

# Effect of different levels of amino acid and vitamins in starter period of broilers on performance and antibody titer production

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## Abstract

This study was conducted to evaluate the effect of vitamin and amino acid levels in starter period on growth performance and immune responses of broilers. A total of 585 one-day-old broilers (Ross 308) were randomly assigned to nine experimental diets, each diet had five replicates of thirteen broilers. Daily feed intake, body weight, and daily weight gain were not significantly affected by amino acid and vitamin levels in each rearing period ( $P>0.05$ ). In overall rearing period the lowest feed conversion ratio was obtained by 85% vitamin level ( $P<0.05$ ). Also, the significant effect was observed between interaction effect of amino acid and vitamin levels ( $P<0.05$ ). The antibody titer against Newcastle Disease and Avian Influenza Viruses were not significantly affected by amino acid and vitamin levels ( $P>0.05$ ). Although, in starter period performance indices by 85% amino acid and vitamin levels were insignificantly higher than other levels. In grower period, performance indices were insignificantly increased by 115% amino acid level and 85% vitamin level ( $P>0.05$ ). The 100% amino acid and 85% vitamin level in finisher period insignificantly increased performance indices ( $P>0.05$ ). In overall rearing period, performance indices were improved by 100% amino acid and 85% vitamin level ( $P>0.05$ ). These results indicated that performance indices was improved by 100% amino acid and 85% vitamin level ( $P>0.05$ ), but immune responses were not significantly affected by amino acids and vitamin levels ( $P>0.05$ ).

**Keywords:** Broiler; Performance; Amino acid; Vitamin; Immunity

## Introduction

Methionine is one of the essential amino acid with at least four main roles; first participation protein synthesis; second, as a glutathione precursor, which reduces reaction oxygen and protects cell from oxidative stress; third, methionine needs for synthesis polyamines such as spermine and spermidine which participate in nucleus and cell division events; fourth, methionine as an important methyl group donor for methylation reactions of DNA and other molecules. (Rubin et al. 2007). Methionine and lysine are the two amino acids that have proven immune regulatory role (Bouyeh, 2012). Some studies reported that methionine constructively affects the immune system, improving both cellular and humoral immune responses (Shini and Bryden, 2005). Methionine supplementation has useful effects on the immune system under various catabolic conditions (Mirzaaghatabar et al. 2011). Some researchers reported that dietary levels of lysine and methionine more than recommended amounts for broilers may increase body weight (El-Wahab et al. 2015; Zhai et al. 2016), body weight gain (Si et al. 2001; El-Wahab et al. 2015), feed conversion ratio, breast meat yield (Si et al. 2001; Bouyeh and Gevorgyan, 2011; Zhai et al. 2016; Walls 1999), abdominal fat deposition, fat content of the breast and thigh muscles carcass efficiency (Bouyeh and Gevorgyan, 2011). Also, recent studies related to broilers shown that methionine and lysine supplementation can stimulate the immune responses of broilers (Mirzaaghatabar et al. 2011; Faluyi et al. 2015; Saleh et al. 2018). Also, previous studies have shown that methionine deficiency can change the relative proportion of T lymphocyte subset (Wu et al. 2012), and inhibit the propagation and differentiation of bursal lymphocytes (Wu et al. 2013).

Therefore, the ratio of macronutrients has a main effect on performance and body composition of broilers after post-hatch (Collin et al, 2003; Swennen et al. 2007) and macronutrients changes will decrease their performance (Kidd et al. 2004). So, the content of crude protein, amino acids, energy, macro and micro elements in pre-starter diet can affect the growth and development of broiler chickens.

Threonine is a third limiting amino acid for broilers and they cannot synthesize it in their body (Ayasan et al. 2009). It was reported that threonine deficiency resulted in decreasing application of total sulfur amino acid and lysine. Because the concentration of threonine is high in Gamma globulin, it affects the immune function (Azzam et al., 2011a, b). Ciftci and Ceylan (2004) reported that threonine requirements of broilers at various age is available. Threonine serves as a variety of function in body; for instance, plays an important role in feather synthesis, precursor of glycine and serine and it is important for mucin production and intestinal health (Najafi et al., 2017).

