

## **EFFECT OF LOW PROTEIN-METHIONINE-AND-LYSINE-SUPPLEMENTED DIETS ON PERFORMANCE, IMMUNE RESPONSE AND CARCASS CHARACTERISTICS IN BROILERS**

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### **Abstract**

Two experiments were conducted to investigate the effect of supplementing low CP diets with methionine and lysine on broiler performance, carcass measure and their immune response against Infectious Bursa Disease (IBD) virus. In Experiment 1, ten diets were formulated. Diet 1 (control diet) contained 23.0% CP and 0.6% methionine. The remaining 9 test diets were formulated to contain 20, 17 and 14% CP each with 0.6, 1.0 and 1.4% methionine in a 3x3 factorial arrangement. Three hundred and twenty 1-d-old broiler chicks were weighed and randomly allocated to the 10 diets with four replicates and eight birds each. In Experiment 2, 10 diets were also formulated. Diet 1 (control diet) contained 23% CP and 1.2% lysine. The rest 9 diets were similar to those in Experiment 1 but lysine was used to supplement the CP at levels 1.2, 1.6 and 2.0% inclusion. One hundred and sixty 1-d-old broiler chicks were weighed and randomly allocated into the 10 diets with four replicates of four birds each. In Experiment 1, Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversion Ratio (FCR) in control and 20% CP diet with 1.0 methionine were similar. Dressed weight, weights of thigh, breast, and drum stick in the control and 1.0% methionine diets were similar. There was no effect of methionine, CP or their interaction on the antibody titre against IBD. In Experiment 2, increasing lysine and decreasing CP significantly ( $P < 0.01$ ) reduced BWG and FI, but 1.6% lysine had similar effect on FCR as the control diet. There was no effect of lysine, CP or their interaction on the antibody titre against IBD virus. Results suggest that supplementation of broiler diets of CP less than 23% with 1.0% methionine and 1.6% lysine resulted in similar performance as control diet and the bird's immune response against IBD virus was not compromised.

**Keywords:** Immune response, Ideal methionine to lysine ratio, Performance indices, Antibodies, Carcass characteristics.

## **EFFET DE LA SUPPLÉMENTATION DES RÉGIMES PAUVRES EN PROTÉINES AVEC LA MÉTHIONINE ET LA LYSINE SUR LA PERFORMANCE, LA RÉPONSE IMMUNITAIRE ET LES CARACTÉRISTIQUES DE CARCASSE DES POULETS DE CHAIR**

### **Résumé**

Deux expériences ont été réalisées dans le but d'étudier l'effet de la supplémentation des régimes faibles en PB avec la méthionine et la lysine sur la performance et les caractéristiques de carcasse des poulets de chair ainsi que leur réponse immunitaire contre le virus de la bursite infectieuse (BI). Dans l'Expérience 1, dix régimes ont été formulés. Le Régime 1 (régime témoin) contenait 23,0% de PB et 0,6% de méthionine. Les 9 régimes d'essais restants ont été formulés de manière à contenir 20 ; 17 ; et 14% de PB chacun, respectivement avec 0,6 ; 1,0 ; et 1,4% de méthionine selon un plan factoriel 3x3. Trois cent vingt poussins de chair âgés d'un jour (1 J) ont été pesés et répartis de manière aléatoire dans les 10 régimes avec quatre répétitions et huit oiseaux chacun. Dans l'Expérience 2, 10 régimes ont été également formulés. Le Régime 1 (régime témoin) contenait 23% de PB et 1,2% de lysine. Les 9 régimes restants étaient similaires à ceux de l'Expérience 1, mais la lysine a été utilisée pour compléter les PB aux niveaux d'inclusion de 1,2 ;

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1,6 et 2,0%. Cent soixante poussins de chair d'un jour ont été pesés et répartis de manière aléatoire dans les 10 régimes avec quatre répétitions de quatre oiseaux chacun. Dans l'Expérience 1, le gain pondéral (GP), la prise alimentaire (PA) et l'indice de consommation (IC) dans le régime témoin et le régime à 20% de PB avec supplément de méthionine à 1,0 étaient similaires. Le poids de carcasse, les poids de cuisse, de poitrine et de pilon dans le régime témoin et les régimes avec supplément de méthionine à 1,0% étaient similaires. La méthionine, les PB ou leur interaction n'ont pas eu d'effet sur le titre de l'anticorps dirigé contre la BI. Dans l'Expérience 2, l'augmentation de la lysine et la réduction des PB ont diminué de manière significative ( $P < 0,01$ ) le GP et la PA, mais la lysine à 1,6% a eu un effet similaire à celui du régime témoin sur l'IC. La lysine, les PB ou leur interaction n'ont pas eu d'effet sur le titre de l'anticorps dirigé contre le virus de la BI. Les résultats laissent entendre que la supplémentation des régimes alimentaires de poulets de chair ayant des PB à moins de 23% avec de la méthionine à 1,0% et de la lysine à 1,6% a produit une performance similaire à celle du régime témoin et que la réponse immunitaire des oiseaux contre le virus de la BI n'a pas été compromise.

