Effect of Stocking Density and Dietary Sulfur Amino Acids on Welfare Indices of Broiler Chicks

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***Abstract*—Stocking density is a key point in broiler welfare and cost of production. This experiment was carried out to investigate effect of three stocking density (12, 16 and 18 birds per m^2) and two levels of sulfur amino acids (S-AA) (100 and 120% requirement) on welfare indices (gait score, breast burn, hock burn, footpad dermatitis, feathering score and tonic immobility) of 390 day-old chickens. Most favorable gait score, footpad dermatitis, hock burn and breast burn obtained using 12 chickens per m^2 at 35 and 42d. Increasing levels of S-AA significantly decreased footpad dermatitis, hock burn and breast burn (P<0.05). Tonic immobility also increased remarkably with increasing of stocking density and 120% S-AA decreased tonic immobility times (P<0.05). Chickens had more feather coverage in 120% S-AA and in density of 12 chickens (P<0.05). In conclusion, lower stocking densities and higher level of sulfur amino acids improved welfare indices of broiler chicks.**

***Index Terms*—broiler chickens, stocking density, sulfur amino acid, welfare**

I. Introduction

Animal welfare is an important issue around the world. The issue of stock density is a key point in animal welfare [1]. One of the important factors in flock yield is flock density. However by increasing flock density, production rate, costs of production, worker, fuel, etc. increase but it can damage and birds and their welfare condition decreased. Along with increase stock density, some performance and welfare indices decline such as: body weight, feed consumption, flock density, leg health and frequencies of tibial dischodroplasia, gait score, carcass bruising and scratching, disturbances or exacerbated mortality related to heat stress increase [2]-[5].

Between different strains of broiler chicks, methionine is considered as the first limiting amino acid, because in diets base on protein plant sources, this amino acid is limited and necessary for feather growth and protein synthesis. Methionine plays key roles in poultry such as: 1) essential amino acid for protein synthesis; 2) methyl donor; 3) involved to polyamine synthesis; 4) sulfur donor [6]. It seems that between essential amino acid, the interaction between methionine and other amino acid is more, because methionine plays a key role in many metabolic chains. Base on resultant experiment, methionine is an excellent promoter for healthy birds. Methionine supplementation improve white blood cell immigration, humoral and cellular immune responses [7], [8], total serum protein enhancement, globulin, antibody response against Newcastle viruses, aspartate amino acid transferase and alanine amino transferase reduction [9], enhancement of total antibody, response to mitogen phytohemaglutenine (probably related to T-cell). Therefore, methionine deficiency leads to reduction of humoral immunity and none-specific immunity in broiler chicks [10].

One of the important structure body of poultry is feather. Feathers act like insulating material and reduce maintenance energy needs, also, feathers prevent to skin abrasions and infection [11]. Number of downgrading birds at slaughtering increase by poor feathering thus net profit reduces. Feathering influenced nutritional factors and environment conditions [12]. Essential amino acids are an example of nutritional factors that affect feathering, sulfur amino acid, valine, lysine and arginine have been shown to affect feather development [13].

Velmurugu *et al.* reported that lymphoid organs weight (spleen and bursa) by increasing stock density decreased which shows their stress [14]. As a results of Onbasilar *et al.* percentage weight of heart, tunic immobility, heterophil to lymphocyte ratio, serum glucose concentration and serum cholesterol levels was significantly higher in 17.5 pound/m^2 compared to 11.9 pound/m^2 [15]. Sorensen *et al.* [16] in order to measurement of leg weakness affected by aging and stock density performed an experiment and reported high stock density even until 4 wk had marketable impact on walking ability, prevalence of leg weakness was less in low stock density. Heckert *et al.* [17] performed an experiment to evaluate the effect of stock density on

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immune response, immunity parameters (lymphoid organs weight, antibody titer against sheep red blood cell, heterophil to lymphocyte ratio at 32 and 42 days of age; they stated by increasing stock density bursa weight and its percentage significantly decreased but this treatment had no effect on other indices.

