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The Influences of Different Stocking Densities on Some Welfare Indicators, Lipid Peroxidation (MDA) and Antioxidant Enzyme Activities (GSH, GSH-Px, CAT) in Broiler Chickens

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Abstract: This study was conducted to investigate the influence of stocking density on some welfare indicators, lipid peroxidation and antioxidant enzyme activities. A total of 1600 Ross-308 3 days old broiler chicks was placed into 25 floor pens (2×2 m) including five replicates from each treatment. Stocking density treatments were 22.5 (47.3 kg bw m^{-2}), 18.75 (41.9 kg bw m^{-2}), 15 (34.3 kg bw m^{-2}), 11.25 (26.7 kg bw m^{-2}), 7.5 (18.1 kg bw m^{-2}) broilers m^{-2}. Data on welfare indicators (litter moisture, hock and food-pad necrosis) and MDA, GSH, GSH-Px, CAT levels were determined on day 42. Body temperature was measured on days 21 and 42. Pen temperature was measured daily during 4-42 days. Higher stocking density increased litter moisture, food-pad and hock necrosis percentages ($p<0.01$). The body temperature of 21st day was found to be similar among the treatments ($p>0.05$), but it reduced significantly parallel to increasing stocking density on day 42 ($p<0.01$). Higher stocking density increased the pen temperature during 22-35 days ($p<0.05$) and 36-42 days ($p<0.01$), but not during 4-21 days ($p>0.05$). Crowding increased serum MDA level ($p<0.01$) and reduced GSH-Px ($p<0.01$), but not changed serum CAT ($p = 0.07$) and GSH ($p = 0.28$) levels. Consequently, the increase of stocking density affected badly on broiler welfare and oxidative stress parameters.

Key words: Broiler, stocking density, welfare, MDA, antioxidant enzyme activities, chickens

INTRODUCTION

In the broiler industry, the major welfare concerns is the effect of high stocking densities on the welfare of the birds, especially during the final weeks of the growing period when the body weight per unit area is high. In most part of the world, the accepted industry standard is to achieve 30-38 body weight m^{-2} (Bessei, 2004). Additionally, in the European Union, stocking density for broiler production is limited with 30 kg m^{-2}. However, a number of welfare measures have been reported to vary with stocking density (Thomas *et al*., 2004; Jones *et al*., 2005; Thaxton *et al*., 2006; Onbasilar *et al*., 2008). High stocking density has been reported to increase ammonia production, litter moisture, leg problems, footpad problems, carcass damages, heat stress and locomotion deformities (Sørensen *et al*., 2000; Dozier *et al*., 2006; Muniz *et al*., 2006). The changes in blood parameters have also been presented, indicating that crowding causes the birds to be susceptible to immune insufficiency (Kuan *et al*., 1992; Erisir and Erisir, 2002) and consecuently may result in an increase in the incidence of diseases and deaths (Cravener *et al*., 1992; Imaeda, 2000). Therefore, the welfare indicators including stocking density in broiler production are considered of economic, social and scientific importance. The modern broiler house enables producers to have great control over the house environment. Birds can be placed at higher densities as long as correct environment (temperature, ventilation, humidity) is provided. If the problems of high stocking density can be mitigated by buildings with good indoor environmental conditions, any recommendations for limiting stocking density should be taken this into account by the major welfare authorities.

Stocking density has been thoroughly investigated for years, but such information could be great interest for utilization at broiler production. The study was aimed to determine the influences of stocking density on litter moisture, hock and food-pad necrosis percentages, body temperature, pen temperature and oxidative stress parameters.

