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Asian Journal of Animal and Veterinary Advances

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Asian Journal of Animal and Veterinary Advances 9 (9): 568-577, 2014
ISSN 1683-9919 / DOI: 10.3923/ajava.2014.568.577

Effects of Dietary Tryptophan Levels and Stocking Density During the Growing-Finishing Phase on Broiler Performance and Immunity

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ABSTRACT

A total of 240 Cobb 500 broiler chicks, 18 day old were allocated to 10 treatments groups, each of which included 4 replicates. Experimental treatments consisted of a 5×2 factorial arrangement with 5 levels of L-tryptophan supplementation and 2 levels of stocking density (11.90 birds m^{-2} as the normal stocking density or 16.66 birds m^{-2} as the high stocking density). Crystalline L-Trp was added to the basal diet at 0.0 (100%, NRC), 0.25 (114%, NRC), 0.50 (128%, NRC), 0.75 (141%, NRC) and 1.00 (156%, NRC) g kg^{-1} diet to obtain the dietary Trp level at 1.8, 2.05, 2.3, 2.55 and 2.8 g kg^{-1}. Increasing L-Trp level in the diet did not affect LBW, BWG and FCR ($p>0.05$). However, feed intake decreased significantly with L-Trp and it was minimized at 0.75 and 1.0 g kg^{-1} diet. Also, increasing L-Trp level had no effects on immunity, plasma total protein and glucose (GLU). However, adding L-Trp at 0.75 or 1.00 g kg^{-1} diet increased liver weight. Also, plasma cholesterol (CHO) levels decreased significantly ($p<0.05$) with L-Trp supplementation and the lowest levels occurred at 0.25 L-Trp (114%, NRC). In addition, plasma triiodothyronine (T3) and thyroxine (T4) levels were higher at 0.75 (141%, NRC) L-Trp supplementation ($p<0.05$). The normal stocking density resulted in better performance ($p<0.05$) compared with the high stocking density. However, stocking density did not affect plasma total protein, total Ig, IgG, IgM, GLU, CHO, T3 and T4 levels. Significant interactions between Trp level and stocking density were observed for plasma levels of CHO. Findings suggest that addition of L-Trp up to 0.25 g kg^{-1} achieve 114% of NRC recommendations of dietary Trp that has a positive effect on decreasing plasma total CHO levels.

Key words: Cholesterol, immunity, L-tryptophan, stocking density

INTRODUCTION

The stocking densities differ between different strains, countries and husbandry systems. However, many producers around the world need to increase stocking densities to maximize profitability. A high stocking density affects performance, welfare, immunity and gut health negatively (Shane, 2000; Heckert *et al*., 2002; Thaxton *et al*., 2006; Estevez, 2007).

Tryptophan (Trp) is an essential amino acid that participates in protein synthesis. Moreover, it has been shown that a deficiency of Trp decreased antibody production in rats (Gershoff *et al*., 1968). In addition, Emadi *et al*. (2010) reported that high levels of Trp have positive effects on systemic immune response and growth performance in broiler chickens. Trp is considered as a precursor of serotonin [5-hydroxytryptamine (5-HT)]. Serotonin (a neurotransmitter) has many functions in the central nervous system to inhibit aggression and modulates stress response, including social and environmental adaptability (Martin *et al*., 2000).

Recently, Houshmand *et al.* (2012) observed significant interactions between protein level and stocking density for BW gain and final BW. Therefore, the aim of this study was to investigate the effects of dietary tryptophan as one of essential amino acids on performance, immune function and stress indicators of broilers chickens during growing phase at different stocking densities.

MATERIALS AND METHODS

This experiment was conducted at Poultry Unit of the Department of Poultry Production, Teaching and Research Farm, Faculty of Agriculture, Mansoura University, Mansoura, Egypt.

