# THE INFLUENCE OF DIFFERENT LIGHTING PROGRAMS, STOCKING DENSITIES AND LITTER AMOUNTS ON THE WELFARE AND PRODUCTIVITY TRAITS OF A COMMERCIAL BROILER LINE

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Running head: *Management factors and welfare of broiler.*
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**Summary.** The purpose of this study was to analyze the effects of lighting program, stocking density and amount of litter on productivity and some welfare indicators of broiler. A total of 684 day-old male broiler chicks were randomly assigned to 12 treatment groups based on the lighting, stocking density and litter amount with three replicates. Data on growth, welfare indicators, meat and litter quality were obtained. The birds at the greater density grew slower ($P<0.001$) and consumed more feed for per kg body weight gain compared to other stocking density groups. Under the conditions of the current study mortality of broilers grown at all densities were found similar. As stocking density increased, litter moisture, litter pH and the incidence of footpad lesion were adversely affected. In general; lighting program and litter amount had no significant effect on growth, meat quality and welfare indicators of broilers. We observed no difference in H/L ratio for the tested main effects lighting program, stocking density and litter amount. Significant interactions were determined between the main factors of some of the traits investigated. The results of this study indicated that increased stocking density higher than 19 birds/$m^2$ adversely affected growth responses, litter quality and incidence of footpad lesions. Therefore, much attention should be given to create better growing conditions especially at higher stocking density by using high quality and more abundant litter material and applying proper managerial practices.
**Keywords:** broiler, management factors, welfare, growth.

# APŠVIETIMO, LAIKYMO TANKIO IR LIZDO DYDŽIO ĮTAKA KOMERCINIŲ BROILERIŲ VEISLIŲ GEROVEI IR PRODUKTYVUMUI

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**Santrauka. A**tlikti tyrimai norint išsiaiškinti apšvietimo, laikymo tankio ir lizdo dydžio įtaką paukščių gerovei ir produktyvumui auginant komercines broilerių veisles. Bandymui atsitiktiniu metodu atrinkti 684 vienos dienos gaidžiukai, suskirstyti į 12 grupių (3 pogrupiai po 1 lizdą kiekvienai grupei) pagal apšvietimą, laikymo tankį ir lizdo dydį. Bandymo metu tirta paukščių augimo sparta, jų gerovė, mėsos ir lizdo kokybė. Nustatyta, kad paukščiai, laikyti mažiausiame plote, t. y. dideliu tankiu, statistiškai augo ženkliai lėčiau ($p<0,001$) ir sulesė daugiau pašaro palyginti su paukščiais, laikytais mažiausiu tankiu. Ištirta, kad broilerių visose grupėse krito maždaug vienodas skaičius ir statistiškai su laikymo tankiu nekoreliavo. Nustatyta, kad, didėjant laikymo tankiui, didėjo paukščių lizdo drėgmė, pH ir paukščių su pažeistomis kojomis procentas. Tyrimai parodė, kad apšvietimas mėsos kokybei, broilerių augimui ir jų gerovei ženklios įtakos nedarė. Ištirta, kad, laikymo tankiui padidėjus iki <19 paukščių/$m^2$, ženkliai sulėtėjo broilerių augimas, pablogėjo bendras sveikatingumas, padidėjo kojų pažeidimų tikimybė. Auginant broilerius rekomenduojama daugiau dėmesio skirti laikymo tankiui ir naudoti kokybiškus lizdų pakratus. Šios sąlygos padidina paukščių priesvorį, pagerina bendrą sveikatingumą ir apsaugo nuo kojų pažeidimų.
**Raktažodžiai:** broileris, laikymo tankis, paukščių gerovė, augimo greitis.

