Effect of stocking density and dietary tryptophan on growth performance and intestinal barrier function in broiler chickens

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ABSTRACT The objective of the present experiment was to investigate the effect of stocking density (SD) and dietary supplementation of crystalline tryptophan (Trp) on growth performance and intestinal barrier function in broiler chickens raised in a floor pen. The experiment was conducted using a completely randomized design with a 2 × 2 factorial arrangement consisting of 2 different SD and 2 supplemental levels of dietary Trp. A total of 1,140 Ross 308 broiler chickens at 21 d of age were allotted to 1 of 4 treatments with 5 replicates. Low SD (9 birds/m^2) and high SD (18 birds/m^2) were achieved by raising different number of birds per identical floor pen (2.0 m × 2.4 m). The basal diet was formulated with no supplemental Trp in diets to meet or exceed nutrient recommendation of the Ross 308 manual. The calculated concentrations of total Trp and digestible Trp in the basal diet were 0.19 and 0.16%, respectively. The other diet was prepared by adding 0.16% crystalline Trp to the basal diet. Diets were fed to birds for 21 d. At the end of the experiment, 2 birds per replicate were euthanized to collect tissue samples for further analyses. Results indicated that there were no interactions between SD and dietary Trp for all measurements. For the main effects, birds raised at a low SD had greater ($P < 0.01$) body weight gain, feed intake, and feed efficiency than those raised at a high SD. However, supplementation of dietary Trp had no effect on broiler performance. Furthermore, there were no main effects of SD and dietary Trp on intestinal barrier functions. In conclusion, broiler chickens raised in a floor pen with a high SD (18 birds/m^2) have decreased growth performance with little changes in intestinal barrier functions. Supplementation of dietary Trp at 0.16% has no positive effect on broiler chickens raised in a floor pen with either a low or high SD.

Key words: broiler chicken, dietary tryptophan, intestinal barrier function, stocking density, tight junction-related gene expression

2019 Poultry Science 98:4504–4508
http://dx.doi.org/10.3382/ps/pez215

INTRODUCTION

Stocking density (**SD**) is one of the major concerns in the broiler industry owing to its direct association with economic feasibility in broiler production. Currently, a high SD is commonly applied to increase the profitability by yielding more chicken meat per fixed raising area (Puron et al., 1995). However, the high SD has been reported to decrease broiler productivity by lowering growth performance and meat quality, and increasing incidence of health problems (Estevez, 2007). As a result, various efforts have been made to ameliorate the negative outcomes of a high SD in broiler chickens; however, few solutions have been yet reported.

Tryptophan (**Trp**) is an essential amino acid for protein synthesis. Moreover, Trp has its own physiological role in the body (Corzo et al., 2005). In particular, Trp is a precursor of serotonin (5-hydroxytryptamine) that plays a role in regulating the central nerve system to inhibit aggressive behavior and to control stress responses in animals (Shen et al., 2012). Therefore, increasing intake of Trp has been hypothesized to improve animal performance by attenuating stress responses (Emadi et al., 2010; Shen et al., 2012). However, the results for additional supplementation of dietary Trp in broiler chickens raised at a high SD have been inconsistent (El-Gogary and Azzam, 2014; Wang et al., 2014). In addition, these previous experiments used battery cages for broiler-rearing facilities (El-Gogary and Azzam, 2014; Wang et al., 2014), and therefore, there is still limited information regarding the effect of additional supplementation of dietary Trp when broiler chickens are raised in the conventional floor pen with a high SD. Moreover, our recent study reported that a high SD of broiler chickens decreased intestinal barrier function, which may be one of reasons for impaired performance of broiler chickens (Goo et al., 2019). However, researchers have not yet investigated the effects of additional supplementation of dietary Trp on intestinal

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Received February 19, 2019.

Accepted March 22, 2019.

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Table 1. Composition, and calculated energy and nutrient content of experimental diets.

