

Performance Hematology and Correlation between Economical Traits under the Effects of Dietary Lysine and Methionine in Broilers

Research Article

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ABSTRACT

Lysine (Lys) and Methionine (Met) as two primary essential amino acids and precursors of carnitine biosynthesis are involved in most of economical traits function in domestic animals. We assessed the impact of dietary Lys and Met on the performance, lipid redistribution, intramuscular fat, carcass quality and especially phenotypic correlations among some studied parameters in broiler chickens. 300 day-old male Ross 308 chicks were randomly divided among 5 treatments, with 4 replicates per treatment. There were 15 chicks in each replicate in a completely randomized design. Same basal diet was supplemented with 5 levels of synthetic Lys and Met in amount of 0, 10, 20, 30 or 40% higher than National Research Council (NRC), 1994 recommendation for starter and grower periods. The collected data were analyzed and determined the correlation coefficient by SAS software and Duncan's test was used to compare the means on a value of ($P < 0.05$). The results indicated that the two highest levels of Lys and Met treatments (30 and 40% more than NRC recommendation) led to significant increase in carcass efficiency, European Production Efficiency Factor (EPEF), blood albumin, breast muscle, heart, spleen, lymphocytes and liver weight ($P < 0.05$), whereas feed conversion ratio (FCR), crude fat contents of breast and thigh muscles and plasma triglyceride were the least in these two treatment groups ($P < 0.05$). Statistical analysis also showed many numbers of significant (at levels of $P < 0.01$ or $P < 0.05$) positive or negative correlations between the studied traits. For example highly positive correlations between carcass efficiency with heart, liver, spleen and breast weights and negative correlations with FCR, abdominal fat, plasma triglyceride, heterophyles breast and thigh fat was observed. As a conclusion of present study, dietary Lys and Met in higher levels of NRC recommendation could influence the parameters relate to performance, fat distribution, carcass quality and immune system in broilers.

KEY WORDS correlation, economical traits, lysine, methionine.

INTRODUCTION

The most important goal of broiler breeding is to increase profitability of broiler meat production. Until the last few decades, most birds were sold whole, but there has been a dramatic increase in the proportion of birds being grown for portioning and further processing (Ewart, 1993). Poultry production and processing technologies have become rap-

idly accessible and are being implemented on a worldwide basis, which will allow continued expansion and competitiveness in this meat sector (Aho, 2001). So, the success of poultry meat production has been strongly related to improvements in growth factors, liveability and carcass quality together with each other especially by increasing breast muscle proportion and reducing abdominal fat pad. Abdominal and subcutaneous fat are being regarded as the

main sources of waste in the slaughterhouse. Because abdominal fat is highly correlated ($r=0.6$ to 0.9) with total carcass lipids, it is used as the main criteria reflecting excessive fat deposition in broilers (Chambers, 1990). Havenstein *et al.* (2003) described that fat in broiler (at 43 d of age) accounts for as much as 10 to 15% of the total carcass weight. Therefore, there is substantial potential to improve feed efficiency and carcass quality by further reducing fatness.

There are a number of studies have been conducted to determine the influences of lysine and methionine as the first two limiting amino acids in practical corn-soybean based diets for broiler chicks. Some researches have suggested that levels of lysine and methionine in excess of NRC (1994) recommendations may result in enhanced performance, especially in regard to breast meat yield (Si *et al.* 2004; Schutte and Pack, 1995; Hicking *et al.* 1990; Moran and Bilgili, 1990), weight gain and feed conversion ratio (Si *et al.* 2001; Gorman and Balnave, 1995) and abdominal fat (Bouyeh and Gevorgyan, 2011a). Some studies else that have been conducted to evaluate the effects of these amino acids in excess of NRC recommendations on laying hens performance, confirmed its effect on egg production, feed conversion ratio, egg weight, egg mass and livability specially in low protein diets (Bouyeh and Gevorgyan, 2011b). Murray *et al.* (1998) found that addition of synthetic amino acids like lysine and methionine at high levels to the diet can stimulate insulin secretion from pancreas and aggregate in plasma which in turn releases amino acids and fatty acids (Sturkie, 1986) from the bodily saved sources and leads to protein synthesis. Moreover, some reports have shown the positive effect of adding more lysine to the diet than required on the chickens suffering different stresses (Ayupov, 1985).

