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Araştırma Makalesi ***Research Article***

Impact of Glutamine in Drinking Water on Performance and Intestinal Morphology of Broiler Chickens under High Stocking Density

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Abstract

This work sought to look into the impacts of glutamine in drinking water on performance, intestinal morphology and corticosterone level of chickens under different stocking densities. A total 300 male chicks randomly were divided to battery cages as 10 birds/(normal stocking density) and 15 birds/(high stocking density). The chicks received feed as (i) control diet and (ii) control diet + 5g glutamine/ liter in drinking water under normal and high stocking density for the whole experiment. On d 42, 3 chicks from each cage were randomly selected and slaughtered to collect blood and duodenal samples. The results showed significant improvement in growth performance and longer villi when chicken were supplemented with glutamine via drinking water. High stocking density impaired performance of chicks in the control diet, but not in chicken supplemented with glutamine under high stocking density condition. Moreover, high stocking density increased the level of corticosterone in the both groups. More interestingly, the rate of mortality significantly decreased in chickens fed with glutamine supplemented diet (2% when supplemented with glutamine and 5.33% without supplementation). In conclusion, glutamine supplementation via drinking water resulted in better growth performance of birds subjected to high stocking density.

Introduction

Over the past few years, the broiler meat market is confronted with high numbers of customers who demand for more products because of lower final price and shorter raring period compared to other meat products. Some issues like sudden death syndrome and other diseases are some common issues that broiler industry is facing (Bessei, 2006). Many of these problems may negatively influence the performance when chicken are stocked in high numbers per square meters. Although, rearing chickens in higher number than optimal number per square meter is still a main purpose of producers. Therefore, it needs to work on this matter and attempt to decrease the negative effects of high stocking density on chicken performance. High stocking density can negatively affect chicken performance via different kinds of stresses such as heat stress (Imaeda, 2000). The normal required space area per chicken is different in each country and is directly dependent on climate condition.

High stocking density has introduced as a factor with negative impacts on welfare and the quantity of products in broiler chickens. Therefore, there is an attempt to estimate the amount of negative effects of high stocking density on chickens' performance by measuring corticosterone (CRT) level in blood. CRT has considered as a blood factor to measure physiological stress (Vachon and Moreau, 2001) and gut health (Sapolsky et al., 2000).

Glutamine works as a nitrogen shuttle, which helps to defend the body from excess levels of ammonia (Labow, 2001). Glutamine is available in tissues, muscles and proteins to provide energy for immune system and help the body under stressful conditions such as injury (Newsholme, 2001). Moreover, glutamine has positive impacts on performance, immune system and gastrointestinal tract (Bartell and Batal, 2007; Dai et al.,

2009; Yi et al., 2001). Several reports showed that glutamine supplementation could improve performance of broiler chickens under stressful or normal conditions (Dai et al., 2009; Soltan, 2009).

The present study was carried out to investigate effects of glutamine in drinking water on growth performance, intestinal morphology and corticosterone level in broiler chickens when were subjected to different stocking densities.

Materials and Methods

Birds, Husbandry and Housing

The project was carried out under department of agriculture at University Putra Malaysia. A total of 300 one-day-old *Cobb* 500 male broiler chicks arrived from the local hatchery. On d-1, the chicks were randomly selected, weighted and divided to 24 equally size battery cages (1m × 1m) (minimum and maximum temperature were, 24 °C to 35 °C, respectively). The relative humidity was between 75 to 90%.