**Introduction.** Intensive farming methods in broiler production have attracted accusations because of poor welfare conditions (Debut, 2005; Bessei, 2006). The major welfare concern in the sector is the high stocking density especially in the final weeks of growing period. The European Union adopted standards for broilers by limiting maximum stocking density (EU press release, 2007). According to this directive maximal stocking density should not exceed 33 kg/m$^2$ unless otherwise stated. In case of application of optimal managerial conditions this value can be augmented to 39 kg/m$^2$ and each member state has to implement this new rule until 2010 (Stevenson et al., 2007; Linden, 2007). In fact, the findings on the broiler welfare and stocking density are not so clear. A large-scale study in Europe on broiler welfare has shown that stocking density did not affect bird behavior (Dawkins et al., 2004). Buijs et al., (2009) reported that stocking density affected different indicators of welfare at different levels and no single critical stocking density could be identified. As a result of greater stocking density litter moisture might be increase and higher levels of litter moisture and ammonia may result with some leg problems such as angle-out legs and unblemished hocks (Thomas et al. 2004; Jones et al., 2005; Campo and Prieto, 2009). The lighting scheme is accepted as a convenient managerial technique for the animal welfare due to some interactions observed between lighting program and some metabolic disorders (Buyse et al., 1998; Decuypere et al., 2005; Petek et al., 2005; Knowles et al., 2008). Exposure to continuous lighting increased the duration of tonic immobility (a measure of psychological stress) in chicks relative to less duration of lighting (Campo and Davilla, 2002). The new EU directive stipulates that lighting in broiler houses must include minimum periods of darkness to allow chickens to rest (EU Press Release, 2007; Stevenson et al., 2007; Linden 2007). There are still some doubts on the functionality of these new regulations since the correlations between management factors and animal welfare are not very well-studied. Only a small number of reports are focused on the interactive effects of stocking density, lighting program and the amount of litter on broiler welfare and production parameters. Although the use of high stocking densities can decrease individual growth the economic benefit per square meter is often higher if the chickens are stocked more densely. 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. However, the relation between stocking density and welfare is much more complex and so more difficult to determine precisely. Present study was done to evaluate the influence of stocking density and the amount of litter on some welfare indicators and productivity traits in a commercial broiler line reared on different lighting program.

**Material and Methods.** In this study, 684 day-old male chicks (Ross PM$_3$) obtained from a commercial hatchery and two separate windowed experimental units were used to perform c*ontinuous or intermittent lighting*. Each experimental units futher divided into 18 floor pens and birds in each experimental units distributed into floor pens according to stocking density (*15, 19 or 23 chicks/m$^2$*), litter amount (*5 or 7.5 kg rice hulls/m$^2$*) and their three replicates. All birds in the experiment received continuous light as 12 h natural day-light plus 12 h artificial light from the hatching day to 3 day old age. At 3 day of age intermittent lighting program consisted of 2 h L : 2 h D after natural day-light was provided for the treatment group whereas for the control birds continuous light was maintained. Standard commercial broiler feed was used during the experiment (220 g/kg of protein and 12.50 MJ/kg of metabolisable energy from 1 to 14 d of age, 200 g/kg of protein and 12.70 MJ/kg of metabolisable energy from 15 to 28 d of age, 180 g/kg of protein and 12.92 MJ/kg of metabolisable energy from 28 to 35 d of age). Starter feed was provided as crumples while the subsequent feeds were in pellet forms. All birds had *ad libitum* access to feed and water. Chicks were vaccinated against Newcastle and Gumboro diseases.