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MATERIALS AND METHODS

Husbandry: The study was conducted using 1600 Ross-308 broiler chickens in a modern broiler house of a commercial broiler firm. The chicks were 3 days old when obtained and divided into treatment groups and sub-divided into five regular replicates according to equal live weight and gender. The study was carry out in 5 stocking densities ((22.5 (47.3 kg body weight (bw) m^{-2}), 18.75 (41.9 kg bw m^{-2}), 15 (34.3 kg b w m^{-2}), 11.25 (26.7 kg bw m^{-2}), 7.5 (18.1 kg bw m^{-2}) broilers m^{-2}). The study house was separated into 25 pens, each of which had an area of 2×2 m. The doors of the pens were designed to be movable, so that bird density could be kept constant by movement of the doors, compensating for any mortality. The house temperature was recorded 26.5 during 4-21 days and 21.48°C during 21-42 days in the morning at 8:00 am by using a digital charts table. Relative humidity was 65-75%. In order to allow the heat to spread equally throughout the building, a tube-shaped canvas system was used in the study house. Rations were prepared according to National Research Council (NRC) standards and given in Table 1. Water and feed were given *ad libitum* and the photoperiod was 24 h day^{-1}. Litter moisture measured the samples, taken from four different parts of each pen on day 42 as the sample weight difference after drying at 80°C for 24 h $original^{-1}$ sample weight×100. Footpad and hock dermatitis (no/yes coloration or lesion) and body temperatures of chickens detected at 5 males and 5 females, selected randomly from each pen. Body temperatures of chickens were measured with a digital thermometer from cloacae on days 21 and 42, when they were hunger. For measured pens temperatures, thermometers that have equal sensitivity were hanged above 17 cm from the litter at central of the each pen. The pens temperatures were measured when the pens were empty and the chicks from per replicate were accommodate to pens homogeneously. Temperatures of the pens were measured everyday at 8:00 am during 4-42 days. Oxidative stress parameters were determined in serum of 6 males and 6 females, whose live weights were close to the group average on day 42.

Table 1: Compositions of the diets

Feed ingredients (%)	Starter	Grower	Finisher
Corn	45.87	36.48	49.71
Wheat	-	15.00	-
Sunflower meal	-	-	7.71
Soybean meal (44 CP)	18.19	9.98	-
Full fat soybean	25.00	26.00	30.00
Poultry by products	4.00	5.00	5.00
Meat bone meal	3.00	2.00	2.00
Vegetable oil	1.30	2.37	2.86
Ground Limestone	0.31	0.61	0.38
Dicalcium Phosphate	1.12	1.06	1.03
$NaHCO_3$	0.02	0.16	0.11
Salt	0.19	0.14	0.17
DL-Methionine	0.33	0.34	3.00
L-Lysine	0.17	0.36	0.23
Choline	0.10	0.10	0.10
Vitamin Premix*	0.15	0.15	0.15
Mineral Premix**	0.10	0.10	0.10
Avatec	0.05	0.05	0.05
Biacid	0.10	0.10	0.10
Total	100.00	100.00	100.00
Nutritional composition (diet (%))			
Dry matter	88.82	87.74	87.55
Crude protein	24.50	22.50	21.00
Crude fiber	3.31	3.33	4.62
Ash	6.34	6.02	5.64
Ether extract	9.38	10.47	12.57
Ca***	1.00	1.00	0.92
P***	0.80	0.74	0.77
Methionine***	0.68	0.66	0.63
Lysine***	1.44	1.42	1.20
ME (MJ kg^{-1})***	12.77	13.27	13.60

*Vitamin premix supplied per kg; vitamin A 12.000 IU; vitamin D_3 5.000 IU; vitamin E 75 IU; vitamin K_3 3 mg; vitamin B_1 3 mg; vitamin B_2 6 mg; niacin 45 mg; calcium d-pantothenat 10 mg; vitamin B_6 7.5 mg; vitamin B_{12} 0.03 mg; folic acid 1 mg; d-biotin 0.15 mg; **Mineral premix supplied/1 kg; Mn 100 mg; Fe 60 mg; Zn 60 mg; Cu 5 mg; Co 0.3 mg; I 1 mg; Se 0.35 mg; ***It was found by calculation

Analyses: Chemical composition of feed ingredients (dry matter, crude protein, ash and ether extract) were determined according to methods defined in AOAC and crude fiber level was determined according to Crampton and Maynard (1983).

