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# Early Growth Response of Broilers to Dietary Lysine at Fixed Ratio to Crude Protein and Essential Amino Acids

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**ABSTRACT :** The effects of dietary lysine (Lys) at a fixed ratio to crude protein (CP) and essential amino acids (EAA) on early growth response of broilers were studied. Four diets were formulated to contain similar metabolizable energy (ME, 2,950 kcal/kg) but contained graded levels of incremental Lys (1.1, 1.2, 1.3 and 1.4%) while also increasing the dietary CP and EAA (methionine, methionine+ cystine, threonine and tryptophan) to maintain a constant ratio with Lys. Each diet was fed at random to 10 replicates of 6 chicks each throughout the experimental period (1-21 d). At the lowest concentration of Lys of 1.1% (19.04% CP), body weight gain (BWG) was lowest and feed conversion ratio (FCR) was poorest. The BWG increased and FCR decreased linearly as dietary Lys increased upto 1.3% (22.5% CP). Lowest feed consumption was observed in the dietary group that contained 1.1% Lys (19.04% CP) in the diet. Increasing the concentration of Lys to 1.2% (20.77% CP), significantly increased the feed consumption. The concentrations of protein, calcium, phosphorus and cholesterol in serum were not influenced by the variation in Lys contents in the diet. The humoral immune response as measured by antibody titre in response to SRBC inoculation was significantly lower in the diets containing 1.1% Lys compared to 1.4%. It is concluded that the Lys requirement of broilers is 1.3% (22.5% CP) during 0 to 21 days of age for eliciting optimum performance when a fixed ratio of Lys to CP (1:17.31) and essential AA is maintained (1:0.47 Met; 1:0.56 Thr; 1:0.17 Try).
(**Key Words :** Lysine, Crude Protein, Essential Amino Acid, Early Growth Response, Broilers)

## INTRODUCTION

Commercial Poultry breeding programmes have been imposing high selection pressure for achieving rapid genetic gain per unit time at the earliest possible ages. Today, the broiler chickens weigh around 2.2 to 2.4 kg at 40 days of age. The magnitude of the growth indicates that each day in the life of broiler chicken is important to achieve the targeted body weight. Considering the high magnitude of growth, there has been considerable research directing towards defining the minimum intake of dietary protein (CP) and amino acids (AA) to reduce the nitrogen in the excreta , thus reducing the nitrogen loss to the environment (Aletor et al., 2000; Plumstead et al., 2007; Waguespack et al., 2009). Nevertheless, difference have existed in the literature with regard to the estimated minimum lysine (Lys) requirement of broilers and this has been attributed to the broiler strain (Kid et al., 2005), proportion of breast meat accretion (Bilgili et al., 1992), environmental factors (Kidd and Fracncher, 2001) and the method of determination of requirement (Plumstead et al., 2007).

Research reports as early as 1948 have suggested that the dietary Lys requirement of broilers is a function of the dietary CP content (Grau, 1948). The relationship between dietary CP and Lys requirements in commercial broiler diets were subsequently noted by Almquist (1952) who stated that the level of an essential AA required for optimum chick performance was a positive linear function of the dietary CP level. NRC (984) has suggested that the dietary CP and amino acid requirements should be expressed as a percentage of the diteray ME contents. This was based on evidence that poultry adjusted their feed intake to differences in the dietary ME density to maintain a constant ME intake. Subsequently, Morris et al. (1999) reported that the requirement of Lys and other essential AA should be expressed as a fixed proportion of the dietary CP content because a deficiency or excess of dietary CP could affect the requirement of the broilers for several essential AA. Many studies thereafter conducted to evaluate the Lys requirement by creating an imbalance of Lys relative to

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other essential AA by continuous supplementation of a single synthetic AA to a basal diet that contained a fixed level of dietary CP and other essential AA which may lead to AA imbalance and can influence the response obtained to Lys. However, only limited information are available on early body weight gain in broilers in relation to Lys and concentration of balanced dietary AA and CP (Lemme, 2003; Quentin et al., 2005). Therefore, the present study was conducted to determine the Lys requirement by increasing the dietary Lys while also increasing the dietary CP and other essential AA as a fixed ratio of dietary Lys.

## MATERIALS AND METHODS

### Birds and management

Day old, commercial broiler (Cobb 400) male chicks (240) were wing banded and randomly distributed into 40 raised-floored stainless steel battery brooder pens with 6 birds per pen. The brooder temperature was maintained at 34±1°C up to 7 days of age and gradually reduced to 26±1°C by 21 days of age. Uniform management and vaccination schedules were followed for all the birds. The experiment was conducted following the guidelines of the Institute Animal Ethics Committee.

