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Effect of Stocking Density and Methionine Levels on Growth Performance and Immunity of Broiler Chicks

Research Article

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ABSTRACT

The experiment was conducted to investigate the effect of stocking density and incremental methionine levels on performance, immune response, carcass traits, and digestive organs of broiler chicks. A total of 390 day-old broiler chicks (Ross 308) were allocated to six dietary treatments and five replicates base on 3 × 2 factorial arrangement in a completely randomized design. Experimental diets included three levels of stocking density (12, 16 and 18 birds/m^2) and two levels of methionine (100 and 120% requirement recommended for Ross 308) applied during 42 days of experiment. The results showed that 18 birds density decreased body weight and weight gain, feed intake and increased feed conversion ratio (P<0.05) in overall rearing period. The 120% methionine level tended to increase body weight at 42 day of age. Stocking density significantly (P<0.05) affected the relative weight of proventriculus, pancreas, gizzard and duodenum. The jejunum relative weight and ileum length of chicks received 120% methionine level significantly increased. Antibody titer against sheep red blood cells (SRBC), Influenza and Newcastle viruses, heterophil to lymphocyte ratio and lymphoid organs except spleen were not affected by dietary treatments (P<0.05). As a conclusion, broiler performance of chicks adversely affected by stocking density and methionine levels had no considerable effect on their performance.

KEY WORDS broiler, immunity, methionine, performance.

INTRODUCTION

Rearing poultry in intensive systems needs the birds with fast growth rate to be raised at high stocking density. Based on the investigations 85 to 90% of performance gains are due to genetic selection, while the 10 to 15% are the results of improvements in nutrition and other management practices (Havenstein *et al*. 2003). Meanwhile, selection for growth rate and feed efficiency cause to a number of negative effects that adversely influence welfare and productivity of poultry (Lara and Rostagno, 2013). Some research has shown that high stocking density demonstrates negative effects on growth and external carcass quality (Feddes *et al.* 2002). Dozier *et al*. (2005) evaluated performance of broilers grown to final body weight of 3.1 kg. By increasing stocking density from 30 to 45 kg/m^2, cumulative growth rate decreased by nearly 6%. Kestin *et al*. (1992) reported that by increasing stocking density lymphoid organs weight (spleen and bursa of fabricius) decreased that is an indication of stress caused by overcrowding. Heckert *et al.* (2002) studied the effect of stocking density on immune response, lymphoid organs weight, antibody titer against sheep red blood cells (SRBC), and heterophil to lymphocyte ratio at 32 and 42 days of age, they observed that increasing stocking density resulted in significant decreases in body weight and relative weight of bursa of fabricius. In combination with fast growth, the requirements for sulphur amino acids, especially methionine are high.

Most of the available feeds for poultry have a relatively low amount of sulphur amino acids, therefore without sulphur amino acids supplementation, it is difficult to reach a good amino acid balance in broiler diets. An imbalanced amino acid composition addition to impaired production, may to affect animal health and welfare through negative effects of immune response (Chen *et al*. 2003; Konashi *et al*. 2000; Kwak *et al*. 1999). Methionine as an essential amino acid at has least four main roles that may directly or indirectly involved in immune system responses, such as: 1) participates in protein synthesis; 2) glutathione precursor, a tripeptide that reduces reactive oxygen species (ROS) and protect cells from oxidative stress; 3) synthesis of polyamines (spermine and spermidine) require methionine that participate in nucleus and cell division events (Bouyeh, 2012).

Ahmed and Abbas (2011) using incremental level of methionine (0, 100, 110, 120 and 130% of recommended NRC (1394)) observed significant effect on feed intake, feed conversion ratio and protein yield, which is in agreement with Pillai *et al*. (2006) who observed the maximum feed consumption, weight gain and feed efficiency by increasing methionine level in broilers diet.

Attia *et al*. (2005), and Swan and Johri (2000) observed that by methionine supplementation white blood cell immigration, humoral and cellular immune responses improved. Also, total serum protein enhancement, globulin, antibody response against Newcastle viruses, aspartate amino acid transferase and alanine amino acid transferase reduction, enhancement of total antibody, response to mitogen phytohemaglutenine (likely related to T-cell) improved by supplementing methionine in broilers diet (Tsiagbe *et al*. 1987). Thus, methionine deficiency may lead to the reduction of humoral immunity and none-specific immunity in broiler chicks (Zhang and Guo, 2008). This study was conducted to investigate the effect of methionine supplementation on performance and immunity of broiler chicks exposed to overcrowding.

