Journal of Applied Veterinary Sciences, 7 (3): 41-49.
ISSN: Online: 2090-3308, Print: 1687-4072
Journal homepage : https://javs.journals.ekb.eg

The effect of Guanidinoacetic Acid Supplementation on Behavior and Welfare of Broiler Chickens Reared at Two Stocking Densities

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ABSTRACT

The experiment was conducted to investigate the potential effects associated with dietary guanidinoacetic acid (GAA) supplementation and different stocking densities on behavioral patterns and welfare indicators of broiler chickens. In total, 364 Arbor-Acres one-day-old mixed-sex broiler chicks were randomly assigned to four treatments of 2 × 2 factorial arrangement in a completely randomized design experiment (seven replicates each). The first treatment (T1): standard stocking density (SSD) reared under 10 birds/m^2 without GAA supplements, treatment 2 (T2): SSD (10 birds /m^2) with GAA supplements at the rate of 0.06% (600 g/ton feed), treatment 3 (T3): high stocking density (HSD) reared under 16 birds/m^2 without GAA supplements, treatment 4 (T4): HSD (16 birds/m^2) with 0.06 % GAA supplements. Scan sampling behavior observations were recorded through video recording cameras and welfare indices were measured using 3 levels scale score. Results of the current study showed a positive increase in foraging and comfort behavior in SSD, while an increase in standing and a decrease in sitting was observed in HSD. Furthermore, GAA resulted in raising foraging behavior in SSD, however, no effect was noted on standing, resting, and comfort behavior. Based on interaction analysis, a higher standing behavior was observed by GAA supplements in the HSD group. Regarding the footpad dermatitis (FPD) and hock burns score, HSD was reported to negatively affect leg health as it significantly increases FPD and hock burns score, however, neither GAA nor interaction between Stocking density x GAA had a significant effect on welfare indices. In conclusion, HSD had a negative impact on the behavior and welfare of broiler chickens as it showed adverse effects on leg health. However, the positive effects of GAA on some behavioral patterns were more pronounced in the SSD group than in the overcrowding conditions.

Original Article:
DOI: HTTPS://DX.DOI.ORG/10.21608/JAVS.2022.133166.1144
Received
Accepted
Published in

Keywords: Behavior, Broilers, Footpad dermatitis, Guanidinoacetic acid, Hock burn, Stocking density, Welfare.

J. Appl. Vet. Sci., 7(3): 41-49

INTRODUCTION

Several poultry producers need to achieve the maximum outcome of broiler industry by increasing the number of birds in a limited floor space (**Rashidi *et al.*, 2019),** but this practice affected negatively the welfare aspects of birds. Stocking density is a significant environmental factor that influences the development of poultry farming, which has caused widespread concern **(Li *et al.*, 2019).** Stocking density is considered an important issue from the economic point of view in broiler industry, recommendations of the optimum stocking density differ widely according to country and organizations **(Bessei, 2006)**. The maximum gainfulness could be achieved by raising the number of birds per unit area if the productive performance, health parameters and welfare aspects of the reared birds have been successfully accomplished **(Chegini *et al.*, 2019).**

Several studies carried out to investigate the effect of overcrowding conditions on broiler chicken welfare indicators and behavior patterns. Welfare indices such as; gait score, hock burn and foot pad dermatitis were found to be negatively affected in overcrowding conditions while improved in standard stocking density (SSD). on the other hand, **Son, (2013)** showed that behavior patterns such as locomotion activity was reduced in high stocking density (HSD). As reported by **Yanai *et al.*, (2018)** higher stocking density increase the mortality rate, the incidence of leg problems and contact dermatitis, also disturbing the resting behavior and negatively affected locomotion and preening behavior.

Physiological stress caused by HSD in broiler, leading to changes in their metabolism and reduced energy required for their activity (**Simsek *et al.*, 2009).** Different strategies have been adopted to alleviate the negative impacts of HSD induced muscle energy expenditure in broilers. Creatine is a key component in energy metabolism, particularly in muscle cells needed for growth and activity. The role of creatine in energy metabolism is presented in donating phosphate groups that can regenerate ATP from ADP at the site of ATP need (**Portocarero and Braun 2021).**This system is called creatine-ATP recycling system and occur only in cells which have a major variability in energy needs like muscle cells for their growth and behavioural activity **(Michiels *et al.*, 2012)**.