Feeding program	0 to 3 wk	4 to 6 wk	
Items (%, unless noted)	Starter diet	Control diet	Diet with supplemental tryptophan
Ingredients			
Corn	54.56	59.36	59.36
Soybean meal	23.66	18.99	18.99
Corn gluten meal	10.40	10.00	10.00
Tallow	4.06	4.73	4.73
Salt	0.30	0.30	0.30
Monodicalcium phosphate	1.71	1.50	1.50
Limestone	1.69	1.55	1.55
DL-Methionine (88%)	0.32	0.29	0.29
L-Lysine-H_2SO_4 (54%)	0.76	0.75	0.75
L-Threonine (98.5%)	0.14	0.13	0.13
L-Tryptophan (98%)	0.00	0.00	0.16
Choline (50%)	0.10	0.10	0.10
Sodium bicarbonate	0.10	0.10	0.10
Vitamin premix[1]	0.10	0.10	0.10
Mineral premix[2]	0.10	0.10	0.10
Celite	2.00	2.00	1.84
Total	100.00	100.00	100.00
Calculated energy and nutrient content[3]			
AME_n (kcal/kg)	3,131	3,203	3,203
CP	21.57	19.51	19.51
Lysine	1.29	1.16	1.16
Methionine + cysteine	1.01	0.93	0.93
Threonine	0.97	0.87	0.87
Tryptophan	0.21	0.19	0.34
Digestible tryptophan	0.18	0.16	0.32
Calcium	1.00	0.90	0.90
Available phosphorus	0.45	0.40	0.40

[1] Provided per kilogram of the complete diet: vitamin A, 13,000 IU; vitamin D_3, 5,000 IU; vitamin C, 330 IU; vitamin E, 80 IU; vitamin K_3, 4 mg; vitamin B_1, 4 mg; vitamin B_2, 10 mg; vitamin B_6, 6 mg; vitamin B_{12}, 20 μg; biotin, 0.2 mg; vitamin B_5, 20 mg; folic acid, 2 mg; niacin, 60 mg; antioxidant, 0.1 mg.

[2] Provided per kg of the complete diet: zinc, 100 mg; manganese, 120 mg; iron, 60 mg; copper, 16 mg; cobalt, 1 mg; iodine, 1.3 mg; selenium, 0.3 mg.

[3] Calculated values from NRC (1994) and Ross 308 manual (Aviagen, 2017).

barrier functions in broiler chickens raised at a high SD in a floor pen.

Therefore, the present experiment was conducted to investigate the effect of SD and additional supplementation of dietary Trp on growth performance and intestinal barrier function in broiler chickens raised in a floor pen.

MATERIALS AND METHODS

Birds, Diets, and Experimental Design

The protocol for the current experiment was approved by the Institutional Animal Care and Use Committee at Chung-Ang University. The experiment was conducted using a completely randomized design with 2 × 2 factorial arrangement consisting of 2 different SD and 2 supplemental levels of dietary Trp. A total of 1,140 Ross 308 broiler chickens at 21 d of age were allotted to 1 of 4 treatments with 5 replicates. This experiment started with 21-d-old birds because the negative outcomes of high SD become noticeable mainly in growing broiler chickens (Wang et al., 2014). Low SD (9 birds/m^2) and high SD (18 birds/m^2) were achieved by raising different number of birds per floor pen (Goo et al., 2019) with an identical floor size (2.0 m × 2.4 m). The area occupied by immobile objects (i.e., 2 feeders and 1 bell drinker) was not included when the floor space was calculated. The commercial-type basal diet was formulated without the use of supplemental Trp to meet or exceed the nutrient recommendation of the Ross 308 manual (Aviagen, 2017). The calculated concentrations of total Trp and digestible Trp in the basal diet were 0.19 and 0.16%, respectively (Table 1), which were close to their recommended concentrations in broiler diets (Aviagen, 2017). The other diet was prepared by adding 0.16% feed-grade crystalline Trp (> 98%; Henan Julong Biological Engineering Co., Ltd., Henan, China) to the basal diet at the expense of celite. The calculated concentrations of total Trp and digestible Trp in the diet supplemented with Trp were 0.34 and 0.32%, respectively. These concentrations in the diet were higher approximately by 1.8-fold and 2.0-fold than the recommendations for total Trp and digestible Trp, respectively (Aviagen, 2017). Prior to the start of the experiment, all chickens were raised under the identical environmental conditions and were fed the