Birds and treatments: Cobb 500 broiler chicks (n = 240), 18 day old, were divided into 10 treatments groups, each of which included 4 replicates (cages). Experimental treatments consisted of a 5×2 factorial arrangement with 5 levels Cobb 500 broiler chicks (n = 240), 18 day old, were divided into 10 treatments groups, each of which included 4 replicates (cages). Experimental treatments consisted of a 5×2 factorial arrangement with 5 levels of dietary L-Trp and 2 levels of stocking density. Experimental diet was formulated to meet or exceed the NRC (1994) nutrition recommendations from 21-42 days of age for all nutrients (Table 1). Crystalline L-Trp was added

Table 1: Composition and nutrient content of the diets (g kg^{-1})

Nutrient content	Composition (g kg^{-1})
Corn	696.00
Soybean meal	165.00
Corn gluten meal	100.00
L-Lys. HCL	3.00
Dl-Meth	0.50
Dicalcium phosphate	16.50
Limestone	13.00
NaCl	3.00
Premix[1]	3.00
Total	1000.00
Nutrient level	**Composition (g kg^{-2})**
Crude protein	195.80
Lysine	10.30
Methionine	4.30
Tryptophan[3]	1.80
Threonine	6.90
Isoleucine	7.70
Valine	9.00
Calcium	9.20
Available phosphorus	4.20
ME (Kcal kg^{-1})	3071.55

[1]Premix provided the following per kilogram of diet: Vitamin A (retinyl acetate): 2654 µg, Vitamin D3 (cholecalciferol): 125 µg, Vitamin E (dl-α-tocopheryl acetate): 9.9 mg, Vitamin K3 (menadione dimethylpyrimidinol): 1.7 mg, Vitamin B1 (thiamin mononitrate): 1.6 mg, Vitamin B12 (cyanocobalamin): 16.7 µg, Riboflavin: 5.3 mg, Niacin (niacinamide) 36 mg, Calcium pantothenate 13 mg, Folic acid: 0.8 mg, d-biotin: 0.1 mg, Choline chloride: 270, BHT: 5.8, Fe (iron sulphate monohydrate): 50 mg, Cu (copper sulphate pentahydrate): 12 mg, I (calcium iodate): 0.9 mg, Zn (zinc oxide): 50 mg, Mn (manganous oxide): 60 mg, Se (sodium selenite): 0.2 mg, Co (cobalt sulphate): 0.2 mg. [2]Calculated from data provided by NRC (1994). [3]The respective diet formulated to contain 1.8, 2.05, 2.3, 2.55 and 2.8 g kg^{-1} tryptophan and the dose titrations were achieved by addition of L-Trp at the expense of soybean meal

to the basal diet at 0.0 (100%, NRC) 0.25 (114%, NRC), 0.50 (128%, NRC), 0.75 (141%, NRC) and 1.00 (156%, NRC) g kg^{-1} diet to obtain the dietary Trp level at 1.8, 2.05, 2.3, 2.55 and 2.8 g kg^{-1}. Birds were reared in battery cages and the length, width and height of each cage were 70, 60 and 40 cm, respectively. Thus, the cage floor area was 0.42 m^2 (70×60 cm). The numbers of birds located in each cage varied, depending on the stocking density. For the normal and high stocking densities, 5 and 7 birds, respectively, were placed in each cage. The stocking density was 11.90 birds m^{-2} as the normal density and 16.66 birds m^{-2} as the high density. The present study was carried out during March to April. The average daily temperature inside the farm ranged from 19.5-23.3°C. The photoperiod was 23L:1D throughout the experiment. Chickens were reared 42 days of age and fed a starter ration from 1-17 days of age (3100 kcal of ME kg^{-1} of diet and 22% CP) and a grower ration from 18-42 days of age (Table 1). Feed in mash form and water (via nipple drinkers) was provided freely.

Broiler performance parameters: Live Body Weight (BW) and Feed Intake (FI) were measured every week based on a replicate. Body Weight Gain (BWG) was calculated on weekly basis throughout the experimental period. The consumed amounts of feeds were recorded every week and corrected Feed Conversion Ratio (FCR) was then calculated. Mortalities and health status were visually observed and recorded daily throughout the entire experimental period.