*Data*

Data on growth and welfare indicators were obtained in the experiment from day-old to 35 d of age. Body weight of birds were determined individually and body weight uniformity was also calculated (% within±10% of BW mean). Feed consumption and mortality were recorded when required or occurred, and the cause of death was classified by visual examination as either metabolic (skeletal disorders or others) or non-metabolic (other causes) based on position and posture. Welfare was assessed through mortality, physiology (blood parameters), pH of drumstick and breast meats, and footpad lesions at the end of the experiment. Body temperature was measured just before slaughtering process by rectal way (The sensor was inserted into the rectum to a depth of 5 cm). Drumstick and breast meat pH was measured on the hot carcass at 0 h and 24 h post-slaughtering. Foot pad lesions were determined after chilling. The visual ranking system was used for scoring footpad lesions where 0 was for no lesion, 1 for mild lesions (lesion ≤7.5 mm) and 2 for severe lesions (lesion >7.5 mm) (Bilgili *et al*., 2006). Blood samples were drawn from 5 birds from each pen by venipuncturing directly into heparinized tubes, just before slaughtering process. Immediately, 2 thin smears were prepared from each blood sample on clean microscope slides and allowed to air-dry. Dried blood smears were stained. The hematocrit was determined by centrifuging the blood in heparinised capillary tubes in a micro hematocrit centrifuge. Haemoglobin concentration was measured spectrophotometrically. Erythrocyte and leukocyte numbers were determined using a haemocytometer Burker chamber (W. Schreck Jena, Germany) after diluting the samples 1:100 in Natt-Herrick solution. The number of heterophils and lymphocytes was determined by examining blood smear stained with May Grünwald-Giemsa stain within 2 days of preparation. The total leukocyte count included heterophils, lymphocytes, monocytes, basophils and eosinophils. About 100 cells were counted for each ratio. H/L ratio was calculated by dividing percentage of heterophils by percentage of lymphocytes. On the 1$^{st}$, 21$^{st}$ and 35$^{th}$ day of age, litter samples were collected from each pen for determination of moisture and pH (AOAC, 1996). The upper 10 cm of the litter was collected and transported to laboratory for pH determination (Mettler Toledo, GmbH, Switzerland). For this purpose, litter was diluted in deionized water in the ratio of 1:5 as described by Miles et al., (2006). To determine the litter moisture, litters from four different locations in each pen were collected, mixed and 100-g sample dried in oven for 48 h at 60 $^0$C, cooled in desiccators before weighing. (Willis et al., 1997).

*Statistical analysis*

The data were analyzed by ANOVA with two levels of lighting program, three levels of stocking density and two levels of amount of litter (Snedecor and Cochran, 1989). Mean separation was performed using Duncan test. All tests were performed in SPSS® computer software 13.00 (SPSS INC, 2004).

**Results.** Data related to the influence of lighting program, stocking density and the amount of litter on growth performance and carcass weight in broiler are presented in Table 1. Live weight, feed conversion ratio and mortality rate in the lighting and litter amount groups were found to be similar. Likewise, stocking density had also no significant influence on mortality as well as feed conversion. Meanwhile, body weight uniformity at densities of 23 birds per m$^2$ was found clearly lover compared to other groups. Litter moisture content and pH increased over the time in all main groups (Table 2). While litter moisture content was found similar in lighting and litter amount groups, clearly higher level of moisture was noted in denser populated group. The lowest percentage of foot-pad lesions was observed from birds reared in continuous lighting, 15 birds/m$^2$ and 7.5 kg litter/m$^2$. The influence of tested factors on the PCV, total white and red blood cell, H/L and body temperature are given in Table 3. Stocking density had significantly important influence on PCV and body temperature ($P<0.01$ and $P<0.05$), while litter amount had only influence on total white cells ($P<0.01$). Interactions of stocking density x litter amount and also lighting program x stocking density x litter amount were found to have influence on total white blood cell ($P<0.05$), total red blood cell and H/L ratio ($P<0.05$, $P<0.001$) respectively. Thus, higher meat pH was obtained in denser groups (Table 4). Similarly, pH of drumsticks was higher in 5/m$^2$ litter amount group. Statistically important differences were calculated for interactions of i) lighting programme x stocking density on pH of drumstick at 24 h post-slaughtering ($P<0.05$), ii) stocking density x litter amount on pH of drumstick at 0 h ($P<0.05$) iii) stocking density x litter amount on pH of breast meat at 24 h post-slaughtering ($P<0.01$) and iv) lighting programme x stocking density x litter amount on pH of breast meat at 0 h ($P<0.01$) and 24 h post-slaughtering ($P<0.05$).

