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ORIGINAL RESEARCH ARTICLE

Effect of stocking density on the welfare, haematology and serum biochemical indices of broiler chickens

*[1]Adeyemo, G. O, [1]Fashola, O.O. and [2]Ademulegun, T.I.

[1]Department of Animal Science, Faculty of Agriculture, University of Ibadan, Nigeria
[2]Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria
*Corresponding author Email: gbemiadeyemo7@gmail.com

ABSTRACT

Influence of stocking density on welfare parameters (Litter temperature and moisture, microbial load, Gait score), haematology and serum biochemical indices (as indicators of condition of stress) on broilers was studied in an eight week feeding trial. A total of 240 one- day old Arbor acre broiler chicks were randomly allotted to three treatments (stocking densities) with 4 replicates each having 20 birds per replicate. The stocking densities rates were 10birds/m², 12birds/m² and 14 birds/ m² respectively. Data obtained were subjected to one way analysis of variance. Serum biochemical indices and microbial load were not significantly (p>0.05) influenced by the treatments. A fraction of haematology, the haemoglobin (Hb) and eosinophil (Eos) were significantly (p<0.05) influenced by different stocking densities. Microbial load, litter moisture and temperature increased linearly with increasing stocking density across the treatments. Blood profile of the broiler chickens indicated no negative effect of stocking density except on Hb and Eos which reflected the packed condition of the birds. In conclusion, stocking density of 10birds/m² can be recommended as birds fared best under this treatment.

Keywords: Broilers, Haematology Serum biochemical indices, Stocking density and Welfare

INTRODUCTION

During the last decades, many developing countries have adopted intensive poultry production in other to meet the demand for animal protein. Intensively kept poultry is seen as a way of rapidly increasing animal protein supplies for rapidly increasing urban population. Modern poultry production occurs primarily in enclosed buildings to protect the birds from weather, predator and the spread of diseases from wild birds. This has allowed farmers to greatly increase production efficiency while significantly reducing the amount of labour required. The modern broiler house enables producers to have great control over the house environment. Birds can be placed at higher densities as long as the correct environment conditions of temperature, ventilation and humidity is provided. Factors to consider when determining stocking density include but are not limited to bird size, feeder space, drinker space, house dimensions, bird welfare, nutrition, breed, performance and economic return. Stocking density is currently expressed as a mass per unit of space rather than numbers of birds being reared in a given area (Thaxton *et al.*, 2006). For many years, the term stocking density indicated the numbers of birds being reared in a given housing area. Today, broilers are reared to target body weight (BW) that far exceeds those of only a decade ago. To account for these large increases in final body weight (FBW), it has become necessary to develop a new expression of stocking density. Specifically, many in the poultry industry now express stocking density as mass per unit of space. The quality of the in-house environment is highly dependent upon litter quality. The litter environment is ideal for bacterial proliferation and ammonia production. Higher mortality, lower meat production, greater incidence of leg disorders, and cannibalism occur at higher stocking densities in broilers. Zulkifli *et al.* (2003) reported that the heterophil to lymphocyte ratio (H: L) is a reliable indicator of avian stress.

Broilers exposed to heat stress in summer showed an increase in heterophils and a decrease in lymphocytes, which leads to an increase in the H: L ratio. Mcfarlane and Curtis, (1989) reported that the H:L ratio increased with heat stress in broiler chicks. Elevation of the H:L ratio with increasing stocking density was reported to indicate that high stocking density in broiler production is stressful. Spinu *et al.* (2003) reported that there were no differences in the H:L ratio between different stocking densities in broiler breeders. The ultimate goal is to maximize meat yield of chicken while preventing production losses due to overcrowding. In many cases, producers have to settle for slightly reduced performance to achieve a satisfactory economic return. Another concern with increased stocking density is broiler welfare. Animal activist groups request that broilers be given more space during grow-out and cite behavioural and physiological stress as the reason (Fairchild, 2005). The objective of this study therefore was to investigate the effect of stocking density on welfare parameters like litter temperature and moisture, microbial load, and haematology and serum biochemical indices of broiler chickens.