Lipid peroxidation: The levels of Malondialdehyde (MDA) were measured in the serum with the tiobarbituric-acid reaction by the method of Placer *et al.* (1966). The quantification of thiobarbituric acid reactive substances was determined by comparing the absorption to the standart curve of MDA equivalents generated by acid catalyzed hydrolysis of 1, 1, 3, 3 tetramethoxypropane. The values of MDA were expressed as nmol g^{-1} protein. Every sample was assayed in duplicate and the assay coefficients of variation for MDA were <3%.

Catalase: The serum catalase activity was measured by Goth (1991). Briefly, 0.2 mL of serum samples was incubated in 1.0 mL substrate (65 μmol mL^{-1} hydrogen peroxide in 50 mM phosphate buffer, pH 7.0) at 37°C for 60 sec. The enzymatic reaction was terminated with 1.0 mL of 32.4 mM ammonium molybdate. Hydrogen peroxide was measured at 405 nm against blank containing all the components except the enzyme on a spectrophotometer (Shimadzu 2R/UV-visible, Tokyo, Japan). The catalase activity was expressed as kU L^{-1}.

Glutathione peroxidase (GSH-Px): The GSH-Px activity was determined according to the method of Lawrence and Burk (1976). The reaction mixture consisted of 50 mM

potassium phosphate buffer (pH 7.0), 1 mM Etilen Diamine Tetra Acetic acid (EDTA), 1 mM sodium azide (NaN_3), 0.2 mM reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH), 1 IU mL^{-1} oxidized Glutathione (GSSG)-reductase, 1 mM GSH and 0.25 mM hydrogen peroxide (H_2O_2). Enzyme source (0.1 mL) was added to 0.8 mL of the above mixture and incubated at 25°C for 5 min before initiation of the reaction with the addition of 0.1 mL of peroxide solution. The absorbance at 340 nm was recorded for 5 min on a spectrophotometer. The activity was calculated from the slope of the lines as micromoles of NADPH oxidized per minute. The blank value (the enzyme was replaced with distilled water) was subtracted from each value. The GSH-Px activity was expressed as IU g^{-1} protein.

Reduced Glutathione (GSH): The GSH content of the serum was measured at 412 nm using the method of Sedlak and Lindsay (1968). The samples were precipitated with 50% trichloroacetic acid and then centrifuged at 1000×g for 5 min. The reaction mixture contained 0.5 mL of supernatant, 2.0 mL of Tris-EDTA buffer (0.2 M; pH 8.9) and 0.1 mL of 0.01 M 5, 5'-dithio-bis-2-nitrobenzoic acid. The solution was kept at room temperature for 5 min and then read at 412 nm on the spectrophotometer. The GSH level was expressed as μmol g^{-1} protein. The protein content in the serum was measured by method of Lowry *et al.* (1951).

Statiscal analyses: Data were subjected to analysis of variance by using SPSS for Windows. Significant differences were further subjected to Duncan's multiple range test. The results were considered as significant when $p<0.05$ and $p<0.01$.