On the other hand, lysine and methionine as precursors of L-carnitine (Borum, 1983) can play important roles in lipid and energy metabolism in poultry. L-carnitine is a natural, vitamin-like substance that acts in the cells as a receptor molecule for activated fatty acids. The major metabolic role of it appears to be the transport of long-chain fatty acids into the mitochondria for β -oxidation (Coulter, 1995). A short age of this substance results primarily in impaired energy metabolism and membrane function (Harmeyer, 2002). In this regard, some researches indicated that carnitine supplementation of diets can be used to augment carnitine supply for use in metabolism, thereby facilitating fatty acid oxidation and reducing the amount of long-chain fatty acids available for storage in adipose tissue (Golzar Adabi *et al.* 2006; Kidd *et al.* 2009). Improvement in weight gain, feed conversion ratio, carcass characteristics or decrease in serum triglyceride in birds fed supplemented L-carnitine reported by researchers such as Vonlettnner *et al.*

(1992) and Xu *et al.* (2003). In this regard, determining the quantities and qualities of relationship among the important traits can help to improve both main section of poultry industry: breeding and production (Gorgani Firozjah *et al.* 2015). This study aimed to estimate phenotypic correlations between some performance, fat and immune related traits as the most economical parameters in broiler chicks under the influence of different levels of dietary lysine and methionine.

MATERIALS AND METHODS

This experiment was conducted at the broiler farm belonged to Islamic Azad University, Rasht branch, using three hundred day-old male broiler chickens (Ross 308) that were selected very carefully in aspects of uniformity in body weight, good appearance, motility, etc., so that the body weight deviation of mean (46 g) was only 0.5 g. The chicks allotted to five experiment groups, each of which included four replicates of 15 birds, performed in a completely randomized design. Same basal diet was supplemented with 5 levels of synthetic lysine (as Lys-HCl) and methionine (DL-methionine) in amount of T1= 0 (control), T2= 10, T3= 20, T4= 30 and T5= 40% higher than NRC (1994) recommendation, regarding with lysine and total sulfur amino acids (TSAA) for broilers. Diets were fed from 1 to 42 d and included starter (1 to 21 d) and grower (22 to 42 d). Nutrient levels of the basal diets were based on the NRC (1994) recommendations. In order to buffer the excess chloride provided by L-Lys HCl, there was added 0.1% NaHCO_3 to both basal diets including starter and grower that were supplied in mash physical form (Table 1). The broiler chickens were maintained in 2×1 m pens, equipped with bell drinkers and hanging tube feeders, feed and water were available *ad libitum*, light schedule, temperature and general management were performed according to Ross 308 (2007). During 42 d experimental period, body weight gain, feed consumption, mortality, feed conversion ratio and European Production Efficiency Factor (EPEF), were recorded weekly, birds were checked twice a day for mortality; dead birds were weighed and the weight was used to adjust feed conversion ratio (FCR) (total feed consumed divided by weight of live birds plus dead birds weight). At 21 and 42 day of age three birds from each pen that were within one-half standard deviation of the overall pen body weights mean and free from visible defects were randomly chosen for blood sampling which collected into a syringe from wing vein and placed into proper tubes. These blood samples were urgently sent to determine triglyceride, cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), uric acid, alkaline phosphatase, lymphocytes, heterophiles and glucose.

At the end of the experiment, after blood sampling, feed but not water was withheld 6 hr. prior to slaughter and then, those three birds of each replicate were processed for carcass characteristics.

Table 1 Composition (g/kg) of basal diets

Ingredient	Starter (0-21 d)	Grower (22-42 d)
Yellow corn	550	620
Dehulled soybean meal	380	320
Corn oil	21.5	14
Dicalcium phosphate	22	18
Oyster shell	14	15.8
Sodium chloride	2	2
Vitamin premix ¹	3	3
Trace mineral mix ²	3	3
L-lysine-HCl	1.2	1.1
DL-methionine (98%)	2.3	2.1
Sodium bicarbonate	1	1
Total	1000	1000
Nutrient		
ME (kcal/kg)	3000	3000
CP (%)	21.90	19.8
Lysine (%)	1.10	1.00
Methionine (%)	0.50	0.40
TSAA (%)	0.90	0.75

¹ Provides per kg of diets: vitamin A: 17500 IU; Cholecalciferol 5000 IU; vitamin E: 25 IU; B₁₂: 0.03 mg; Riboflavin: 15 mg; Niacin: 75 mg; Choline: 700 mg; Folic acid: 1.5 mg; Pyridoxine: 6.25 mg; Biotin: 0.127 mg and Thiamine: 3.05 mg.

