



Category: Research Article

Effect of Stocking Density at Brooding Stage on Performances and Stress Response of Broiler Chickens

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ARTICLE DETAILS

Article History

Published Online:

30th December 2020

Keywords

Growth performance, Feed conversion ratio, Tonic immobility

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ABSTRACT

This study aims to assess the performance and the stress response of broilers reared at different stocking densities (SD) during the brooding period. One hundred and forty-four, Cobb500, day-old broiler chicks were randomly stocked at three SDs (T1 = 40, T2 = 80, and T3 = 120 chicks/m²) with three replicates in a completely randomised design for 1-10 days. During the brooding period, daily body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were measured. The stress response was assessed by tonic immobility duration (TI) and blood glucose level (BGL). During the growing period (11-42 days), FI and BWG were measured. On the 42nd day, birds were slaughtered, and the carcass and meat quality traits were assessed. TI duration and BGL were significantly higher ($p < 0.05$) in T3 (257.40 ± 6 s and 280.55 ± 3 mg/dm³, respectively), and T1 recorded the lowest (40.86 ± 6 s and 232.44 ± 3 mg/dm³, respectively) at the end of the brooding period. Significantly higher BWG (2.73 ± 0 kg) and significantly lower FCR (1.35 ± 0) were observed in T1. T1 recorded a higher profit per bird (LKR 462.95 ± 13) compared to T2 (LKR 374.80 ± 13) and T3 (LKR 372.62 ± 13). Further, different stocking densities resulted in similar carcass and breast meat quality characteristics. It is concluded that a lower stocking density (40 birds/m²) during the brooding period can lower the stress response, enhance the growth performance, and profit from broiler production.

1. Introduction

Poultry meat production's performance has been closely linked to growth performances and carcass characteristics of broiler birds. Supply with proper environmental conditions and nutrients is essential for the present-day commercial broiler strains targeted for the intensive production systems [1]. Any deviation from optimum conditions will lead to reduced performance in terms of profitability [2]. To achieve satisfactory performances, housing management in poultry production is essential, and by that, specific floor space for a bird is allocated to have a comfortable environment.

Stocking density is a significant component of the environment that plays a critical role in determining a bird's well-being [3]. Depending on local legislation, production systems, and target body weight, broilers are housed at different stocking densities in modern broiler production systems [4]. In a high stocking density situation, airflow at bird level is often decreased, leading to a reduced dissipation of body heat into the air. Further, high stocking density results in poor air

quality due to insufficient air exchange and increased ammonia concentration. Moreover, higher stocking density decreases access to feed and water by creating a competitive environment. Further, it increases the risk of health-related problems such as leg problems and disease susceptibility [5]. Hence, the overall effect of high stocking density in broiler chickens can reduce growth rate and carcass quality while having a high feed conversion ratio and mortality.

Stocking density could be a critical factor during the brooding period of broiler chicks. The brooding period is a critical phase of a bird's life, and during this period immature thermo-regulatory system transit to a mature system. Therefore, providing a comfortable and healthy environment during brooding is crucial to getting the birds off to a good start. It is possible to speculate that reduced development or damage caused during this crucial period may reduce the performance later in life.

Studies have been conducted to determine the effect of stocking density on the performances and the welfare of broilers [6, 7]. However, the focus of these studies was on the growing stage rather than the brooding period. Further, a limited number of studies were only conducted to find out the effect of stocking density at the brooding stage on the performances of broilers, and these studies were discontinued at the end of the brooding cycle, or the results may be outdated with the fast-changing genetic makeup of the present-day broilers [4]. Moreover, there are recommendations for stocking densities in broilers that are different by country depending on the production systems and target body weight, aiming to minimise fixed costs and maximise profitability. Hence, the recommendations are given by the broiler breeder companies [6,8] may not be applicable in the local condition. Therefore, farmers and researchers use different stocking densities during the brooding period. However, the effect of these different densities has not been evaluated. Hence, the present study's objectives were to assess the effect of stocking density at the brooding period on the production performance, the stress response, meat quality, and carcass characteristics of broilers and on the profit potential of the broiler production.

2. Material and Methods

Fieldwork and laboratory analysis were conducted at Livestock farm and Animal Science Laboratory in the Faculty of Agriculture, Rajarata University of Sri Lanka, Puliyankulama, Anuradhapura, respectively.

