



Original Research

Response of Feeding Supplemental Choline Chloride on Immune Response of Broiler Chickens Fed Diets Containing Different Levels of Methionine

Rupjyoti Hazarika*, Chandra Deo, M. A. Wani and Sajad A. Sheikh

Avian Nutrition and Feed Technology Division, CARI, Izatnagar, Bareilly-243122,
Uttar Pradesh, INDIA

*Corresponding author: hazarikarupjyoti@gmail.com

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Abstract

An experiment was conducted to evaluate the response of feeding different levels of supplemental choline on Cell Mediated Immune Response (CMI), Humoral Immune Response (HI) and weight of different immune organs of broiler chickens fed on diets containing different levels of methionine. A six weeks (0-6wks) feeding trial was conducted involving three levels of methionine (100%, 90% and 80% of (NRC, 1994) each with three levels of choline (100%, 110% and 120% of (NRC, 1994) through 3x3 factorial experiments. Day old broiler chicks (n=360) were distributed randomly in to 45 groups of 8 chicks each. Each test diet was offered ad libitum to five replicated groups of 8 chicks each, which were housed in battery brooders cages with uniform managemental condition. At 21st days of experiment CMI, HI was assessed and immune organs weight was assessed at 21 and 42 days of age. Results indicated that supplementation of choline to different diets that are adequate and deficient in methionine had no significant effect on CMI and HI responses. Significant ($P<0.01$) reduce in the weight of bursa, spleen and thymus was observed on 21st day and 42 day of age as methionine levels was decreased in diets. With choline supplementation, the weight of bursa ($P<0.01$) and thymus ($P<0.05$) was increased significantly on 21st day of age. Based on the result, it could be concluded that methionine and choline content in diet had significant effect on immune organs weight in broiler chickens.

Key words: Choline, Immunity, Methionine

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Introduction

Methionine is an essential and first limiting amino acid in corn-soya based diet. Therefore, synthetic DL-methionine is supplemented to meet the bird's dietary requirements of methionine. But due to high cost of methionine it is essential to look for alternate sources for this amino acid. Out of various functions of methionine one of the major functions in biological systems is methyl group sparing activity (Pesti *et al.*,



1981; Chamruspollert *et al.*, 2002). Reduction in quantity of supplemental DL- methionine by fortifying the diet with methyl donors may reduce the dietary requirement of the amino acid. As a methyl donor, choline's methyl group is available after the conversion of choline to betaine in the liver. Growing chickens can use betaine inter-changeably with choline for the methylation function, but it cannot replace choline to prevent perosis (Igwe *et al.*, 2015). There is an interrelationship between choline and methionine i.e., choline donate methyl group which provide one-carbon unit in the conversion of homocysteine to methionine (Zeisel and Blusztajn, 1994). Many studies showed that choline can spare methionine requirement (Keshavaraz and Austic, 1985; Waldroup *et al.*, 2006). Swain and Johri (2000) who observed that higher value of leucocyte migration inhibition was found in chicks fed on diet containing 6.5 g/kg methionine and 1300 mg/kg choline, indicating a significantly improved cellular immune response. But limited information is available the supplementary role of choline on immunity in broiler chickens in methionine deficient diets. Therefore, the present study has been undertaken to study the effects of supplementing choline in diets containing different levels of methionine on CMI, HI and immune organs weight of broiler chickens.

Materials and Methods

The biological trial was conducted at the Division of Avian Nutrition and Feed Technology, Central Avian Research Institute (CARI), Izatnagar, Bareilly U. P. India. Day old broiler chicks (n=360) of same hatch with uniform body weight were randomly selected, wing banded, weighed individually and distributed into 45 groups with 8 chicks each and were reared in battery brooder cages, following uniform managerial conditions. Nine experimental diets with three levels of methionine –NRC(1994) 100% (0.50%), 90% (0.45%) and 80% (0.40%) during starting phase (0-3wks) and NRC (1994) 100% (0.38%), 90% (0.34%) and 80% (0.30%) during finishing phase (4-6wks) of age with three levels of choline NRC (1994) 100% (1300 mg/kg), 110% (1430mg/kg) and 120% (1560mg/kg) during starter phase and NRC(1994) 100% (1000mg/kg), 110% (1100mg/kg) and 120% (1200mg/kg) during finishing phase were formulated through factorial (3×3) experiment. The ingredients and nutrient composition of basal diet during starting (0- 3 weeks) and finishing period (4-6 weeks) have been presented in Table1. Each of such test diet was offered adlibitum as mash to five replicated groups (40birds/ treatment) of chicks from 0-6wks of age. The cell mediated immune response was assessed by injecting PHA-P (1 mg/ml PBS) to five birds per treatment intra-dermally in between third and fourth inter digital spaces at 21st day of age and response was calculated by measuring the web swelling after 24 hrs of PHAP injection and footpad index was calculated to show *in vivo* response to PHA-P (Corrier and Deloach, 1990).