MATERIALS AND METHODS

Animals, management and treatments

Three hundred and ninety day-old mixed sex broiler chicks (Ross 308) were used in this study. The chicks were housed in pens 1.2 × 1.2 m in size with wood shavings flooring. The chicks were randomly assigned to 6 dietary treatments (Table1) each replicated five times based on a completely randomized design in a factorial arrangement. Water and mash feed were provided *ad-libitum*. Lighting program was 23 h light and 1 h darkness. Experimental diets included: 3 levels of stocking density (12, 16 and 18 broilers in each meter square) and 2 levels of methionine (100 and 120% of Ross requirement).

At the arrival time of broilers temperature was set to 35 ˚C and then 2 ˚C decreased weekly till 26 ˚C and kept constant to end of the experiment.

Performance parameters and carcass traits

Body weight (BW), weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) were recorded at the end of each rearing period. At 42 days of age, 2 birds closest to the mean weight of each replicate were selected, weighed and killed. Parameters such as carcass weight, weight of abdominal fat, proventriculus, gizzard, pancrease, liver, lymphoid organs (bursa of fabricius and spleen), weight and length of duodenum, jejunum, ileum and cecum were measured.

Immune response parameters

At 28 days, 2 birds per each replicate were selected and 1 ml of 1% rinsed sheep red blood cell (SRBC) was injected through their brachial vein and 5 day later (33 day) bloods were taken from them and centrifuge to assay antibody titer against SRBC by the micrometer procedure of Wegmann and Smithies (1966).

Titers were expressed as the $\log_2$ of the reciprocal of the highest dilution giving visible hemagglutination. At 33 days, 2 birds per each replicate were selected and blood samples of them were taken and centrifuged at 3000 × g for 10 min for antibody titers measurements against Newcastle and Influenza viruses using hemagglutination inhibition test.

At 33 day, 2 birds per each replicate were selected and blood samples were taken through brachial vein, then colored with Geimsa method and heterophil and lymphocyts were measured under light microscope.

Statistical analysis

Data were analyzed using ANOVA and general linear model (GLM) procedure of SAS (2008) appropriate for a factorial (2×3) arrangement in a completely randomized design with the main effect of stocking density and methionine level. Means were compared using least significant difference (LSD) test and were considered to be significant different at $P < 0.05$.

RESULTS AND DISCUSSION

Performance, carcass traits, digestive organ weight and intestinal length

The effect of methionine levels and stocking density on performance parameters of broiler chicks presented in Table 2. Stocking density significantly influenced body weight at 42 day ($P<0.001$), weight gain at 1-42 day ($P<0.01$) and feed intake at 1-42 day ($P<0.001$).

Table 1 Ingredients and composition of the diet in different rearing periods

Diet composition (%)	Starter (1-14 d)[1]	Grower (15-28 d)[1]	Finisher (29-42 d)[1]
Corn	53.87	56.67	58.97
Soybean meal (42% CP)	40	36.7	34.7
Soybean oil	1.84	3	3
Dicalcium phosphate[2]	1.9	1.67	1.52
Calcium carbonate	1.14	0.93	0.9
DL-methionine	0.32	0.24	0.16
L-lysine	0.18	0.6	0
Salt	0.3	0.3	0.3
Vitamin premix[3]	0.2	0.2	0.2
Mineral premix[4]	0.2	0.2	0.2
Calculated composition			
Metabolizable energy (kcal/kg)	2870	3000	3035
Crude protein (CP) (%)	21.91	20.7	20
Lysine (%)	1.36	1.18	1.08
Lysine + methionine (%)	1.01	0.9	0.82
Calcium (%)	1	0.86	0.8
Available phosphorous (%)	0.47	0.43	0.4

[1] Note: in all of the diet methionine requirement provided 100% and 120% more than Ross requirement.
[2] Dicalcium phosphate contained: 16% phosphorous and 23% calcium.
[3] Vitamin premix per kg of diet: vitamin A (retinol): 2.7 mg; vitamin D_3 (cholecalciferol): 0.05 mg; vitamin E (tocopheryl acetate): 18 mg; vitamin K_3: 2 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Panthothenic acid: 10 mg; Pyridoxine: 3 mg; Cyanocobalamin: 0.015 mg; Niacin: 30 mg; Biotin: 0.1 mg; Folic acid: 1 mg and Choline chloride: 250 mg.
[4] Mineral premix per kg of diet: Fe ($FeSO_4.7H_2O$, 20.09% Fe): 50 mg; Mn ($MnSO_4.H_2O$, 32.49% Mn): 100 mg; Zn (ZnO, 80.35% Zn): 100 mg; Cu ($CuSO_4.5H_2O$): 10 mg; I (KI, 58% I): 1 mg and Se ($NaSeO_3$, 45.56% Se): 0.2 mg.