Ojano and Waldroup [18] determined 0.38, 0.44 and 0.05% methionine levels influenced on growth and meat yield of broiler chicks, also mortality rate decreased by increasing methionine level. Ahmed and Abbas [19] using different level of methionine 0, 100, 110, 120 and 130% recommended by NRC, significant impact observed on feed intake, feed conversion ratio and protein yield, this results in agree with Pillai *et al.* [20] that they showed the maximum feed consumption, weight gain and feed efficiency by increasing methionine level was reached.

Since birds welfare is influenced by nutritional and environmental factors and in most of time these parameters mixed together happened in poultry husbandry but unfortunately number of literature in this category is scared; for this reason the present study was performed to investigate the effect of three levels of stocking density and two levels of sulfur amino acid on welfare indices of broiler chicks.

II. Materials and Methods

A. Bird Rearing and Treatments

A total 390 day-old chicks (Ross 308) were weighed on arrival time and randomly assigned to six experimental treatments with five replicates. Water and feed were provided *ad-libitum* throughout the trial, diets were provided in mash form in starter phase (1-14 d); grower (15-28 d) and finisher (29-42 d). Lighting was prepared 23 h/d. Experimental diets included of three levels density (12, 16 and 18 broiler in each meter square) and two levels sulfur amino acid (100% and 120% of Ross requirement). At the arrival time temperature was set on 35 ℃ then 2 ℃ decreased each week till 26 ℃ and fix kept by end of experiment.

Measuring welfare parameters: Welfare indices including walking ability, feather growth, chest burns, hock buns and foot pad lesions at 35 and 42 days of age and tonic immobility were determined at 42 days of age. According to methods described by kestin *et al.* [21] and Thomas *et al.* [22] walking ability assessed. Score 1: the bird had a slight defect that was difficult to define precisely; score 2: indicated a definite and indefinable defect in gait; score 3: indicated that the bird had an obvious gait defect that affected ability to move about; score 4: indicated that the bird had a severe gait defect; score 5: indicated complete lameness (totally immobile). The measurement was made on an individual basis by allowing the bird to walk in a home pen in area about 2m×4m. The observer maintained a distance of about 2m. The hock burns were determined, score 1: no burns; score 2: mild burns; score 3: severe burns. According to methods described by Gyles *et al.* [23] feather cover of chickens was evaluated, score 1: relatively large amount of skin showing; score 2: medium amount of skin showing and score 3: almost complete feather cover with small amount of skin showing. Foot pad lesions measured using three point scale, score 1: no lesions; score 2: mild lesions; score 3: severe lesions.

Tonic immobility test: A total of 120 birds (10 birds per each treatment) at 35 and 42 d were tested individually for duration of Tonic Immobility (TI). The birds were carried to a separate room (no visual contact with other birds) and subjected to TI measurements. TI was induced by laying the bird down on its right side and gently restraining it by hand for 15s. Then the hand was removed, and the experimentalist retreated approximately 1 m out of sight of the bird and remained silent. The time was measured from withdrawal of the hand until the bird straightened up. If the bird straightened up in less than 10 s, it was restrained repeatedly. If TI was not induced after 3 attempts, the duration of TI was considered 0s. If the bird did not straighten up within 10 min, it was removed and given the maximum duration of 600s. The number of inductions required to attain TI was also recorded for each bird.

Statistical analysis: The data were subjected to analysis of variance procedure appropriate for a factorial (2×3) arrangement in a completely randomized design with the main effects of stock density and sulfur amino acids. Means were compared using LSD test and were considered to be significant at $P<0.05$.