MATERIALS AND METHODS

Experiment was conducted at Poultry Unit of teaching and research farm, University of Ibadan, Ibadan, Nigeria. The rearing house was previously washed and disinfected, allowed to dry and rest before arrival of birds. Birds were vaccinated with Gumboro and Lasota twice during the period of the experiment. Two hundred and forty, 1- day old Arbor acre chicks were randomly allotted to three treatments (stocking densities) with four replicates, each replicate had twenty birds. Treatments 1, 2 and 3 had 80 birds each which were designated for stocking densities 10 birds/m^2, 12 birds/m^2 and 14 birds/m^2 respectively. The sizes of the pens were 3.00m^2, 2.67m^2 and 2.43 m^2 respectively. The birds in all three (3) treatments were allocated with equal space of feeders and drinkers. The birds were reared for eight weeks.

Gait Score

The bird's ability to walk was scored on a six point scale: The descriptions of the scores are as follows: Gait Score 0: The birds walked normally with no detectable abnormality it was dexterous and agile. Typically, the birds pick up the foot up and down smoothly and each foot was brought under the centre of gravity of the bird as it walked; Gait Score 1: The bird had slight defect which was difficult to define precisely for example the bird takes and unduly large stride which the observer may not recognise the exalt cause, produced and uneven gait; Gait Score 2: The bird had a definite and identifiable defect in its gait but the lesion did not hinder it from moving or competing for resources, move ability acceleration and speed were not seriously compromised; Gait Score 3: The birds had an obvious gait defect which compromised its moveability. The defect could the form of a limp, jerky or unsteady strut, the bird often prefer to swat when to force to move. Moveability, acceleration and speed were affected; Gait Score 4: The bird had a severe gait defect, it was still capable of walking only with difficulty when forced otherwise it swatted at the first available opportunity. Moveability, acceleration were severely affected; Gait Score 5: The bird was incapable of sustained walking on its feet. Locomotion is only possible with assistance of the wings or by crawling on its shanks. Normal gait score is represented by gait score 0, Unsteady gait score covers gait score 2 - 4, while paralyzed gait score is gait score 5. The scores obtained for the three different categories were pooled per treatment and converted to percentages.

Data Collection

The feed intake and the weight gain were taken weekly. The feed conversion ratio was calculated by dividing the daily feed intake by the daily weight gain. The incidence of mortality was also recorded daily. The litter from 12 pens were sampled separately in four replicates. Prior to sampling, the litter was mixed and 10g of litter was collected aseptically into a sterile universal bottle this was done using methods described by Barrow and Feltham (1993). Media used was prepared according to the manufacturers specifications.

Table 1: Gross composition of starter and finisher diets fed to birds in three different stocking densities.

	Experimental Diets	
	Starter (%)	Finisher (%)
Maize	49.00	58.50
Wheat offal	5.00	2.00
FFSB	8.65	15.40
GNC	25.00	15.00
Fish Meal	4.00	0.00
Palm oil	3.00	3.81
Bone meal	3.00	3.00
Oyster shell	1.50	1.50
Salt	0.25	0.25
Broiler premix	0.25	0.25
DL-Methionine	0.10	0.11
L- Lysine	0.25	0.18
Total	100.00	100.00
Calculated Analysis		
CP (%)	23.06	19.92
ME (kcal/kg)	3083.00	3217.00
L- Lysine (%)	1.20	1.04
DL- Methionine (%)	0.45	0.40

FFSB- Full fat soyabean, GNC-Groundnut cake, CP- Crude protein, ME- Metabolisable energy.

This standard plate's technique was used in the microbial load determination. 1g of litter described above was used for serial dilution in sterile 15ml test tube containing 9ml of 0.1% sterile peptone H_2O and vortexes serial dilutions level. Appropriate dilution of 10^3 and 10^5 were inoculated on plate count agar. This was inoculated at 37°c for 18hrs. Discrete colonies on plates were counted using a colony counter and estimated in log_{10} CFU/ml. Walking ability was scored according to method by Kestin *et al.* (1992). Gait score were designated using three different categories of walking ability ranked as Normal, unsteady and paralysed. For the purpose of determination of the quality of litter, temperature of litter was measured on days 28 and 56, on three measuring locations/points in the pen and the average temperature of litter was based on each pen. At the end of the trial, in the same way, samples of litter were taken for determination of the moisture content, by method described by AOAC (1991).

Haematology and Serum Biochemical Indices
At the end of the experiment, 12 birds (one bird per replicate) were selected and bled by the jugular vein using hypodermic needle with syringe. Blood was drained into 2 differently labelled bottles for haematological and serum metabolite investigations. The blood samples for haematological parameters were collected into bottles pre-treated with EDTA (Ethylene Diamine Tetra Acetic acid), anti coagulant. Blood samples for biochemical indices were collected into another sample bottle without EDTA. Haematology and Serum biochemical indices investigated were Packed Cell Volume (PCV), Red Blood Cell (RBC) White blood Cell, haemoglobin (Hb), eosinophil, heterophil to lymphocyte ratio (H: L), Heterophil, Basophil, Lymphocyte, Monocytes, Cholesterol, Glucose and Total protein. Feed offered were analysed for Ash Content, Crude Protein (CP), Ether Extract (EE) and Crude Fibre (CF) according to the method described by AOAC 2002.