Table 2. Effects of stocking density (SD) and additional supplementation of dietary tryptophan (Trp) on growth performance of broiler chickens.

Treatment		Growth performance[1]		
SD (birds/m^2)	Trp (%)	BWG (g)	FI (g)	G:F
9	0.16	1,883	3,291	572
	0.32	1,884	3,248	580
18	0.16	1,583	3,036	521
	0.32	1,631	3,054	534
SEM (n = 5)		33.8	37.0	7.8
Main effect				
SD (birds/m^2)				
9		1,884	3,269	576
18		1,607	3,045	528
SEM (n = 10)		23.9	26.2	5.5
Trp (%)				
0.16		1,733	3,163	547
0.32		1,758	3,151	557
SEM (n = 10)		23.9	26.2	5.5
Effect (*P*-value)				
SD		<0.001	<0.001	<0.001
Trp		0.475	0.742	0.202
SD × Trp		0.499	0.409	0.759

[1] BWG, body weight gain; FI, feed intake; G:F, feed efficiency (gain:feed, g/kg).

same starter diets for 20 d (Table 1). On 21 d of age, all chickens were weighed and separated to 20 pens. From 21 to 42 d, experimental diets and water were provided ad libitum. A 24-h lighting schedule was used throughout the entire experiment. All experimental pens were placed in an environmentally controlled room. The average room temperature was maintained at 20.4 ± 2.8°C during overall experimental period. Body weight gain (**BWG**) and feed intake (**FI**) were recorded at the end of the experiment. The feed efficiency (**G:F**, g/kg) was calculated as BWG divided by FI after adjusting for mortality (Kim et al., 2017).

Sample Collection and Analysis

At the conclusion of experiment, 2 birds per replicate with body weight (**BW**) close to the average of each pen were euthanized by CO$_2$ asphyxiation. One bird was used to analyze mucosal gene expression in the jejunum, whereas the other bird was used to analyze intestinal permeability by a Ussing chamber. Jejunal mucosa were collected by a gentle scraping, frozen in liquid nitrogen, and stored at −85°C prior to gene expression analysis. The expression of tight junction-related genes including zonula occludens-1 (***ZO-1***), occludin (***OCLN***), claudin-1 (***CLDN-1***), and junctional adhesion molecule B (***JAM-2***) in the jejunal mucosa was analyzed by a quantitative real-time PCR based on the methods of Shin et al. (2018). All primers for tight junction-related genes were presented previously (Goo et al., 2019). In addition, intestinal permeability was determined by transepithelial electrical resistance (**TER**) value in a dual-channel self-contained Ussing chamber system (U2500, Warner Instruments, Hamden, CT). The detailed procedure was reported previously (Goo et al., 2019).

Statistical Analysis

All data were analyzed by 2-way ANOVA in a completely randomized design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The replicate was an experimental unit for all analyses. The model included the effects of SD, dietary supplementation of Trp, and their interaction. Outliers were checked using the UNIVARIATE procedure of SAS (Seo et al., 2018), but no outliers were identified. The LSMEANS procedure was used to calculate mean values. Significance for statistical test was set at $P < 0.05$.