Table 1. **Influence of tested factors on growth performance**

| Factors | Body weight | BW uniformity | FCR | Mortality (%) | | |
|---|---|---|---|---|---|---|
| | g | % | | Metabolic* | Other | Total |
| Lighting | | | | | | |
| Continuous (C) | 1710±30 | 52.6 | 1.68 | 1.16 | 0.08 | 1.24 |
| Intermittent (I) | 1768±31 | 51.3 | 1.65 | 1.16 | 1.00 | 2.16 |
| Stocking Density/m$^2$ | | | | | | |
| 15 | 1782±42$^a$ | 53.7 | 1.64 | 0.50 | 2.00 | 2.50 |
| 19 | 1816±36$^a$ | 55.4 | 1.66 | 1.00 | 0.25 | 1.25 |
| 23 | 1618±32$^b$ | 49.3 | 1.71 | 2.00 | 0.50 | 2.50 |
| Litter Amount/m$^2$ | | | | | | |
| 5 | 1745±31 | 50.8 | 1.66 | 1.00 | 1.50 | 2.50 |
| 7.5 | 1732±30 | 54.8 | 1.68 | 1.16 | 0.33 | 1.49 |
| Lighting (L) x Stocking Density (SD) x Litter Amount (LA) | | | | | | |
| C-15-5 | 1654±80 | 50.0 | 1.64 | 0.00 | 3.00 | 3.00 |
| C-15-7.5 | 1764±86 | 61.5 | 1.66 | 1.00 | 0.00 | 1.00 |
| C-19-5 | 1803±70 | 68.4 | 1.68 | 0.00 | 0.00 | 0.00 |
| C-19-7.5 | 1754±71 | 47.4 | 1.65 | 1.00 | 0.00 | 1.00 |
| C-23-5 | 1662±67 | 42.9 | 1.70 | 3.00 | 1.00 | 4.00 |
| C-23-7.5 | 1622±66 | 45.5 | 1.75 | 2.00 | 1.00 | 3.00 |
| I-15-5 | 1844±93 | 50.0 | 1.62 | 1.00 | 4.00 | 5.00 |
| I-15-7.5 | 1866±80 | 53.3 | 1.63 | 0.00 | 1.00 | 1.00 |
| I-19-5 | 1842±75 | 41.2 | 1.64 | 2.00 | 1.00 | 3.00 |
| I-19-7.5 | 1866±75 | 64.7 | 1.65 | 1.00 | 0.00 | 1.00 |
| I-23-5 | 1667±64 | 52.2 | 1.67 | 1.00 | 0.00 | 1.00 |
| I-23-7.5 | 1521±60 | 56.5 | 1.71 | 2.00 | 0.00 | 2.00 |
| ANOVA** | | | | | | |
| Stocking density | *** | - | n.s | - | - | n.s |
| SEM | 21.74 | - | 0.01 | - | - | 0.01 |

SEM: Pooled standard error of mean. *Mortality due to leg abnormalities ** All the other main and interactive effects were found to be unsignificant

**Discussion.** In this study, increased stocking density adversely affected cumulative body weight gain (P<0.001) and uniformity. A mean suppression of 198 g on cumulative body weight gain occurred when the stocking density increased from 19 to 23 birds per $m^2$. Supporting these results Feddes et al., 2002, Dozier et al., 2006 and Estevez, 2007 reported that increased stocking density resulted in decreased body weight gain. The adverse effect for body weight uniformity was particularly evident in the group of animals housed at stocking density of 23 /$m^2$ and litter amount of 5 kg/$m^2$. In our study, similar to body weight gain and body weight uniformity, feed conversion ratio and total mortality were also negatively influenced at higher stocking densities at the end of the 35 d period (Table 1). Although Renden et al., 1996 indicated that restricted lighting programs generally have beneficial effects on mortality total mortality in all groups was low and there was no statistical difference among the groups. Important part of mortality observed in the groups may be linked to leg abnormalities resulted from metabolic problems. Dawkins et al., 2004 reported that not only stocking density but also housing conditions is important for broiler performance and animal welfare. In higher densities environmental managing factors should be optimized to provide higher living and welfare standards.