Blood sampling and laboratory analyses: Four birds of each treatment slaughtered and blood samples were collected at the end of experiment. The spleen, bursa, gizzard, livers, pancreas and intestine of each bird were collected and weighed. Blood samples were obtained via vena cava puncture and fresh blood was collected in heparinized tubes and centrifuged at 4000 rpm for 15 min. Plasma obtained was stored at -20°C until analysis. Plasma samples were tested colorimetrically using commercial kits according to the procedures outlined by the manufactures, for determination of total protein (Fales, 1982), cholesterol (Allain *et al*., 1974) and glucose (Trinder, 1969). Plasma T3 and T4 were determined by RIA technique using Gamma-Coat 125I RIA Kits, Chinical Assay, Cambridge, Medical Diagnostics, Boston, MA, as reported by Akiba *et al*. (1982).

Immunity parameters: The spleen and bursa of each bird were collected and weighed. Plasma total Ig and IgG were analyzed according to Meurman *et al*. (1982). IgM was calculated by the difference between total Ig and IgG.

Statistical analyses: All statistical analyses were performed using SPSS Version 16.0 for Windows (SPSS Inc., Chicago, IL) and data was subjected to 2 way ANOVA. Values were considered statistically different at $p = 0.05$. When significant differences were found ($p<0.05$), Tukey *post hoc* tests were performed. The main effects of Trp levels were further tested for linear, quadratic and cubic responses using orthogonal contrasts.

RESULTS AND DISCUSSION

Broiler performance: The effects of stocking density and Trp Levels on the weight gain, feed intake and FCR of broiler chickens at 42 days of age are presented in Table 2. Increasing L-Trp level in the diet did not affect LBW, BWG and FCR ($p>0.05$). However, feed intake, decreased significantly with L-Trp and it was minimized at 0.75 and 1.0 g kg^{-1} diet. This result is in

Table 2: Effect of Trp levels and stocking density on growth performance of broilers at 42 days of age

Item	Final BW (kg)	BWG (kg)	Feed intake/bird (kg)	FCR
Trp levels (g kg^{-1})				
0.0 (100%, NRC, 1994)	1.82	1.34	2.59^{a}	1.93
0.25 (114%, NRC)	1.78	1.30	2.49^{ab}	1.91
0.50 (128%, NRC)	1.74	1.26	2.49^{ab}	1.97
0.75 (141%, NRC)	1.72	1.24	2.44^{b}	1.97
1.00 (156%, NRC)	1.71	1.22	2.44^{b}	2.0
SEM	0.05	0.05	0.03	0.08
Contrast p-valne				
Trp	0.52	0.53	0.04	0.93
Linear	0.08	0.09	0.004	0.47
Quadratic	0.74	0.71	0.24	0.82
Cubic	0.99	0.99	0.67	0.89
Stoking density				
Normal	1.82^{a}	1.33^{a}	2.67^{a}	2.00^{a}
High	1.69^{b}	1.22^{b}	2.31^{b}	1.89^{b}
SEM	0.03	0.03	0.02	0.05
p-value	0.009	0.032	0.00	0.11
Interaction (p-value)	0.57	0.56	0.31	0.75

[1]Data are means of 4 replications with 5[2] or 7[3] birds per replicate according to stocking density

agreement with Li (2000) who reported that the requirement of the total Trp was 0.18-0. 19% from 3-6 weeks of age. However, Corzo *et al.* (2005a) recommended that total Trp needs were 0.20, 0.21 and 0.22% for feed intake, body weight gain and feed conversion, respectively. Edmonds and Baker (1987) found that a 4% excess of tryptophan reduced the weight gain of broilers by 57%. This result is in agreement with Rosebrough (1996) who reported that low protein levels (12%) supplemented with tryptophan excess caused a reduction in feed intake which did not happen with diets high in crude protein (30%) for 28 day old male broilers. However, Duarte *et al.* (2013) reported that the tryptophan levels did not significantly affect feed intake. In contrast, Peganova and Eder (2003) reported that feed consumption was 6% higher in broiler chickens that received a high concentration of dietary Trp. In general, animals decrease their feed consumption when fed diets in which the protein content is very low, very high or deficient in an indispensable amino acid or in which the protein pattern is grossly distorted from the amino acid needs of the animal (Anderson, 1977). This depression in feed intake has been attributed to changes in free amino acids in body fluids which give rise to signals monitored in the Central Nervous System (CNS) (Rogers and Leung, 1977).