The aim of the study was to evaluate the levels of methionine, lysine and threonine requirements and vitamin supplement in starter periods on performance, Newcastle and Influenza Disease of broiler chicks.

## Materials and methods

### *Experimental design and bird husbandry*

The experiment was conducted according to methods approved by the animal care and use committee of the Department of Animal Science, Islamic Azad University, Isfahan (Khorasgan) Branch, Isfahan, Iran. A total of 585 one-day-old broilers (Ross 308) were randomly assigned to nine experimental diets consisting of five replicates of thirteen broilers each pen. All the birds had ad-libitum access to feed and water throughout the experiment. At day one the temperature was set at 33°C, and then it was reduced by 2.5°C each week until reaching 24°C.

### *Experimental diets*

Nine experimental diets were formulated in this experiment to contain 85, 100 and 115% amino acid, and 85, 199 and 115% vitamin requirement of Ross 308 in starter (Table 1), grower and finisher (Table 2) periods. The amino acids and vitamins were provided from Evonik Degussa and ADISSEO, respectively. The broilers received the experimental diet from zero to 10 days of age, and after that broilers received common diets. The experimental diets were as follow: A) Basal diet+100% amino acid+100% vitamin; B) Basal diet+100% amino acid+115% vitamin; C) Basal diet+100% amino acid+85% vitamin; D) Basal diet+115% amino acid+100% vitamin; E) Basal diet+115% amino acid+ 115% vitamin; F) Basal diet+115% amino acid+ 85% vitamin; G) Basal diet+85% amino acid+100% vitamin; H) Basal diet+85% amino acid+ 115% vitamin; I) Basal diet+85% amino acid+85% vitamin.

### *Performance measurement*

The performance parameters such as body weight (BW), average daily gain (ADG), daily feed intake (DFI) and feed conversion ratio (FCR) were measured at the end of each rearing period.

### *Immune response*

At 32 days of age two broilers per replicate were randomly selected and blood sample was taken from the brachial vein and centrifuged to obtain serum. Antibody titers against Newcastle and Influenza Viruses were measured using Hemagglutination Inhibition (HI) test (Jahanian, 2009).

*Statistical analysis*

The obtained results were analyzed using one-way ANOVA of SAS software (SAS, 2009), significant differences ( $P < 0.05$ ), among treatment means were determined using the Duncan multiple range test.

**Table 1** Ingredients and nutrient composition of the experimental starter (0-10 d) diet

Item (%)	100% Amino acid			115% Amino acid			85% Amino acid		
	A	B	C	D	E	F	G	H	I
Corn	49	49	49	48.5	48.5	48.5	49.5	49.5	49.5
Soybean meal	38	38	38	38.1	38.1	38.1	37.9	37.9	37.9
Wheat	8	8	8	8	8	8	8	8	8
Soybean oil	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97
CaCO <sub>3</sub>	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Monocalcium phosphate	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Salt	0.18	0.18	0.18	0.13	0.13	0.13	0.21	0.21	0.21
Choline chloride	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine	0.25	0.25	0.25	0.42	0.42	0.42	0.08	0.08	0.08
DL-Methionine	0.34	0.34	0.34	0.43	0.43	0.43	0.23	0.23	0.23
L-Threonine	0.1	0.1	0.1	0.19	0.19	0.19	0.01	0.01	0.01
Phytase	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
NaHCO <sub>3</sub>	0.08	0.08	0.08	0.17	0.17	0.17	0.02	0.02	0.02
Vitamin premix <sup>1</sup>	0.1	0.115	0.85	0.1	0.11	0.85	0.1	0.11	0.85
Mineral premix <sup>2</sup>	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Calculated nutrients									
Metabolizable energy (Kcal/Kg)	2850	2850	2850	2850	2850	2850	2850	2850	2850
Crude protein (%)	22	22	22	22	22	22	22	22	22
Lysine (%)	1.35	1.35	1.35	1.48	1.48	1.48	1.21	1.21	1.21
Methionine+Cysteine(%)	1	1	1	1.1	1.1	1.1	0.9	0.9	0.9
Threonine (%)	0.9	0.9	0.9	1	1	1	0.8	0.8	0.8
Calcium (%)	1	1	1	1	1	1	1	1	1
Available phosphorous (%)	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48

<sup>1</sup> Provided per kilogram of diet: Vitamin A 12000; Cholecalciferol 5000 IU; Vitamin E 45 IU; Vitamin K 2.4 mg; Thiamin 2.6 mg; Riboflavin 6.6 mg; Pantothenic acid 25 mg; Niacin 55 mg; Cholin chloride 500 mg; Biotin 0.1 mg; Folic acid 1.5 mg; Pyridoxine 5.5 mg; Vitamin B12 0.015 mg; BHT 1 mg.

