were bled by brachial vein puncture. Approximately 3 mL of blood were collected from each bird and placed in tubes containing lithium-heparin as anticoagulant. These tubes were kept on ice and transferred to the laboratory where they were centrifuged at 1500g for 10 minutes and then the plasma was removed and passed to Eppendorf tubes (250 μl) to be stored at -20ºC for necessary analysis. All blood samples were taken within 3 min of the bird's capture in order to minimize the effects of sampling on plasma corticosterone (Littin and Cockrem, 2001) and to measure basal plasma corticosterone (CS). Plasma corticosterone concentrations were determined by radio-immune analysis (Inmunochem TM Double antibody, corticosterone 125 RIA kits from MP Biomedicals Inc.). The mean intra and interassay coefficients of variation were 4.4-10.3% and 6.5-7.2% respectively and all the samples were replicated.

The same samples were used to measure the activity of certain plasma enzymes such as CK, AST, glucose (GLU), and alanine transferase (ALT). All of these enzyme and glucose plasma activities were measured by IDEXX VetTest® Chemistry Analyzer and were replicated.

For the determination of heterophils/lymphocytes ratio (H/L ratio), one drop of whole blood of each bled chicken was smeared on a glass slide. The smears were stained with May-Grümwald and Giemsa stains (Lucas and Jamroz, 1961) after immediate 3-min methanol fixation. One hundred leucocytes were counted on each slide and H/L ratio was calculated by dividing the number of heterophils by that of lymphocytes.

Rectal temperature was measured in all these bled birds on days 27 and 28 and on days 38 and 39, before and after the bleeding, to evaluate the susceptibility of the animals to management. A digital thermometer was used and inserted approximately 2 cm into the animal's rectum.

This protocol has been revised and accepted by the Animal Welfare Committee of the IVIA (Instituto Valenciano de Investigaciones Agrarias).

Statistical Analysis

All data were subjected to an analysis of variance before and after the simulated heat wave according to the following model:

$$Y_{ij} = \mu + D_i + \varepsilon_{ij} \qquad (1)$$

Where Y_{ij} is the response variable at a certain moment; D_i is the effect of stocking density (i=15 or 20 chickens/m^2); ε_{ij} is the error (j being the number of individuals). Analyses were performed by Mixed Models Procedure (proc MIXED) using SAS System® (SAS Institute 2001) with repeated measurements and the experimental unit was the pen. Tukey adjustments were used for post-hoc comparisons. Logarithmic transformations for corticosterone levels and H/L ratio were used for analysis of variance, but the back-transformed data are reported.

Chi squared test was used to assess the effect of stocking density on mortality but mortality rates did not differ.

3. RESULTS AND DISCUSSION

Mortality rates did not differ between the two studied stocking densities and the mean rate during the experimental period (from day 29 to 36) was 0.9%.

The remaining indicators considered in this study are shown in Table 1 and Table 2. As results show, before the heat wave took place stocking density had not lead to any significant effect on the parameters. After this heat wave was produced, differences between the two studied stocking densities were not statistically significant either and the response to the experimental period was the same. Nevertheless, rectal temperature behaved in a different way and in the first sampling on days 27 and 28, no statistical differences were found between the two studied densities, whereas on days 38 and 39, differences appeared (p=0.0344): those birds housed at 15 birds/m^2 showed a higher temperature than those housed at 20 birds/m^2. Conversely, the increase of temperature after bleeding and management in the second sampling was the same in both stocking densities.

The lack of differences between the two studied stocking densities before the simulated heat wave was performed, means that the starting point of the experiment was the same in both densities.

Once the heat wave took place, results relative to weight showed that birds continued growing in the studied conditions and stocking density had no influence on it. Furthermore, CS levels agreed with basal levels for broilers and hens stated by de Jong *et al*. (2001) in both samplings but no significant difference was found.

Regarding the rest of the measured parameters, no difference was found between the two studied stocking densities after the experimental period for AST, GLU, ALT and

CK, so the proposed reduction in stocking density did not lead to any improvement in the physiological conditions of the birds.