III. Results and Discussion

According to results of an experimental diets and treatments in Table I, at 35 days breast burns was not observed in none of experimental diets, while with stock density increasing walking score significantly increased and highest score related to 18 birds/m^2. Foot pad and hock burns increased by increasing stock density. The highest foot pad burns was for 18 birds/m^2 and highest hock burns was for 16 birds/m^2. According to Cheetsaz [24] statements hock burns and foot pad burns have direct contact with each other, therefore by increasing stock density the problems of foot will be increased because of high stock density and litter moisture increases. Accordance with Dozier *et al.* [25] and Mayne [26], wet litter and foot problems simultaneously increases with high density. Base on Feddes *et al.* [1], best poultry performance will be achieved in 10 pieces per m^2. So that with increasing level of methionine, all of the welfare indices significantly decreased ($P<0.05$).

TABLE I. Effect of Stock Density and Sulfur Amino Acids Levels on Welfare Indices at 35 Days

Stock density	Gait score	Foot pad burns	Hock burns score	Breast burns
12 birds/m^2	$0.93^b \pm 0.09$	$0.02^c \pm 0.01$	0^b	0
16 birds/m^2	$1.86^a \pm 0.11$	$0.42^b \pm 0.07$	$0.75^a \pm 0.08$	0
18 birds/m^2	$2.07^a \pm 0.10$	0.73 ± 0.10	$0.73^a \pm 0.10$	0
S-AA level				
100%	$1.84^a \pm 0.09$	$0.63^a \pm 0.08$	$0.63^a \pm 0.08$	$1.60^a \pm 0.08$
120%	$1.41^b \pm 0.09$	$0.16^b \pm 0.03$	$0.16^b \pm 0.03$	$0.27^b \pm 0.04$

$^{a-b}$ Values in the same column not sharing a common superscript differ significantly ($P<0.05$).
S-AA: Sulfur amino acids

The results of experimental diets on welfare indices at 42 days are summarized in Table II, by increasing stock density welfare indices (walking ability, foot pad burns, hock burns and breast burns) compared to 12 birds/m^2 significantly increased (P<0.05). When methionine level increased, foot pad burns, breast burns significantly decreased, walking ability decreased although this reduction was not significant. The results of fear reaction (tonic immobility) different ages are presented at in Table III and Table IV.

TABLE II. EFFECT OF STOCK DENSITY AND SULFUR AMINO ACIDS LEVELS ON WELFARE INDICES AT 42 DAYS

Stock density	Gait score	Foot pad burns	Hock burns score	Breast burns
12 birds/m^2	1.32^b±0.11	0.03^c±0.02	0^c±0	0^b±0
16 birds/m^2	1.70^a±0.12	0.90^b±0.11	0.28^b±0.05	0.07^b±0.03
18 birds/m^2	1.86^a±0.11	1.33^a±0.12	0.67±0.09	0.23^a±0.04
S-AA level				
100%	1.69±0.09	1.03^a±0.10	0.47^a±0.07	0.14^a±0.03
120%	1.56±0.09	0.48^b±0.07	0.17^b±0.03	0.06^b±0.02

$^{a-b}$ Values in the same column not sharing a common superscript differ significantly (P<0.05).
S-AA: Sulfur amino acids

TABLE III. EFFECT OF STOCK DENSITY AND SULFUR AMINO ACIDS LEVELS ON FEAR REACTION AT 28 DAY

Stock density	Tonic immobility	
	Try	Duration (Sec)
12 birds/m^2	2.51a±0.07	155.23b±13.01
16 birds/m^2	2.05b±0.08	281.08a±17.19
18 birds/m^2	1.82b±0.09	305.60a±18.12
S-AA level		
100%	1.94±0.07	289.68a±15.32
120%	2.30a±0.06	205.63b±12.68

$^{a-b}$ Values in the same column not sharing a common superscript differ significantly (P<0.05).
S-AA: Sulfur amino acids

TABLE IV. EFFECT OF STOCK DENSITY AND SULFUR AMINO ACIDS LEVELS ON FEAR REACTION AT 42 DAYS

Stock density	Tonic immobility	
	Try	Duration (Sec)
12 birds/m^2	2.38^a±0.07	147.40^b±12.55
16 birds/m^2	1.98^b±0.09	283.03^a±18.48
18 birds/m^2	1.82^b±0.08	312.83^a±17.49
S-AA level		
100%	1.97±0.07	292.70^a±15.61
120%	2.15^a±0.07	203.56^b±12.94