As reported previously by Elfadil et al., 1996 and Simsek et al., 2009, high stocking density increased the litter moisture and pH, and the incidence of foot-pad lesions (Table 2). The response was more pronounced as stocking density was as high as 23 bird/$m^2$ of floor space. The increase in foot-pad lesions was probably a reflection of the poor litter quality associated with high stocking density. Improper litters not only cause bad odors affecting the bird's respiratory system, but also result with intestinal disorders. Food-pad lesions may create opportunity for fungi and bacteria to enter the body which may lead to condemnation of the carcass. The proportion of birds having no foot pad lesions between continuous and intermittent lighting or 5 and 7.5 kg/$m^2$ litter groups were similar. The number of birds having no pad lesions was decreased with the increased stocking density (P<0.05). The variability of litter pH as well as increased stocking density might also have been attributable to the amount of caked litter in the pens. Caked litter corresponds to high litter moisture or areas where litter becomes anaerobic, which suppresses ammonia volatilization (Carr et al., 1990). In the middle and end-of experiment the litter moisture in all groups were found higher than ideal litter moisture which should be maintained between 20-25% (Glebocka, 2008). Moisture content of litter exceeding 35% is detrimental and correlated with higher fecal corticosteroid (stress hormone) which can increase the mortality (Butcher and Miles 2009). Lighting and litter treatments had not significantly affected the moisture and pH of litter.

Table 2. **Litter and foot quality measurements of male broilers subjected to various lighting program, stocking density and amount of litter**

| Factors | Litter Moisture (%) | | | Litter pH | | | Incidence of footpad lesions (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 d | 15 d | 35 d | 0 d | 15 d | 35 d | None | Mild | Severe |
| Lighting | | | | | | | | | |
| Continuous (C) | 17.6 | 51.5 | 61.6 | 6.68 | 6.43 | 7.65 | 73.3 | 4.4 | 22.3 |
| Intermittent (I) | 24.6 | 53.6 | 62.8 | 6.61 | 7.26 | 8.65 | 61.0 | 24.5 | 14.5 |
| Stocking Density/$m^2$ | | | | | | | | | |
| 15 | 18.0 | 46.4 | 55.5 | 6.58 | 6.49 | 8.12 | 79.4 | 1.1 | 19.6 |
| 19 | 20.6 | 53.2 | 64.2 | 6.71 | 6.69 | 8.14 | 73.7 | 13.1 | 13.1 |
| 23 | 24.7 | 58.0 | 68.8 | 6.62 | 7.34 | 8.15 | 48.4 | 26.2 | 20.3 |
| Litter amount/$m^2$ | | | | | | | | | |
| 5 | 22.0 | 54.5 | 63.3 | 6.68 | 6.75 | 8.14 | 65.0 | 19.1 | 15.9 |
| 7.5 | 20.3 | 50.6 | 61.1 | 6.62 | 6.94 | 8.16 | 69.3 | 9.9 | 20.9 |
| Lighting x Stocking Density x Litter Amount | | | | | | | | | |
| C-15-5 | 13.5 | 41.7 | 58.00 | 6.77 | 6.39 | 7.64 | 80.4 | 0.0 | 19.6 |
| C-15-7.5 | 12.4 | 53.8 | 53.1 | 6.38 | 5.96 | 7.51 | 78.3 | 2.2 | 19.6 |
| C-19-5 | 17.3 | 56.2 | 65.0 | 6.75 | 5.90 | 7.84 | 73.8 | 2.6 | 23.6 |
| C-19-7.5 | 15.8 | 52.2 | 62.3 | 6.75 | 6.29 | 7.08 | 73.8 | 5.2 | 21.0 |
| C-23-5 | 24.8 | 50.7 | 67.0 | 6.68 | 6.56 | 7.70 | 66.7 | 13.3 | 20.0 |
| C-23-7.5 | 21.8 | 54.3 | 64.5 | 6.72 | 7.47 | 8.15 | 66.7 | 3.3 | 30.0 |
| I-15-5 | 24.4 | 50.3 | 53.7 | 6.58 | 6.80 | 8.84 | 80.4 | 2.2 | 17.4 |
| I-15-7.5 | 21.9 | 39.9 | 49.9 | 6.66 | 6.82 | 8.93 | 78.3 | 0.0 | 21.7 |
| I-19-5 | 25.2 | 58.9 | 64.6 | 6.62 | 7.19 | 8.69 | 73.7 | 26.3 | 0.0 |
| I-19-7.5 | 24.0 | 45.5 | 64.9 | 6.72 | 7.39 | 8.66 | 73.7 | 18.4 | 7.9 |
| I-23-5 | 26.5 | 69.0 | 71.7 | 6.62 | 7.63 | 8.12 | 15.0 | 70.0 | 15.0 |
| I-23-7.5 | 25.7 | 58.1 | 72.1 | 6.48 | 7.71 | 8.64 | 45.0 | 30.0 | 25.0 |