2.1 Birds and Experimental Design

A total of 144, day-old, Cobb500 broiler chicks were purchased from a commercial hatchery (Nova Breeders Farm, Matale) and transported to the experimental location. The chicks were randomly assigned to three stocking densities as treatments, designated T1, T2, and T3 in a Completely Randomised Design (CRD). Three stocking densities at the brooding stage were as follows,

- T1 = Stock 40 chick in 1 m²
- T2 = Stock 80 chicks in 1 m²
- T3 = Stock 120 chicks in 1 m²

Each treatment was replicated three times, and the number of chicks was maintained in a replicate according to the relevant stocking density in a constant area.

2.2 Brooding Management

Before the chicks' arrival, cages, feeders, and drinkers were thoroughly cleaned. At the reception of chicks, the average body weight was measured. A glucose solution was provided after arrival to reduce

stress. Chick tonic[®] (Farm Chem (Pvt) Ltd, Sri Lanka), and vitamin E (Selvite-E[®] Dymac (Pvt) Ltd, Sri Lanka) were provided at 3rd, 4th, 5th, and 6th days. During the brooding period, commercial broiler starter feed was provided, and access to feed and water was not restricted. Temperature, lighting, and ventilation were controlled, and birds were reared under good hygienic conditions. Electric bulbs were used to provide the initial heating and lighting, and paddy husk was used as the litter material. Brooding management was practised up to ten days (10) of the age of chicks.

2.3 Broiler Management Practices

After completion of the brooding period, birds were stocked in separate cages according to treatments and replicates. However, until the slaughtering age, birds were reared under the same conditions. All the birds were vaccinated against infectious bursal disease (IBD) at 7 and 14 days. The broiler starter diet was continued up to day 21, and afterwards, it was changed to the broiler finisher diet, which was continued until slaughtering (42nd day). Throughout the study period, access to feed and water was not restricted. All other general management practices were followed until the birds were slaughtered.

2.4 Slaughtering of Birds

At the end of the expected period, three birds were randomly selected from each replicate at 10 and 42 days of age, and they were slaughtered. *Ante-mortem* inspections were done by visual observations. The birds were fasted for 12 hours. They were held in killing cones, and major blood vessels were severed within 10 seconds and kept for complete bleeding for at least 2 minutes. Scalding was done, and then a cut was made at the end of the abdomen, and the abdominal cavity was opened, and the digestive tract, respiratory tract, and heart were removed. Similarly, the liver was removed, and the gall bladder was peeled away. Gizzard was cleaned, and the inner layer was removed. Further, the spleen, thymus gland, and bursa were carefully removed and weighed.

2.5 Data Collection

The growth performances of the broiler birds were assessed by feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), live weight (LW), and dressing percentage (DP). The given amount of feed and the remained amount of feed per pen were measured and FI was calculated daily throughout the study period. During the brooding period, BWG was measured daily, and FCR was calculated daily. After the brooding period, BWG and FCR were calculated weekly. LW and carcass weights were recorded and the DP was calculated.

The birds' stress response was measured by performing the tonic immobility test, measuring blood glucose level and the weights of the lymphoid organs, and calculating mortality percentage. According to the method described by Ghareeb et al. [9], the tonic immobility test was performed. A blood glucose monitoring system (FreeStyle, Optimum) was used, and the blood glucose level was measured according to the manufacturer's instructions. The weight of lymphoid organs (bursa, spleen, and thymus) was measured. The daily mortality was recorded during the study period.

Carcass and breast meat quality characteristics were evaluated, and the weight of the internal organs (liver, gizzard, and heart) was measured to interpret the carcass characteristics. Further, according to the methods described by Karunanayaka et al. [10] meat colour, pH, and water holding capacity (WHC) were measured as meat quality characteristics in breast muscle.

The cost-benefit analysis was done to evaluate the cost-effectiveness of the assessed stocking densities. Hence, the feed cost and the other expenses (chick, labour, electricity, water, and medicine) were recorded separately for the brooding and growing period, and the total cost per bird was calculated. Total revenue was calculated by multiplying the carcass weight of chickens by the unit price.

2.6 Statistical Analysis

Data on growth performances, stress responses, carcass, and meat quality parameters were analysed using One-way Analysis of Variance (ANOVA) procedure in Statistical Analysis Software (SAS), Version 9.0 [11]. Means were compared with Turkey's Standardized Range Test (TSRT). Statistical significance was declared at $p < 0.05$.

3. Results and Discussion

3.1 Growth Performances

Effect of stocking density on FI, BWG, and FCR of the broilers at brooding (1-10 days) and growing (11-42 days) periods are presented in Table 1.