Birds at normal stocking density (11.90 birds m^{-2}) resulted in better performance ($p<0.05$) compared with the high stocking density (16.66 birds m^{-2}). This indicates a greater degree of stress on the performance. As shown in Table 2, broiler LBW, BWG, feed intake and FCR were affected negatively by high stocking density. Similarly, other researchers (Elwinger, 1995; Thomas *et al.*, 2004; Muniz *et al.*, 2006) indicated that increasing the number of birds per unit depresses growth rate and feed intake. In contrast, Buijs *et al.* (2009) reported that at 39 day of age LBW was not significantly different between birds reared at different stocking densities (6, 15, 23, 33, 35, 41, 47 and 56 kg m^{2}). Also, FCR was affected adversely by high stocking density. Similarly, Houshmand *et al.* (2012) reported that during the grower phase (day 22-42), broilers raised at a high density had an inferior FCR compared with birds housed at a normal density. However, the

FCR found in this study are in disagreement with the findings of other researchers have been concluded that there was no significant effect of stocking density on FCR of broilers (Feddes *et al*., 2002; El-Deek and Al-Harthi, 2004; Galobart and Moran, 2005; Türkyilmaz, 2008). The difference in management and hygiene may have been responsible for the observed discrepancy between the different studies. The reduction in production performance due to high stocking density could be related to lots of reasons. A reduction in the airflow at the bird level which occurred at the high stocking density, could decrease the dissipation of body heat to the air. Moreover, a reduction in access to water and feed, enhancement ammonia and an unfavorable air quality because of insufficient air exchange (Feddes *et al*., 2002). In addition, Dozier III *et al*. (2005) reported that the negative effect of a high stocking density on broiler growth rate is double as the chicks progressed in BW. Under stressors, the behavioral patterns of birds will be changed and consequently, their energy consumption will increase (Zulkifli and Azah, 2004). This study found that stocking density did not affect mortality.

No interaction was noted between L-Trp supplementation and stocking density in broiler performance. These findings are in agreement with others related to lysine and ME. Zuowei *et al*. (2011a) reported that lysine requirement of broilers is not altered by stocking density. In addition, Zuowei *et al*. (2011b) reported that no significant interaction between the diet ME level and the stocking density, suggesting that the ME requirement was not changed by the stocking density.

Mortality ranged between 1.0-2% during whole experiment and was not related to any treatment. It was not statistically different among groups. Previous studies reported the same findings (Sekeroglu *et al*., 2011; Türkyilmaz, 2008; Ravindran *et al*., 2006; Buijs *et al*., 2009; Skomorucha *et al*., 2009).

Liver, gizzard and pancreas weights: Liver, gizzard, pancreas and intestine weights are presented in Table 3. The weights of the gizzard, pancreas and intestine remained unaltered by the dietary inclusion of L-Trp. However, adding L-Trp at 0.75 or 1.00 g kg^{-1} diet increased liver weight. When treatments affect organ weights, this may be used as an indication that organ function also is affected. Increased liver weight is always regarded as one indicator of stress conditions.

The weights of the liver, gizzard, pancreas and intestine remained unaltered by the dietary inclusion of stocking density. Similarly, other reported that there were no significant difference in liver and gizzard weight ($p>0.05$) due to stocking density (El-Deek and Al-Harthi, 2004; Sekeroglu *et al*., 2011). No interaction was noted between L-Trp supplementation and stocking density in organ weight.