RESULTS AND DISCUSSION

When influences of stocking density on litter moisture were examined (Table 2), litter moisture, foot-pad and hock necrosis percentages were found to be higher ($p<0.001$) in crowding groups. It is generally accepted that litter moisture increases at high stocking densities (Thomas *et al.*, 2004; Dozier *et al.*, 2006). The higher incidences of hock and footpad lesions are related to partly higher moisture levels and poorer quality of litter at high densities (Sørensen *et al.*, 2000; Onbasilar *et al.*, 2008). The incidences of food-pad necrosis of the present study were 88, 82, 32, 18, 0% and hock necrosis 64, 34, 24, 12, 0% for stocking densities 22.5, 18.75, 15, 11.25, 7.5 broilers m^{-2}, respectively. The results of the study clearly demonstrated that there were a proportional relationship among stocking density, litter moisture, food-pad and hock necrosis percentages. In addition, the relatively high ratio of hock necrosis in 22.5 broilers m^{-2} group indicated that the broilers in this group could not move enough and consequently had to lie down. Similarly, the prevalence of hock and food-pad increased linearly with increasing stocking density (5, 10, 15 and 20 birds m^{-2}) as reported by Thomas *et al.* (2004), relating poorer litter quality and the increased time the birds spend sitting in contact with the litter. According to Table 2, body temperature of 42nd day was lower as stocking density increased ($p<0.01$). It was thought that high stocking density limited to broiler chickens for natural behaviours such as walking ability, pecking and scratching, especially as the chickens get older. In agree with this consideration, Sørensen *et al.* (2000) reported that at higher stocking densities, bird movement was more constrained on the 6 and 7 weeks of age. The lower body temperature might be related with reducing activity in higher density groups. In addition, in dense groups the litter was wet and was thus cold, broilers had to lie down on their chests and the litter smudged the feathers and moisturized them could be another factor that reduced body temperature. Although, Shlomo (2004) reported that parallel to ammonia increase in the environment, body temperature increased, in the present study, body temperature was found to below in dense groups.

When the influences of different stocking densities on pen temperatures were examined (Table 3), the pens temperatures amoung the groups were not important during 4-21 days ($p>0.05$).

However, the pen temperatures increased paralel to increasing stocking density during 22-42 days ($p<0.01$). This indicated that the increase in broiler numbers in unit area significantly increased the temperature of the environment. Moreover, temperature differences became more significant with the growth of the broilers, especially during 36-42 days.

Lipid peroxidation, an indicative of stress is an autocatalytic mechanism leading to oxidative destruction of cellular membranes (Cheeseman, 1993). Malondialdehyde (MDA) is the main final product of lipid peroxidation and has been often used for determining oxidative damage (Sevanian and Mcleod, 1997), which is indicated by high levels of MDA. The serum MDA levels among the stocking density groups of the present study were different significantly ($p<0.01$). Especially, the MDA level of the group 22.5 broilers m^{-2} was higher than other groups (Table 4). This finding indicated that crowding enhanced oxidative destruction and caused MDA generation. Living organisims are able to adapt to oxidative stress by including the synthesis of antioxidant enzymes and repair systems. Antioxidant enzymes such as Catalase (CAT), Glutathione (GSH), Glutathione

Table 2: The influences of different stocking densities on litter moisture, foot health and body temperature

Properties	22.50 birds m^{-2} 47.3 kg bw m^{-2}	18.75 birds m^{-2} 41.9 kg bw m^{-2}	15 birds m^{-2} 34.3 kg bw m^{-2}	11.25 birds m^{-2} 26.7 kg bw m^{-2}	7.5 birds m^{-2} 18.1 kg bw m^{-2}	P (statistical significance)
Litter moisture (%)	21.01±1.09^{a}	21.95±0.95^{a}	20.88±1.78^{ab}	18.27±0.74^{bc}	15.26±0.76^{c}	0.003
Hock necrosis (%)	64.00±12.88^{a}	34.00±7.44^{b}	24.00±10.29^{b}	12.00±3.74^{bc}	0.000±0.00^{c}	0.000
Footpad necrosis (%)	88.00±3.74^{a}	82.00±5.83^{a}	32.00±9.00^{b}	18.00±6.63^{bc}	0.000±0.00^{c}	0.000
Body temperature (°C)						
21st day	40.63±0.06	40.75±0.07	40.56±0.06	40.77±0.06	40.82±0.07	0.069
42nd day	40.59±0.06^{b}	40.52±0.05^{b}	40.48±0.07^{b}	40.72±0.05^{a}	40.77±0.05^{a}	0.006