Table 1. Effect of stocking density and time on the variables measured in broiler chickens in the first sample (days 27-28) (mean±sd)

	15 birds/m^2	20 birds/m^2	Sig level
Weight (g)	1386[a]±17	1379[a] ±17	NS
H/L ratio	0.19[a]±0.02	0.21[a]±0.02	NS
CS (ng/ml)	7.09±1.10	6.42±1.10	NS
ALT (U/L)	20.32[a]±0.69	20.90[a]±0.73	NS
AST (U/L)	204.04[a]±2.97	201.99[a]±3.07	NS
CK (U/L)	2016.02[a]±73.73	2053.17[a]±77.27	NS
Glucose (mg/dl)	236.25[a]±2.04	229.68[a]±2.11	NS
RT	41.48[a]±0.03	41.45[a]±0.03	NS

[a,b] means in the same row with different superscript differ significantly between them (P>0.05). H/L: heterophil/lymphocyte; CS: corticosterone; ALT: alanine transferase; AST: aspartate transaminase; CK: certain kinase; RT: rectal temperature before bleeding;

In parallel to this, stocking density seemed to have no effect on the animals' response to chronic stress either, as the H/L ratio was not significantly different, so no positive effect of the reduction in stocking density was observed in H/L ratio, it means, in the stress of the animals from a long term point of view. Nevertheless, the lack of differences between the two studied stocking densities in H/L ratio, CS and GLU is in accordance with Thaxton *et al*. (2006) and Dozier *et al*. (2006) who did not find any significant differences working with densities ranging between 20 and 55 kg/m^2 and 20 and 40 kg/m^2 respectively.

Table 2. Effect of stocking density and time on the variables measured in broiler chickens in the second sample (days 38-39) (mean±sd)

	15 birds/m^2	20 birds/m^2	Sig level
Weight (g)	2077[a] ±23	2035[a]±23	NS
H/L ratio	0.38[a]±0.02	0.37[a]±0.02	NS
CS (ng/ml)	7.92[a] ±1.10	6.24 ±1.10	NS
ALT (U/L)	19.65[a]±0.73	17.69[a]±0.87	NS
AST (U/L)	264.90[a]±10.52	266.02[a]±12.62	NS
CK (U/L)	3857.91[a]±180.72	3541.00[a]±223.79	NS
Glucose (mg/dl)	212.08[a]±2.69	214.23[a]±3.21	NS
RT	41.75[a]±0.05	41.61[b]±0.05	0.0344
ΔT	0.1733[a]±0.04	0.1952[a]±0.04	NS

[a,b] means in the same row with different superscript differ significantly between them (P>0.05). H/L: heterophil/lymphocyte; CS: corticosterone; ALT: alanine transferase; AST: aspartate transaminase; CK: certain kinase; RT: rectal temperature before bleeding; ΔT: increase of rectal temperature before and after bleeding

Moreover, the values of CS, ALT and GLU seem to be confusing, as they were lower after the simulated heat wave than before it, whereas H/L ratio (indicator of chronic stress), AST and CK (indicators of tissue damage) increased, although these increases and decreases require further research with control and treated groups.

Finally, the rectal temperature in the second sampling ranges between 40.5 to 42^0C. These temperatures are within the range of normal body temperature of broilers hence no visible rectal temperature difference was noticed. In addition, the increase of rectal temperature before and after the blood sampling was the same in both densities, so any sort of stress induced hyperthermia (SIH) has to be ruled out.

In conclusion, as Vale *et al*. (2008) suggested, characterizing a heat wave, as well as its effects on broiler production and welfare needs further exploration. In this sense, present results show that the studied decrease in stocking density had no beneficial effects when broilers had to cope with a heat wave at the end of the production period, since of housing the animals at 15 or 20 chickens/m^2 did not lead to any differences in H/L ratio, AST, CK, CS and GLU.

In addition, the plasma CS and GLU behavior in this work indicate that they are not good stress indicators in the studied conditions, as results are confusing, and it would be necessary to carry out other studies in order to confirm this theory. The same occurs with ALT, which despite being an indicator of tissue damage, does not seem to be useful in this study.

Nevertheless, it should be deeply studied whether higher or lower densities than chosen in the present work have an effect under the studied conditions, as the heat waves and prolonged high temperatures are a real problem in certain areas of the Mediterranean area in summer and attempts have to be made to reduce their impact.

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