Experimental Treatments

The experiment was designed as a 2x2 factorial arrangements with or without glutamine via drinking water under normal or high stocking density with 6 replicates for each density. Birds were raised at stocking densities of 0.100 m^2/bird (10 birds /cage) or 0.067 m^2/bird (15 birds/cage). The diets prepared as control diet with (5 g or 0.5%) or without glutamine solved in drinking water per litter from 1-42 d under normal or high stocking density. The cages were equipped with movable walls to change the size of cages in case of mortality and keep the density as 0.100 m^2/bird and 0.067 m^2/bird, respectively. The reason of using only one level of glutamine was based on other obtained researches results that showed under normal conditions 0.5-1% glutamine in diet has positive impacts on chicken performance (Soltan, 2009; Yi et al., 2001). Therefore, for this study, 0.5% of glutamine was added in drinking water, which will be equal to 1% in diet (feed) as broiler chicken normally drinks 2 times more water than feed. Moreover, 5 g glutamine/liter was added during the whole experiment in drinking water to provide more accessibility to glutamine according to the chicken's age. The starter diets were prepared in mash form for the first 3 weeks (1-21d) and finisher for the last 3 weeks (22-42d) of the experiment. The nutrient composition presented in Table 1.

Growth Performance

At day 1 (before chickens placed in the cages) and d 42, chicks and feed weighted to measure the feed conversion ratio (FCR). The mortality rate was recorded daily.

Table 1. Composition of dietary treatments (%).

Ingredients	Starter (1 to 21 d)	Grower (22 to 42 d)
Corn	61.40	64.70
Soybean meal	28.30	24.50
Fish meal	5.00	4.00
Palm oil	2.00	3.50
Dicalcium phosphate	1.30	1.30
Calcium carbonate	1.20	1.20
Vitamin premix[1]	0.30	0.30
Mineral premix[2]	0.30	0.30
DL-Methionine	0.20	0.20
Total	100	100
Nutrients Composition (calculated values)		
ME (kcal/kg)[3]	3000	3100
Crude Protein	21.5	19.5
Calcium	1.00	0.96
Available Phosphorus	0.46	0.44
Methionine	0.57	0.54
Lysine	1.16	1.02
Sodium	0.13	0.13

[1]Supplied per kilogram of diet: vitamin A: 4,500 IU; vitamin D3: 1000 IU; vitamin E, 50 mg; vitamin K, 1.5 mg; vitamin B12, 0.02 mg; vitamin B2, 3 mg; pantothenic acid, 5 mg;

[2]per kg of diet; zinc, 40 mg; iron, 80 mg; iodine, 80 mg; manganese, 60 mg; copper, 8 mg; selenium, 0.2 and cobalt, 0.4 mg.

[3]Metabolizable Energy

Corticosterone (CRT)

At the end of the experiment, 18 birds per treatment were selected and slaughtered to collect blood. Plasma was separated and stored at -80°C to measure CRT level. The CRT was measured by a commercial ELISA kit (Cayman, Michigan, USA). Briefly, standards were prepared as 500 ng/mL, 166.7ng/mL, 55.6 ng/mL, 18.5ng/mL, 6.17ng/mL, respectively. 50 µl of standards and 50µl of samples were loaded to specific wells. After loading, 50µl AChE tracer was loaded to all wells except TA (total activity) and blank wells. 50 µl antiserum was loaded to wells except for TA, blanks and NSB (Non-specific binding). Then, the plate incubated for 2 hours at room temperature on an orbit shaker. All wells were washed 5 times with wash buffer. 200 µl Ellman's reagent was loaded to all wells. 5 µl tracer was added to TA well. The plate covered and kept in a dark place on an orbit shaker for 60 minutes. Finally, micro-plate reads at 450 nm.

Intestinal Morphology

The duodenal segments (18 birds per treatment) were flushed with phosphate buffer and fixed in 10% formalin buffer. Each tissue was cut into 5 sections (10 mm) and placed into a tissue cassette. The tissues were processed by dehydration following a series of graded alcohols, with xylene, and embedded in paraffin. Paraffin sections (5-µm thickness) were mounted onto slides. The slides were stained using routine procedures for Mayer's hematoxylin and eosin (Baddeley et al., 1986). The villi length (from the tip of the villi to the villus crypt junction) and crypt depth (depth of the invagination between adjacent villi) were measured using a light microscope (Olypmus, Tokyo, Japan) according to Bancroft and Gamble, (2002). Two villi lengths and crypt depth were measured per sample.