RESULTS

Growth Performance

No interaction between SD and additional supplementation of dietary Trp was observed for growth performance of broiler chickens (Table 2). During the experiment, birds raised at a high SD had less ($P < 0.01$) BWG, FI, and G:F than those raised at a low SD. However, additional supplementation of Trp in diets had no beneficial effect on growth performance of broiler chickens, regardless of SD.

Intestinal Barrier Function

No interaction between SD and additional supplementation of dietary Trp was observed for TER values as a measure of intestinal permeability (Table 3). In addition, TER values were not affected by different SD and additional supplementation of dietary Trp. There were no interactive and main effects of SD and additional supplementation of dietary Trp on the expressions of the tight junction-related genes including

Table 3. Effects of stocking density (SD) and additional supplementation of dietary tryptophan (Trp) on transepithelial electrical resistance (TER) values and tight junction-related gene expressions in the jejunum of broiler chickens.

Treatment			Gene expression[1]			
SD (birds/m^2)	Trp (%)	TER (Ω/cm^2)	*ZO-1*	*OCLN*	*CLDN-1*	*JAM-2*
9	0.16	361	0.93	0.98	0.85	0.86
	0.32	360	1.07	1.26	0.90	0.89
18	0.16	336	0.72	1.73	0.72	0.93
	0.32	315	0.89	1.54	0.95	1.07
SEM (n = 5)		34.9	0.150	0.322	0.150	0.213
Main effect						
SD (birds/m^2)						
9		361	1.00	1.12	0.88	0.88
18		325	0.81	1.64	0.84	1.00
SEM (n = 10)		24.7	0.106	0.228	0.106	0.151
Trp (%)						
0.16		349	0.83	1.35	0.79	0.89
0.32		338	0.98	1.40	0.93	0.98
SEM (n = 10)		24.7	0.106	0.228	0.106	0.151
Effect (*P*-value)						
SD		0.328	0.222	0.128	0.783	0.574
Trp		0.757	0.327	0.882	0.358	0.695
SD × Trp		0.772	0.909	0.476	0.549	0.795

[1] *ZO-1*, zonula occludens-1; *OCLN*, occludin; *CLDN-1*, claudin-1; *JAM-2*, junctional adhesion molecules B.

ZO-1, *OCLN*, *CLDN-1*, and *JAM-2* in the jejunal mucosa of broiler chickens.

DISCUSSION

Many previous experiments have reported that a high SD decreased the BWG, FI, and G:F of broiler chickens compared with a low SD (Zuowei et al., 2011; Cengiz et al., 2015), which was in agreement with the current observation. The reasons for these negative outcomes have been associated with various factors such as decreasing FI by restricted movements and increasing stress responses by crowding and unfavorable environmental conditions (Feddes et al., 2002; Goo et al., 2019).

Dietary Trp has a unique role in affecting the behavior and stress responses of animals by stimulating the serotonin synthesis (Shen et al., 2012). Therefore, we hypothesized that additional supplementation of dietary Trp may ameliorate stress responses in broiler chickens raised at a high SD in a floor pen. However, additional supplementation of dietary Trp had no beneficial effect on broiler performance in this experiment. This result disagrees with Wang et al. (2014), who reported that the concentrations of dietary Trp at 1.5-fold greater (0.27%) than NRC requirement (0.18%; NRC, 1994) improved feed efficiency in broiler chickens raised at a high SD (15.4 birds/m^2). In contrast, El-Gogary and Azzam (2014) reported that increasing concentrations of dietary Trp from 0.18 to 0.28% had no effect on growth performance of broiler chickens raised at the high SD (16.7 birds/m^2). These inconsistent results are likely attributed to differences in the rearing facilities used in the respective studies (battery cages vs. floor pens) and other variations in experimental conditions, because the optimal SD for broiler chickens is affected by animals (e.g., sex and genotype) and environmental factors (e.g., ambient temperature, ventilation, and floor materials; Bessei, 2006; Estevez, 2007).