² Provides per kg of diet: Zinc: 100 mg; Manganese: 120 mg; Copper: 10 mg; Iron: 75 mg; Iodine: 2.5 mg; Selenium: 0.15 mg and Calcium: 130 mg.

After weighing the carcass pieces, thigh (biceps femoris) and breast (pectoralis major) muscles, without skin were taken, chopped, ground and frozen at -20 °C until further analyses. After thawing, tissues were extracted with 2:1 chloroform: methanol. Total lipids were extracted as described by Folch *et al.* (1957) and cholesterol content of these tissues was determined enzymatically by the method of Allain *et al.* (1974), as modified by Sale *et al.* (1984). For evaluation the fatty acids profile of the muscles, it was used a gas chromatograph (not shown its results in this paper). The weight of breast and thigh (with leg) muscles, calculated as carcass weight percentage. At the end, data were analyzed by software, the partial correlation coefficients among the traits were estimated, using the software SAS, version 6.12 (SAS, 1999), fitting the same values of lysine and methionine. Path analysis was used by expanding the matrix of partial correlation in coefficients which give the direct influence of one trait on another, regardless the effect of the other traits.

RESULTS AND DISCUSSION

Main effects of treatments on the studied traits

Table 2, shows statistical comparison between the means of traits.

The effects of different dietary levels of lysine and methionine on the most number of parameters were significant ($P < 0.01$ or $P < 0.05$). For example, live body weight (at d 42), absolute weight gain, EPEF, carcass weight, (as a percentage of carcass weight) and blood albumin attained the highest value in T4.

There was a linear reduction of abdominal fat with increasing dietary lysine and methionine and also decrease in some other fat related parameters, so that reduce about 45% in plasma triglyceride, 50% in abdominal fat, 35% in breast fat content and 27% in thigh muscle fat in the highest level of lysine and methionine group in comparison with the control group was observed (not shown in the Table). Whereas the means of breast muscle and blood albumin were significantly higher in T4 and T5 experiment groups (Table 2). The trends like this, were observed in regard to bursa (bursa of fabricius), Lymphocytes, heterophyles, heterophyles/lymphocytes ratio (known as a stress criteria) which showed the better result by the two highest levels means T4 and T5 experiment groups (not shown in the Table). This result can be caused by two separate effects of lysine and methionine: 1) as two amino acids in high levels can tend to release amino acids from bodily save sources following stimulate pancreas for further secretion insulin into blood and so stimulate tissues synthesis and 2) adding of these two amino acids to diets as precursors of L-carnitine, could be used to augment carnitine supply for use in metabolism, thereby facilitating fatty acid oxidation and so reducing the amount of long-chain fatty acids available for storage in adipose tissues. There is a positive correlation between consumption lysine and methionine with the level of serum carnitine (Krajčovičková, 2000). Beside, methionine participates in protein synthesis as an essential amino acid and is also as a glutathione precursor that helps to protect cells from oxidative stress, and is required for the synthesis of polyamines (spermine and spermidine), which take part in nucleus and cell division processes and also, methionine is the most important methyl group donor for methylation reaction of DNA and other molecules (Jankowski *et al.* 2014).

On the other hand lysine is also an essential amino acid that is necessary to produce proteins like antibodies, so adequate dietary levels of these amino acids are needed to support optimum performance of immune system. Some poultry nutritionists use the level recommended by NRC as a guideline in establishing their own amino acid requirements regardless of location, health or environmental conditions. There are few research works relate to the effect of lysine and methionine on immune system. Several studies demonstrated that methionine and lysine constructively affect the immune system improving both cellular and humeral immune response.

