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Research Article

Effects of Stocking Density on Performance and Immunity in Ross 308 Broiler Chickens

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Abstract

To investigate the effect of stocking density on productive traits, blood parameters and immunity, 1305 Ross 308 male broiler chicks (initial weight: 45±1.5 g) were used as a completely randomized design with 2 treatments and 15 replications. Treatments included 1) 12 chicks per pen 2) 18 chicks per pen (1.2x1.2 m^2). The results were recorded in three periods of 0-24, 25-42 and 0-42 d. From 0 to 24 d of age, the stocking density had no significant effect on feed intake and body weight gain (P>0.05), but feed conversion ratio significantly decreased in 12 chicks density group (P<0.05). Feed conversion ratio, body weight gain and feed intake were improved significantly in 12 chicks compared to 18 chicks density for 25-42 and 0-42 periods (P<0.05). The production per m^2 was significantly higher in 18 chicks density group at 0-42 d period (P<0.05). High stocking density caused to increase the moisture content of bedding in 4-6th weeks of age (P<0.05). Stocking density had no significant effect on mortality percentage (P>0.05). Also the relative weights of spleen, bursa of fabricius, abdominal fat, thigh and breast were not affected by experimental treatments (P>0.05). Increasing the number of chicks per m^2 caused to increase heterophile count and decrease lymphocyte count and increase heterophile to lymphocyte ratio (P<0.05). Newcastle's titer was not affected by treatments (P>0.05). Given that performance indices were better under low stocking density, but live body weight per m^2 was significantly higher in 18 chicks density group (32%), it's seems economically 18 chicks per m^2 is the best stocking density.

Keywords: *Broiler chickens, Immune system, Performance traits, Stocking density*

Ross 308 Broiler Tavuklarda Yerleşim Sıklığının Performans ve Bağışıklığa Etkileri

Öz

Yerleşim sıklığının üretim özellikleri, kan parametreleri ve bağışıklığa etkilerini incelemek amacıyla, 1305 adet Ross 308 erkek broiler civciv (ön ağırlık: 45±1.5 g) rastgele dizaynda, 2 uygulama ve 15 tekrar olmak üzere kullanıldı. Uygulamalar 1: her 2 kafes için 12 civciv ve 2: her kafes (1.2x1.2 m^2) için 18 civciv olarak gerçekleştirildi. Sonuçlar 0-24, 25-42 ve 0-42 gün olmak üzere üç periyotta incelendi. 0-24 günlük periyotta yerleşim sıklığı yem tüketiminde ve vücut ağırlık kazanımında anlamlı bir etkiye neden olmazken (P>0.05) yem konversiyon oranı 12 civciv sıklık grubunda anlamlı derecede düştü (P<0.05). Yem konversiyon oranı, vücut ağırlık kazanımı ve yem tüketimi 12 civciv grubunda 18 civciv grubu ile karşılaştırıldığında 25-42 ve 0-42 gün periyotlarında anlamlı derecede iyileşme gösterdi (P<0.05). Her m^2 için üretim 18 civciv sıklık grubunda 0-42 gün periyotta anlamlı derecede daha yüksekti (P<0.05). Yüksek yerleşim sıklığı 4-6. haftalarda altlığın nem miktarının artmasına neden oldu (P<0.05). Yerleşim sıklığı mortalite yüzdesinde anlamlı bir etkiye neden olmadı (P>0.05). Dalak, bursa Fabricius, abdominal yağ, but ve göğüs görece ağırlıkları deneysel uygulamalardan etkilenmedi (P>0.05). Civciv sayısını m^2 başına artırmak heterofil sayısında artmaya ve lenfosit sayısında azalmaya neden olurken heterofil lenfosit oranı artma gösterdi (P<0.05). Newcastle titresi uygulamalardan etkilenmedi (P>0.05). Bu performans endekslerine göre; düşük yerleşim sıklığı daha iyi sonuçlar verirken, 18 civciv sıklık grubunda m^2 başına canlı vücut ağırlık daha yüksek olması sebebiyle ekonomik olarak her m^2'ye 18 civciv en iyi yerleşim sıklığı olarak gözükmektedir.