The intestinal barrier plays an important role in maintaining the health of the gastrointestinal tract and even of the entire animals (Anderson and Van Itallie, 1995). Impaired intestinal barrier function has been considered one of the potential reasons for decreased productive performance and increased incidences of health problems in animals (Lambert, 2009). Stress may be the main stimulator damaging the intestinal barrier function (Lambert, 2009; Goo et al., 2019). Our previous experiment reported that increasing SD from 15.2 to 30.4 birds/m^2 linearly decreased intestinal barrier function (i.e., increased intestinal permeability) when broiler chickens were raised in battery cages (Goo et al., 2019). However, the current experiment showed no effect of a high SD on intestinal permeability and tight junction-related gene expressions in the jejunal mucosa. The reason for this observation is unclear; however, it is likely related to less SD used in the current experiment (i.e., 18.0 birds/m^2) compared with our previous experiment (i.e., 30.4 birds/m^2). In addition, birds raised in the floor pen may be less susceptible to stressful conditions than those raised in the cage because deep litters in the floor pen provide more comfort to birds (Bilal et al., 2014). It is suggested, therefore, that broiler chickens raised at the SD of up to 18.0 birds/m^2 in a floor pen may have little change in intestinal barrier function. Thus, our observation for decreased broiler performance at a high SD may not be a consequence of impaired intestinal barrier function.

Additional supplementation of dietary Trp has been reported to affect intestinal barrier function in pigs although no data are available in broiler chickens. Liu et al. (2017) reported that additional supplementation of 0.20% Trp in diets promoted tight junction-related protein expression, especially for *ZO-1* in finishing pigs, suggesting that dietary Trp may act as a functional amino acid to improve intestinal barrier function. On the contrary, Li et al. (2016) observed increased intestinal permeability in addition to decreased tight junction-related gene expression when weanling pigs were fed diets supplemented with 0.20% Trp. No clear reason has been elucidated for these inconsistent results among previous experiments. However, our results indicate that additional supplementation of dietary Trp has no beneficial effect on intestinal barrier function in broiler chickens, regardless of SD in the floor pen. To our knowledge, our experiment was the first to report the effect of additional supplementation of dietary Trp on intestinal barrier function in broiler chickens raised in a floor pen with a high or low SD.

CONCLUSION

Broiler chickens raised in a floor pen with a high SD (18 birds/m^2) during 21 to 42 d of age have decreased growth performance than those raised at a low SD (9 birds/m^2). However, intestinal barrier function in broiler chickens is not affected by the high SD in the condition of the present experiment. No positive effect of additional supplementation of dietary Trp at 0.16% is observed for broiler chickens raised in a floor pen, regardless of SD.

ACKNOWLEDGMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (NRF-2016R1C1B1009323).

REFERENCES

Anderson, J. M., and C. M. Van Itallie. 1995. Tight junctions and the molecular basis for regulation of paracellular permeability. Am. J. Physiol. 269:G467–475.

Aviagen. 2017. Ross 308 AP Broiler: Nutrition Specifications. Ross Breeders Limited, Newbridge, Midlothian, Scotland, UK.

Bessei, W. 2006. Welfare of broilers: a review. Worlds Poult. Sci. J. 62:455–466.

Bilal, K., S. Mehmood, M. Akram, S. Imran, A. W. Sahota, K. Javed, J. Hussain, and H. Ashfaq. 2014. Growth performance of broilers under two rearing systems in three different housing zones in an environmentally controlled house during winter. J. Anim. Plant Sci. 24:1039–1044.

Cengiz, O., B. H. Koksal, O. Tatli, O. Sevim, U. Ahsan, A. G. Uner, P. A. Ulutas, D. Beyaz, S. Buyukyoruk, A. Yakan, and A. G. Onol. 2015. Effect of dietary probiotic and high stocking density on the performance, carcass yield, gut microflora, and stress indicators of broilers. Poult. Sci. 94:2395–2403.