When considering the brooding period, there was a significant difference ($p < 0.05$) in feed intake among different treatments (Table 1). According to the results, when increasing the stocking density, the average feed intake was decreased. However, after the brooding period, providing the uniform conditions for all treatments, there was no significant difference ($p > 0.05$) in FI among the three treatments.

At the brooding period, the body weight gain results indicated a significant difference ($p < 0.05$) among the three treatments. The reduction of feed intake observed with increasing stocking density may be the underlying reason for reducing body weight gain. Results from day 11 to 42 showed a significant difference ($p < 0.05$) among three treatments, and the highest gain was recorded for T1.

The result of the total study period was the same as the result of the growing period. According to the study results, it can be suggested that higher stocking density during the brooding period decreases the average body weight gain.

During the brooding period, the FCR of the birds in different treatments were not different ($p > 0.05$) (Table 1) and during the growing period, the FCR of the birds in different treatments were significantly different ($p < 0.05$). When considering the total study period, there was a significant difference ($p < 0.05$) among the three treatments. T1 recorded the lowest value, which may be attributed to recording higher body weight gain than feed intake.

Table 1: Average feed intake, body weight gain and feed conversion ratio of broilers raised at different stocking densities during the brooding period

Period	Parameter	Treatments			p-value	SEM
		T1	T2	T3		
Brooding Period	FI (g)	320.44 ^a	308.08 ^a	242.88 ^b	0.00	6.56
	BWG (g)	293.93 ^a	286.17 ^a	258.09 ^b	0.01	6.09
	FCR	1.09	1.07	0.94	0.10	0.04
Growing Period	FI (g)	3313.87	3454.40	3227.67	0.22	82.73
	BWG (g)	2460.62 ^a	2222.26 ^b	2141.79 ^b	0.02	36.21
	FCR	1.35 ^b	1.55 ^a	1.50 ^{ab}	0.00	0.03
Total Period	FI (g)	3634.38 ^{ab}	3762.53 ^a	3470.69 ^b	0.01	79.75
	BWG (g)	2738.03 ^a	2508.43 ^b	2412.13 ^b	0.00	43.28
	FCR	1.33 ^b	1.55 ^a	1.44 ^a	0.02	0.03

Data presented as mean \pm SEM (Standard Error of the Mean). ^{a,b,c} means within the same row with different superscripts are significantly different ($p < 0.05$). *The treatments were, T1= 40 birds stocked at 1 m², T2= 80 birds stocked at 1 m², T3= 120 birds stocked at 1m² at the brooding period (1 – 10 days)

BWG and FCR results of the present study agreed with the findings of several previous studies [4, 6, 13] which showed birds at lower stocking densities at the brooding period gained more weight. The higher stocking density per unit area in intensive poultry production systems is to lower the production costs [1]. However, it may cause stress to the birds, which finally affects their productivity. Further, the physical access to the feeders is limited due to increased stocking density and the competition between birds to get to the feeder [12]. This may be affected negatively on the feed intake and thereby possibly reduce the broiler birds' growth rate [12]. Further, in a high stocking density situation, heat stress for the birds can be increased due to the reduction of the airflow at the bird's level. Hence, when birds are subjected to these chronic stressors including poor air quality due to inadequate air exchange, increased ammonia, and reduced access to feed and water, it can reduce growth rate, feed efficiency, and liveability [6, 14].

3.2 Stress Responses

The bursa, spleen, and thymus weights of the broilers were not different ($p>0.05$) at the end of the brooding period (Table 2). Further, the weights of the bursa and spleen were similar ($p>0.05$) at the end of the study period among the three treatments (T2). According to the results of this study, it can be suggested that the stocking density of the brooding period does not significantly affect the lymphoid organ weights. Pope [15] reported that the weights of the lymphoid organs (spleen, bursa, and thymus) might decrease in response to higher levels of stress and those are commonly assessed as measures of immune status in poultry. According to the findings of Heckert et al. [16] and Thaxton et al. [17], high stocking densities can be stressful and negatively affect broiler immunity.

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In contrast to the present study's findings, Qaid et al. [4] reported that the higher the stocking density, the lower the weights of the lymphoid organs in an age-dependent manner. This implies that, under stressful conditions, birds are more suffered, resulting in less developed lymphoid organs. The average weights of lymphoid organs in the present study were agreed with previous studies, which showed that the relative weights of lymphoid organ weights are not affected by stocking density at the market age [8, 16].