Table 2 Effect of methionine levels and stocking density on growth performance of broiler chicks in overall growth period

Performance	Treatment							
	Stocking density (SD, bird/m^2)			Methionine level (ML, %)		Source of variation (significance)		
	12	16	18	100	120	SD	ML	SD × ML
Body weight (g) 42 day	2670^a±30.20	2718^a±36.88	2516^b±28.41	2620±38.72	2649±2.20	***	NS	NS
Weight gain (g/d) 1-42 day	62.0^a±0.95	63.2^a±0.77	58.9^b±0.7	61.4±0.93	61.3±0.67	**	NS	NS
Feed intake (g/d) 1-42 day	109.1^b±0.82	114.8^a±0.87	108^b±0.7	110.2±1.09	110.9±0.84	***	NS	NS
FCR 1-42 day	1.76±0.02	1.82±0.03	1.83±0.02	1.79±0.02	1.81±0.02	NS	NS	NS

FCR: feed conversion ratio.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05); ** (P<0.01) and *** (P<0.001).
NS: non significant.

Methionine levels and interaction effect of stocking density and methionine levels on performance parameters of broilers chicks were not significant (P>0.05).

Lowest body weight, weight gain, feed intake and highest feed conversion ratio (FCR) was observed in 18 birds density (P>0.05). 120% methionine level numerically increased body weight at 42 day and FCR at 1-42 day of age. As observed in Table 3, stocking density significantly affected weight of carcass (P<0.001), liver and heart (P<0.05).

Methionine levels only affected abdominal fat (P<0.05), and the interaction effect of stocking density and methionine level was significantly affected carcass weight (P<0.05).

In 18 birds density, carcass weight significantly (P<0.001) and heart numerically decreased compare with 12 birds, while liver weight in 18 birds density compare to 12 birds significantly increased (P<0.05).

Abdominal fat in 100% methionine level was significantly lower than 120% methionine level.

Table 4, illustrates the results of methionine levels and stocking density on digestive organ weight and intestinal length.

Stocking density significantly affected weight of pancreas, cecum (P<0.05), jejunum and ileum (P<0.01); methionine levels significantly affected cecum weight (P<0.01) and ileum length (P<0.05).

Table 3 Effect of methionine levels and stocking density on carcass traits of broiler chicks (live weight percentage) on day 42

Performance	Treatment							
	Stocking density (SD, bird/m^2)			Methionine level (ML, %)		Source of variation (significance)		
	12	16	18	100	120	SD	ML	SD × ML
Carcass	65.33^a±0.23	64.36^b±0.46	62.96^b±0.29	64.42±0.38	64.00±0.26	***	NS	*
Liver	2.15^b±0.05	2.16^b±0.03	2.34^a±0.06	2.25±0.05	2.18±0.03	*	NS	NS
Heart	0.59^a±0.01	0.52^b±0.01	0.57^{ab}±0.02	0.55±0.01	0.57±0.01	*	NS	NS
Abdominal fat	1.11^a±0.06	0.97^{ab}±0.07	0.89^b±0.05	1.07^a±0.05	0.91^b±0.05	*	*	NS

FCR: feed conversion ratio.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05); ** (P<0.01) and *** (P<0.001).
NS: non significant.

Table 4 Effect of methionine levels and stocking density on digestive organ weight and intestinal length on day 42