### Diets

Prior to the formulation of the diets, maize and soybean meal was subjected to CP and total AA analyses (AOAC, 2006). Four diets were formulated to contain similar ME (2,950 kcal/kg) but contained graded levels of incremental lysine (1.1, 1.2, 1.3 and 1.4%) while also increasing the dietary CP and other EAA (methionine, methionine+cystine, threonine and tryptophan) to maintain a constant ratio with Lys. The concentrations of CP (19.04, 20.77, 22.5 and 24.23%), methionine (0.51, 0.56, 0.61 and 0.66%), methionine+cystine (0.78, 0.85, 0.92 and 0.99%), threonine (0.61, 0.67, 0.72 and 0.78%) and tryptophan (0.17, 0.20, 0.22, 0.23%) were increased progressively with increase in Lys, respectively. The ratio of CP to lysine (17.31) and lysine to other amino acids (Lys/Met - 0.47; Lys/Met+Cys - 0.71; Lys/Thr - 0.56 and Lys/Trp - 0.17) were kept constant in all the diets using an ideal amino acid profile (Baker et al., 2002). Each diet was fed at random to 10 replicates of 6 chicks each throughout the experimental period.

**Table 1.** Ingredient and nutrient composition of experimental diets (%)

| Ingredient | Lysine (%) | | | |
|---|---|---|---|---|
| | 1.1 (D1) | 1.2 (D2) | 1.3 (D3) | 1.4 (D4) |
| Maize | 64.25 | 58.42 | 52.59 | 47.60 |
| Soybean meal | 30.19 | 35.13 | 40.04 | 44.25 |
| Salt | 0.4 | 0.4 | 0.4 | 0.4 |
| Dicalcium phosphate | 2.24 | 2.21 | 2.19 | 2.17 |
| Shell grit | 0.87 | 0.84 | 0.81 | 0.78 |
| DL- methionine | 0.23 | 0.25 | 0.28 | 0.31 |
| L-lysine HCl | 0.105 | 0.074 | 0.043 | 0.036 |
| Choline chloride 50% | 0.1 | 0.1 | 0.1 | 0.1 |
| Vegetable oil | 1.17 | 2.13 | 3.08 | 3.89 |
| Vitamin premix[1] | 0.03 | 0.03 | 0.03 | 0.03 |
| Mineral premix[2] | 0.12 | 0.12 | 0.12 | 0.12 |
| Toxin Binder | 0.1 | 0.1 | 0.1 | 0.1 |
| Antibiotic | 0.05 | 0.05 | 0.05 | 0.05 |
| | Nutrient composition (%) | | | |
| ME (kcal/kg) | 2,950 | 2,950 | 2,950 | 2,950 |
| Protein | 19.04 | 20.77 | 22.50 | 24.20 |
| Lysine | 1.1 | 1.2 | 1.3 | 1.4 |
| Methionine | 0.51 | 0.56 | 0.61 | 0.66 |
| Methionine+cystine | 0.78 | 0.85 | 0.92 | 0.99 |
| Threonine | 0.61 | 0.67 | 0.72 | 0.78 |
| Tryptophan | 0.17 | 0.20 | 0.22 | 0.23 |
| Av. phosphorous | 0.50 | 0.50 | 0.50 | 0.50 |
| Calcium | 1.0 | 1.0 | 1.0 | 1.0 |

[1] Supplies per kg diet: Vitamin A, 16,500 IU; vitamin $D_3$, 3,200 ICU; vitamin E, 12 mg; vitamin K, 2 mg; vitamin $B_1$, 1.2 mg; vitamin $B_2$ 10 mg; vitamin $B_6$, 2.4 mg; vitamin $B_{12}$, 12 μg; niacin, 18 mg; pantothenic acid, 12 mg;
[2] Mn, 90 mg; Zn, 72 mg; Fe, 60 mg; Cu, 10 mg ; I, 1.2 mg.

**Table 2.** Effect of dietary lysine concentration on performance of broiler chickens

| Lys, CP (%) | Body weight gain (g) | Feed consumption (g) | FCR | Mortality (%) |
|---|---|---|---|---|
| 1.1 (19.04) | 617[c] | 827[b] | 1.34[a] | 1.66 |
| 1.2 (20.77) | 717[b] | 918[a] | 1.28[b] | 3.33 |
| 1.3 (22.5) | 751[a] | 916[a] | 1.22[c] | 1.66 |
| 1.4 (24.23) | 758[a] | 902[a] | 1.19[c] | 1.66 |
| SEM | 7.05 | 13.70 | 0.01 | - |
| p-value | 0.001 | 0.011 | 0.001 | - |

[a, b, c] Means with different superscripts in a column differ significantly.
Lys = Lysine; CP = Crude protein; FCR = Feed conversion ratio.