Immunity: In this study, the immune organ weight and the concentrations of immunoglobulins in plasma were assayed to investigate the effects on the immune system. Immune organs, including the spleen and bursa of Fabricus, have a very important role in the immune response of chicks, especially the spleen (Mast and Goddeeris, 1999). Bursa and spleen weights are presented in Table 4. The weights of the burasa and spleen remained unaltered by the dietary inclusion of L-Trp or stocking density. In contrast with this result, Tabiri *et al*. (2002) suggested that the feeding of chicks on a low Trp diet could, in part, feasibly lessen the impairment of immune function imposed by heat stress in broiler chickens. Lymphoid organ weights are an indicator of immunity in poultry. Ravindran *et al*. (2006) reported that relative weights of spleen and bursa decreased as density increased (16, 20 and 24 birds m^{-2}). Heckert *et al*. (2002) showed that bursa weight/BW ratios

Table 3: Effect of Trp levels and stocking density on weight of digestive organs[1]

	Weight (g)			
Item	Liver	Gizzard	Pancreas	Intestine
Trp levels (g kg^{-1})				
0.0 (100%, NRC, 1994)	45.190^{b}	34.58	3.32	95.08
0.25 (114%, NRC)	45.460^{ab}	35.18	4.33	82.42
0.50 (128%, NRC)	52.980^{ab}	37.50	4.50	85.77
0.75 (141%, NRC)	57.610^{a}	34.56	4.16	79.62
1.00 (156%, NRC)	56.780^{a}	38.52	4.08	91.50
SEM	2.880	2.49	0.45	4.21
Contrast p-valne				
Trp	0.009	0.66	0.42	0.26
Linear	0.001	0.35	0.33	0.60
Quadratic	0.630	0.87	0.12	0.11
Cubic	0.160	0.49	0.43	0.99
Stoking density				
Normal	49.840	35.57	4.09	87.37
High	53.370	36.56	4.06	87.36
SEM	1.720	1.51	0.27	3.57
p-value	0.170	0.65	0.93	0.99
Interaction (p-value)	0.120	0.25	0.72	0.52

[1]n= 4 broilers/group

Table 4: Effect of Trp levels and stocking density on immunity[1]

	log[2]			Weight (g)	
Item	Total Ig	IgG	IgM	Spleen	Bursa
Trp levels (g kg^{-1})					
0.0 (100%, NRC, 1994)	6.108	4.217	1.892	2.54	1.17
0.25 (114%, NRC)	5.967	4.050	1.917	2.52	1.55
0.50 (128%, NRC)	7.050	4.825	2.225	2.55	1.07
0.75 (141%, NRC)	5.625	3.800	1.825	2.57	1.04
1.00 (156%, NRC)	6.092	4.133	1.958	2.15	0.89
SEM	0.320	0.230	0.110	0.31	0.25
Contrast p-valne					
Trp	0.140	0.130	0.310	0.92	0.36
Linear	0.730	0.590	0.910	0.52	0.18
Quadratic	0.350	0.410	0.320	0.53	0.51
Cubic	0.530	0.580	0.510	0.67	0.31
Stoking density					
Normal	6.300	4.260	2.030	2.37	1.19
High	6.030	4.140	1.890	2.56	1.09
SEM	0.210	0.150	0.070	0.19	0.14
p-value	0.400	0.580	0.210	0.56	0.61
Interaction (p-value)	0.940	0.830	0.980	0.79	0.67

[1]n = 4 broilers/group

decreased significantly at high stocking density but no effect on the relative weight of the spleen. In this study no significant differences ($p>0.05$) were observed in bursa or spleen weight of the birds due to stocking density. Others are in agreement with these findings (Buijs *et al*., 2009;

Table 5: Effect of Trp levels and stocking density on plasma indices of broilers[1]

Item	Glucose (mg dL^{-1})	Total protein (g dL^{-1})	Cholesterol (mg dL^{-1})	T3 (ng mL^{-1})	T4 (ng mL^{-1})
Trp levels (g kg^{-1})					
0.0 (100%, NRC, 1994)	222.91	4.75	233.01^{a}	2.84^{b}	15.27^{b}
0.25 (114%, NRC)	215.00	4.72	189.08^{b}	3.49^{ab}	19.10^{ab}
0.50 (128%, NRC)	236.25	4.01	196.80^{b}	2.93^{b}	16.30^{b}
0.75 (141%, NRC)	207.50	4.99	197.38^{b}	3.66^{a}	20.38^{a}
1.00 (156%, NRC)	228.75	4.71	208.68^{ab}	3.39^{ab}	18.65^{ab}
SEM	10.54	0.25	5.91	0.17	0.93
Contrast p-valne					
Trp	0.36	0.11	0.00	0.01	0.005
Linear	0.90	0.81	0.04	0.02	0.01
Quadratic	0.83	0.22	0.00	0.40	0.23
Cubic	0.53	0.46	0.41	0.70	0.78
Stoking density					
Normal	226.33	4.54	206.29	17.63	3.22
High	217.83	4.73	203.69	18.25	3.31
SEM	6.66	0.16	3.74	0.58	0.10
p-value	0.37	0.40	0.63	0.46	0.54
Interaction (p-value)	0.39	0.32	0.007	0.87	0.91