At the end of the brooding period, there was a significant difference ($p<0.05$) among three treatments in the tonic immobility test results (Table 3). It can be concluded that tonic immobility duration was increased with the stocking density during the brooding period. Due to the provision of the same stocking density to all treatments, at the slaughtering age (42 days) there was no significant difference ($p>0.05$) among the three treatments. It was observed that the tonic immobility duration was increased with age.

Siegal [18] reported that different stressors could increase stress hormone release; corticosterone and elevate the corticosterone concentration in the plasma.

Table 2: Relative weights of lymphoid organs of broilers raised at different stocking densities during the brooding period

Age at sampling	Lymphoid organ (% live weight)	Treatments [*]			p-value	SEM
		T1	T2	T3		
10 days	Bursa	0.22	0.21	0.27	0.28	0.02
	Spleen	0.11	0.10	0.11	0.59	0.00
	Thymus	0.62	0.53	0.52	0.44	0.05
42 days	Bursa	0.06	0.08	0.07	0.72	0.01
	Spleen	0.60	0.60	0.63	0.92	0.00

Data presented as mean \pm SEM (Standard Error of Mean). *The treatments were, T1= 40 birds stocked at 1 m², T2= 80 birds stocked at 1 m², T3= 120 birds stocked at 1m² at the brooding period (1 – 10 days)

Table 3: The duration taken for tonic immobility reaction by broilers raised at different stocking densities during the brooding period

Age at test	Duration (s) to complete the reaction in treatments [*]			p-value	SEM
	T1	T2	T3		
10 days	40.86 ^c	126.80 ^b	257.40 ^a	0.00	6.01
42 days	338.80	312.13	320.80	0.11	7.54

Data presented as mean \pm SEM (Standard Error of Mean).

*The treatments were, T1= 40 birds stocked at 1 m², T2= 80 birds stocked at 1 m², T3= 120 birds stocked at 1m² at the brooding period (1 – 10 days).^{a,b,c} means within the same row with different superscripts are significantly different ($p < 0.05$).

Further, Bedánová [19] observed that plasma corticosterone positively correlated with the fear-behaviours of the chickens, which means a longer tonic immobility reaction. Hence, during the brooding period, high stocking density may act as a stressor, and due to that, the birds showed fear behaviours, measured as tonic immobility.

At the end of the brooding period, the blood glucose levels (mg/dm³) were reported as 232.44, 262.89, and 280.55 for treatments 1, 2, and 3, respectively. Accordingly, a significant difference ($p < 0.05$) in blood glucose level was recorded at the end of the brooding period. The results indicated that the blood glucose level was increased with increasing stocking density. A similar finding was reported by Virden and Kidd [20], but Dozier et al. [21] found that there was no relationship between blood glucose level and the stocking density of the poultry birds. On the other hand, Summers [22] reported that when poultry birds are subjected to stressors, adrenaline is being secreted and it mobilised the glycogen reserves of the body, resulting in lower blood glucose levels.

3.3 Carcass and Meat Quality Characteristics

The mortality percentages of the brooding period were not significantly different ($p > 0.05$) among the three treatments (Figure 1). This finding was agreed with Qaid et al. [4], who reported that the brooding period's mortality percentage was not influenced by stocking density or broiler chicks' age.

There was no significant difference ($p > 0.05$) observed in the organ weights except the gizzard and dressing percentage among the three treatments; however, there was a significant difference ($p < 0.05$) among the three treatments in carcass weight (Table 4). It suggests no significant effect of stocking density at the brooding period to the final carcass characteristics. However, possible to affect the final carcass weight by affecting the final yield of the birds.

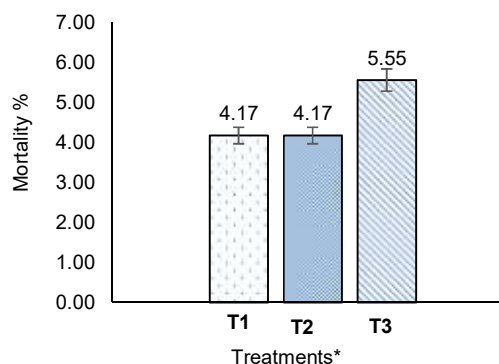
Table 4: Carcass characteristics and relative organ weights of broilers raised at different stocking densities during the brooding period

Carcass Characteristics	Treatments [*]			p-value	SEM
	T ₁	T ₂	T ₃		
Dressing %	72.23	72.28	73.03	0.09	0.23
Carcass weight (kg)	2.01 ^a	1.84 ^b	1.78 ^b	0.00	30.52
Liver (%)	1.54	1.56	1.59	0.81	0.05
Heart (%)	0.38	0.39	0.40	0.75	0.01
Gizzard (%)	0.72 ^a	0.90 ^b	0.80 ^{ab}	0.09	0.05
Abdominal fat (%)	1.60	1.73	1.88	0.54	0.17

Data presented as mean \pm SEM (Standard Error of Mean).