**Mots-clés :** Réponse immunitaire ; Ratio méthionine/lysine idéal ; Indices de performance ; Anticorps ; Caractéristiques de carcasse

## Introduction

Nutrition is a critical determinant of immune responses, and malnutrition is the most common cause of immunodeficiency worldwide. Nutrients can influence the maturity of the immune system (Cook, 1991; Latshaw, 1991). The quality of dietary protein is an essential factor, which influences the synthesis of immunoglobulins and their function. Protein-energy malnutrition is associated with a significant impairment of cell-mediated immunity, phagocytic function, complement system, secretory immunoglobulin A antibody concentration, and cytokine production (Chandra, 1997). Increasing dietary crude protein content will increase daily feed consumption and weight gain of broilers (Jahanian, 2009). Diets low in crude protein content without adequate amino acid supplementation will negatively affect the immunologically performance of the birds. Their ability to fight infection will be reduced and consequently will affect performance (weight gain, feed intake and feed conversion ratio) and carcass quality of the birds. Antibodies being proteins, deficiency of any essential amino acid particularly in the growing phase in chicken results in poor immune competence (Latshaw, 1991). Protein deficiency inhibited antibody production and the development of antibody producing cells in response to T-dependent antigens (Carlomagno et al., 1980). According to the report of Jahanian (2009), diets low in crude protein decreased ( $P < 0.001$ ) relative weights of thymus and bursa of Fabricius. Skin

reaction to phytohaemagglutinin P was also impaired when the diets were low in crude protein. Nutritional strategy aimed at reducing the crude protein requirement of broilers is the partial replacement of intact protein (e.g., soybean meal) with crystalline free amino acids, by which excess dietary amino acids are minimized in relation to their requirements, bringing the dietary protein closer to ideal protein and in turn decreasing the dietary CP content.

The development of amino acid supplementation allows meeting the essential amino acid needs at low protein levels (Dirain and Waldroup, 2002). Schutte (1987), Stillborn and Waldroup, (1988) reported that if low-CP diets were supplemented with adequate amounts of essential and nonessential amino acids to meet the nutritional requirements of birds, performance can be produced similar to that observed with the conventional standard (high CP) diets. Research has suggested that levels of lysine and methionine in excess of NRC (1994) recommendation may result in enhanced performance, especially in regards to breast meat yield (Schutte and Pack, 1995). Adequate dietary levels of methionine and lysine are needed to support immune response, optimum growth and carcass yield of fast-growing commercial broilers. Information on the exact requirement of methionine and lysine needed for optimal immune response of broilers is still scanty. This study describes two experiments conducted to determine the effect of supplementation of methionine and lysine to graded CP levels on performance,

carcass measures and immune response to IBD virus in broilers.

## Materials and methods

### *Diets and animals*

Ten diets were formulated: diet 1 (control diet) contained 23% CP and 0.6% methionine (Experiment 1) and 23% CP and 1.2% lysine (Experiment 2). The nine other experimental diets consisted of 20, 17 and 14% CP each with 0.6, 1.0 and 1.4% methionine (Experiment 1) and same level of CP but 1.2, 1.4 and 1.6% lysine (Experiment 2). The composition diets for Experiment 1 is shown in table 1 and composition of diets for Experiment 2 is shown in table 2. In Experiment 1 320 one-day-old broiler chicks (Arbor Acre strain, CHI Ltd, Ajanla Farms, Ibadan) were wing-branded, weighed (Blocked by weight) and randomly allotted to 40 pens of 4 replicates with 8 birds per replicate. A group of 4 replicates was fed any of the 10 experimental diets in a factorial arrangement (4 levels of CP and 3 levels of methionine). The birds were housed in a well illuminated and ventilated poultry house. Feed and water were provided ad libitum. The chicks were vaccinated against Infectious Bursal Disease (IBD) virus on d 10 posthatch via drinking water. The birds were fed the diets for 42 days. In Experiment 2, 160 one-day-old broiler chicks (Arbor Acre strain, CHI Ltd, Ajanla Farms, Ibadan) were wing-branded, weighed and randomly allocated to the 10 diets on weight basis with 4 replicate pens and 4 birds per replicate. The chicks were also vaccinated against IBD virus at d 10 post-hatch. The study lasted 35 days.

### *Sample collection, antibody determination, carcass measures and chemical analysis*

The proximate composition of the diets was determined according to the methods of AOAC (2000). In both studies, blood samples were collected from two chicks per pen through the jugular vein at weekly interval pre and post vaccination for period of the study. Serum was separated by centrifugation (8000 revolution per minute for 5 minutes) and antibodies specific for IBD was detected in the sera using an ELISA kit (ProFLOKR,

Synbiotics Corporation, CA, USA) according to manufacturer's instructions. One hundred microliters of each sample were used for the assay. Absorbance was measured at 405nm using an ELISA reader (SUNRISE Absorbance Reader, TECAN) by standard procedures, (Snyder et al., 1988). For carcass measure, two birds per replicate were randomly selected, weighed, sacrificed and defeathered. Carcass parts were weighed and their weights expressed as percent of body weight to determine the effect of the experimental diets on the cut parts.

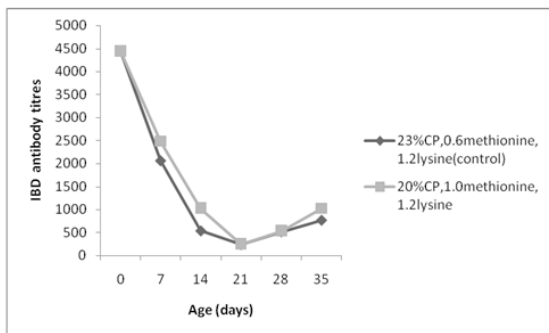
### *Statistical analysis*

Data in both studies were analysed by the two-way analysis of variance procedure appropriate for completely randomized design with a factorial arrangement using the General Linear Models (GLM) procedure of SAS (SAS, 1999). Means were further separated using Duncan Multiple range test.