$^{a-b}$ Values in the same column not sharing a common superscript differ significantly (P<0.05).
S-AA: Sulfur amino acids

When number of birds per pen increased, the number of try and duration significantly decreased and increased, respectively (P<0.05). Minimum number of try and most duration was observed in 18 pieces per pen. By increasing number of birds from 12 to 16 and 18, number of try. As observed, by increasing methionine level, number of try significantly increased while duration significantly decreased (P<0.05). Contemporary with increasing number of birds, the number of try significantly decreased (P<0.05) and duration significantly increased (P<0.05). Fewest number of try was observed in 18 pieces per m^2 and more duration was belong to this density. Duration in 120% methionine level compared to 100% methionine significantly decreased (P<0.05).

Only feather covering in tail influenced by sulfur amino acid level. At 35 days of age feather cover in all parts of body except breast area significantly influenced by stock density (P<0.05). The effect of dietary treatments on feather indices at different ages are presented in Table V and Table VI, stocking density had significant effect on feather covering at 28 and 35 days, so in 12 birds/m^2 more feather cover was observed than other treatments. The more feather cover in this density is related to more feed consumption than other densities. In 16 and 18 densities wet litter increased and markedly influenced on feather cover especially in legs and breast but feather rate in other parts of body were weak. Pourreza [27] stated, feather growth is influenced by environment condition. For ideal growth of feather, moisture, sufficient temperature, protein and essential amino acid are needed.

TABLE V. EFFECT OF STOCK DENSITY AND SULFUR AMINO ACIDS LEVELS ON FEATHER COVERING AT 28 DAYS

Stock density	Feather covering score			
	Back	Tail	Wing	Total
12 birds/m^2	2.51^a±0.08	1.90±0.08	2.66^a±0.09	2.58^a±0.07
16 birds/m^2	2.25^b±0.09	1.86±0.08	2.37^b±0.09	2.32^b±0.08
18 birds/m^2	2.13^b±0.08	1.70±0.07	2.27^b±0.09	2.11^b±0.07
S-AA level				
100%	2.31±0.07	1.7b±0.06	2.43±0.07	2.27±0.07
120%	2.29±0.07	1.94^a±0.06	2.42±0.07	2.40±0.06

$^{a-b}$ Values in the same column not sharing a common superscript differ significantly (P<0.05).
S-AA: Sulfur amino acids

TABLE VI. EFFECT OF STOCK DENSITY AND SULFUR AMINO ACIDS LEVELS ON FEATHER COVERING AT 35 DAYS

Stock density	Feather covering score			
	Back	Tail	Wing	Total
12 birds/m^2	4.11^a±0.08	3.56^a±0.09	4^a±0.09	3.96^a±0.08
16 birds/m^2	3.35^b±0.10	3.18^b±0.10	3.31^b±0.11	3.35^b±0.09
18 birds/m^2	3.22^b±0.10	2.67^c±0.10	3.12^b±0.10	3.08^c±0.08
S-AA level				
100%	3.40^b±0.08	2.93^b±0.08	3.21^b±0.09	3.16^a±0.07
120%	3.71^a±0.08	3.34^a±0.09	3.73^a±0.08	3.76^b±0.07

$^{a-b}$ Values in the same column not sharing a common superscript differ significantly (P<0.05).
S-AA: Sulfur amino acids

In conclusion, In order to achieve best performance and welfare, the ideal stock density should be considered, therefore, 12 birds/m^2 is acceptable. In terms of nutrition, increases levels of sulfur amino acids positive effect on foot pad burns, hock burns and breast burns. In 12 birds/m^2 density, feather covering, and tonic immobility duration is acceptable.

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