Anahtar sözcükler: *Broiler tavuk, Bağışıklık sistemi, Performans özellikleri, Yerleşim sıklığı*

INTRODUCTION

One of the common subjects in poultry breeding is the proper stocking density. The excessive increase of broiler chick's density may reduce welfare, health, body weight, feed intake, feed efficiency, flock's uniformity and increase

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skeletal disorders and mortality [1]. On the other hand, increasing density will reduce fixed costs and increase production per area unit, and by increasing density to reach the critical point, profitability will also increase. It is difficult to determine a proper stocking density because of different environmental conditions and equipment, chick comfort criteria and the used strain genetic in experiments [2]. Reducing the air quality of poultry houses, in particular increasing ammonia, and reducing access to water and feed can reduce performance under high density condition. The reduction of performance by increasing stocking density can be due to reduced feed intake and thus Decrease received nutrients for maximum growth [3]. By increasing density, the number of birds per unit of floor space increases thus higher efficiency can be obtained, but this economic profit may be at the expense of reduced performance, hygiene and comfort of the bird [4]. In a research [5] investigated the effects of stocking density, chick's sexuality and the amount of metabolizable energy on performance and leg problems in broiler chickens, they stated that the stocking density have severe effects on the growth rate of male broiler chickens before 35 d of age, and male chickens need more space than females to reach marketing age. Based on the findings of this study, there was a significant interaction between stocking density, sexuality and age of chicks on performance, as high density decreased body weight gain and increased feed conversion ratio, and male chicks had a significant increase in feed intake, weight gain and reduced feed conversion ratio in comparison with female chicks. Also they expressed that the prevalence and severity of leg problems are related to stocking density, chick's sexuality and type of the diet.

In a similar study two levels of density (10 and 16 chicks per m^2) were examined, lower density increased body weight, improved feed conversion ratio and the Newcastle titer significantly [6]. Ventura *et al*.[7] studied the effect of stocking density and sleeping wood on leg health, fear and performance of broiler chickens. They stated that increasing stocking density has negative effect on leg health and chicks reared under high density (18 chicks) had severe lesions in their legs and rabbit joint. The aim of this study was to investigate the effect of stocking density on performance and immunity traits of broiler chickens and compare the density of 18 and 12 chicks per m^2.

MATERIAL and METHODS

Birds and Treatments

A total of 1305 male broilers (Ross 308) were used as a completely randomized design with 2 treatments and 15 replications. Treatments included 2 levels of density (12 and 18 chicks per m^2). The experimental pen dimensions were 1.2 in 2.4 meters, therefore 35 chicks (for 12 density) and 52 chicks (for 18 density) were reared in each pen. All birds had freely access to feed and water throughout the experiment and bird management was according to Ross strain standards and was the same for all treatments. Feed intake and body weight gain were recorded for 1-25, 25-42 and 1-42 d of age and also mortality was recorded by hen day method.

Diets

Corn and soybean based diets were formulated according to Ross 308 nutritional recommendation *(Table 1)*.

Feed ingredients and diets were analyzed for dry matter [8], protein [8], digestible amino acids [9], crude fiber [9], calcium [10], phosphorus [10] and crude fat [10]. The chemical analysis of feeds is presented in *Table 1*.

Performance Traits

Performance traits including body weight gain, feed intake and feed conversion ratio were recorded for 1-24, 25-42 and 1-42 d of age. Then feed intake and feed conversion ratio data were corrected based on mortality and hen day.

Slaughtering and Carcass Analysis

At the end of experiment, 2 birds close to average weight from each experimental unit were selected, weighed and were slaughtered according to the regulations approved by the Animal Protection Committee of Islamic Azad University, Science and Research Branch Protocol [11]. Then the relative weights (% of BW) of bursa of fabricius, abdominal fat, breast, thighs and spleen were recorded.

Bedding Moisture Measurement

To evaluate bedding moisture, 1 kg combined sample was formed by mixing collected samples from 6 areas of each pen (around the plate, the drinkers and end of the pens) weekly [12]. Each sample was weighed accurately and then dried in an oven at 105°C for 24 h. Then, the moisture content was calculated from the subtraction of initial weight from dried sample weight and expressed as a percentage of the initial weight [10].

Evaluation of Heterophile to Lymphocyte Ratio and Bursa and Spleen Organs

At 41 d of age, two chickens were selected from each replicate and 2 mL blood were taken from each chicken to evaluate the heterophile to lymphocytes ratio, and the number of heterophile to lymphocyte was determined by staining on a slide. After slaughter, the spleen and bursa were separated, weighed and expressed as a percentage of carcass weight [1].

Newcastle Antibody Titer by HI Method

At 41 d of age, blood sampling was carried out from wing vein of two chickens of each replicate and the titer of Newcastle antibody was determined in them [13].