Table 3: The influences of different stocking densities on pen temperature

Properties (days)	22.50 birds m^{-2} 47.3 kg bw m^{-2}	18.75 birds m^{-2} 41.9 kg bw m^{-2}	15 birds m^{-2} 34.3 kg bw m^{-2}	11.25 birds m^{-2} 26.7 kg bw m^{-2}	7.5 birds m^{-2} 18.1 kg bw m^{-2}	P (statistical significance)
04-14	28.66±0.55	28.36±0.38	28.64±0.53	28.10±0.54	28.03±0.38	0.833
15-21	25.80±0.31	25.70±0.12	26.26±0.71	25.16±0.53	25.33±0.23	0.427
22-28	26.48±0.52^{a}	25.82±0.11^{ab}	25.98±0.25^{a}	24.83±0.42^{bc}	24.62±0.36^{c}	0.014
29-35	24.05±0.61^{a}	24.86±0.34^{a}	24.14±0.48^{a}	23.66±0.46^{b}	23.41±0.55^{b}	0.018
36-42	22.91±0.33^{a}	21.56±0.31^{a}	21.98±0.39^{a}	19.49±0.54^{b}	19.83±0.58^{b}	0.008

Table 4: The influences of different stocking densities on oxidative stress parameters

Properties	22.50 birds m^{-2} 47.3 kg bw m^{-2}	18.75 birds m^{-2} 41.9 kg bw m^{-2}	15 birds m^{-2} 34.3 kg bw m^{-2}	11.25 birds m^{-2} 26.7 kg bw m^{-2}	7.5 birds m^{-2} 18.1 kg bw m^{-2}	P (statistical significance)
MDA	0.69±0.02^{a}	0.64±0.02^{ab}	0.61±0.01^{b}	0.61±0.01^{b}	0.60±0.01^{b}	0.006
GSH	0.08±0.00	0.08±0.00	0.08±0.00	0.09±0.00	0.09±0.00	0.281
GSH-Px	8.07±0.05^{c}	8.21±0.08^{b}	8.45±0.09^{ab}	8.50±0.06^{a}	8.69±0.09^{a}	0.003
CAT	34.77±1.62	38.37±2.04	41.84±2.43	41.53±1.43	41.57±1.89	0.076

*The results were considered as significant when p-values were <0.05 and 0.01; * $^{a-c}$Mean values with different superscripts within a line differ significantly; *Mean±SE

Peroxidase (GSH-Px) play a vital role in antioxidant defence mechanisms (Seven *et al*., 2009). However, higher stocking density reduced serum GSH-Px level ($p<0.01$), but not changed serum CAT ($p = 0.07$) and GSH ($p = 0.28$) levels in the present study. This may be explained by the activity of antioxidant enzymes in inhibition of increased lipid peroxidation enhancing free radical in tissues (Nakazawa *et al*., 1996; Seven *et al*., 2009). Similarly, Kijparkorn and Angkanaporn (2003) reported that treatment with stocking density 18 birds m^{-2} had a trend to give higher H/L ratios, higher MDA than 9 birds m^{-2}. In the other resarches, about the effects of stocking densitiy on selected welfare indicators, Siegel (1960) found that increased population density caused the adrenal glands of chicks to hypertrophy. Muniz *et al.* (2006) confirmed that higher stocking density increased cortex percentage of bursal follicles in broiler on the 6th week. Later researches showed that higher population density increased plazma corticosterone level (Pesti and Howarth, 1983), percentage of heart, serum glucose and cholesterol levels (Onbasilar *et al*., 2008).

CONCLUSION

Higher stocking density increased litter moisture, hock and food-pad necrosis percentages, temperature of the pen and reduced body temperature. Crowding also enhanced MDA generation and reduced antioxidant enzyme activities. The higher MDA and the lower GSH-Px levels in 22.5 broilers m^{-2} group showed that the birds in the group were exposed to stress significantly.

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