None: No lesion present; Mild:lesion<7.5 mm; Severe:lesion>7.5 mm

Table 3. **PCV, Total white and Red Blood Cell, H/L and body temperature of broilers in the groups**

| Factors | PCV (%) | TRBC ($x10^6/mm^3$) | TWBC ($x10^3/mm^3$) | H/L | BT ($C^o$) |
|---|---|---|---|---|---|
| Lighting | | | | | |
| Continuous (C) | 36.1±0.8 | 3.19±0.10 | 8.97±0.01 | 1.35±0.19 | 41.3±0.11 |
| Intermittent (I) | 36.4±0.9 | 3.27±0.11 | 8.37±0.02 | 1.75±0.18 | 41.5±0.10 |
| Stocking Density/$m^2$ | | | | | |
| 15 | 38.6±1.0 | 3.33±0.13 | 8.28±0.03 | 1.89±0.21 | 41.4±0.13 |
| 19 | 33.6±1.1 | 3.05±0.12 | 8.80±0.02 | 1.51±0.22 | 41.7±0.12 |
| 23 | 36.7±1.0 | 3.30±0.11 | 8.93±0.01 | 1.26±0.20 | 41.2±0.12 |
| Litter amount /$m^2$ | | | | | |
| 5 | 36.4±0.8 | 3.03±0.10 | 9.22±0.02 | 1.56±0.18 | 41.4±0.10 |
| 7.5 | 36.1±0.9 | 3.43±0.09 | 8.12±0.01 | 1.54±0.19 | 41.4±0.12 |
| Lighting (L) x Stocking density (SD) x Litter amount (LA) | | | | | |
| C-15-5 | 38.2±2.1 | 2.97±0.25 | 8.05±0.02 | 0.46±0.44 | 41.9±0.24 |
| C-15-7.5 | 39.4±2.0 | 3.37±0.24 | 6.85±0.04 | 2.04±0.43 | 40.7±0.26 |
| C-19-5 | 31.6±2.1 | 2.69±0.24 | 12.40±0.03 | 1.44±0.41 | 41.7±0.24 |
| C-19-7.5 | 33.6±2.2 | 3.45±0.23 | 7.35±0.02 | 1.05±0.42 | 42.0±0.25 |
| C-23-5 | 38.0±1.9 | 3.47±0.22 | 9.80±0.01 | 1.57±0.43 | 41.0±0.22 |
| C-23-7.5 | 36.0±2.0 | 3.16±0.21 | 9.35±0.02 | 1.47±0.41 | 42.0±0.23 |
| I-15-5 | 40.6±2.1 | 3.29±0.22 | 11.35±0.01 | 4.05±0.42 | 41.3±0.24 |
| I-15-7.5 | 36.0±1.9 | 3.68±0.23 | 6.85±0.02 | 0.95±0.43 | 42.0±0.26 |
| I-19-5 | 32.0±2.0 | 2.48±0.24 | 6.30±0.03 | 1.05±0.44 | 41.4±0.23 |
| I-19-7.5 | 37.0±2.2 | 3.57±0.25 | 9.15±0.02 | 2.48±0.45 | 42.0±0.22 |
| I-23-5 | 38.0±2.1 | 3.27±0.26 | 7.40±0.03 | 0.79±0.46 | 42.0±0.21 |
| I-23-7.5 | 34.8±2.2 | 3.32±0.27 | 9.15±0.04 | 1.19±0.46 | 41.3±0.22 |
| ANOVA* | | | | | |
| Stocking density | ** | n.s | n.s | n.s | * |
| Litter | n.s | ** | n.s | n.s | n.s |
| Lx SD | n.s | n.s | n.s | * | n.s |
| SD x L | n.s | * | n.s | n.s | n.s |
| L x SD x LA | n.s | n.s | * | *** | n.s |
| SEM | 0.61 | 0.07 | 0.04 | 0.13 | 0.07 |