[1]n = 4 broilers/group

Tong *et al*., 2012; Houshmand *et al*., 2012). The effects of stocking density and Trp Levels on immunoglobulins are presented in Table 4. From the nutritional viewpoint, amino acids affect the synthesis of effector molecules (immunoglobulins, nitric oxide, lysozyme and complement). Gershoff *et al*. (1968) observed that a deficiency of Trp decreased antibody production in rats. In current study, adding L-Trp has no affect on total Ig, IgG or IgM. In the process of protein anabolism and proteolysis, the serum protein level is always an indicator of the protein metabolism and immunity function situation *in vivo*. Avian total protein contains albumin and α, β and γ-globulin (Lumeji, 1997), thus, high concentrations of total protein are associated with significant increases in levels of serum albumin and globulin (Hunt and Hunsaker, 1965). Tyler *et al*. (1996) suggested that the IgG concentration increases as the total protein concentration increases. L-Trp did not affect levels of plasma total protein (Table 5). In this study, stocking density had no affect on levels of plasma immunoglobulins (Table 4). Tong *et al*. (2012) showed that no significant difference was noted in the immunological parameters due to different stocking density. However, Houshmand *et al*. (2012) concluded that the normal stocking density resulted in higher antibody titer against Newcastle disease compared with the high stocking density. These data suggest the stocking density 16.66 birds m^{-2} did not suppress immune system but suppress broiler performance compared with 11.9 birds m^{-2}. No interaction was noted between L-Trp supplementation and stocking density in immunity.

Blood parameters: The effects of stocking density and Trp Levels on the blood chemicals are presented in Table 5. There was no significant effects of L-Trp on glucose. However, plasma cholesterol (CHO) decreased significantly ($p<0.05$) response to L-Trp and the lowest levels occurred at 0.25 L-Trp g kg^{-1}. There was interaction between L-Trp supplementation and stocking density in plasma cholesterol (CHO). Also, Corzo *et al*. (2005a, b) showed that blood plasma cholesterol showed a linear decrease with increasing dietary Trp. No significant effects of stocking

density on plasma total protein. Abudabos *et al.* (2013) showed that serum total protein did not change by stocking density. However, other disagreements with current result (Sekeroglu *et al.*, 2011; Tong *et al.*, 2012) reported that level of blood total protein increased by increasing stocking density. In addition, plasma triiodothyronine (T3) and thyroxine (T4) levels were higher at 0.75 (141%, NRC) L-Trp supplementation ($p<0.05$) (Table 5). In rats, Alfonso *et al.* (2001) also reported that L-Glu increased serum T3 and T4 and they suggested that the importance of EAAs in the regulation of hormone secretion from the pituitary-thyroid axis.

No significant effects of stocking density on stress indicators (cholesterol or glucose). These results are in agreement with other studies who reported no evidence of physiological stress resulting from a high stocking density (Dozier III *et al.*, 2006; Thaxton *et al.*, 2006; Buijs *et al.*, 2009; Houshmand *et al.*, 2012). Also, stocking density did not affect plasma levels triiodothyronine (T3) and thyroxine (T4). Similarly, Tong *et al.* (2012) reported that no effects were found on T3 or T4 due to stocking density.

CONCLUSION

The findings of present study suggest that addition of L-Trp up to 0.25 g kg^{-1} achieve 114% of NRC recommendations of dietary Trp has a positive effect on decreasing plasma total CHO levels.

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