Because of its instability and high cost, creatine may not be suitable for use as a feed supplement and can be substituted by guanidinoacetic acid (GAA) supplements **(Ostojic 2015; Vranes *et al.*, 2017).** GAA is formed from glycine and arginine amino acids in the kidney and methylated by S-adenosylmethionine to creatine in the liver. As reported by **Michiels *et al.*, (2012)** GAA supplementation resulted in improving the activity of broiler chickens. CreAmino®, a granulated product of GAA, considered an immediate precursor of creatine for energy supply **(Boney *et al.*, 2019)**.

Several authors, **Nasiroleslami *et al.*, (2018); Amiri *et al.*, (2019)** investigated the effects of GAA supplementation on stress indicators in broiler under heat or cold stress and declared that GAA has ameliorating effect of stressful conditions exposed by broiler chickens**.** in addition **Michiels *et al.*, (2012)** declared that GAA supplementation resulted in improving the activity of broiler chickens. To the best of our knowledge, there is no comprehensive study has yet been carried out to investigate the effect of dietary supplementation of GAA on behavioral patterns and welfare indices of broiler chickens reared under HSD. So, the aim of this study is to declare the ameliorative effect of Guanidinoacetic acid (GAA) supplement at the rate of 0.06% on the behavior and welfare of broiler chickens reared under HSD (16 birds / m^2).

MATERIALS AND METHODS

Ethical statement:

The experimental protocol of the current study was approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Cairo University, Egypt (IACUC approval No. Vet CU28/4/2021/311).

Birds, management, and dietary treatments:

The experiment was carried out in the Poultry Research Unit of Veterinary Hygiene and Management department, Faculty of Veterinary Medicine, Cairo University, Egypt. A total of 364 one-day-old, mixed sex Arbor-Acres chicks were purchased from a commercial hatchery. Chicks were individually weighed and randomly assigned into 4 experimental groups with a 2 × 2 factorial arrangement of treatments. Each treatment was subdivided into 7 replicates .The first treatment (T1): n= 70, standard stocking density (SSD) 10 birds/m^2 without GAA supplements, treatment 2 (T2): n= 70, SSD (10 birds / m^2) with GAA supplements at the rate of 0.06% (600 g/ton feed), treatment 3 (T3): n= 112, high stocking density (HSD) 16 birds / m^2 without GAA supplements, treatment 4 (T4): n= 112, HSD (16 birds/m^2) with 0.06 % of GAA supplements. The birds of each replicate were housed in a separate floor pen (1 x 1 m) with 7-10 cm layer of clean wood shaving.

The environmental temperature was set at 32°C in the first three days of brooding period then gradually reduced till reaching 24 °C and maintained until the end of the study, the relative humidity was ranged from 45-65%. Birds were exposed to a continuous lighting program of a daily 24-h light /0-h dark cycle till the end of the experiment. Corn-soya bean meal-based diet in the form of crumble (starter) and pellets (grower and finisher) diet was supplied ad libitum. Basal diets (Table 1) were formulated to meet the nutritional requirements of the broiler chicks (Arbor Acres) according to the strain catalog recommendation **(Aviagen, 2019).**

Table 1: Composition and calculated nutrients profile of the basal diet used in experiment:

Ingredients (%)	Arbo-starter 0-14 day	Arbo-grower 15-28 day	Arbo-finisher 29-35 day
Corn	51.61	57.60	61.57
Soybean meal (48% crude protein)	34.39	28.66	25.09
Corn gluten meal	6.81	6.58	5.61
Soya oil	2.17	2.5	3.4
Limestone	1.4	1.34	1.23
Dicalcium phosphate.$2H_2O$	1.35	1.12	1
L-Lysine HCL	0.37	0.35	0.3
Salt	0.3	0.3	0.3
DL-Methionine	0.24	0.21	0.19
Sodium bicarbonate	0.23	0.23	0.24
Premix blank (1)	0.2	0.2	0.2
L-Arginine	0.13	0.12	0.09
Choline-Cl 60%	0.09	0.1	0.11
L-Threonine	0.09	0.07	0.05
Zylam 100g	0.01	0.01	0.01
Quantum blue 100g	0.01	0.01	0.01
CreAmino® (2)	0.6	0.6	0.6
Total	100	100	100
Calculated energy and nutrient content (3):			
AME (kcal/kg)	2900	3000.26	3100.58
Crude protein (%)	24.18	21.93	19.97
Lysine (%)	1.37	1.22	1.09
Methionine+Cystiene (%)	1.03	0.94	0.86
Threonine (%)	0.98	0.87	0.78
Tryptophan (%)	0.27	0.23	0.21
Calcium (%)	0.96	0.87	0.79
Available phosphorous (%)	0.38	0.33	0.3

(1) Provided per kilogram of the complete diet: vitamin A, 12,000 IU; vitamin D_3, 2,000 IU; vitamin E, 15mg; vitamin K_3, 1.5mg; vitamin B_1, 0.5mg; vitamin B_2, 2.5mg; vitamin B_6, 1.25mg; vitamin B_{12}, 10mcg; niacin, 17.5mg; pantothenic acid, 5mg; folic acid, 0.5mg; biotin, 50mcg; iron, 22mg; copper, 2.5mg; zinc, 37.5mg; manganese, 31mg; selenium, 0.113mg; cobalt, 0.05mg; iodine, 0.65mg; antioxidant(BHT), 0.25mg.