Table 1: Ingredients and nutrients composition of basal diets for broiler chicks used during starter (0-3wks) and finisher (4-6wks) of age

Ingredients (%)	Starter (0-3wks)	Finisher (4-6wks)
Maize	55.54	68.24
MBM	5	5
Soyabean	38	25.6
Limestone	0.8	0.6
DCP	0.1	0
Salt	0.3	0.3
TM. Premix ¹	0.1	0.1
Vitamin Premix ²	0.15	0.15
B comp	0.02	0.02
Total	100	100
Nutrient Composition		
Analysed (%DM)		
Crude Protein	22.08	18.03
Calcium	1.12	1
Total phosphorus	0.74	0.69
Calculated		
E:P ratio	132.61	169.2
M Energy (Kcal/kg)	2928	3051
Available P (%)	0.45	0.41
Lysine (%)	1.28	0.99
Methionine (%)*	0.36	0.3
Threonine (%)	0.93	0.74
Choline (mg/kg)*	1300	1000
Cost (Rs/kg)	20.65	18.85

Trace mineral premix supplied mg/kg diet: Mg 300, Mn 55, I 0.4, Fe 56, Zn 30, and Cu 4. 2. The vitamin premix supplied per kg diet: Vit. A 2.475 mg, Vit. D₃ 0.03 mg, Vit. K 1mg, Vit. E 40IU, Vit. B₁ 2mg, Vit B₂ 4mg, Vit. B₁₂ 10mg, Niacin 60 mg, Pantothenic acid 10 mg, Choline 500 mg. * Variable in experimental diet.

Humoral immune response was assessed by injecting sheep RBC at 28th days of age to five birds per treatment and the serum antibody titer was assessed after five days of post-immunization from serum of immunized birds by HA test (Van der Zijpp, 1983 ; Siegel & Gross, 1980). At 21st and 42nd days of age, five birds per treatment in each of the age group were selected randomly and slaughtered as per standard methods, for evaluation of immune organs weight; namely- bursa, spleen and thymus. The data obtained from various parameters were analysed statistically for mean, standard errors and analysis of variance by Snedecor and Cochran (1989) using Statistical Package for Social Science (SPSS) 16th version and comparison of mean was tested by using Tukey test.

Results

The cell mediated immune and humoral immune response did not differed significantly due to interaction between methionine and choline levels in the diets (Table 2).

Table 2: Cellular and humoral immunity of broiler chickens fed different levels of methionine and choline

Treatment	Cellular and Humoral Immunity		
Methionine (% NRC,1994)	Choline (% NRC,1994)	Foot Pad Index	HA Titre(log2)
Interaction effect (Methionine X Choline)			
100	100	0.7	8
	110	0.7	8
	120	0.8	8.4
90	100	0.6	7.8
	110	0.7	7.6
	120	0.7	8
80	100	0.6	7.2
	110	0.6	7.2
	120	0.6	7.4
Pooled SEM		0.028	0.214
Methionine	100	0.74	8.13
	90	0.66	7.8
	80	0.64	7.27
Choline	100	0.67	7.67
	110	0.68	7.6
	120	0.69	7.93
Probabilities	Interaction	NS	NS
	Methionine	NS	NS
	Choline	NS	NS

NS-Non-significant

The mean immune organ weight such as bursa, spleen and thymus at 21st and 42nd day of age did not differ significantly due to the interaction between methionine and choline levels in the diets (Table 3). However, significant differences were observed on various immune organ weights on 21st and 42nd day of age due to different level of methionine in the diets. Significant (P<0.01) linear decreased in weight of bursa and thymus with gradual decrease in methionine concentration in diets from 100 to 80% at 21 days of age. However, weight of spleen was significantly (P<0.01) lower at 80% methionine than those recorded at higher levels of methionine in the diets on 21st day of age. At 42nd day of age, significantly (P<0.01) higher bursa, spleen and thymus weight were observed at 100% methionine than those recorded at other dietary levels of it. The mean weight of bursa and thymus differed significantly due to different level of choline in the diets on 21st days of age. However, weight of spleen was not significantly influence due to different levels of methionine at 21st day of age. Higher (P<0.01) weight of bursa was recorded at 120% choline than those observed in other dietary levels on 21st day of age. Significantly (P<0.05) lower thymus weight was noticed at 100% dietary choline than that recorded at 120% choline in the diet. However, thymus weight recorded at 110% choline was found intermediary on 21st day of age. The mean organs weight (bursa, spleen and thymus) did not differ significantly due to different levels of choline in the diets on 42nd day of age.