Statistical analysis: All statistical analysis was performed using the one way ANOVA of Statistical Analysis System (SAS, 1996). Three treatments were arranged in 4 replications in a completely randomized design. Means for measurements showing significant differences in the analysis of variance were tested using the Duncan option.

RESULTS

Proximate composition of the starter and finisher diets are presented in Table 2. The values obtained in the dry matter (DM), Crude protein (CP), Crude fibre (CF), Ash and Ether extract (EE) for starter diet was higher than the values obtained in finisher diet except Nitrogen free extract (NFE). The effect of stocking density on some welfare parameters are presented in Table 3. There were no significant differences ($p>0.05$) among the parameters observed in this study except the litter moisture and litter temperature. In litter moisture parameters observed in week four for treatment 3 (40.54%) was significantly higher ($p<0.05$) compared to treatments 1(33.75%) and 2 (31.00%). In week eight of the experiment, the parameters were not significantly influenced ($p>0.05$) by the stocking density. The result indicated that litter temperature was significantly influenced ($p<0.05$) by the stocking density in which treatment 1 (33.83%) and treatment 2 (33.23%) were significantly higher ($p<0.05$) than treatment 3 (31.40%). In week four treatment 3 (34.17%) recorded the highest value followed by treatment 1 (33.00%) and treatment 2 (32.53%) respectively.

The effect of stocking density on haematological and serum biochemical parameters of broiler chickens is presented in Table 4. There were no significant differences ($p>0.05$) among the parameters observed except Eosinophils (Eos) and Haemoglobin (Hb). Haemoglobin (hb) values obtained for treatment 2 (27.00g/dL) and treatment 3 (29.00g/dL) were similar but compared with values obtained in treatment 1 (12.33g/dL) they were significantly higher ($p<0.05$).

Table 2: Proximate composition of starter and finisher diets fed to birds in three different stocking densities

	Experimental Diets	
Parameters	Starter (%)	Finisher (%)
DM (%)	92.14	91.07
CP (%)	25.03	21.88
CF (%)	4.00	1.00
ASH (%)	9.30	6.75
EE (%)	13.82	13.41
NFE (%)	49.82	59.12

DM- Dry matter, CP- Crude protein, CF- Crude fibre, EE- Ether extract, NFE- Nitrogen free extract.

Table 3: The effect of different stocking densities on welfare parameters (litter moisture, litter temperature, microbial assay and gait score)

Parameters	Treatments			
	1	2	3	SEM
Litter temperature (°c)				
At week 4	33.83[a]	33.23[a]	31.40[b]	0.42
At week 8	33.00[ab]	32.53[b]	34.17[a]	0.31
Litter Moisture (%)				
At week 4	33.75[b]	31.00[b]	40.54[a]	1.78
At week 8	45.26	44.74	47.62	1.01
Microbial load (CFU/ml)	5.98	6.22	6.22	0.09
Normal Gait Score (%)	73.81	86.61	90.63	3.87
Unsteady Gait Score (%)	26.19	13.39	56.25	9.71
Paralyzed Gait Score (%)	0.00	0.00	3.13	1.04

[a,b] means in the same row having different superscript are significantly different ($p<0.05$). SEM means standard error of mean.

Eosinophil (Eos) values of 4.00%, 4.67% and 10.67% were obtained for treatments 1, 2 and 3 respectively with the value for birds on treatment 3 being significantly higher and treatment 1 had the lowest. Serum biochemical parameters observed in the study were not significantly influenced by stocking density.

DISCUSSION

Gait score represents a subjective method of evaluating the walking ability of birds. It was observed that there was no problem with gaits of chickens in any of the treatments. The performance of the birds in gait score in this study were not influenced by the stocking density which corroborated the findings of Ravindran *et al.* (2006) who reported that stocking density did not exert any influence on the leg of chickens. Frequency of certain score per treatments, however, did not indicate any effect of stocking density, as well as the age which reflects the effect of body weight of chickens on the ability to walk. The treatment with the lowest space had the highest value followed by the next in line in term of space and then the control that indicated the normal gait score. Similar trends were observed in paralyzed gait score. Sorensen *et al.* (2000) agrees with our observation he reported poorer walking ability in birds reared at higher densities which could be attributed to constrained mobility and reduced opportunity for activity, especially as birds approach the end of the grow out phase. The parameters observed on litter moisture at half way time of trials and the microbial load were not influenced while litter moisture at the end of study and litter temperature at middle and, end of experiment were influenced by stocking density.