(2) Guanidinoacetic acid (96% guanidinoacetic acid, AlzChem Trostberg GmbH, Germany) was added at the rate of 0.06% (600 g/ton feed) in the form of CreAmino® powder on the basal diet.

(3) Calculated values from NRC and Arbor Acres manual **(Aviagen, 2019)**, AME; apparent metabolizable energy.

Behavioral observations:

Observation of the behavior patterns was carried out from the 2nd week until the 5th week. Behavior observation was done according to the method of **Dixon (2020)** , using Eco-Tech smart cameras all pens were video recorded, scan sampling was carried out one day per week, one scan for 20 consecutive seconds per hour for the 24 hour to obtain 24 scan samples per one day every week, for each behavior noted the same 10 seconds were rewound again to avoid interruption through a still video image. According to **Dixon** and **Copper (2010)**, the scan sampling method used in the current study gives a logical approximation of daily time budgets when compared with continuous recording periods. **Nielsen *et al.*, (2011)** defined behavior patterns as shown in Table (2).

Table 2: Behavior patterns and their description:

Behavior	Description
Standing	Stand up without any movement.
Sitting/lying	Sitting or lie down on breast.
Walking	Walking or running without any discernible activity.
Foraging	Pecking the floor (including feeding fodder).
Comfort	Preening, wing and leg stretching, body shaking, and scratching.

Some welfare indices measurement:

At the end of experimental trial, 14 birds of each treatment were examined for presence of footpad dermatitis and hock burns. The severity of lesion were scored using a three-point scale (1 = no burns; 2= mild burns; 3 = severe burns) according to **Thomas *et al.*, (2004)**.

Statistical analysis:

Two-way ANOVA was used to determine the effect of stocking density (SD) and Guanidinoacetic acid (GAA) on the selected parameters of broiler. General linear model procedures were applied using SPSS ver.20. *P*-values less than 0.05 were significant for the main effects including stocking density, GAA and interaction between them. Results were presented as means ± SE.

RESULTS

Behavior observations:

The effects of the stocking densities and dietary GAA (Guanidinoacetic acid) supplementation at the rate of 0.06% on the proportion of broiler chicken behavior are given in Table (3). The effect of high stocking density (HSD) was significant ($P<0.05$) resulted in increase of standing behavior (+ 0.007; $P =0.02$), decrease of sitting behaviour (-0.055; $P =0.03$). Moreover, HSD resulted in significant reduction in foraging behavior (-0.009; P =0.01), comfort behavior (-0.015; P =0.02) and locomotor activity (-0.006; $P =0.04$) than standard stocking density (SSD). Guanidinoacetic acid supplementation resulted in significantly increase foraging behavior in SSD supplemented group (+0.003; $P =0.002$) than SSD unsupplemented group (T1), while no significant effect was observed in HSD treatments. However, there was no significant effect on standing, resting, locomotion and comfort behavior. Significant interactions between stocking densities and dietary GAA supplementation were observed for standing behaviour, foraging behavior and comfort behaviour ($P < 0.05$).

According to the interaction analysis, it should be noticed that for the HSD, significant effect ($P < 0.05$) of GAA found, as showed by higher standing behaviour (T4: 0.024 *vs* T3: 0.016); however in the comparison between the SSD (T1 *vs* T2); this effect was not significant ($P > 0.05$). In addition, the foraging behavior did not significantly affected ($P > 0.05$) in HSD treatments by GAA supplementation, but increased in (T2: 0.026 *vs* T1: 0.019) in SSD treatments, furthermore, comfort behaviour significantly reduced ($P < 0.05$) in HSD groups by GAA supplements (T4: 0.073 *vs* T3: 0.081), whereas this reduction was not seen in the SSD groups (T1 *vs* T2).

Table (4) displayed the foot pad dermatitis and hock burns score in the tested groups. The different stocking densities significantly affected the foot pad dermatitis and hock burns of the broiler chicken. Where the higher score (+ 0.35; $P =0.03$) for footpad dermatitis and (+0.39; $P =0.03$) for hock burns noted at high stocking density (HSD) groups compared with standard stocking density (SSD) groups. No significant effect was noticed by GAA and interaction between Stocking density x GAA.