*The treatments were, T1= 40 birds stocked at 1 m², T2= 80 birds stocked at 1 m², T3= 120 birds stocked at 1m² at the brooding period (1 – 10 days).^{a,b,c} means within the same row with different superscripts are significantly different ($p < 0.05$).

*The treatments were, T1= 40 birds stocked at 1 m², T2= 80 birds stocked at 1 m², T3= 120 birds stocked at 1m² at the brooding period

**Figure 1: Mortality percentage of the broilers allocated for different stocking densities during the brooding period**

The present study results were agreed with Dozier et al. [21], who reported that, though the carcass weight varies with the stocking density, some of the quality parameters such as carcass yield, abdominal fat content are not affected. Further, it may be assumed that lower carcass weights at higher stocking density may be linked to the reduction in feed intake [21], which occurred in the present study.

There was no significant difference ($p > 0.05$) in meat quality parameters; colour, pH, and water holding capacity among the three treatments (Table 5). These results indicated that the brooding period's stocking density does not significantly affect the meat quality characteristics of broilers. Similarly,

Sekeroglu et al. [23] also reported that there was no significant difference in carcass yield, pH, meat colour, and internal organ weights of different stocking density groups of broilers at age 0-21 and 0-42 days.

Table 5: Meat quality characteristics of broilers raised at different stocking densities during the brooding period

Meat quality characteristics	Treatments [*]			p-value	SEM
	T1	T2	T3		
Colour (L*)	54.13	54.34	56.50	0.39	1.25
pH	6.01	5.97	5.94	0.72	0.05
WHC (%)	69.88	72.58	72.58	0.48	1.73

Data presented as means \pm SEM (Standard Error of Mean). *The treatments were, T₁= 40 birds stocked at 1 m², T₂= 80 birds stocked at 1 m², T₃= 120 birds stocked at 1 m² at the brooding period (1 – 10 days)

3.4 Cost-Benefit Analysis

Table 6: Total cost, total revenue, and profit per bird of different treatments

Parameter/bird	Treatments [*]			p-value	SEM
	T1	T2	T3		
Cost	503.18 ^{ab}	512.12 ^a	485.57 ^b	0.04	5.68
Income	966.13 ^a	886.67 ^b	858.19 ^b	0.00	14.89
Profit	462.95 ^a	374.55 ^b	372.62 ^b	0.00	13.98

Data presented as mean \pm SE (Standard Error of Mean). ^{a,b,c} means within the same row with different superscripts are significantly different ($p < 0.05$). *The treatments were, T₁ = 40 birds stocked at 1m², T₂ = 80 birds stocked at 1m², T₃ = 120 birds stocked at 1m² at the brooding period (1 – 10 days).

Cost refers to feed, labour, electricity, medicine, and water costs separately for the brooding period and growing period. The cost-benefit analysis of this experiment was shown in Table 6. The cost of inputs such as chicks, labour, medicine, electricity, water, and other expenditures was similar among all treatments. However, due to the differences in the birds' feed intake, feed cost was different among the three treatments. Therefore, the total cost was approximately varying. There was a significant difference ($p < 0.05$) among the three treatments in total cost, total revenue, and profit. T2 expended the highest cost compared to the other treatments due to the birds' higher feed intake. According to the carcass weights shown in Table 5, T1 recorded the highest revenue compared to T2 and T3, respectively.

4. Conclusion

The results indicated that broilers' performances affected by the stocking density at the brooding period, though, birds received a similar condition in their latter part of life. Further, high stocking density at the brooding period can be a stressor for the broiler birds, and it may determine the final profit of the production by affecting the yield of the bird. Though the brooding period's stocking density was a stressor for the birds, it did not influence meat quality and the carcass characteristics. Hence, a lower stocking density (40 birds/m²) during the brooding period lowered the stress response, enhanced the growth performance, and profit of broiler production.

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