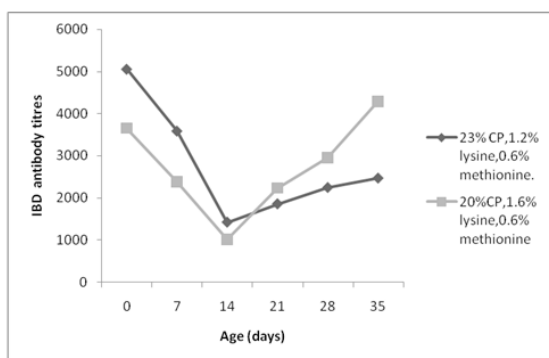
## Results

### *Experiment 1 (Methionine supplementation)*

The analysed nutrient composition for diets in Experiment 1 is shown in table 3 and the analysed composition for diets in Experiment 2 is shown in Table 4. Diets contained CP levels of between 14.2-22.7% and crude fibre levels of 2.0-6.0%. The results of performance characteristics are shown in Tables 5. Body weight gain and feed intake were significantly reduced by decreasing CP levels and increasing methionine level beyond 1.0%. There was no significant effect on performance of the birds on either 0.6 or 1.0% methionine diet (Table 6). Results of the effect of methionine, crude protein and their interaction on carcass parts are presented in Table 7. There was no significant effect of 0.6 and 1.0% methionine levels and 23 and 20% CP on carcass parts. Methionine above 1.0% significantly ( $P < 0.05$ ) decreased weight of carcass parts. Decrease in CP level beyond 20%, significantly ( $P < 0.05$ ) reduced dressed weight, drum stick and thigh. Table 8 shows the result of antibody titre against IBD. There was no significant effect of CP, methionine or their interaction on IBD antibody titre. Nevertheless, birds on the 20% CP and 1.0% methionine had higher antibody



**Figure 1:** Infectious bursal disease virus antibody titre in broilers on 23% crude protein, 0.6% methionine with 1.2% lysine and 20% crude protein, 1.0% methionine with 1.2% lysine



**Figure 2:** Infectious bursal disease virus antibody titre in broilers on 23%CP, 1.2%lysine with 0.6%methionine and 20%CP, 1.6% lysine with 0.6%methionine

titre compared to birds on the control and other diets as further shown in figure 1.

*Experiment 2 (Lysine supplementation)*

Crude protein in the diets was between 14.41 and 22.54% while the crude fibre value ranged from 3.0 to 4.0% (Table 4). The results of performance of the birds are shown in Table 9 and the effect of CP, lysine and their interactions on body weight gain, feed intake and feed conversion ratio are as presented in Table 10. Increasing lysine levels significantly ( $P<0.05$ ) lowered body weight gain, feed intake and feed conversion ratio. Crude protein significantly ( $p<0.05$ ) decreased the parameters as well. There was no significant difference in the feed intake of birds on 23 and 20% crude protein diets. Interaction between CP and lysine significantly affected feed intake

but not weight gain and feed conversion ratio. The results of weights of carcass parts are shown in Table 11. Increasing lysine levels significantly ( $P<0.05$ ) lowered the weight of thigh, drumstick and breast. There was no significant difference in the weight of thigh in birds on 23 and 20% CP diets. Interaction of lysine and crude protein had no significant effect on any of the carcass parts.

The results of the effect of CP, lysine and their interactions on antibody titre against IBD virus are as shown in Table 12. Lysine levels had no significant effect on the antibody titre. The highest antibody titre was produced in birds on the 17% CP diet. Interaction between CP and lysine had no effect on the antibody titre in the birds. Figure 2 shows the effect of antibody titre against Infectious bursal disease virus in broilers on 23% CP, 1.2% lysine with 0.6% methionine control diet and broilers on 20% CP, 1.6% lysine with 0.6% methionine diet, showing that birds on 20% CP, 1.6% lysine with 0.6% methionine diet had better immune response than birds on the control diet and other diets in the study.

**Discussion**

The NRC (1994) requirements for amino acids and crude protein are designed to support maximum growth and production in healthy birds kept under ideal conditions. The recommended levels for methionine in poultry depend on species, stage of production, environmental condition and amount of energy in feed. The low concentration of methionine in high-protein corn-soybean diets has led to wide use of synthetic methionine supplementation in poultry feed. An improvement in broiler performance when methionine was added to a corn-soybean diet was reported by Virtanen and Rosi (1995) and Hesabi et al. (2006). In the present study, birds on 20% CP with 1.0% methionine had better weight gain, and feed conversion ratio than birds on 23% CP with 0.6% methionine control diet and the other experimental diets. This is in support of earlier reports of (Lipstein et al., 1975; Schutte, 1987; Parr and Summers, 1991; Deschepper and De Groote, 1995; Yamazaki et al., 1996, 1998 and Aletor et al., 2000) that reduced crude protein-

**Table 1:** Gross composition (g/100gDM) of experimental diets. (Study 1)

|                         |               |               |               |               |               |               |               |               |               |               |
|-------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Crude protein           | 23            | 20            | 20            | 20            | 17            | 17            | 17            | 14            | 14            | 14            |
| Methionine              | 0.6           | 0.6           | 1.0           | 1.4           | 0.6           | 1.0           | 1.4           | 0.6           | 1.0           | 1.4           |
| Lysine                  | 1.2           | 1.2           | 1.2           | 1.2           | 1.2           | 1.2           | 1.2           | 1.2           | 1.2           | 1.2           |
| <b>Ingredients</b>      |               |               |               |               |               |               |               |               |               |               |
| Maize                   | 36.00         | 45.50         | 45.50         | 45.50         | 48.00         | 48.00         | 48.00         | 56.00         | 56.00         | 56.00         |
| Soybean meal            | 41.00         | 32.00         | 32.00         | 32.00         | 21.00         | 21.00         | 21.00         | 12.00         | 12.00         | 12.00         |
| Wheat Offal             | 13.00         | 12.00         | 12.00         | 12.00         | 20.00         | 20.00         | 20.00         | 21.00         | 21.00         | 21.00         |
| Palm oil                | 5.00          | 5.00          | 5.00          | 5.00          | 5.00          | 5.00          | 5.00          | 5.00          | 5.00          | 5.00          |
| Dicalcium phosphate     | 2.14          | 2.40          | 2.22          | 2.00          | 2.50          | 2.45          | 2.05          | 2.50          | 2.19          | 2.00          |
| Limestone               | 2.09          | 2.22          | 2.00          | 1.82          | 2.35          | 2.00          | 2.00          | 2.09          | 2.00          | 1.79          |
| *Vitamin-mineral premix | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          |
| Salt                    | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          |
| Met                     | 0.27          | 0.30          | 0.70          | 1.10          | 0.34          | 0.74          | 1.14          | 0.38          | 0.78          | 1.18          |
| Lysine                  | -             | 0.08          | 0.08          | 0.08          | 0.31          | 0.31          | 0.31          | 0.53          | 0.53          | 0.53          |
| <b>Total</b>            | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> |