Parameters*	Stocking density (SD, bird/m^2)			Methionine level (ML, %)		Source of variation (significance)		
	12	16	18	100	120	SD	ML	SD × ML
Proventriculus	0.38^b±0.009	0.39^{ab}±0.008	0.41^a±0.01	0.39±0.007	0.39±0.0009	*	NS	NS
Gizzard	1.35±0.02	1.39±0.008	1.40±0.03	1.34^b±0.02	1.42^a±0.02	NS	*	NS
Pancreas	0.24^b±0.008	0.26^{ab}±0.01	0.28^a±0.009	0.25±0.005	0.27±0.009	*	NS	NS
Duodenum	0.60^b±0.01	0.70^a±0.02	0.73^a±0.02	0.68±0.01	0.67±0.01	*	NS	**
Jejunum	1.98^b±0.08	2.02^b±0.05	2.29^a±0.09	2.01^b±0.07	2.19^a±0.05	**	NS	NS
Ileum	1.52^b±0.07	1.79^a±0.06	1.80^a±0.07	1.77±0.07	1.63±0.04	**	NS	NS
Cecum	0.52^b±0.02	0.53^a±0.06	0.59^a±0.07	0.58^a±0.02	0.52^b±0.01	*	**	*
Length of intestine (cm)								
Duodenum	35^a ±0.83	38^b ±0.88	37^b ±0.46	37±0.55	38±0.73	**	NS	**
Jejunum	85±0.07	87±2.16	89±2.09	86±1.74	88±1.69	NS	NS	NS
Ileum	93±1.27	93±1.52	93±1.51	95^a±1.27	91^b±0.91	NS	*	NS
Cecum	42±0.43	42±0.60	42±0.83	42±0.41	42±0.60	NS	NS	NS

* Live weight percentage.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05); ** (P<0.01) and *** (P<0.001).
NS: non significant.

Interaction effect of stocking density and methionine level affected duodenum weight and length (P<0.01), and cecum weight (P<0.05).

Immune response, lymphoid organs and heterophil to lymphocyte ratio

Base on the information of methionine levels and stocking density in Table 5, spleen weight only affected by stocking density (P<0.05); also, spleen weight in 18 birds density compare to 16 birds density significantly increased (P<0.05). None of the other parameters affected by methionine levels. Interaction effect of methionine levels and stocking density influenced on Newcastle and influenza titer and bursa of fabricius weight (P<0.05). Heterophil to lymphocyte ratio was not significantly affected by methionine levels and stocking density, but the ratio in 18 birds density and 120% methionine level numerically increased.

Performance, carcass traits, digestive organ weight and intestinal length

In present study it was observed that 18 birds stocking density significantly decreased body weight and weight gain (P<0.01), but feed intake and FCR numerically decreased in this treatment.

The effect of stocking density in recent study is in line with Shanawany (1988), who observed a decrease in average feed intake with increasing stocking density. This reduction may because of low feeder space for each bird. So, lower feed intake caused lower weight gain in high density treatments.

The FCR enhancement in higher stocking density may because of the competitive situation for feed intake and likely increase their activities (Thaxton *et al.* 2006).

Although, methionine has a role in protein synthesis, methylation reactions and is a methyl donor (Pillai *et al.* 2006), but increasing methionine level in this experiment did not significantly affect performance parameters and slightly increased body weight and FCR. Inconsistent with present study Hesabi *et al.* (2006) stated that broiler fed with incremental methionine level improved FCR. It seems that, adding methionine to broiler diet with higher protein level can be effective.

Based on the information in Table 3 carcass weight in 12 birds density compared to 16 and 18 birds density was significantly higher (P<0.001). It may because of higher feed intake in low stocking density. In accordance with present result, Dozier *et al.* (2005) and Dozier *et al.* (2006) stated that increasing stocking density decreased carcass weight.

Table 5 Effect of methionine levels and stocking density on immune response, lymphoid organs and heterophil to lymphocyte ratio

Variable	Stocking density (SD, bird/m^2)			Methionine level (ML, %)		Source of variation (significance)[1]		
	12	16	18	100	120	SD	ML	SD × ML
Newcastle (log_2)	4.0±0.02	4.3±0.19	4.2±0.19	4.4±0.14	4.1±0.16	NS	NS	*
Influenza (log_2)	3.9±0.25	3.9±0.23	3.4±0.16	3.7±0.16	3.7±0.20	NS	NS	*
Sheep red blood cells (SRBC)	8.7±0.27	8.6±0.24	8.6±0.22	8.8±0.21	8.5±0.18	NS	NS	NS
Lymphoid organs (% live body weight)								
Bursa of fabricius	0.06±0.006	0.08±0.008	0.07±0.006	0.07±0.007	0.07±0.004	NS	NS	*
Spleen	0.13^{ab}±0.006	0.11^{b}±0.004	0.14^{a}±0.013	0.12±0.007	0.12±0.008	*	NS	NS
Heterophil/lymphocyte	0.46	0.45	0.48	0.45±0.02	0.48±0.03	NS	NS	NS

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05); ** (P<0.01) and *** (P<0.001).
NS: non significant.