Table 2 Effects of lysine and methionine on some performance related parameters of the broilers

Variable	Amount of dietary lysine and methionine (based on TSAA) relative to NRC recommendation					Significant
	Groups					
	T1-control (NRC)	T2 (1.1 NRC)	T3 (1.2 NRC)	T4 (1.3 NRC)	T5 (1.4 NRC)	
Live body weight (day 42)	2989.6±65.80 ^a	2949.2±70.65 ^a	2878.5±66.78 ^{ab}	2999.7±62.87 ^a	2757.3±57.07 ^b	*
Absolute gain of live weight (g)	2943.6±65.12 ^a	2902.7±69.90 ^a	2833.0±66.12 ^{ab}	2953.2±62.25 ^a	2711.3±56.50 ^b	*
EPEF	388±6.45 ^b	385±7.12 ^b	372±7.10 ^b	440±6.25 ^a	389±8.15 ^b	**
Carcass weight (g)	2116.5±47.32 ^b	2131.5±45.35 ^b	2109.0±47.32 ^b	2316.5±49.78 ^a	2110.0±41.65 ^b	**
Breast muscle weight (%) (with bone)	34.67±0.11 ^b	36.65±0.12 ^b	35.60±0.23 ^b	38.12±0.16 ^a	39.10±0.15 ^a	*
Thigh muscle weight (%) (with bone)	37.22±3.23	40.82±2.95	36.95±3.25	37.65±3.87	33.67±3.13	NS
Abdominal fat (%)	0.91±0.01 ^a	0.85±0.01 ^{ab}	0.84±0.01 ^b	0.67±0.01 ^c	0.44±0.01 ^d	**
Blood alkaline phosphatase (IU/L)	1125±48.56	1098±62.37	1112±89.47	1178±75.19	1169±87.21	NS
Blood albumin (g/dL)	1.64±0.14 ^b	1.58±0.11 ^b	1.62±0.14 ^b	1.82±0.16 ^a	1.79±0.12 ^a	*
Heart weight (%)	0.605±0.003 ^c	0.617±0.004 ^c	0.710±0.03 ^b	0.810±0.04 ^a	0.827±0.03 ^a	**

EPEF: European Production Efficiency Factor and TSAA: total sulfur amino acids.

* (P<0.05) and ** (P<0.01).

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

NS: non significant.

It was reported that methionine and lysine requirements for optimal immunity are higher than for optimal growth (Tsiagbe *et al.* 1987; Swain and Johri, 2000; Shini *et al.* 2005; Khalil *et al.* 2010).

Also it is reported that restriction of sulfur amino acids (SAA) results in severe lymphocyte depletion in intestinal tissues (Swain and Johri, 2000).

Correlation coefficients between the studied traits

Table 3 and 4 show the correlation coefficients between some of the studied traits which were more important or had more significant correlations with the other traits. As it is shown in the tables, some of correlation data are positive and others are negative, and also some of them are significant at statistical level of 0.01 or 0.05 and others non-significant. These correlation coefficients which are separated here into three groups include performance, lipid and immune related traits, indicate the quality and quantity of relationship between the traits under the influences of dietary lysine and methionine as experimental treatments.

Performance related traits

There was some significant correlations between the traits relate to performance with each other or with other traits (Table 3 and 4) which are classified into two following groups including positive and negative correlations at level of (P<0.05 or P<0.01).

Positive correlations

As it is shown in Table 3 and 4, there were some significant positive correlations between following parameters:

The correlations between feed conversion ratio (FCR) and some traits such as breast fat (r=0.683), thigh fat (r=0.526), plasma triglyceride (r=0.585) and abdominal fat pad (r=0.620).

These results show that the higher amount of breast and thigh fat, plasma triglyceride or abdominal fat pad tends to the higher FCR and so, the lower feed efficiency. This result can be acceptable because production of fat in the body is usually in companion with the more metabolic costs than other products such as protein to synthesis the body tissues.

With regard carcass efficiency, it was observed positive correlation with spleen weight (r=0.899), liver weight (r=0.800) and heart weight (r=0.915). It can be concluded that increasing in mentioned above organ weights tend to higher carcass efficiency. This result may be due to the positive effect of stronger heart, liver and spleen on health and performance of the bird.

Negative correlations

Negative correlations between FCR and heart weight (r=-0.660), shows that the higher amount of heart weight (as a percentage of the carcass weight) could decrease FCR. In regard with carcass efficiency, it observed significant negative correlations with some traits such as breast fat (r=-0.634), thigh fat (r=-0.641), plasma triglyceride (r=-0.680), and abdominal fat (r=-0.763). This result emphasize the negative effect of fat content on carcass efficiency of the broilers.