Corzo, A., M. T. Kidd, J. P. Thaxton, and B. J. Kerr. 2005. Dietary tryptophan effects on growth and stress responses of male broiler chicks. Br. Poult. Sci. 46:478–484.

El-Gogary, M., and M. Azzam. 2014. Effects of dietary tryptophan levels and stocking density during the growing-finishing phase on broiler performance and immunity. Asian J. Anim. Vet. Adv. 9:568–577.

Emadi, M., F. Jahanshiri, K. Kaveh, M. Hair-Bejo, A. Ideris, and A. Alimon. 2010. Tryptophan stimulates immune response in broiler chickens challenged with infectious bursal disease vaccine. J. Anim. Vet. Adv. 9:610–616.

Estevez, I. 2007. Density allowances for broilers: Where to set the limits? Poult. Sci. 86:1265–1272.

Feddes, J. J., E. J. Emmanuel, and M. J. Zuidhoft. 2002. Broiler performance, body weight variance, feed and water intake, and carcass quality at different stocking densities. Poult. Sci. 81:774–779.

Goo, D., J. H. Kim, H. S. Choi, G. H. Park, G. P. Han, and D. Y. Kil. 2019. Effect of stocking density and sex on growth performance, meat quality, and intestinal barrier function in broiler chickens. Poult. Sci. 98:1153–1160.

Kim, J. H., H. Jung, F. M. Pitargue, G. P. Han, H. S. Choi, and D. Y. Kil. 2017. Effect of dietary calcium concentrations in low non-phytate phosphorus diets containing phytase on growth performance, bone mineralization, litter quality, and footpad dermatitis incidence in growing broiler chickens. Asian-Australas. J. Anim. Sci. 30:979–984.

Lambert, G. P. 2009. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. J. Anim. Sci. 87:E101–E108.

Li, X. L., M. Jiang, Z. Ruan, S. M. Mi, X. Wu, K. Yao, X. Xiong, Y. Zhou, and Y. L. Yin. 2016. Tryptophan increases intestinal permeability and decreases intestinal tight junction protein expression in weanling piglets. J. Anim. Sci. 94:87–90.

Liu, W., S. Mi, Z. Ruan, J. Li, X. Shu, K. Yao, M. Jiang, and Z. Deng. 2017. Dietary tryptophan enhanced the expression of tight junction protein ZO-1 in intestine. J. Food Sci. 82:562–567.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Puron, D., R. Santamaria, J. C. Segura, and J. L. Alamilla. 1995. Broiler performance at different stocking densities. J. Appl. Poult. Res. 4:55–60.

Seo, S., S. Jeon, and J. K. Ha. 2018. Guidelines for experimental design and statistical analyses in animal studies submitted for publication in the Asian-Australasian Journal of Animal Sciences. Asian-Australas. J. Anim. Sci. 31:1381–1386.

Shen, Y. B., G. Voilque, J. D. Kim, J. Odle, and S. W. Kim. 2012. Effects of increasing tryptophan intake on growth and physiological changes in nursery pigs. J. Anim. Sci. 90:2264–2275.

Shin, J. E., J. H. Kim, D. Goo, G. P. Han, F. M. Pitargue, H. K. Kang, and D. Y. Kil. 2018. Effect of dietary supplementation of betaine on productive performance, egg quality, and jejunal tight junction-related gene expression in laying hens raised under hot environmental conditions. Livest. Sci. 214:79–82.

Wang, B., Z. Min, J. Yuan, B. Zhang, and Y. Guo. 2014. Effects of dietary tryptophan and stocking density on the performance, meat quality, and metabolic status of broilers. J. Anim. Sci. Biotechnol. 5:44.

Zuowei, S., L. Yan, L. Yuan, H. Jiao, Z. Song, Y. Guo, and H. Lin. 2011. Stocking density affects the growth performance of broilers in a sex-dependent fashion. Poult. Sci. 90:1406–1415.