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Effect of Stocking Density on Intestinal Histology and Ileal Bacterial Count in Broilers

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ABSTRACT

This study was executed to assess the effect of stocking density on carcass processing yield, small intestine morphometric measurements and ileal bacterial count in female Ross 308 from 0-30 day of age. A total of 96 female Ross chicks were randomly distributed in a randomized complete block design among 12 cages with three dietary stocking densities based on final body weight: low (28.0 kg m^{-2}), medium (37.0 kg m^{-2}) and high (40.0 kg m^{-2}) which was equivalent to 0.050, 0.037 and 0.030 m^2/bird, respectively. Results revealed that heavier breasts were obtained from birds which had subjected to the low stocking density ($p<0.001$). Total small intestine lengths and weights from birds which were subjected to the low density were the longest and the heaviest as compared to the other two groups. Birds in the low and high densities had longer villi in the duodenum and jejunum than did the group in the medium density. Ileal *Clostridium perfringens* (*C. perfringens*) and gram negative *Bacilli* counts in the low density birds tended to be the lowest among all groups ($p<0.05$). Based on presented evidences it can be concluded that increasing the stocking density of chicks from 28 to 40 kg of BW m^{-2} resulted in poor performance and could jeopardize their welfare.

Key words: Stocking density, carcass yield, intestinal histology, ileal bacterial count, Ross

INTRODUCTION

The ultimate goal of poultry producers worldwide is to maximize kg of chicken produced per m^2 while preventing production losses due to overcrowdings to achieve a satisfactory economic return. At higher stocking density rate the economic return per bird decreased while, total kg produced per unit of space increased (Shanawany, 1988; Cravener *et al*., 1992), resulting in higher profits. However, stocking density and economic return relationship is not linear. Puron *et al*. (1995) showed that the relationship was valid only up to a certain point but when the density was excessive bird performance declined resulting in similar meat production per m^2 at high and low densities.

Stocking density of broilers is an important issue in poultry industry nowadays since it affects the behavioral and physiological status of the birds and thus the performance. Consumers want

high welfare standards to be applied which will result in high quality products (Sundrum, 2001). The effect of stocking density on broiler's production is well documented, the majority of the reports showed an increase in growth rate and performance of broilers as the stocking density rate decreased (Dozier *et al*., 2005; Skrbic *et al*., 2009; Abudabos *et al*., 2013). However, little has been published concerning the influence of stocking density on morphometrics measurements and bacterial count in the small intestine as an indication for poultry welfare.

Therefore, the objective of this study was to study the influence of different stocking density rates on carcass yield, small intestine morphometric measurements and ileal bacterial count in broilers.

MATERIALS AND METHODS

Animals, husbandry and treatments: Ross 308 chicks were obtained from a commercial hatchery (Al-Wadi Poultry Farm Co., Riyadh, Saudi Arabia). Chicks were sexed, grouped by weight in such a way as to reduce variation in mean body weight and a total of 96-0 day old female broiler chicks were utilized. Chicks were allotted to twelve cages in a four-deck cage system and received the experimental diets in electrically heated battery brooders with raised wire floors. The chicks had been vaccinated for Marek's disease, Newcastle and Infectious Bronchitis. Birds were maintained at 24 h light schedule.

Birds were randomly assigned to 3 treatments based on a final body weight, low (28.0 kg m^{-2}), medium (37.0 kg m^{-2}) and high (40.0 kg m^{-2}) stocking densities which were equivalent to 0.050, 0.037 and 0.030 m^2/bird, respectively. Experimental pens contained 6, 8 and 10 chicks for the treatments low, medium and high, respectively. Feed and water were provided *ad libitum*. A typical starter (0-16 day) and finisher (17-30 day) diets based on corn and SBM diets were formulated in mashed form according to Table 1 which met or exceeded the recommendations in commercial practice in Saudi Arabia. Ambient temperature and relative humidity were continuously recorded at 3 h interval using two data loggers (HOBO Pro Series, Model H08-032-08, Onset Co., USA). The average temperature and relative humidity for the whole period were 24.95°C±0.26 (SD) and 26.63% ±3.30 (SD), respectively. The study was conducted under a protocol approved by King Saud University and complies with the current laws of Saudi Arabia.

Carcass measurements: At day 25, five birds per treatment were selected, after euthanasia, feather, heads, necks and shanks were removed and the remaining carcasses were dissected to breast and leg quarter and were weighed. The percentage of yield of each part was calculated on the basis of dressed weight.