Table 1. *Ingredients of basal diets (%)*

Ingredients %	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Ground corn	47.43	54.27	59.27
Soybean meal (45%)	39.50	32.75	28.16
Soybean oil	1.09	1.00	1.00
Wheat	7.00	7.00	7.00
DL-methionine	0.35	0.27	0.25
L-lysine hydrochloride	0.27	0.20	0.16
Threonine	0.14	0.09	0.07
Choline chloride 60%	0.02	0.02	0.02
D-calcium phosphate	1.99	1.57	1.35
Calcium carbonate	1.16	1.12	1.06
Sodium bicarbonate	0.27	0.17	0.22
NaCl	0.22	0.17	0.16
Mineral supplement[1]	0.25	0.23	0.20
Vitamin supplement[2]	0.25	0.23	0.20
Bentonite	-	0.85	0.80
Phytase5000 (Biochem,Germany)	0.00	0.01	0.01
Salinomycin	0.05	0.05	0.05
Total	100	100	100
Nutrient			
Crude protein	22.64	20.00	18.8
Energy (kcal/kg)	2877	2936	2995
Calcium	1.03	0.91	0.83
Available phosphorus	0.51	0.44	0.39
Sodium	0.18	0.14	0.14
Crude fiber	3.08	3.84	3.68
Digestible methionine	0.65	0.54	0.50
Digestible methionine + Cysteine	0.95	0.82	0.76
Digestible lysine	1.3	0.95	0.98
Digestible threonine	0.89	0.75	0.67

Vitamin and mineral supplements per kilogram of diet provide the following amounts: [1] Mineral: I, 0.43 mg; Cu, 13.56 mg; Zn, 29.3 mg; Se, 6.57 mg; Mn, 88.51 mg; Fe, 17.28 mg; [2] Vitamins: vitamin A, 15600 IU; vitamin D_3,6750 IU; vitamin E, 120 IU; vitamin K_3, 4.8 mg; vitamin B_1, 3.84 mg; vitamin B_2, 10.32 mg; vitamin B_3, 72 mg; vitamin B_5, 20.4 mg; vitamin B_6, 6.48 mg; vitamin B_{12}, 0.021 mg; vitamin B_9, 2.75 mg; Biotin 0.36 mg

Statistical Analysis

Data were subjected to the GLM procedure for ANOVA in a completely randomized design. Differences among means were separated with the LSMEANS option of SAS (2002) [14]. Statistical significance was considered at $P \leq 0.05$. The statistical model was as follows:

$$Y_{ij} = \mu + A_i + e_{ij}$$

The components of this model are:

Y_{ij} = The measured value of each observation

μ = mean

A_i = Effect of treatment

e_{ij} = Experimental error

RESULTS

Table 2 show the effect of two levels of density (12 and 18 chicks) on the performance of broiler chickens in different periods. Increasing the density from 12 chicks to 18 per m^2 significantly reduced the weight gain and feed intake, over 25-42 d and 1-42 d of age, also significantly increased feed conversion ratio for all periods ($P<0.05$).

As shown in *Table 3*, increasing density caused to a significant increase in production per area unit ($P<0.05$).

In this study, carcass parameters did not affected by increasing the number of chicks per area unit ($P>0.05$) *(Table 4)*.

Table 2. *Effect of stocking density on body weight gain, feed intake and feed conversion ratio of broiler chickens in different growth periods (g)*

Period	Treatments	Body Weight Gain	Feed Intake	Feed Conversion Ratio
(1-25 d)	12 chicks density	1299.70	1884.40	1.45[b]
	18 chicks density	1269.90	1867.20	1.47[a]
	SEM	11.61	13.60	0.005
	P value	0.0801	0.379	0.0203
(25-42 d)	12 chicks density	1552.1[a]	3082.80[a]	1.99[b]
	18 chicks density	1273.7[b]	2760.00[b]	2.17[a]
	SEM	26.74	33.01	0.036
	P value	0.0001	0.0001	0.0001
(1-42 d)	12 chicks density	2851.9[a]	4967.20[a]	1.74[b]
	18 chicks density	2543.6[b]	4627.20[b]	1.81[a]
	SEM	23.93	37.65	0.018
	P value	0.0001	0.0001	0.0135

Table 3. *Effect of stocking density on live weight of broiler chickens per m² at the end of experiment (kg/m²)*

Treatments	Production per m²
12 chicks density	34.70[b]
18 chicks density	45.90[a]
SEM	0.39
P value	0.0001

Table 4. *Effect of stocking density on some carcass parameters (% of BW)*

Treatments	Spleen	Bursa	Abdominal Fat	Thigh	Breast
12 chicks density	0.13	0.06	1.08	19.00	26.50
18 chicks density	0.12	0.05	1.15	19.30	25.90
SEM	0.0056	0.0049	0.059	0.1500	0.3400
P value	0.544	0.673	0.389	0.062	0.228