**Parameters studied**

Individual body weight of chicks and replicate-wise feed intake were recorded at weekly intervals. Feed conversion ratio was calculated as the ratio between feed consumed and weight gained.

On day 18, about 3 ml of blood was collected from brachial vein from one bird from each replicate in each dietary treatment (10 birds/treatment). Subsequently serum was separated by centrifuging the blood at 5,000 rpm for 10 min and the levels of Ca (AOAC, 1990), P (Fiske and Subba Row, 1925) and protein (Doumas et al., 1971) and cholesterol (Zlatkis et al., 1953) and were estimated.

By repeated centrifugation (3,000 rpm/10 min) and washing (3/4 times) of about 10 ml sheep blood, SRBC was harvested. The SRBC was diluted with normal saline to get a concentration of 0.5% RBC. Subsequently, 0.1 ml of 0.5% SRBC suspension was inoculated into wing vein of one bird from each replicate in each dietary treatment (10 birds/ treatment) at 14th day. At 5th day post inoculation, blood was collected from the wing vein of the SRBC inoculated bird. Antibody titres against SRBC were measured using 0.75% SRBC suspension (in NSS) by haemagglutination test procedure (Wegmann and Simthies, 1966). The reciprocal of the highest dilution where there was complete agglutination was considered as titre.

**Statistical analysis**

Data were subjected to statistical analysis under completely randomized design employing one-way analysis of variance (Snedecor and Cochran, 1989). The means of different treatments were compared with Duncan's multiple range tests (Duncan, 1955). Significance was considered at $p<0.05$ levels.

## RESULTS

The performance of broilers fed diet containing different concentrations of Lys is presented in Table 2. At the lowest concentration of Lys of 1.1% (19.04% CP), body weight gain (BWG) was lowest and feed conversion ratio (FCR) was poorest. The BWG increased and FCR decreased linearly as dietary Lys increased upto 1.3% (22.5% CP). No further improvement in BWG and FCR could be notice there after (1.4% Lys; 24.23% CP). Lowest feed consumption was observed in the dietary group containing 1.1% Lys (19.04% CP) in the diet. Increasing the concentration of Lys to 1.2% (20.77% CP), significantly increased the feed consumption by the birds. No difference in the feed consumption was noticed amongst the dietary group containing 1.2 to 1.4% Lys in diets. The mortality was 1.66, 3.32, 1.66 and 1.66% in the 1.1, 1.2, 1.3 and 1.4% Lys containing diets, respectively and was within the limit.

The concentrations of serum biochemical parameters such as protein, calcium, phosphorus and cholesterol were not influenced by the variation in Lys contents of the diet (Table 3). The humoral immune response as measured by antibody titre in response to SRBC inoculation was significantly lower in the diets containing 1.1% Lys

**Table 3.** Serum biochemical profile and immune response in broiler chickens as influenced by dietary lysine concentrations

| Lys, CP (%) | Protein (g/dl) | Cholesterol (mg/dl) | Calcium (mg/dl) | Phosphorous (mg/dl) | Antibody titre ($log_2$) |
|---|---|---|---|---|---|
| 1.1 (19.04) | 6.55[a] | 198.72[a] | 7.28[a] | 6.47[a] | 7.16[b] |
| 1.2 (20.77) | 5.68[a] | 172.44[a] | 8.35[a] | 7.19[a] | 8.00[ab] |
| 1.3 (22.5) | 6.91[a] | 221.55[a] | 7.28[a] | 7.68[a] | 7.50[ab] |
| 1.4 (24.23) | 5.65[a] | 191.77[a] | 8.85[a] | 7.62[a] | 8.33[a] |
| SEM | 0.419 | 12.603 | 0.304 | 0.322 | 0.183 |
| p-value | 0.664 | 0.614 | 0.167 | 0.549 | 0.010 |

[a, b, c] Means with different superscripts n a column differ significantly.
Lys = Lysine; CP = Crude protein; FCR = Feed conversion ratio.

compared to 1.4%. It was intermediate in groups fed 1.2 and 1.3% Lys diets.