Thus, with increasing body weight of broilers in low stocking density, the amount of body muscle and blood circulation need to be more and heart weight was increased that is accordance with Onbasilar *et al.* (2008).

Liver weight in 18 birds density was significantly higher than 12 and 16 birds density. The research of Feddes *et al.* (2002), Cravener *et al.* (1992), Martrenchar *et al.* (1997) and Proudfoot *et al.* (1982) support the findings obtained in present study.

Abdominal fat in lower stocking density was significantly lower than high stocking density (P<0.05), while it was expected to be lower in high stocking density. Similar to the present findings Velmurugu *et al.* (2006) observed that increasing density lowered abdominal fat weight. This discrepancy may be due to differences in the age of slaughter. Increase methionine level had no significant effect on carcass traits, but abdominal fat significantly decreased by 120% methionine level. Similarly, OjanoDirain and Waldroup (2002) stated that increases methionine level from 0.38 to 0.44% did not significantly decrease abdominal fat. Also, Rostagno *et al.* (1995) reported that methionine supplementation in broilers diet have a direct correlation with lowering abdominal fat, that is accordance with observations of present study.

Presumably, methionine by stimulating oxidative stress through carnitine synthesis has a role in fat metabolism that is cause to abdominal fat reduction.

As mentioned previously, duodenum, jejunum, ileum and cecum weights significantly increased in 16 and 18 birds density. It can be assume that there is a diverse relation between digestive organs and body weight that by increasing digestive organs weight, body weight decreased relatively, because digestive organs are stated based on live weight percent of live weight. By increasing or decreasing digestive organs weight, carcass weight and live weight will change proportionally.

Incremental methionine level, percentage weight of gizzard and jejunum significantly increased (P<0.05), while cecum weight and ileum length significantly decreased (P<0.05).

Jejunum is a place for protein absorption and increasing jejunum and ileum length will enhance absorption area.

Immune response, lymphoid organs and heterophil to lymphocyte ratio

As shown in Table 5 antibody titer against Newcastle, Influenza and SRBC as an immune response, and bursa of fabricius as a lymphoid organ and heterophil to lymphocyte ratio as a stress index were not affected by stocking density; also, mentioned parameters were not affected by methionine level. Spleen weight decreased as bird density increased from 16 birds to 18 birds (P<0.05).

In accordance with obtained results, Dozier *et al.* (2006) observed that increasing stocking density resulted in an increased heterophil to lymphocyte ratio. Similar to the results in present study, Heckert *et al.* (2002) reported that humoral immune response parameters such as SRBC, heterophil to lymphocyte ratio, and lymphocyte blastogenesis were not different across densities. Another consistent result to current study is Palizdar *et al.* (2016), who observed that by increasing stocking density up to 18 birds/m^2, immune response such as SRBC, IgG and IgM titers did not affected. Also, Mehmet Kenan (2008) concluded that different stocking density did not make a significant different in heterophil to lymphocyte ratio. Fidan and Nazligul (2013) reported that heterophil to lymphocyte ratio on 656.0, 492.0 and 393.6 cm^2 cage floor spaces/hen groups were 0.78, 0.87 and 0.90, respectively (P>0.05).

Increasing spleen weight with higher stocking density in recent experiment may be results of high density per m^2. Similarly, Thomas *et al.* (2004) reported that increasing density enhance spleen weight, but was inconsistent with results of present study about bursa of fabricius. Muniz *et al.* (2006) stated that higher densities increased bursa cortical follicles in sixth week of age.

Methionine levels did not significantly affect immune response. Although, methionine has a role in immune performance of broilers, but this effect was not observed in present study and need to do more study about the effect of methionine level on broiler immunity.

CONCLUSION

As a conclusion, 18 birds/m^2 compare to two other densities decreased fee intake, body weight, weight gain and feed intake; also, increased FCR in overall rearing period (P<0.05). Body weight in 120% methionine tended to increase. The relative weight of proventriculus, pancreas, gizzard and duodenum considerably affected by stocking density, and significantly increased in 18 birds/m^2 (P<0.05). Relative weight of jejunum and ileum length of chicks received 120% methionine significantly increased (P<0.05). Immune responses and heterophil to lymphocyte ratio were not affected by dietary treatments (P>0.05).

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