Table 5. *Effect of stocking density on some blood parameters and mortality percentage in the intervals of 1-25 and 25-42 d*

Treatments	Heterophile/Lymphocyte	Heterophile (%)	Lymphocyte (%)
12 chicks density	0.52[b]	33.10[b]	63.60[a]
18 chicks density	0.71[a]	40.60[a]	57.00[b]
SEM	0.044	1.703	1.610
P value	0.007	0.0034	0.0082
Treatments	**Mortality Percent (1-25)**	**Mortality Percent (25-42)**	
12 chicks density	1.52	1.90	
18 chicks density	1.91	1.67	
SEM	0.64	0.79	
P value	0.676	0.427	

According to the data presented in *Table 5*, increasing stocking density, significantly increased heterophile percentage, heterophile to lymphocyte ratio and decreased lymphocyte percentage (P>0.05). Results also show that stocking density has not any effect on mortality in 1-25 and 25-42 d of age (P>0.05).

According to *Fig. 1*, increasing the stocking density increased the moisture content of the bedding material in the 4th, 5th and 6th weeks of breeding (P<0.05). At the third week, the difference in moisture between two groups was close to significant (P=0.0621) *(Fig. 1)*.

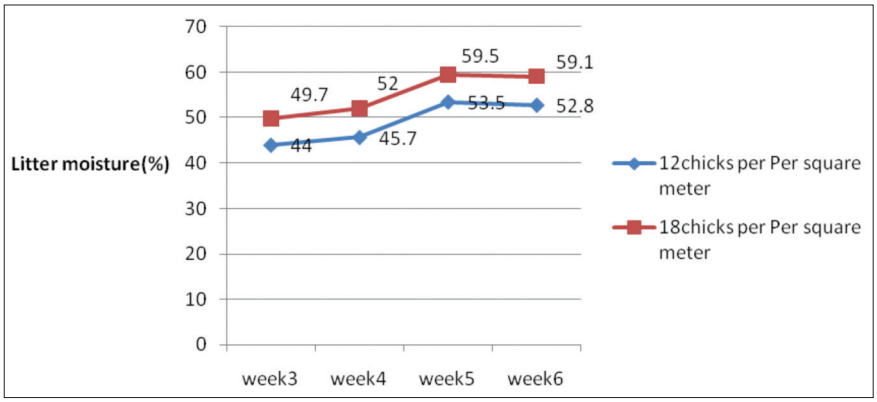

Fig 1. Effects of stocking density on litter moisture in different weeks

DISCUSSION

In this experiment, the performance traits including body weight gain, feed intake and feed conversion ratio, in 12 chicks per m^2 group were significantly better than 18 chicks group. Low density (12 chicks) provided the best environmental conditions for chicks and allowed the chicks to have the best feed consumption without any competition, followed by the best body weight gain and feed conversion ratio. Considering that about 32% in live body weight was obtained in 18 chicks density and according to some researchers however, at higher densities, the performance per bird decreases, but the kilogram of produced meat per m^2 increases and leads to more economic profit [15,16]. In another study Puron *et al.*[2] showed that increasing stocking density caused to gain more amount of meat produced per m^2. However, the relationship between density and economic efficiency is not a linear relationship. According to their statement this relationship is valid only to a certain extent, because under high density condition, the performance of the chick is reduced, and if the density exceeds from 17 male and 19 female chicks per m^2, finally the weight of the produced meat will be the same in both densities. These results indicate that if the stocking density exceeds the above limit, flock profitability and bird welfare will disturb [17]. A number of researchers have reported that the lower stocking density (11.9) compared to higher density (23.8) significantly improved body weight gain, but the production per m^2 was significantly higher for high density groups [18]. A number of studies have shown the improvement of performance traits in lower stocking density in broiler chickens [19-21], while others reported no effect [22] or even negative effects of density on the performance of broiler chickens [18]. In the present study, decreasing daily weight gain and feed intake is consistent with some studies [23-25]. The results of performance characteristics are consistent with some studies, including the study of Nahashon *et al.*[26] that they were investigated the effect of stocking density on performance and carcass characteristics of broiler chicks in Guinea. For this purpose, they used four treatments of 10.7, 12, 13.6 and 15.6 chicks per area unit. Feed intake in 10.7 density group was significantly higher than other groups also the average weight gain of chickens in 15.6 chicks density was significantly lower than other treatments. In total, there was a significant difference in feed conversion ratio and high differences for carcass efficiency of chicks in 12 and 13.6 treatments compared to other treatments. Therefore, according to the findings of this research, broiler chickens in Guinea [27] showed the best performance in 13.6 and 12 density groups. One of the factors affecting the reduction of performance in high stocking density is reducing feed intake and thus reducing nutrient intake for maximum growth [3]. Also Sekerogla *et al.*[27] expressed that 13 chicks per area unit density compared to 17 and 19 has led to the best performance conditions. In the present experiment the stocking density had no significant effect on carcass parameters, which is consistent with [19], they reported that stocking density was ineffective on mortality, breast muscle size and carcass quality. The percentage of carcass, thigh, breast and abdominal fat was not affected by stocking density, which is consistent with the study of other researchers [4,21,28,29].