Table 3: Effect of stocking density and GAA supplementation on behavioral patterns proportion:

Stocking density (birds/m^2)	GAA (g/ton feed)	Group	Standing	Sitting	Locomotion	Foraging	Comfort
SSD	0	T1	0.012[b]	0.790	0.019	0.019[b]	0.089[a]
SSD	600	T2	0.013[b]	0.780	0.020	0.026[a]	0.095[a]
HSD	0	T3	0.016[b]	0.740	0.014	0.014[c]	0.081[b]
HSD	600	T4	0.024[a]	0.720	0.013	0.013[c]	0.073[c]
Pooled SEM			0.001	0.004	0.001	0.001	0.002
Stocking density (birds/m^2)							
SSD (10 birds/m^2)			0.013[b]	0.785[a]	0.020[a]	0.023[a]	0.092[a]
HSD (16 birds/m^2)			0.020[a]	0.730[b]	0.014[b]	0.014[b]	0.077[b]
GAA (g/ton feed)							
0			0.014	0.765	0.017	0.017[b]	0.085
600			0.019	0.750	0.017	0.020[a]	0.084
p value							
SD			0.020	0.030	0.042	0.013	0.019
GAA			0.663	0.112	0.281	0.002	0.132
SD x GAA			0.006	0.078	0.994	0.016	0.003

SSD: standard stocking density, HSD: high stocking density, SD: stocking density, GAA: guanidinoacetic acid
a,b,c different superscript letters in the same column indicates significant difference ($P< 0.05$), Pooled SEM: total SEM

Table 4: Effect of stocking density and GAA supplementation on welfare indicators (Footpad dermatitis and Hock burns score):

Stocking density (birds/m^2)	GAA (g/ton feed)	Group	Footpad dermatitis	Hock burns
SSD	0	T1	1.43	1.43
SSD	600	T2	1.14	1.37
HSD	0	T3	1.57	1.57
HSD	600	T4	1.71	2.00
Pooled SEM			0.08	0.09
Stocking density (birds/m^2)				
SSD (10 birds/m^2)			1.29[b]	1.40[b]
HSD (16 birds/m^2)			1.64[a]	1.79[a]
GAA (g/ton feed)				
0			1.50	1.50
600			1.43	1.69
p value				
SD			0.025	0.030
GAA			0.646	0.093
SD x GAA			0.172	0.396

SSD: standard stocking density, HSD: high stocking density, SD: stocking density, GAA: guanidinoacetic acid
[a,b,c]: different superscript letters in the same column indicates significant difference ($P< 0.05$) , Pooled SEM: total SEM

DISCUSSION

Health status, welfare, and harmony of broilers with the surrounding environment could be reflected by several indicators such as behavioral patterns and physical condition of the birds **(Erasmus, 2017)**. Floor space per bird is very crucial welfare factor which affects and determines the welfare indicators of broiler chickens (**Thomas *et al.*, 2004).** Raising stocking density leading to lower scores of walking ability (gait score), foot pad lesions and hock burns (**Skrbic *et al.*, 2011)**. Moreover, locomotion, resting and preening behavior negatively affected in broiler reared under overcrowding conditions **(Yanai *et al.*, 2018).** Guanidinoacetic acid (GAA) used in this study is considered the precursor of creatine which used in energy metabolism. and may had an improving effect of the broiler chickens activities **(Michiels *et al.*, 2012)**. So the aim of our study to find out how to alleviate the deleterious effect of overcrowding conditions on behavior and welfare of bird by adding 0.06% GAA to the basal diet.

The results of the current study agreed with **Buijs *et al.*, (2010); Ma *et al.*, (2020)**; who reported that resting, walking and preening reduced in high stocking density (HSD) while standing behaviour increased. These findings could be owed to the negatively affected leg health condition in HSD resulted in less movement and reduced preening. Also comfort behaviour pattern such as wing and leg stretching need a fair space to be done by birds but HSD didn't provide this space. **Lewis and Hurnik, (1990); Hall, (2001)** agreed with our study in reduction of resting behavior in HSD, and this could be attributed to the increased opportunities of birds for disturbed rest in overcrowding conditions. Also, **Thomas *et al.*, (2011)** showed the same results in decreasing foraging behavior and this may be related to the restricted space for pecking floor in HSD. **Martrenchar *et al.*, (1997) a**dditionally found that the activity of birds was reduced in HSD, so locomotion and foraging were decreased as well. However, **Bailie *et al.*, (2018) and Son (2013)** disagreed with the current results as they found no significant effect of stocking density on resting, foraging and preening behavior.