SEM: Pooled standard error of mean. TRBC:Total Red Blood Cell,
TWBC: Total White Blood Cell, BT: Body Temperature
*All the other main and interactive effects were found to be insignificant.

In this study, we observed no difference in H/L ratio for the tested main effects lighting program, stocking density and litter amount. Nevertheless, statistically important difference was calculated for interaction of lighting x stocking density x litter amount (P<0.001). As outlined previously, if increased H/L ratio would be accepted an indication of chronic stress (Maxwell, 1993; Hocking et al., 1996) animals from intermittent lighting and high stocking density groups would not suffer from chronic stress. Our finding is concurrent with the findings of Lien et al. (2007) who emphasized that H/L ratios were not affected by light intensity or photoperiod and Blair et al. (1993) who reported that lighting (directly 23L:1D or increasing from 6L:18D to 23L:1D) had no influence on H/L ratio in broilers. Also, Campo and Davila, 2002 did not find significant differences in the H/L ratio among 3 lighting regimens (23L:1D, 14L:10D, and 18.5L:5.5D) in hens from 3 breeds. Nevertheless, Vo et al. (1998) found that continuous lighting increased the percentage of heterophils and decreased the lymphocytes compared to 16L:8D and 12L:12D lightings. Main groups of stocking density and amount of litter affected PCV and total white cell of birds, respectively. Body temperature of animals from different treatments was not exhibited clear difference. Statistical importance was calculated for the interaction of lighting x stocking density x litter amount on total red cell, H/L and body temperature (P<0.021, P<0.001, P<0.005). Similarly, stocking density x litter amount interaction was significant for total white cell (P<0.05). Significant (P<0.05) stocking density x amount of litter interaction stated that high stocking density decreased total white blood cell in 5 kg litter/$m^2$ and not in 7.5 litter/$m^2$ litter condition. Lighting x stocking density x litter amount interaction calculated for total white cell and H/L ratio (P<0.05, P<0.001) revealed that lower stocking density increased total white cell and H/L especially in intermittent lighting with 5 kg/$m^2$ litter.