Statistical Analysis

All data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute Inc., 2003). Pen served as the experimental unit for performance parameters and bird as the experimental unit for histology and corticosterone parameters. All results were analyzed based on glutamine levels, stocking density, and their interactions. When the interactions were significant, comparisons were made within each experimental variable. When significant effects were found, comparisons among multiple means were modelled by Tukey's test. Mortality data were analyzed using the chi-square test. Statistical significance was declared at a probability of $P<0.05$.

Table 2. Mean (±SEM) weight gain (g), feed intake (g), FCR and mortality rate of chickens supplemented with or without glutamine in drinking water (Normal density n=120; High density n=180).

	Density				Effects, P-Value		
	Normal Stocking Density		High stocking density				
	Control	Glutamine	Control	Glutamine	Glutamine (G)	Density (D)	G x D
Weight Gain (g)	1944±12^{b}	2283±87^{a}	1823±77^{c}	2202±55^{a}	0.0001	0.001	0.01
Feed Intake (g)	3810±14	4061±13	3804±11	3894±89	0.41	0.66	0.68
FCR	1.95±0.09^{a}	1.77±0.03^{b}	2.08±0.15^{a}	1.76±0.14^{b}	0.03	0.64	0.37
Mortality (%)	2.00^{b}	0.66^{c}	3.44^{a}	1.33^{b}			

^{a,b,c} Means values within a row-subgroup with no common superscripts are different at P≤0.05.

Table 3. Mean (±SEM) level of corticosterone (CRT) in chicks with or without glutamine in drinking water (n=18 birds per treatment).

	Density				Effects, P-Value		
	Normal Stocking Density		High Stocking Density				
	Control	Glutamine	Control	Glutamine	Glutamine (G)	Density (D)	G x D
CRT (ng/ml)	1.24±0.09 ^{b}	1.11±0.05^{b}	1.71±0.17^{a}	1.63±0.13^{a}	0.83	0.0002	0.19

^{a,b} Means values within a row-subgroup with no common superscripts are different at P≤0.05.

Table 4. Mean (±SEM) levels of villi length (µm) and crypt depth (µm) of chickens supplemented with or without glutamine in drinking water (n=18 birds per treatment).

	Density				Effects, P-Value		
	Normal Stocking Density		High Stocking Density				
	Control	Glutamine	Control	Glutamine	Glutamine (G)	Density (D)	G x D
Villi Length (µm)	1185±12[b]	1261±30[a]	1084±24[c]	1239±32[a]	0.001	0.005	0.04
Crypt Depth (µm)	138±13	142±15	130±19	134±12	0.39	0.08	0.97

[a,b,c] Means values within a row-subgroup with no common superscripts are different at P≤0.05.

Results

Growth Performance

Observations of this study showed significant differences (P≤0.05) between glutamine levels and diet × density for weight gain, feed intake and FCR during 6 weeks of the experiment (Table 2). High stocking density did not affect (P>0.05) performance of chicks when chickens were supplemented with glutamine. However, control diet without glutamine was affected negatively (P≤0.05) by high stocking density. More interestingly mortality rate was significantly (P≤0.05) (2%) decreased in glutamine group in both densities compared to the control diet without glutamine (5.33%).

Blood Parameters

There was no substantial difference (P>0.05) between diet × density for CRT. The data of the corticosterone is presented in Table 3. The results of CRT showed no significant differences for the diets in the same density, but level of CRT was dramatically (P≤0.05) increased in high stocking density.

Morphometric Variables

The intestine morphology results showed significant differences (P≤0.05) between diet × densities for villi length (Table 4). Glutamine caused longer villus length in the both densities. However, control diet caused a shorter villus length compare to glutamine group in the both densities, and shorter under high stocking density. The crypt depth was not significantly different between the densities and the treatments with or without glutamine in drinking water.