Energy depletion compromises the birds' welfare and reduces their ability to deal with additional stress (**Zhang *et al*., 2009**). GAA used in the current study had a vital role in creates creatine inside the body which had a direct impact in protein accretion by improving the availability of ATP for myosin. (**Portocarero and Braun, 2021).** No comparable data in the available literature on the effects of supplementary GAA on behaviour and welfare of broilers raised under HSD conditions. However **Zhang *et al.*, (2014)** reported that creatine supplementation alleviated the stress upon birds during transportation for long time and it positively reflected on welfare and poultry industry. Several studies investigated the influence of GAA in ameliorating the stressful conditions on broiler through increasing the antioxidant capacity and muscle growth (**Nasiroleslami *et al.*, 2018; Wang *et al.*, 2012)**. From the overall point of view, the results of GAA supplementation on behavioural patterns revealed that, GAA supplements significantly increased foraging in standard stocking density (SSD) supplemented group (T2) than SSD unsupplemented group (T1), while no such effect was observed after GAA supplementation in high stocking density conditions, these results could be related to the higher net energy of GAA for production and activity, which could be linked to an increased ATP buffering capacity due to increased phosphocreatine hydrolysis in muscle (**Fosoul *et al.*, 2018**). This energy used by birds in different activities such as foraging behavior in SSD groups which had more space for these activities compared with HSD groups. Regarding the different behavioural patterns, the statistical analysis showed an interaction between the two examined parameters (GAA supplementation and stocking density).

From the overall view of behavioral results and based on the effect of stocking density, GAA treatment showed a significant increase in standing behavior in HSD + GAA treatment compared to HSD without GAA treatment, but, it was not significantly different in SSD treatments, these results may be interpreted by the higher energy supply provided by GAA treatment to supply muscle creatine resulting in more activity of birds **(Michiels *et al.*, 2012)**. On the other hand, the effect of GAA supplementation showed no significant difference on comfort behaviour in two stocking density circumstances, however SSD +GAA treatment exhibited higher comfort behaviour than HSD +GAA group and this may be attributed to the more space provided in the SSD treatment to express their normal comfort behavior compared with HSD treatments and this result in line with **Ma *et al.*, (2020)** that attributed the reduction in preening behavior due to poor leg condition in HSD.

In recent decades the concerns about farm animal welfare have been increased substantially in industrialized countries. Intensive rearing strategies which used to increase the productivity of farm animal have increased dramatically at the same time and this reflected negatively on some aspects of growth performance, welfare and health status **(Goo *et al.*, 2019; Heidari & Toghyani, 2018; Jobe *et al.*, 2019; Xiong *et al.*, 2020)**. Footpad dermatitis and hock burns have the negative impacts on chickens welfare resulted from wet litter and high level of ammonia in overcrowding environment **(Mohiti-Asli *et al.*, 2016; Rashidi *et al.*, 2019b)**. High stocking density conditions negatively affected welfare indicators such as footpad dermatitis and hock burns as reported by (**Thomas *et al.*, 2004; Bailie, Ijichi and O'Connell 2018)**. Similar findings were reported by **Son (2013)** who reported a bad score of footpad and hock burn in broiler reared in HSD compared with SSD. These results could be attributed to less resting and more standing times for birds which resulted in very close contact of foot with the wet litter having high moisture and ammonia contents. **De Jong *et al.*, (2012)** although revealed that overcrowding conditions adversely affected leg health, walking ability and disruption of rest. In addition **Ibrahim *et al.*, (2017)** found that ammonia burns severely increased in high stocking density resulting in hock burns and footpad dermatitis. On the other hands, GAA supplementation or interaction between stocking density and GAA reported no significant difference on welfare indices neither SSD nor HSD treatments.

CONCLUSION

The present study concluded that overcrowding condition revealed negative impacts on behavior and welfare of broiler chicken such as reduction in resting, foraging and comfort behaviour, higher standing behaviour and elevation of footpad dermatitis and hock burns score, which could be reflected negatively on health status and productive performance. However, a partial beneficial effect of guanidinoacetic acid (GAA) supplementation at the rate of 0.06% was found in some behavioural patterns especially in standard stocking density. The potential effect of GAA in broiler chicks may depend on the usage dosage. Further studies should be carried out on different concentration of GAA to alleviate the negative impacts of high stocking densities on broiler chickens.

Declaration of Conflicting Interests:

The authors declare that they have no competing interests.

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