Histopathology and morphometric measurements: The small intestine was removed aseptically, weighed, then was separated into duodenum, jejunum and ileum and for each part measurements of length and weight were taken. A 2-cm-long samples from each portion of the small intestine (i.e., duodenum, jejunum and ileum) were collected for histology measurements. Samples were fixed in phosphate-buffered formalin for at least 48 h, after which they were embedded in paraffin. Sections of 5 mm were cut and stained with haematoxylin and eosin for measurements of height and width which were based on at least 4 well-oriented villi per section per broiler. The measurements were done by using an IX71 Inverted Olympus Microscope (Eyepiece: WH10X, Objective Lens: 4X). Cellsens Digital Imaging Software for Research Application software was used for calculations.

Table 1: Dietary ingredients and chemical composition of the starter and finisher diets

	Experimental diet	
Parameters	Starter	Finisher
Ingredients (g kg^{-1})		
Corn	542.60	564.00
Soybean meal	361.00	341.00
Palm oil	54.00	59.00
Dicalcium phosphate	23.00	20.00
Ground limestone	7.20	7.00
DL-methionine	2.30	1.00
Salt	3.00	3.00
Vitamin premix[1]	2.50	2.50
Trace mineral mix[2]	0.50	0.50
Choline chloride 60	1.00	0.50
Na Bicarbonate	2.90	1.50
Calculated analysis		
Metabolizable energy (kcal kg^{-1})	3100.00	3150.00
Crude protein (%)	22.00	21.00
Lysine (%)	1.20	1.10
Methionine (%)	0.55	0.40
Threonine (%)	0.84	0.81
Sulfur amino acids (%)	0.90	0.75
Calcium (%)	1.00	0.90
Non phytate P	0.45	0.40

[1]Vitamin-mineral mix is supplied in the following per kg of diet: Retinyl acetate, 3.41 mg; cholecalciferol, 0.07 mg; DL-α-tocopheryl acetate, 27.5 mg; menadione sodium bisulphate, 6 mg; riboflavin, 7.7 mg; niacin, 44 mg; pantothenic acid, 11 mg; cyanocobalamin, 0.02; choline, 496 mg; folic acid, 1.32 mg; pyridoxine HCl, 4.82 mg; thiamine mononitrate, 2.16 mg; D-biotin, 0.11 mg; [2]Mineral-mix is supplied in the following per kg of diet: manganese, 67 mg; zinc, 54 mg; copper, 2 mg; iodine, 0.5 mg; iron, 75 mg; and selenium, 0.2 mg

Enumeration and identification of bacterial cells: Ileal digesta contents were aseptically emptied in a new sterile bag and kept in ice until time of analyses. Samples were diluted in 0.9% saline and 0.1 mL of each sample was plated on duplicates by using selective agar media for enumeration. Tryptose Sulfite-Cycloserine (TSC) agar media was used for *C. perfringens* enumeration (Oxoid CM587 with the addition of SR88 and SR47). Colonies on TSC agar that were suspected to be *C. perfringens* were plated secondarily on blood agar (Garrido *et al*., 2004). Enterobacteriaceae were isolated on MacConkey agar (Oxoiod CM7) after an incubation time of 24 h in an aerobic atmosphere at 37°C (Garrido *et al*., 2004). Enterobacteriaceae and *Salmonella* were identified by API 20E (bioMérieux, Craponne, France). The strips were inoculated, incubated at 37°C for 24 h and interpreted as recommended by the manufacturer. Reactions were recorded and identifications were determined by using a computer program [API Lab Plus software version 3.2.2 (bioMérieux)]. Results were expressed as $\log_{10}$ colony-forming units per gram of ileal digesta (log CFU g^{-1}).

Statistical analysis: Data were analyzed by using the general linear model procedure of SAS Institute Inc. (2002-2003). A cage constituted the experimental unit. Three treatments were replicated 4 times in a randomized complete block design. Means for measurements showing significant differences in the analysis of variance were tested using the PDIFF option. Means±Standard error of the Mean (SEM) are presented in the tables and differences were considered statistically significant at $p<0.05$.