Discussion

Glutamine has been presented as a supplementation that can improve performance of chicks even in infection conditions (Newsholme, 2001). High stocking density has shown as a negative factor in growth performance (Bessei, 2006). Suggested by Estevez (2007) that 0.07 m^2/bird is minimum space for chicks under hot conditions.

The present results confirmed with Houshmand et al. (2012) that growth performance not affected by density in battery cages. Some studies (Ayazi, 2014; Bartell and Batal, 2007; Dai et al., 2009; Dai et al., 2011; Fasina et al., 2010; Salmanzadeh and Shahryar, 2013; Soltan, 2009; Yi et al., 2001) confirmed that glutamine has a positive function on villi length and in follow can improve performance of chicks. In fact, improvement of small intestine is positively in connection with a greater absorption, because of increasing in surface area. However, Jazideh et al. (2014), Ebadiasl (2011), Sakamoto et al. (2006) and Maiorka et al. (2000) showed that there were not significant differences when chickens were supplemented with glutamine in feed compare to the control diet. The reason of differences between current study and these studies may come back to duration of rearing time, the way of supplementing with glutamine (mixed in feed not drinking water) and rearing environment (control temperature room or in cages). The current results showed that chickens were supplemented with glutamine (5 g/liter) via drinking water improved performance and even high stocking density did not adverse performance. This could be because of better accessibility of chicks to glutamine via drinking water. Glutamine has a function in gut health (Menconi et al., 2013), which can decrease the percentage of mortality and less chance for infections (DeMarco et al., 2003; Nascimento et al., 2014).

The result of CRT is in line with Beuving and Vonder (1978) and Vachon and Moreau (2001) showed that CRT could increase in response to physiological stresses. Moreover, long time exercise and struggling to have access to feed can be another reason to increase the level of CRT (Dawkins, 2003). Under stressful environments, synthesis of CRT will happen by activation of the hypothalamus-pituitary-adrenal axis (Sapolsky et al., 2000). In the current study, high stocking density might be increased competition among the chickens to have access to feed during rearing time and enhance the level of stress (Pettit-Riley and Estevez, 2001; Sanotra et al., 2001).

The intestine morphology results showed significant interaction (P>0.05) between diet × densities for villi

length (Table 4). Glutamine caused longer villus length in the both densities. Salmanzadeh and Shahryar (2013) showed higher villi length when chickens were subjected to heat stress. Longer villi length can increase absorption in intestine and in follow can cause better growth performance. However, the control diet had a shorter villus length compare to glutamine diet in the both densities, but, shorter in high stocking density. The crypt length was not significantly different between the densities and treatments.

The result of the current study on intestinal morphology is in agreement with Mitchell and Carlisle (1992). Burkholder et al. (2008) reported that stressful conditions had a negative impact on intestinal morphology. However, differences between our results and other studies on glutamine may arise from the positive impact of glutamine on the immune system (Menconi et al., 2013) and enhance absorption of nutrients in the gut (Salmanzadeh and Shahryar, 2013) when the chicks were supplemented via drinking water. Moreover, accessibility of chickens to glutamine via drinking water can support these results that why the both densities when were supplemented with glutamine had approximately the same villus length. Higher villus length was observed in many studies that used glutamine in diet (Bartell and Batal, 2007; Yi et al., 2005). Glutamine can cause longer villus length in early age (first two weeks) (Yi et al., 2001), may help chicks to have better absorption in intestine. The results of the experiment gives insights into a potential supplemental method using glutamine in drinking water to modulate chicken growth performance and villi length under high stocking density.

Conclusions

In conclusion, under high stocking density condition of this study, glutamine had a positive impact on growth performance and intestinal morphology, but it did not affect corticosterone level of birds reared in battery cages. Moreover, survivability increases when chicks were supplemented with glutamine via drinking water even in high stocking density.

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