Litter moisture content of the treatment with the highest density was higher than for the other treatments which corroborated the report of Sorensen *et al.*, 2000 who stated that higher stocking density increases litter moisture. Increase in stocking density leads to moisture content and litter temperature increments which was similar to the results obtained by Elwinger (1995), Dozier *et al.* (2005), Dozier *et al.* (2006), Mendes *et al. (2004),* who recorded, increased stocking density increasing the litter temperature and moisture. Increase temperature and moisture content of litter, changes the physical and microbiological characteristics of the litter which are important in relation to the incidence of contact dermatitis caused by bacterial infections, especially *Staphylococcus aureus* and *Escherichia coli* (Butterworth, 1999).

In this study, increasing density gave higher microbial load on litters which is contrary to what Thaxton *et al* (2003) reported that the number of flocks housed on the same litter did not significantly alter the microbial population of the litter and the microbial population of the litter did not increase as the number of birds increased. Haemoglobin and eosinophils were influenced across the different stocking densities while other blood parameters were not Gross and Siegel, (1983); Altan *et al,* (2000); Puvadolpoid and Thaxton, (2000); Islam *et al*, (2004) reported that the Heterophil : Lymphocyte ratio, leucocytes changes are recognised as stress indicators. In general, birds respond to stress by decreasing the number of lymphocyte, heterophils, eosinophils and monocytes in circulation. Differences were recorded in heterophil to lymphocyte ratio and values across treatments fall between the low and optimal degrees. Gross and Siegel (1983) suggested reference values for heterophil to lymphocyte ratio of about 0.2, 0.5 and 0.8 which are characterized as Low, Optimal and High degrees of stress respectively. It can therefore be concluded that birds even at the highest density level of 14 birds/ m^2 in this work were not stressed beyond the level that will compromise production.

Table 4: Effect of different stocking densities on haematology and serum biochemical indices of broiler chickens

Parameters	Treatments			
	1	2	3	SEM
Haematological parameters				
PCV (%)	29.67	27.00	29.00	0.88
RBC (10^6/mm^3)	4.42	3.86	4.36	0.22
WBC (10^3/mm^3)	22.30	21.23	22.95	1.72
Hb (g/dL)	12.33^b	27.00^a	29.00^a	2.78
Heterophils (%)	32.00	24.67	27.00	0.17
Eosinophils (%)	4.00^b	4.67^{ab}	10.67^a	1.40
Basophils (%)	0.67	0.00	0.00	0.15
Lymphocytes (%)	59.00	68.33	58.33	2.94
Monocytes (%)	4.33	4.33	4.00	0.22
H:L	0.56	0.37	0.49	0.19
Serum metabolites (g/dL)				
Cholesterol	93.97	91.61	101.42	7.12
Total protein	3.09	3.21	3.38	0.19
Glucose	142.83	141.64	148.51	8.41

^{a,b} means in the same row having different superscript are significantly different ($p<0.05$)
SEM means standard error of mean. PCV (Packed cell volume), RBC (Red blood cell), WBC (White blood cell) and H:L (Heterophil:Lymphocyte ratio), Hb (Haemoglobin)

The serum biochemical parameters observed were similar but birds with the lowest space recorded the highest value of cholesterol and glucose. This agrees with the work of Thaxton *et al.* (2006), who reported that stocking density did not result in a recognizable trend in glucose and cholesterol. Stucchi *et al.* (1991) and Ozbey *et al.* (2007) affirmed that physical activities has a significant role in cholesterol level that is lowering the stocking density reduces serum total cholesterol. But in this study, cholesterol increased with the lowest stocking density though it was not significant at 5% level of probability and this could be attributed to reduction in metabolic activities due to lower stress factors because the broilers had more space to themselves.

CONCLUSION

Blood profile of the broiler chickens indicated no negative effect of stocking density except on haemoglobin and eosinophils which reflected the packed condition of the birds. In conclusion, stocking density of 10 birds/m^2 is recommended as birds fared best under this treatment.

CONFLICT OF INTEREST

No conflict of interest exist with this work

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