RESULTS

The mean percentages of carcass parts are documented in Table 2. Stocking density had no effect on dressing percentage or leg quarter yield ($p>0.05$). Breast muscle yield was influenced by stocking density; heavier breasts were obtained from birds which were subjected to the low density (38.6%) as compared to the medium (36.5%) or high (35.5%) densities ($p<0.001$). No difference in breast muscle yield was noticed between birds which were subjected to the medium or high density.

The data related to intestinal morphology and histology of broilers at 25 day of age is shown in Table 3. As to the analysis of intestinal mucosa morphometrics as a function of stocking density, total small intestine for low, medium and high density (147.0, 139.9 and 138.5 cm, respectively) and duodenal length (17.8, 15.8 and 17.4%, respectively) showed significant differences due to treatment ($p<0.01$, 0.05, respectively). Conversely, jejunum and ileum lengths were not affected by treatment ($p>0.05$). Small intestine from birds which were subjected to the low density was the longest as compared to the other two groups. Absolute total intestine weight followed the same trend; heavier weights were obtained from birds which were subjected to the low density ($p<0.01$)

Table 2: Effect of stocking density on parts yield as percentages of broiler dressed weight

	Stocking density				
Part yield (%)	Low	Medium	High	SEM	p-value
Dressed	69.6	68.2	66.9	±1.15	NS
Breast	38.6[a]	36.5[b]	35.5[b]	±0.44	***
Leg quarter	42.0	41.2	41.9	±0.66	NS

[1]Breast and leg quarter were expressed as percentage of the carcass weight, [ab]Means in the row with different superscripts differ significantly (***$p<0.001$, NS: Not significant)

Table 3: Effect of stocking density on intestinal morphology and histology of broilers

	Stocking density				
Intestinal morphology	Low	Medium	High	SEM	p-value
Intestine length (cm)	147.0[a]	139.9[b]	138.5[b]	±1.49	**
Duodenum length (%)	17.8[a]	15.8[b]	18.4[a]	±0.51	*
Jejunum length (%)	39.9	42.0	40.9	±1.08	NS
Ileum length (%)	42.5	42.8	40.6	±0.84	NS
Intestine weight (g)	59.9[a]	51.1[b]	47.2[b]	±1.97	**
Duodenum weight (%)	15.2	16.2	18.0	±0.74	NS
Jejunum weight (%)	46.9	43.9	47.6	±1.82	NS
Ileum weight (%)	38.0[ab]	39.8[a]	34.4[b]	±1.23	*
Intestine weight (g cm^{-1})	0.44[a]	0.36[ab]	0.28[b]	±0.04	*
IRW[1] (g/100 g BW)	6.0[a]	4.8[b]	5.6[ab]	±0.25	*
Villus height (μm)					
Duodenum	9537.7[a]	8170.0[b]	9282.9[a]	±254.1	**
Jejunum	9112.5[a]	7295.9[b]	9637.7[a]	±355.7	***
Ileum	5180.5[a]	4821.6[ab]	4574.0[b]	±178.4	*
Villus width (μm)					
Duodenum	1124.5[b]	2001.2[a]	1391.0[b]	±141.7	**
Jejunum	1265.0	1146.8	1163.7	±104.5	NS
Ileum	1157.0[a]	964.0[ab]	783.4[b]	±84.0	*

[1]IRW: Intestine relative weight, [abc]Means in the row with different superscripts differ significantly (*$p<0.05$, **$p<0.01$, ***$p<0.001$, NS: Not significant)

Table 4: Effect of stocking density on ileal bacterial count in broilers

	Stocking density				
	Mean (log CFU g^{-1})				
Bacteria	Low	Medium	High	SEM	p-value
C. perfringens	4.1[b]	4.7[a]	4.4[a]	±0.08	**
Bacilli[1]	4.0[b]	4.4[ab]	4.5[a]	±0.10	*

[1]Gram negative *Bacilli*, [abc]Means in the row with different superscripts differ significantly (*p<0.05, **p<0.01, NS: Not significant)

as compared to the medium and high density (0.44, 0.36 and 0.28 g cm^{-1}, respectively). Ileal weight was heavier for birds which were subjected to the low and medium densities as compared to the high density ($p<0.05$). There were no differences due to treatment in duodenal and jejunal weights ($p>0.05$). Heavier intestine weight (g cm^{-1}) and intestine relative weight (g/100 g BW) were obtained from birds which were subjected to the low density stocking rate ($p<0.05$) as compared to the medium density (6.0 and 4.8 g/100 g BW, respectively).