**Table 2** Ingredients and nutrient composition of the grower (11-24 d) and finisher (25-40d) diets

Item (%)	Grower	Finisher
Corn	57.3	57.3
Soybean meal	36.5	30
Soybean oil	2.4	2
CaCO <sub>3</sub>	1.6	1.5
Monocalcium phosphate	1.1	0.9
Salt	0.36	0.35
Choline chloride	0.1	0.1
L-Lysine	0.1	0.14
DL-Methionine	0.28	0.24
L-Threonine	0.05	0.03
Phytase	0.005	0.005
Vitamin premix <sup>1</sup>	0.1	0.1
Mineral premix <sup>2</sup>	0.1	0.1
Calculated nutrients		
Metabolizable energy (Kcal/Kg)	2900	2950
Crude protein (%)	20.7	18.44
Lysine (%)	1.18	1.05
Methionine+Cysteine(%)	0.92	0.82
Threonine (%)	0.82	0.72
Calcium (%)	0.95	0.85
Available phosphorous (%)	0.475	0.42

<sup>1</sup> Provided per kilogram of diet: Vitamin A 12000; Cholecalciferol 5000 IU; Vitamin E 45 IU; Vitamin K 2.4 mg; Thiamin 2.6 mg; Riboflavin 6.6 mg; Pantothenic acid 25 mg; Niacin 55 mg; Cholin chloride 500 mg; Biotin 0.1 mg; Folic acid 1.5 mg; Pyridoxine 5.5 mg; Vitamin B12 0.015 mg; BHT 1 mg. <sup>2</sup> Provided per kilogram of diet: Fe 50 mg; Zn 85 mg; Mn 90mg; I 1 mg; Cu 10 mg; Se 0.25 mg.

## Results

### Growth performance

As shown in Table 3, in different rearing periods dietary amino acid and vitamin levels had no effect on daily feed intake (DFI), body weight (BW), daily weight gain (DWG), feed conversion (FCR) of broilers ( $P < 0.05$ ). Also, there was no significant interaction effect on performance parameters of broilers at different rearing periods ( $P > 0.05$ ).

### Immune response

As presented in Table 4, antibody titer production against Newcastle Disease and Avian Influenza were not significantly affected by different amino acid and vitamin levels ( $P > 0.05$ ). Also, there was no significant interaction effect of vitamin and amino acid levels on antibody titer production ( $P > 0.05$ ).

**Table 3.** Effect of amino acids and vitamins levels on performance of broilers

1-10 d	Amino acid levels			Vitamin levels			Interaction AA×Vit
	85%	100%	115%	85%	100%	115%	
DFI	27.47±0.31	26.61±0.34	26.97±0.22	27.03±0.29	26.89±0.30	27.13±0.33	NS
BW	245±3.20	243±2.77	244±2.16	245±2.02	243±3.16	243±2.89	NS
DWG	20.19±0.31	20.0±0.28	20.10±0.21	20.25±0.19	20.03±0.31	20.01±0.28	NS
FCR	1.36±0.01	1.33±0.01	1.34±0.01	1.33±0.01	1.34±0.01	1.36±0.01	NS
11-24 d							
DFI	72.21±0.84	72.88±0.76	74.45±0.68	72.99±0.82	72.95±0.73	73.60±0.86	NS
BW	846±7.53	857±10.22	865±5.99	865±7.42	850±8.54	853±8.57	NS
DWG	42.91±0.48	43.87±0.62	44.38±0.41	44.27±0.48	43.32±0.56	43.57±0.53	NS
FCR	1.68±0.02	1.66±0.02	1.68±0.02	1.65±0.01	1.69±0.02	1.69±0.02	NS
25-40 d							
DFI	147.16±0.97	147.08±2.03	149.60±1.68	148.96±1.33	146.72±2.21	148.17±1.13	NS
BW	2165±19.1	2199±31.42	2184±29.70	2228±23.67	2156±32.1	2165±21.64	NS
DWG	82.47±1.44	83.92±1.56	82.42±1.83	85.15±1.33	81.67±1.93	81.99±1.38	NS
FCR	1.79±0.03	1.76±0.02	1.82±0.03	1.75±0.02	1.81±0.04	1.81±0.02	NS
1-40 d							
DFI	90.29±0.43	90.10±1.00	91.58±0.79	90.89±0.71	90.05±0.97	91.06±0.66	NS
DWG	53.05±0.47	53.92±0.79	53.53±0.74	54.62±0.59	52.84±0.80	53.05±0.54	NS
FCR	1.70±0.01	1.67±0.02	1.71±0.02	1.66 <sup>b</sup> ±0.01	1.71 <sup>ab</sup> ±0.02	1.72 <sup>a</sup> ±0.02	NS