## DISCUSSION

Dietary protein is used by the broilers for many functions, the most important being accretion as broiler meat. It has been proven that chicken require a specific and balance of dietary EAA and sufficient nitrogen for synthesis of non-EAA (NEAA), rather than CP *per se* (NRC, 1994). Previous research with feeding of low CP diets to broiler decreased the nitrogen excretion in the excreta (Aletor et al., 2000; Aftab et al., 2006), thus reducing the nitrogen pollution from the intensive poultry farming. It has also been observed that feeding such low CP -AA supplemented diets reduced the performance of the broilers (Aftab et al., 2006; Waguespack et al., 2009). Thus, it is suggested to maintain minimum intake of dietary CP, crystalline AA supplementation is required to support the optimum growth potential (Plumstead et al., 2009). Now the question arises, what is the extent to which the concentration of dietary CP is reduced without affecting broiler performance? In the present study, at the lowest concentration of Lys (1.1%) and CP (19.0%), BWG was lowest and FCR was poorest. A linear increase in body weight and decrease in FCR was observed as dietary Lys increased to 1.3% (22.5% CP) and with no further improvement thereafter. This was in agreement with the findings of the earlier workers (Velu et al., 1971; Abebe and Morris, 1990). Lowest feed consumption was observed in group containing 1.1% Lys (19.04% CP) in the diet and increased significantly by increasing concentration of Lys to 1.2% (20.77% CP). This was in line with the report of Aftab et al. (2006). No difference in the feed consumption was noticed amongst the dietary group containing 1.2 to 1.4% Lys in diets. Concomitant to the findings of the present study, Plumstead et al. (2007) reported that dietary Lys, beyond 1.19% (1.19-1.47%) had no effect on feed intake of broilers during 0 to 21 days of age.

Many reports are available in the literature indicating compromised live weight gain and feed efficiency in broilers when fed on Low CP (LCP) diets (Pinchasov et al., 1990; Moran et al., 1992; Si et al., 2004). The possible explanation for the negative effect of LCP diets on performance suggested were insufficiency of non-specific nitrogen for the synthesis of NEAA, tendency of broilers to reduce voluntary feed intake on LCP diets, altered EEA/NEAA ratio, insufficiency of some of the EAA, insufficient synthesis of glycine to fulfill the need of fast growing broilers and efficiency of utilization of AA from a free source vs. intact dietary protein for body protein accretion. In the present study the feed intake also depressed in the LCP diet (19.04% CP; 1.1% Lys) and the depressed growth of broilers in the LCP diet could be explained due to reduced feed intake. Others have also shown a reduced feed intake when broiler fed LCP diets (Si et al., 2004; Jiang et al., 2005).

Selection for maximum growth has resulted in a conflict with magnitude of the immune response (Maatman et al., 1993), which may be due to accommodation of all the physiological demands with the limited resources available for birds (Siegel et al., 1982). Certain non-genetic factors such as dietary nutrient concentration, which influence growth can modify or alter the expression of the genes responsible for immunoresponsiveness (Klasing and Barnes, 1988; Katanbaf et al., 1988) by altering the magnitude of antibody production and maturity of immune system (Cook, 1991; Latshaw, 1991). Antibodies are proteins, therefore any deficiency of essential amino acids particularly during the growing chicken results in poor immune competence (Latshaw, 1991). Lysine is one of the amino acids which can influence the magnitude of antibody response (Praharaj et al., 2002; Kidd, 2004). This could be the reason that lowest immune response was observed in the diet containing 1.1% Lys. This was similar to our earlier findings (Panda et al., 2010). Probably, 1.10% Lys in starter was not sufficient to stimulate optimum antibody production, thereby, a lower immune response was observed at the suboptimal concentration of the limiting amino acid (Kidd, 2004; Panda et al., 2010).

From the above study it was found that Lys requirement of the broiler during 0 to 21 days of age is 1.3% (22.5% CP). It was observed that Lys requirement of the broiler increased when dietary CP content was increased. Several workers also suggested that the Lys requirement of broilers increased when dietary CP was increased (Morris et al., 1987; Surisdiarto and Farrel, 1991; Plumstead et al., 2007). Research reports as early as 1948 have suggested that the dietary Lys requirement of broilers was also a function of the dietary CP content (Grau, 1948). In the present study along with the dietary Lys, the concentrations of other indispensible amino acids such as Met/Met+Cys, Thr and Try were also increased to keep a constant ratio with Lys. The increased response to Lys when concentration of other indispensible amino acids increased suggested that balancing of Lys to other amino acid in the diet is also important to achieve a desired response (Gous and Morris, 1985; Abebe and Morris, 1990). Plumstead et al. (2007) suggested that to prevent an imbalance of amino acid in the diets and obtain broiler performance, the Lys requirement should be expressed as a percentage of dietary CP. In the present study, the same principle was applied and a balance of all other amino acids was maintained and broilers responded positively to the incremental lysine upto 1.3% (22.5% CP). Thus, it is concluded that at a fixed ratio of Lys to CP (1:17.31), Lys requirement of broiler is 1.3% (22.5%

CP) during 0 to 21 days of age for eliciting optimum performance.

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