Duodenal and jejunal villus height were influenced by treatment and followed the same trend ($p<0.01$, 0.001), heights for both sections were higher for birds which were subjected to low and high densities as compared to the medium density. The values were (9537.7, 8170.0 and 8170.0 μm for duodenum) and (5180.5, 4821.6 and 4574.0 μm for ileum) for the low, medium and high density, respectively. Ileal villi height and width from the low density group were higher than those obtained from the high density group ($p<0.05$).

Duodenal and ileal villi width were influenced by treatment ($p<0.01$, 0.05). Birds which were subjected to the low density had wider ileal villi as compared to the birds from the high density (1157.0 vs. 783.4 μm, respectively). While, jejunal villi widths were similar among all treatments ($p>0.05$).

Data related to ileal bacterial count in broilers at 25 day of age are presented in Table 4. Ileal *C. perfringens* and gram negative *Bacilli* counts in the low density birds tended to be lowest among all groups (4.1 and 4.0 log CFU g^{-1}, respectively for the low density; 4.7 and 4.4 log CFU g^{-1}, respectively for the medium density and 4.4 and 4.5 log CFU g^{-1}, respectively for the high density). Similar *C. perfringens* and gram negative *Bacilli* counts were found in the medium and high stoking groups.

DISCUSSION

Breast muscle yield was reduced by 8.0% as stocking density increased from low to medium or high which could be explained by the 16.0% reduction in body weight gain at 30 day of age in the high stocking density (data not shown). According to Skrbic *et al.* (2008) low stocking density provides more intensive growth for broilers and higher absolute yield of processed carcass. Low density allows for better body development, carcass conformation and higher shares of carcass parts which contain more meat, especially breast (Skrbic *et al.*, 2009). These results are in accordance with previous reports (Garcia *et al.*, 2002; Chmelnicna and Solcianska, 2007; Abudabos *et al.*, 2013). Dozier *et al.* (2006) concluded that increasing stocking density beyond 30 kg of BW m^{-2} adversely affects growth responses and meat yield of broilers grown to 1.8 kg. Contrarily (Dozier *et al.*, 2005) found that breast meat yields relative to body weight were not affected as density increased from 30 to 45 kg m^{-2}. However it is important to mention that

birds in Dozier *et al.* (2005) study were grown to 49 day of age and equivalent densities were calculated on 3.2 kg of final body weight, which was much higher than in current study.

The small intestine is the main site for digestion and absorption of nutrients. Long villi are usually equated with excellent gut health, high absorptive efficiency and healthier intestinal tract of chickens (Sims *et al.*, 2004; Alfaro *et al.*, 2007). The reduction in ileal villi height and width between the low and the high densities (11.7 and 32.2%, respectively) which was reported in this experiment might be associated with the presence of a challenge. According to Boleli *et al.* (2002) the villi height in the small intestine epithelium is positively related to absorption capacity and its growth is regulated by 2 cytologic processes: cell turnover and cell extrusion. Nutrient digestion and absorption rates are directly influenced by cell proliferation and differentiation rates, the area of surface for digestion and absorption depend on villi height and width (Franco *et al.*, 2006). On the other hand, cell lesions caused by bacteria, such as *C. perfringens*, may increase cell extrusion rates and reduce villus height.

Clostridium perfringens infection of broilers may cause impairment of production performance (Lovland and Kaldhusdal, 2001). The subclinical disease associated with necrotic enteritis decreases digestion, absorption and reduces weight gains (Kaldhusdal *et al.*, 2001). Wilson *et al.* (2005) suggested that the growth suppressing effect of intestinal bacteria was due to the production of toxic metabolites that irritate the gut mucosa, thereby inhibiting nutrient absorption. In this experiment, an increase in the prevalence of *C. perfringens* (6.8%) and gram negative *bacilli* (11.1%) was noted with the increase in the stocking densities from low to high. The changes in *C. perfringens* and gram negative *bacilli* counts appear in parallel to observed changes in ileal height and width. Burkholder *et al.* (2008) showed that acute stressors in poultry can cause changes in the normal microbiota and epithelial structure, which may lead to increased attachment of pathogenic bacteria.

In conclusion, these data indicated that increasing the stocking density of broiler chickens from 28-37 or 40 kg of BW m^{-2} influenced breast muscle yield and increased the stress which was measured by ileal morphometric measurements and bacterial count.

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