**Table 4.** Effect of amino acids and vitamins levels antibody production against Newcastle Disease and Avian Influenza Viruses (Log<sub>2</sub>)

Antibody response	Amino acid levels			Vitamin levels			Interaction AA×Vit
	85%	100%	115%	85%	100%	115%	
Newcastle Disease	4.53±0.18	4.77±0.16	4.33±0.13	4.50±0.19	4.43±0.13	4.70±0.16	NS
Avian Influenza	3.13±0.11	3.10±0.12	2.93±0.14	2.93±0.12	3.13±0.13	3.10±0.12	NS

## Discussion

### Performance

Similarly, Sigolo et al (2019) observed that 10 and 20% methionine surpluses did not affect broiler growth performance at starter, grower and overall rearing period. While, in finisher period 10% methionine and 20% lysine surplus increased average daily feed intake, average daily energy intake, average daily protein intake, and average daily gain. Wallis (1999) illustrated that feed intake of broilers significantly increased by increasing levels methionine. In agreement with these findings, Cengiz et al (2008) reported that a methionine surplus had no effect on growth performance of broilers, but the lysine surplus decreased the pre-slaughtered body weight. It was suggested that free synthetic amino acid should have higher availability than amino acids in intact proteins (Ciftci and Ceylan, 2004; Abbasi et al. 2014; Sigolo et al. 2017).

The results of Bouyeh and Gevorgyan (2011) are in contrast to recent findings, they showed that supplemental methionine and lysine in broilers diet improved feed conversion ratio. El-Wahab et al (2015) reported that additional methionine and lysine increased weight of broilers. The discrepancies between literatures might be

due to the use of different experimental conditions. Li et al (2001) stated that nutritional shortage in early period after post-hatch development induced constant negative effect on body weight at the end of rearing period. According to obtained results, performance parameters of broilers were insignificantly improved by 85% vitamin level compare to other vitamin levels. It may conclude that vitamin premix used in poultry production provides more vitamin than minimum recommended levels (Maiorka et al. 2002; Rath et al. 2000).

### *Immunity*

Increased haemagglutination inhibition (HI) antibody titre was gained by high levels of methionine, which is not in line with present study. Also, the IgG levels against Newcastle Disease Viruse was significantly increased ( $P<0.05$ ) at high levels of methionine (Mirzaaghatabar et al. 2011). Swain and Johri (2000) reported that leukocyte migration and antibody titre was increased by methionine supplementation.

Bouyeh (2012) stated that methionine and lysine play role in antibody synthesis, so adequate amount of both are needed for immune system function. Sigolo et al (2019) found that immune responses against Newcastle Disease Viruses of broilers fed surplus lysine tended to increase.

### *Conclusion*

In conclusion, amino acid and vitamin levels had no significant effect on performance parameters and Antibody titer production against Newcastle Disease and Avian Influenza Diseases. The 85% vitamin level compare to other levels was optimum for growth performance in different rearing period and overall rearing period. Further research is needed to understand how amino acid and vitamin levels in starter period will affect the performance parameters and antibody titer production at the grower and finisher rearing periods.

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