Lipid related traits

It was observed some significant correlations between the traits relate to lipids with each other or with other traits (Table 3 and 4) which are classified into two following groups including positive and negative correlations.

Positive correlations

Table 3 and 4 shows some significant positive correlations between breast fat with some traits such as thigh fat (r=0.823) and plasma triglyceride (r=0.880).

Table 3 Correlations between the studied traits

Traits	Breast fat	Thigh fat	Breast cholesterol	Thigh cholesterol	Plasma triglyceride	Lymphocytes (%)	Heterophyles (%)	Spleen weight	Bursa weight
Breast fat	1	0.823**	-0.398	-0.454*	0.880**	-0.741**	0.838**	-0.669**	-0.348
Thigh fat	0.823**	1	-0.359	-0.364	0.899**	-0.573**	0.748**	-0.736**	-0.234
Breast cholesterol	-0.398	-0.359	1	0.951*	-0.447*	0.241	-0.451*	0.499*	0.032
Thigh cholesterol	-0.454*	-0.364	0.951*	1	-0.447*	0.355	-0.495*	0.416	0.084
Plasma triglyceride	0.880**	0.899**	-0.447*	-0.447*	1	-0.638**	0.683**	-0.792**	-0.269
Lymphocytes (%)	-0.741**	-0.573**	0.241	0.355	-0.638**	1	-0.654**	0.246	0.360
Heterophyles (%)	0.838**	0.748**	-0.451*	-0.495*	0.683**	-0.654**	1	-0.495*	-0.376
Spleen weight	-0.669**	-0.736**	0.499*	0.416	-0.792**	0.246	-0.495*	1	0.075
Bursa weight	-0.348	-0.234	0.032	0.084	-0.269	0.360	-0.376	0.075	1
Live body weight	0.306	0.474*	0.021	0.020	0.021	-0.126	0.212	-0.276	-0.208
FCR	0.683**	0.526*	-0.468*	-0.462*	0.585**	-0.432	0.484*	-0.685**	-0.017
Carcass efficiency	-0.634**	-0.641*	0.429	0.315	-0.680**	0.135	-0.469*	0.899**	-0.033
Breast weight	-0.604**	-0.694**	0.256	0.228	-0.711**	0.317	-0.638**	0.657**	0.258
Thigh weight	0.250	0.363	-0.299	-0.263	0.376	-0.146	0.001	-0.278	0.172
Abdominal fat	0.781**	0.863**	-0.388	-0.335	0.938**	-0.479*	0.546*	-0.885**	-0.229
Liver weight	-0.417	-0.539*	0.326	0.189	-0.652**	-0.038	-0.148	0.796**	-0.118
Heart weight	-0.543*	-0.595**	0.441	0.307	-0.700**	0.117	-0.313	0.893**	-0.057
Glucose	-0.215	-0.271	0.058	-0.070	-0.364	-0.060	-0.084	0.653**	-0.030

FCR: feed conversion ratio.
* (P<0.05) and ** (P<0.01).

Table 4 Correlations between the studied traits (continue)