The evaluation of blood parameters in this experiment showed that the heterophil to lymphocyte ratio in 18 chicks density was higher than 12 chicks density, which was consistent with Kuan *et al.*[30], they reported that with 10.5, 14, 17.5 and 20.8 chicks per m^2 densities, from 2-6 weeks of age, the treatment of 20.8 chicks per m^2 decreased feed intake and improved feed conversion ratio without affecting the growth efficiency. After the sixth week, the highest daily weight gain and feed intake and the lowest feed conversion ratio were observed in the lowest stocking density group, whereas the highest stocking density showed the lowest body weight gain and feed intake and the highest feed conversion ratio and after 4 weeks, also the heterophile to lymphocyte ratio was increased due to stress.

In another study Dozier *et al.*[17] examined the effect of stocking density on growth rate and stress indices in male broiler chickens up to 1.8 kg weight and showed that by increasing stocking density up to 35 d of age, body weight gain, feed intake and feed conversion ratio decreased significantly and the bedding moisture content increased,

which caused to an increase in foot injuries. Also, the results of their study indicated that increasing the stocking density to more than 30 kg body weight per m^2 until the chick's weight reached 1.8 kg had a negative effect on the growth and production rate of poultry meat, while physiological stress indicators (such as plasma corticosterone, glucose, cholesterol, heterophile and lymphocyte) have not changed. Previous studies have evaluated the immunity response and blood parameters of broiler chickens at 3 stocking density (15, 20 and 25 chicks/m^2) in summer [13] and they reported that stocking density had no significant effect on measured blood parameters such as heterophile to lymphocyte ratio and Newcastle titer.

Thaxton *et al*.[30] investigated the stocking density, adaptation and physiological conformity of broiler chickens to environmental conditions through three experiments. They measured stress creator indices including plasma corticosterone, glucose, cholesterol and heterophile to lymphocyte ratio on 49th d of breeding period. In the first experiment, the stocking density consisted of 20, 25, 30, 35, 40, 45, 50 and 55 kg of live weight per m^2, and in the second and third experiments were 30, 35, 40 and 45. The stocking density was calculated based on the final weight of 3.3 kg and finally, linear analysis was used to evaluate the role of stocking density on each physiological parameter. The results showed that stocking density had no effect on adaptation and physiological stress indicators of chickens. In line with this research, Uzum *et al*.[31] stated that when stocking density was 12 and 18 chickens per m^2 under heat stress condition (32-35°C), overall, body weight gain and feed intake were decreased significantly in 18 chicks density, and the heterophile to lymphocyte ratio was lower in 12 chicks density. In the present study, the effect of density on blood parameters, carcass efficiency and internal organs percentage such as spleen, bursa of fabricius, abdominal fat, thigh and breast were not significant. These results were consistent with Zuowei *et al*.[5] and Petek *et al*.[32]. Various factors can effect on the reduction of performance under high stocking density, such as reducing air quality, increasing the ammonia gas and decreasing access to water and feed.

Under the condition of this experiment, the negative effects of stocking density on growth performance traits were quite obvious and the greatest negative effect of stocking density was on reducing feed intake and increasing the moisture content of bedding, although the heterophile to lymphocyte ratio increased in 18 chicks density. The results were obtained when all birds were kept in pens until the end of experimental period. Production per m^2 is one of the important factors in choosing the best stocking density. In high stocking density (18 chicks) the live weight of chick was over 45 kg/m^2 and 32% more than low density (12 chicks). On the other hand, in this experiment, density did not have a significant effect on mortality. Therefore, due to the amount of production per m^2 and the expiring price of chicken, high density had an economic explanation. It seems that if at 30-35 d of age we took a part of chickens out of the pens (decreasing density), by increasing feed intake and compensatory growth, it could also have a positive effect on the results of study. Of course, these assumptions can be the foundation of other researches.

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