CP-crude protein \*Premix supplied the following information kg of diet: Vitamin A (12,500,000 I.U), Vit D3 (2,500,000 I.U), Vit E (40,000mg) Vitamin K3 (2,000mg), Vit B, (3,000mg), Vit B2 (5,500mg), Naicin (55,000mg), calcium panthothenate (11,500mg) Vit B6 (5000mg) Vit B12 (25mg), choline chloride (500, 000mg), folic acid (1,000mg), Biotin (80mg), Mn (120,000,mg), Fe (100,000mg), Zn (80,000mg), Cu (8,500mg), I (1,500mg) Co (300mg), Se (120mg).

**Table 2:** Gross Composition (g/100gDM) of experimental diets. (Study 2)

|                          | 1             | 2             | 3             | 4             | 5             | 6             | 7             | 8             | 9             | 10            |
|--------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Crude protein            | 23            | 20            | 20            | 20            | 17            | 17            | 17            | 14            | 14            | 14            |
| Lysine (%)               | 1.2           | 1.2           | 1.6           | 2.0           | 1.2           | 1.6           | 2.0           | 1.2           | 1.6           | 2.0           |
| Methionine (%)           | 0.6           | 0.6           | 0.6           | 0.6           | 0.6           | 0.6           | 0.6           | 0.6           | 0.6           | 0.6           |
| <b>Ingredients</b>       |               |               |               |               |               |               |               |               |               |               |
| Maize                    | 32.00         | 44.00         | 44.00         | 44.00         | 50.00         | 50.00         | 50.00         | 57.00         | 57.00         | 57.00         |
| Soybean meal             | 40.00         | 32.00         | 32.00         | 32.00         | 22.00         | 22.00         | 22.00         | 12.00         | 12.00         | 12.00         |
| Wheat offal              | 18.00         | 13.00         | 13.00         | 13.00         | 17.00         | 17.00         | 17.00         | 20.00         | 20.00         | 20.00         |
| Palm oil                 | 5.00          | 5.00          | 5.00          | 5.00          | 5.00          | 5.00          | 5.00          | 5.00          | 5.00          | 5.00          |
| Dicalcium phosphate      | 2.10          | 2.45          | 2.23          | 2.00          | 2.29          | 2.00          | 1.94          | 2.00          | 2.00          | 1.75          |
| Limestone                | 2.14          | 2.60          | 2.40          | 2.20          | 2.50          | 2.36          | 2.00          | 2.50          | 2.08          | 1.90          |
| *Vitamin-minerals premix | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          |
| Salt                     | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          | 0.25          |
| Methionine               | 0.26          | 0.30          | 0.30          | 0.30          | 0.34          | 0.34          | 0.34          | 0.38          | 0.38          | 0.38          |
| Lysine                   | -             | 0.15          | 0.57          | 1.00          | 0.37          | 0.80          | 1.22          | 0.62          | 1.04          | 1.47          |
| <b>Total</b>             | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> |

\*Premix supplied the following information kg of diet: Vitamin A (12,500,000 I.U), Vit D3 (2,500,000 I.U), Vit E (40,000mg) Vitamin K3 (2,000mg), Vit B, (3,000mg), Vit B2 (5,500mg), Naicin (55,000mg), calcium panthothenate (11,500mg) Vit B6 (5000mg) Vit B12 (25mg), choline chloride (500, 000mg), folic acid (1,000mg), Biotin (80mg), Mn (120,000,mg), Fe (100,000mg), Zn (80,000mg), Cu (8,500mg), I (1,500mg) Co (300mg), Se (120mg).

**Table 3:** Proximate composition (g/100gDM) of experimental diets. (Study 1)

|               | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    |
|---------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Crude protein | 23    | 20    | 20    | 20    | 17    | 17    | 17    | 14    | 14    | 14    |
| Methionine    | 0.6   | 0.6   | 1.0   | 1.4   | 0.6   | 1.0   | 1.4   | 0.6   | 1.0   | 1.4   |
| Dry matter    | 92.60 | 92.00 | 92.60 | 91.20 | 92.23 | 92.40 | 91.58 | 91.20 | 91.10 | 91.42 |
| Crude protein | 22.68 | 20.93 | 20.67 | 20.35 | 17.75 | 17.38 | 17.23 | 14.16 | 14.88 | 14.80 |
| Ash           | 10.00 | 10.00 | 9.50  | 9.00  | 8.00  | 7.00  | 7.50  | 8.00  | 9.00  | 8.50  |
| Ether extract | 7.00  | 7.00  | 8.00  | 7.00  | 8.50  | 8.00  | 7.50  | 8.00  | 8.50  | 7.00  |
| Crude fibre   | 3.00  | 2.00  | 4.00  | 4.00  | 4.00  | 6.00  | 4.00  | 4.00  | 4.00  | 5.00  |
| NFE           | 47.23 | 60.07 | 57.83 | 59.65 | 61.75 | 62.62 | 63.77 | 65.84 | 63.62 | 64.70 |