Table 4. **Influence of the treatments on pH of drumstick and breast parts of meat**

| | Drumstick | | Breast | |
|---|---|---|---|---|
| Factors | Hot carcass | 24 h later | Hot carcass | 24 h later |
| Lighting program | | | | |
| Continuous (C) | 6.40±0.022 | 6.06±0.019 | 6.61±0.030 | 5.84±0.020 |
| Intermittent (I) | 6.33±0.022 | 6.11±0.018 | 6.53±0.029 | 5.84±0.021 |
| Stocking Density/$m^2$ | | | | |
| 15 | 6.33±0.027 | 6.03±0.023 | 6.57±0.036 | 5.81±0.025 |
| 19 | 6.33±0.026 | 6.12±0.022 | 6.60±0.035 | 5.82±0.024 |
| 23 | 6.43±0.027 | 6.12±0.024 | 6.57±0.035 | 5.89±0.025 |
| Litter amount /$m^2$ | | | | |
| 5 | 6.41±0.023 | 6.14±0.018 | 6.60±0.030 | 5.85±0.018 |
| 7.5 | 6.33±0.021 | 6.04±0.019 | 6.56±0.031 | 5.83±0.019 |
| Lighting (L) x Stocking Density (SD) x Litter Amount (LA) | | | | |
| C-15-5 | 6.34±0.054 | 6.08±0.046 | 6.45±0.071 | 5.71±0.05 |
| C-15-7.5 | 6.46±0.053 | 6.02±0.045 | 6.73±0.072 | 5.90±0.04 |
| C-19-5 | 6.40±0.052 | 6.17±0.044 | 6.71±0.073 | 5.90±0.04 |
| C-19-7.5 | 6.25±0.052 | 6.00±0.045 | 6.52±0.070 | 5.77±0.05 |
| C-23-5 | 6.51±0.054 | 6.06±0.046 | 6.68±0.071 | 5.89±0.05 |
| C-23-7.5 | 6.41±0.053 | 6.06±0.044 | 6.57±0.072 | 5.86±0.04 |
| I-15-5 | 6.29±0.052 | 6.08±0.043 | 6.66±0.070 | 5.81±0.03 |
| I-15-7.5 | 6.24±0.053 | 5.92±0.046 | 6.45±0.072 | 5.82±0.05 |
| I-19-5 | 6.35±0.054 | 6.18±0.045 | 6.58±0.071 | 5.80±0.04 |
| I-19-7.5 | 6.33±0.052 | 6.12±0.045 | 6.58±0.070 | 5.80±0.05 |
| I-23-5 | 6.53±0.053 | 6.28±0.045 | 6.55±0.069 | 5.99±0.05 |
| I-23-7.5 | 6.27±0.054 | 6.09±0.046 | 6.49±0.074 | 5.80±0.04 |
| ANOVA* | | | | |
| Stocking density | * | ** | n.s | n.s |
| Litter amount | * | *** | n.s | n.s |
| Lx SD | n.s | * | n.s | n.s |
| SD x L | * | n.s | n.s | ** |
| L x SD x LA | n.s | n.s | ** | * |
| SEM | 0.015 | 0.013 | 0.021 | 0.014 |

SEM: Pooled standard error of mean

Rapid pH decline and early onset of rigor mortis may affect poultry meat quality and result lower possessing yields, increased cooking losses, and reduced juiciness (Baracho et al., 2006). Data related to the pH of drumstick and breast meat at 0 h and 24 h post-slaugtering indicated that higher stocking density had no significant effect on meat pH (Table 4). Nevertheless, interactions of lighting programme x stocking density x amount of litter had significant influence on breast meat pH, especially in continuous lighting and 5 kg/$m^2$ ($P<0.01$, $P<0.05$).

**Conclusion.** It can be concluded from the present study that increased stocking density that are above 19 birds/$m^2$ adversely affected growth responses of birds and altered litter quality but did not have influence on physiological stress indicators. Therefore, attentions should be given to create better growing conditions such as more appropriate litter material like wood shavings, more bedding material per unit area in combination with proper managerial practices such as intermittent lighting. In the same time, it is very important to determine the relationship between stocking density, economic benefit 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. Educating companies and producers regarding the impact of environmental quality on broiler health and welfare is also important.

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