Traits	Live body weight	FCR	Carcass efficiency	Breast weight	Thigh weight	Abdominal fat	Liver weight	Heart weight	Glucose
Breast fat	0.306	0.683**	-0.684**	-0.604**	0.250	0.781**	-0.417	-0.543*	-0.215
Thigh fat	0.474*	0.526*	-0.641**	-0.694**	0.363	0.863**	-0.539*	-0.595**	-0.271
Breast cholesterol	0.021	-0.468*	0.429	0.256	-0.299	-0.388	0.326	0.441	0.058
Thigh cholesterol	0.020	-0.462*	0.315	0.228	-0.263	-0.335	0.189	0.307	-0.070
Plasma triglyceride	0.509*	0.585**	-0.680**	-0.711**	0.376	0.938**	-0.652**	-0.700**	-0.364
Lymphocytes (%)	-0.126	-0.432	0.135	0.317	0.146	-0.479	-0.038	0.117	-0.060
Heterophyles (%)	0.212	0.484*	-0.469*	-0.638**	0.001	0.546**	-0.148	-0.313	-0.084
Spleen weight	-0.276	-0.685**	0.899**	0.657**	-0.278	-0.885**	0.796**	0.893**	0.653**
Bursa weight	-0.208	-0.017	-0.033	0.258	0.172	-0.229	-0.118	-0.057	-0.030
Live body weight	1	-0.012	-0.231	-0.293	0.286	0.509*	-0.337	-0.266	-0.145
FCR	-0.012	1	-0.730**	-0.280	0.203	0.620**	-0.423	-0.660**	-0.287
Carcass efficiency	-0.231	-0.730**	1	0.600**	-0.276	-0.763**	0.800**	0.915**	0.634**
Breast weight	-0.293	-0.280	0.600**	1	-0.203	-0.684**	0.598**	0.554*	0.429
Thigh weight	0.286	0.203	-0.276	-0.203	1	0.398	-0.514*	-0.278	-0.120
Abdominal fat	0.509*	0.620**	-0.763**	-0.684**	0.398	1	-0.737**	-0.767**	-0.505*
Liver weight	-0.337	-0.423	0.800**	0.598**	-0.514*	-0.737**	1	0.870**	0.655**
Heart weight	0.266	-0.660**	0.915**	0.554*	-0.278	-0.767**	0.870**	1	0.610**
Glucose	-0.145	-0.278	0.634*	0.429	-0.120	-0.505*	0.655**	0.610**	1

FCR: feed conversion ratio.
* (P<0.05) and ** (P<0.01).

In regard with breast muscle cholesterol, there were some significant positive correlations with thigh muscle cholesterol ($r=0.951$) and spleen weight ($r=0.499$). Correlation between abdominal fat with breast fat ($r=0.781$), plasma triglyceride ($r=0.938$), live body weight ($r=0.509$) was also observed.

Negative correlations

Negative significant correlations between breast fat with some traits including Lymphocytes ($r=-0.741$), spleen weight ($r=-0.669$), breast weight ($r=-0.604$) and heart weight ($r=-$

0.543) indicates the negative effects of excess body fat contents on these traits. Breast cholesterol had also negative correlations with heterophyles ($r=-0.451$) and FCR ($r=-0.468$) which may evaluate the negative effect of high content of tissues cholesterol on immune system.

Immune related traits

Some significant correlation coefficients was observed between the traits relate to immune system of the broilers with each other or with other traits (Table 3 and 4) which can be

classified into two following groups including positive and negative correlations.

Positive correlations

As it is shown in table 3 and 4, there were some significant positive correlations between heterophyles (%) with some traits such as breast fat ($r=0.838$), thigh fat ($r=0.748$), plasma triglyceride ($r=0.683$) and abdominal fat ($r=0.546$). In regard with spleen weight, it was observed positive correlations with carcass efficiency ($r=0.899$), breast weight ($r=0.675$) and blood glucose ($r=0.653$).

Negative correlations

Here were some significant negative correlations between lymphocytes (%) with some traits such as breast fat ($r=-0.741$), and plasma triglyceride ($r=-0.638$). Negative correlation between heterophyles and spleen weight ($r=-0.495$) was also observed. Spleen weight with breast fat, thigh fat, plasma triglyceride, FCR and abdominal fat were also significant (Table 3 and 4) which may due to negative effects of high content of tissues and plasma fat on the broiler immune system.

CONCLUSION

The results obtained from this study implicate that increasing lysine and methionine could reduce abdominal fat content, breast and thigh crude fat and plasma triglyceride and improve feed conversion ratio, breast muscle yield, carcass efficiency (as the most important economical traits in broiler chicks) and some immune relate traits. Investigation on the correlations indicates a close relationship between the lipid content of studied body organs and plasma (especially breast muscle fat, thigh muscle fat, plasma triglyceride and abdominal fat pad) with the most numbers of studied traits relate to performance, carcass characteristics and also immune system of the broilers, so that for example, increasing in fat content of the body tend to suppressing those traits (negative correlation) under the effects of higher levels of lysine and methionine (more than NRC recommendations), and also results reported here support the hypothesis that it is possible to produce poultry meat with different fat content by supplementation lysine and methionine in excess of ordinary levels. So, it is suggested that amount of lysine and methionine in higher levels of NRC (1994) recommendations may result in enhance economical trait performance in broilers.

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