NFE=Nitrogen Free Extract

**Table 4:** Proximate composition (g/100gDM) of experimental diets (Study 2).

|               | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    |
|---------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Crude protein | 23    | 20    | 20    | 20    | 17    | 17    | 17    | 14    | 14    | 14    |
| Lysine        | 1.2   | 1.2   | 1.6   | 2.0   | 1.2   | 1.6   | 2.0   | 1.2   | 1.6   | 2.0   |
| Dry matter    | 92.00 | 90.50 | 91.50 | 91.50 | 90.50 | 91.50 | 91.50 | 92.50 | 91.50 | 92.50 |
| Crude protein | 22.54 | 20.59 | 20.22 | 20.02 | 17.22 | 17.21 | 17.11 | 14.57 | 14.41 | 14.57 |
| Ash           | 7.00  | 7.00  | 9.00  | 5.00  | 7.00  | 8.00  | 6.00  | 9.00  | 6.00  | 7.00  |
| Ether extract | 10.00 | 11.00 | 10.00 | 11.00 | 11.50 | 12.00 | 12.00 | 14.00 | 13.00 | 14.00 |
| Crude fibre   | 4.00  | 3.00  | 4.00  | 3.00  | 4.00  | 3.00  | 4.00  | 3.00  | 4.00  | 3.00  |
| NFE           | 56.46 | 58.41 | 56.78 | 60.98 | 60.28 | 59.79 | 60.89 | 59.43 | 62.59 | 61.43 |

NFE-Nitrogen Free Extract

amino acid supplemented diets improved growth and feed consumption of broilers. Fasuyi and Aletor (2005) also reported that better performance can be obtained with adequate supplementation of essential amino acids especially methionine which has been identified to be in marginal quantities in most poultry diets. Garlich (1985) found that feed conversion was better when diets were supplemented with methionine. As reduction in crude protein exceeded 3 percentage units and methionine inclusion was above 1.0%, there was a reduction in the growth performance of the birds. Excess methionine has been reported to decrease average daily feed intake and negatively affect growth performance (Katz and Baker, 1975; Harter and Baker, 1978 and Han and Baker, 1993). According to the work of Haper et al. (1984) and Nawaz et al. (2006) many amino acids, when fed in excess to growing chickens cause symptoms of toxicity such as decreased feed intake, weight gain and increased mortality.

In study two, birds on 20% CP with 1.2

or 1.6% lysine had similar body weight gain and feed conversion as those on the control (23% CP with 1.2% lysine) diet. This is in agreement with findings of Labadan et al. (2001) that lysine requirements of broilers are higher in low protein diets for maximum weight gain and feed efficiency. At normal CP level, high lysine content has been reported to increase growth rate in broilers (Holsheimer and Veerkamp, 1992). This is also in agreement with the findings of Zarate et al. (2003) that formulation with commercially available purified essential amino acids to meet broiler requirements not only improves their overall balance but also allows for reduction in CP while improving the overall performance of broiler birds. Corzo et al. (2005) found that lysine supplementation significantly improved the live body weight and feed conversion efficiency of broilers, while Si et al. (2001) evaluated the relationship of dietary lysine in diets for broilers and found that increase in the level of essential amino acids resulted in significant improvements in feed conversion. As reduction in crude

protein exceeded 3 percentage units and lysine inclusion was above 1.6%, the performance of the birds was poorer has also been reported by Haper et al. (1984) and Nawaz et al. (2006). Broilers do not seem to do as well when intact protein is reduced to a greater extent and replaced with crystalline essential amino acids (Pinchasov et al., 1990).

In the methionine study, results of carcass measures showed no significant differences between the carcass measures of birds on 23% CP and 0.6% methionine (control diet) and birds on 20% CP supplemented with any of the methionine levels. This is consistent with the report of Hickling et al. (1990) who suggested that amino acid levels in excess of NRC recommendations improved breast meat yield of broilers. The observed significant (P<0.05) reduction in weight of carcass parts in response to CP levels lower than 20% and at 1.4% methionine is in agreement with the findings of Nawaz et al. (2006) of significant decrease in carcass weight of broilers fed low CP diets. Dai (2002) had reported that animals require not only free amino acids, but also small peptides to support growth, and the peptides may be the essential nutrients, and the low-protein, amino acid supplemented diet may have been deficient of these small peptides.

Similarly results of carcass measures in the lysine study showed no significant differences in the carcass parts of birds on 23 and 20% CP at 1.2% lysine inclusion. This is consistent with the report of (Kamran et al., 2004) that dietary protein level for broilers could be reduced from 23 to 20%, with beneficial effects on growth performance and carcass characteristics and increased economic returns. Hesabi et al. (2008) also evaluated the effects of different levels of lysine and methionine on carcass characteristics in broilers and suggested that additional methionine and lysine may improve performance and carcass traits in broiler chicks. Sibbald and Wolynetz (1986) reported that increasing dietary lysine levels has been reported to increase carcass protein retention. In the present study, lowering the dietary CP level below 20% and increasing lysine level above 1.6% significantly reduced carcass weight (p<0.05). This agrees with the findings of Nawaz et al. (2006) who observed