Table 3: Immune organs weight at 21days and 42days of age of broiler chickens fed different levels of methionine and choline

Treatments		Organ weight (%body wt.) at 21 days			Organ weight (%body wt.) at 42 days		
Methionine (%NRC,1994)	Choline (%NRC,1994)	Bursa	Spleen	Thymus	Bursa	Spleen	Thymus
Interaction effect (Methionine x Choline)							
100	100	0.35	0.16	0.37	0.26	0.25	0.34
	110	0.37	0.15	0.39	0.27	0.26	0.35
	120	0.41	0.16	0.42	0.3	0.26	0.38
90	100	0.33	0.14	0.34	0.24	0.23	0.3
	110	0.34	0.15	0.34	0.24	0.25	0.29
	120	0.35	0.15	0.35	0.25	0.25	0.31
80	100	0.3	0.12	0.29	0.22	0.2	0.27
	110	0.32	0.13	0.3	0.23	0.21	0.29
	120	0.31	0.14	0.32	0.24	0.23	0.3
Pooled SEM		0.006	0.003	0.007	0.005	0.005	0.007
Methionine	100	0.38r	0.15q	0.39r	0.28q	0.26q	0.35q
	90	0.34q	0.15p	0.34q	0.24p	0.24q	0.30p
	80	0.31p	0.13p	0.30p	0.23p	0.21p	0.29p
Choline	100	0.33l	0.14	0.33l	0.24	0.22	0.3
	110	0.34l	0.14	0.34lm	0.25	0.24	0.31
	120	0.36m	0.15	0.36m	0.26	0.25	0.33
Probabilities	Interaction	NS	NS	NS	NS	NS	NS
	Methionine	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
	Choline	P<0.01	NS	P<0.05	NS	NS	P<0.01

pqr (Methionine), lmn (Choline); Value bearing different superscripts within a column differ significantly (P<0.05), (P<0.01), NS-Non-significant

Discussion

In present experiment, no significant variation was observed in cellular and humoral immunity due to different levels of methionine and choline in the diets. The present results get support from earlier workers. Tsiagbe *et al.* (1987) observed that choline supplemented in chicken diet did not affect the humoral or cellular immune responses either alone or in combination with methionine. Similarly, El-Shenway and Mosaad Soltan (2015) reported that choline supplementation had no significant effect on differential leukocytic counts with slight improvement of neutrophil percentage, especially with 50% inclusion of herbal choline, which reflected higher immune response. They also reported that with choline supplementation there was non-significant improvement in antibody production in broiler chicks at 14th, 21st and 42nd day of age by about 11.9%, 32.7% and 28.8% respectively, when compared with broiler chicks group fed on the basal diet without choline supplementation. Contrary to present finding, Swain and Johri (2000) who

observed that higher value of leucocyte migration inhibition was found in chicks fed on diet containing 6.5 g/kg methionine and 1300 mg/kg choline, indicating a significantly improved cellular immune response. Lin *et al.* (1996) stated a synergistic effect of methionine and choline on the immune response of broilers at concentrations higher than the recommendations of BIS, 1992. Gholami *et al.* (2015) reported that immunoglobulins M, G, and total antibody titre values against SRBC were similar among the experimental groups on 28th and 42nd day of age revealing that the state of activity of the immune system was not affected by betaine and choline *In-ovo* administration at different concentrations. The results pertaining to immune organs weight at 21st day and 42nd day of age showed non-significant difference due to interaction of methionine and choline.

However, significant differences ($P < 0.01$) were observed on immune organs weight such as bursa, spleen and thymus at both 21st day and 42nd day of age due to different level of methionine in the diet. Significantly ($P < 0.01$) lower weight of immune organs observed when the methionine level was decreased from 100% to 80%. The development of the primary immune organs (thymus and bursa of fabricius) may be easily affected than the secondary immune organs (spleen) by methionine level in the diet (Konashi *et al.*, 2000; Deng *et al.*, 2006 and Sahu *et al.*, 2014). Authors also suggested that in methionine deficiency, there was congestion in cortex and medulla of thymic lobule; and lymphocytes in the medulla was remarkably decreased in number and are loosely arranged. Similarly, Ruan *et al.* (2017) reported that methionine can increase the relative weight of bursa of fabricius and spleen in chicken; but methionine deficiency can lead to immune organs dysplasia, and decrease the relative weight of thymus, spleen and bursa of fabricius. Methionine deficiency decreased lymphocytes in lymphoid follicles with thinner cortices and wider medullae in the bursa. Methionine deficiency reduced the lymphocytes significantly in white pulp and red pulp (Wu *et al.*, 2012).

In the present results choline supplementation showed a significant difference in weight of bursa of fabricius ($P < 0.01$) and thymus ($P < 0.05$) on 21st day of age. The result got supported from the earlier workers. El-Shenway and Mosaad Soltan (2015) found that synthetic choline supplementation in broiler diet slightly improved chicken immune response through increasing relative weight of bursa of fabricius, spleen and thymus when compared with group fed on the basal diet without choline supplementation. Gholami (2016) reported that 0.375 mg soluble choline showed positive effect on lymphoid organ (spleen) in chicks.

Conclusion

Based on the results, it could be concluded that a dietary supplementation of choline chloride to methionine adequate and methionine deficient diets had positive effect on the immune organs' weight at 21st days of age but choline had no significant influence on cell mediated and humoral immune response.

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