**Table 5:** Performance of broilers on low crude protein methionine supplemented diets (n=4 replicates of 8 birds each)

|                             | 1                    | 2                    | 3                   | 4                    | 5                    | 6                     | 7                   | 8                    | 9                    | 10                  |            |
|-----------------------------|----------------------|----------------------|---------------------|----------------------|----------------------|-----------------------|---------------------|----------------------|----------------------|---------------------|------------|
| Crude protein(g/100g)       | 23                   | 20                   | 20                  | 20                   | 17                   | 17                    | 17                  | 14                   | 14                   | 14                  |            |
| Methionine (g/100%)         | 0.6                  | 0.6                  | 1.0                 | 1.4                  | 0.6                  | 1.0                   | 1.4                 | 0.6                  | 1.0                  | 1.4                 |            |
| <b>Parameters</b>           |                      |                      |                     |                      |                      |                       |                     |                      |                      |                     | <b>SEM</b> |
| Initial weight              | 40.2                 | 40.4                 | 40.3                | 40.1                 | 40.2                 | 40.5                  | 40.2                | 40.2                 | 40.2                 | 40.2                |            |
| Final weight (g/b/ 35 days) | 753.13 <sup>ab</sup> | 740.63 <sup>ab</sup> | 775.06 <sup>a</sup> | 678.13 <sup>bc</sup> | 741.96 <sup>ab</sup> | 692.19 <sup>abc</sup> | 625.45 <sup>c</sup> | 722.50 <sup>ab</sup> | 715.63 <sup>ab</sup> | 475.25 <sup>d</sup> | <0.0001    |
| Weight gain(g/b/d)          | 20.37 <sup>ab</sup>  | 20.00 <sup>ab</sup>  | 20.99 <sup>a</sup>  | 18.23 <sup>bc</sup>  | 20.05 <sup>ab</sup>  | 18.62 <sup>abc</sup>  | 16.72 <sup>c</sup>  | 19.49 <sup>ab</sup>  | 19.30 <sup>ab</sup>  | 12.32 <sup>d</sup>  | <0.0001    |
| Feed intake (g/b/d)         | 47.05 <sup>a</sup>   | 44.46 <sup>a</sup>   | 44.29 <sup>a</sup>  | 43.13 <sup>a</sup>   | 45.18 <sup>a</sup>   | 44.82 <sup>a</sup>    | 37.08 <sup>b</sup>  | 45.98 <sup>a</sup>   | 46.34 <sup>a</sup>   | 34.56 <sup>b</sup>  | <0.0001    |
| Feed conv. ratio            | 2.31 <sup>bcd</sup>  | 2.22 <sup>de</sup>   | 2.10 <sup>e</sup>   | 2.36 <sup>bc</sup>   | 2.26 <sup>cd</sup>   | 2.41 <sup>b</sup>     | 2.22 <sup>de</sup>  | 2.36 <sup>bc</sup>   | 2.41 <sup>b</sup>    | 2.82 <sup>a</sup>   | <0.0001    |

<sup>a,b,c,d</sup>Means within the row with different superscripts are significantly different (p<0.05)  
 SEM-Standard error of mean. P-Probability value.

**Table 6:** Effect of methionine, crude protein, and their interaction on performance of broilers on low crude protein methionine supplemented diets (n=4 replicates of 8 birds each)

| Treatment                    | Final weight (g/b)   | Weight gain (g/b)   | Feed intake (g/b)  | FCR               |
|------------------------------|----------------------|---------------------|--------------------|-------------------|
| <b>Methionine (g/100g)</b>   |                      |                     |                    |                   |
| 0.6                          | 739.55 <sup>a</sup>  | 19.98 <sup>a</sup>  | 45.67 <sup>a</sup> | 2.29 <sup>b</sup> |
| 1.0                          | 727.63 <sup>a</sup>  | 19.64 <sup>a</sup>  | 45.15 <sup>a</sup> | 2.31 <sup>b</sup> |
| 1.4                          | 591.61 <sup>b</sup>  | 15.76 <sup>b</sup>  | 38.26 <sup>b</sup> | 2.47 <sup>a</sup> |
| SEM                          | 14.82                | 14.82               | 25.68              | 0.02              |
| <b>Crude protein(g/100g)</b> |                      |                     |                    |                   |
| 23                           | 753.13 <sup>a</sup>  | 20.37 <sup>a</sup>  | 47.05 <sup>a</sup> | 2.31 <sup>b</sup> |
| 20                           | 731.27 <sup>ab</sup> | 19.74 <sup>ab</sup> | 43.96 <sup>b</sup> | 2.23 <sup>b</sup> |
| 17                           | 686.53 <sup>bc</sup> | 18.47 <sup>bc</sup> | 42.36 <sup>b</sup> | 2.30 <sup>b</sup> |
| 14                           | 636.46 <sup>c</sup>  | 17.03 <sup>c</sup>  | 42.30 <sup>b</sup> | 2.53 <sup>a</sup> |
| SEM                          | 18.96                | 18.96               | 32.85              | 0.03              |
| <b>Factorial Effects</b>     | <b>P (ANOVA)</b>     |                     |                    |                   |
| Methionine                   | <0.0001              | <0.0001             | <0.0001            | <0.0001           |
| Crude Protein                | 0.0018               | 0.0018              | 0.2371             | <0.0001           |
| Met x CP                     | 0.0046               | 0.0045              | 0.0026             | <0.0001           |

Means within the same column with different superscripts are significantly different ( $p < 0.05$ )

**Table 7:** Effect of methionine, crude protein, and their interaction on carcass measure (g/100g liveweight)

| Treatment                     | Dressed weight       | Drum stick          | Thigh               | Breast              | Back                | Wing               |
|-------------------------------|----------------------|---------------------|---------------------|---------------------|---------------------|--------------------|
| <b>Methionine (g/100g)</b>    |                      |                     |                     |                     |                     |                    |
| 0.6                           | 514.59 <sup>a</sup>  | 73.88 <sup>a</sup>  | 84.38 <sup>a</sup>  | 145.38 <sup>a</sup> | 130.81 <sup>a</sup> | 78.08              |
| 1.0                           | 491.71 <sup>a</sup>  | 70.88 <sup>a</sup>  | 78.83 <sup>a</sup>  | 143.54 <sup>a</sup> | 125.25 <sup>a</sup> | 72.25              |
| 1.4                           | 369.79 <sup>b</sup>  | 52.88 <sup>b</sup>  | 61.13 <sup>b</sup>  | 103.75 <sup>b</sup> | 95.46 <sup>b</sup>  | 54.33 <sup>b</sup> |
| SEM                           | 21.29                | 3.05                | 3.19                | 6.77                | 5.84                | 3.56               |
| <b>Crude Protein (g/100g)</b> |                      |                     |                     |                     |                     |                    |
| 23                            | 536.25 <sup>a</sup>  | 77.13 <sup>a</sup>  | 89.75 <sup>a</sup>  | 149.38              | 131.25              | 76.13              |
| 20                            | 476.83 <sup>ab</sup> | 69.88 <sup>ab</sup> | 78.21 <sup>ab</sup> | 137.29              | 118.33              | 69.04              |
| 17                            | 442.83 <sup>b</sup>  | 64.00 <sup>b</sup>  | 73.21 <sup>b</sup>  | 127.25              | 118.29              | 67.88              |
| 14                            | 449.21 <sup>b</sup>  | 62.68 <sup>b</sup>  | 71.13 <sup>b</sup>  | 126.79              | 114.75              | 66.46              |
| SEM                           | 27.24                | 3.90                | 4.08                | 8.66                | 7.47                | 4.56               |
| <b>Factorial</b>              | <b>Effects</b>       | <b>P (ANOVA)</b>    |                     |                     |                     |                    |
| Methionine                    | 0.0002               | 0.0002              | 0.0002              | 0.0003              | 0.0006              | 0.0003             |
| Crude Protein                 | 0.6323               | 0.3563              | 0.3327              | 0.6737              | 0.9721              | 0.8668             |
| Met x CP                      | 0.0688               | 0.0855              | 0.0578              | 0.0644              | 0.0825              | 0.0793             |

<sup>a,b</sup>Means within the same column with different superscripts are significantly different ( $p < 0.05$ )

**Table 8:** Effect of CP, Methionine and their interaction on Antibody titre against IBD

| Treatment                     | Antibody titre |
|-------------------------------|----------------|
| <b>Methionine (g/100g)</b>    |                |
| 0.6                           | 895.8          |
| 1.0                           | 981.4          |
| 1.4                           | 950.9          |
| SEM                           | 80.08          |
| <b>Crude Protein (g/100g)</b> |                |
| 23                            | 768.2          |
| 20                            | 1025.8         |
| 17                            | 830.4          |
| 14                            | 1014.4         |
| SEM                           | 102.45         |
| Factorial Effect              | P (ANOVA)      |
| Methionine                    | 0.9324         |
| Crude Protein                 | 0.2369         |
| Met x CP                      | 0.5488         |

significant decrease in carcass responses of broiler fed low CP diets. Metabolically, excess lysine cannot be used efficiently because the receptors for lysine become saturated.

In chickens, it has been shown that shortage or excess of dietary protein (Payne et al., 1990) or amino acids (Bhargava et al., 1971b) alters immune responses. Amino acids are needed to trigger such a response, which consists in clonal proliferation of lymphocytes and establishment of germinative centers in the bursa of Fabricius to refine immunoglobulin affinity. Amino acids are also involved in the recruitment of new bone marrow monocytes and heterophils and synthesis of effector molecules (immunoglobulins, nitric oxide, lysozyme) and communication molecules such as cytokines and eicosanoids (Rubin et al., 2007). In study one, the results of the immune response showed that there were no significant differences between the antibody titre of the birds on 23% CP, 0.6% methionine control diet and birds on the other experimental diets. However, numerically birds on 20% CP and 1.0% methionine diet had higher antibody titre value (1174.6) against IBD virus, compared with those on the control diet (768.2). Tsiagbe et al. (1987) suggested that the requirement for

**Table 9:** Performance of broilers on low crude protein methionine supplemented diets (n=4 replicates of 8 birds each)

|                             | 1                    | 2                    | 3                   | 4                    | 5                    | 6                     | 7                   | 8                    | 9                    | 10                  | SEM   | P-Value |
|-----------------------------|----------------------|----------------------|---------------------|----------------------|----------------------|-----------------------|---------------------|----------------------|----------------------|---------------------|-------|---------|
| Crude protein(g/100g)       | 23                   | 20                   | 20                  | 20                   | 17                   | 17                    | 17                  | 14                   | 14                   | 14                  |       |         |
| Methionine (g/100%)         | 0.6                  | 0.6                  | 1.0                 | 1.4                  | 0.6                  | 1.0                   | 1.4                 | 0.6                  | 1.0                  | 1.4                 |       |         |
| <b>Parameters</b>           |                      |                      |                     |                      |                      |                       |                     |                      |                      |                     |       |         |
| Initial weight              | 40.2                 | 40.4                 | 40.3                | 40.1                 | 40.2                 | 40.5                  | 40.2                | 40.2                 | 40.2                 | 40.2                |       |         |
| Final weight (g/b/ 35 days) | 753.13 <sup>ab</sup> | 740.63 <sup>ab</sup> | 775.06 <sup>a</sup> | 678.13 <sup>bc</sup> | 741.96 <sup>ab</sup> | 692.19 <sup>abc</sup> | 625.45 <sup>c</sup> | 722.50 <sup>ab</sup> | 715.63 <sup>ab</sup> | 475.25 <sup>d</sup> | 26.81 | <0.0001 |
| Weight gain(g/b/d)          | 20.37 <sup>ab</sup>  | 20.00 <sup>ab</sup>  | 20.99 <sup>a</sup>  | 18.23 <sup>bc</sup>  | 20.05 <sup>ab</sup>  | 18.62 <sup>abc</sup>  | 16.72 <sup>c</sup>  | 19.49 <sup>ab</sup>  | 19.30 <sup>ab</sup>  | 12.32 <sup>d</sup>  | 26.82 | <0.0001 |
| Feed intake (g/b/d)         | 47.05 <sup>a</sup>   | 44.46 <sup>a</sup>   | 44.29 <sup>a</sup>  | 43.13 <sup>a</sup>   | 45.18 <sup>a</sup>   | 44.82 <sup>a</sup>    | 37.08 <sup>b</sup>  | 45.98 <sup>a</sup>   | 46.34 <sup>a</sup>   | 34.56 <sup>b</sup>  | 46.46 | <0.0001 |
| Feed conv. ratio            | 2.31 <sup>bcd</sup>  | 2.22 <sup>de</sup>   | 2.10 <sup>e</sup>   | 2.36 <sup>bc</sup>   | 2.26 <sup>cd</sup>   | 2.41 <sup>b</sup>     | 2.22 <sup>de</sup>  | 2.36 <sup>bc</sup>   | 2.41 <sup>b</sup>    | 2.82 <sup>a</sup>   | 0.04  | <0.0001 |

<sup>a,b,c,d</sup>Means within the row with different superscripts are significantly different (p<0.05)  
 SEM-Standard error of mean. P-Probability value

**Table 10:** Effect of CP, lysine and their interaction on performance of broilers (n=4 replicates of 4 birds each)

| Treatment                     | Final Weight (g/b)   | Weight Gain (g/b)  | Feed Intake (g/b)  | Feed Conversion Ratio |
|-------------------------------|----------------------|--------------------|--------------------|-----------------------|
| <b>Lysine (g/100g)</b>        |                      |                    |                    |                       |
| 1.2                           | 8331.56 <sup>a</sup> | 21.36 <sup>a</sup> | 58.68 <sup>a</sup> | 2.98 <sup>b</sup>     |
| 1.6                           | 727.08 <sup>b</sup>  | 18.33 <sup>b</sup> | 54.29 <sup>b</sup> | 3.30 <sup>b</sup>     |
| 2.0                           | 562.50 <sup>c</sup>  | 13.68 <sup>c</sup> | 48.45 <sup>c</sup> | 3.82 <sup>a</sup>     |
| SEM                           | 24.61                | 24.17              | 9.86               | 0.14                  |
| <b>Crude Protein (g/100g)</b> |                      |                    |                    |                       |
| 23                            | 1035 <sup>a</sup>    | 27.12 <sup>a</sup> | 62.75 <sup>a</sup> | 2.31 <sup>c</sup>     |
| 20                            | 920 <sup>b</sup>     | 23.87 <sup>b</sup> | 63.39 <sup>a</sup> | 2.74 <sup>bc</sup>    |
| 17                            | 710.42 <sup>c</sup>  | 17.85 <sup>c</sup> | 55.30 <sup>b</sup> | 3.21 <sup>b</sup>     |
| 14                            | 422.92 <sup>d</sup>  | 9.73 <sup>d</sup>  | 41.37 <sup>c</sup> | 4.37 <sup>a</sup>     |
| SEM                           | 31.48                | 30.91              | 12.61              | 0.18                  |
| <b>Factorial Effect</b>       | <b>P-ANOVA</b>       |                    |                    |                       |
| Lysine                        | 0.0001               | 0.0001             | 0.0001             | 0.0001                |
| Crude Protein                 | 0.0001               | 0.0001             | 0.0001             | 0.0001                |
| Lys x CP                      | 0.0316               | 0.0323             | 0.0001             | 0.4317                |

<sup>a,b,c,d</sup> means within the row with different superscripts are significantly different ( $p < 0.005$ ) g/b- gram / bird

**Table 11:** Effect of CP, lysine and their interaction on broiler carcass parts (g/100gliveweight)

| Treatment                     | Dressed weight      | Drum stick         | Thigh              | Breast              | Back                | Liver              | Lung              |
|-------------------------------|---------------------|--------------------|--------------------|---------------------|---------------------|--------------------|-------------------|
| <b>Lysine (g/100g)</b>        |                     |                    |                    |                     |                     |                    |                   |
| 1.2                           | 437.81 <sup>a</sup> | 67.50 <sup>a</sup> | 72.03 <sup>a</sup> | 129.53 <sup>a</sup> | 96.78 <sup>a</sup>  | 25.03 <sup>a</sup> | 3.97 <sup>a</sup> |
| 1.6                           | 370.83 <sup>b</sup> | 58.17 <sup>b</sup> | 64.63 <sup>b</sup> | 106.00 <sup>b</sup> | 89.33 <sup>a</sup>  | 25.21 <sup>a</sup> | 3.92 <sup>a</sup> |
| 2.0                           | 281.67 <sup>c</sup> | 41.38 <sup>c</sup> | 49.63 <sup>c</sup> | 73.33 <sup>c</sup>  | 67.08 <sup>b</sup>  | 20.04 <sup>b</sup> | 2.96 <sup>b</sup> |
| SEM                           | 13.23               | 2.43               | 2.44               | 3.83                | 4.00                | 1.41               | 0.24              |
| <b>Crude Protein (g/100g)</b> |                     |                    |                    |                     |                     |                    |                   |
| 23                            | 552.50 <sup>a</sup> | 85.13 <sup>a</sup> | 89.38 <sup>a</sup> | 175.25 <sup>a</sup> | 121.75 <sup>a</sup> | 28.25 <sup>a</sup> | 5.50 <sup>a</sup> |
| 20                            | 481.67 <sup>b</sup> | 76.08 <sup>b</sup> | 80.38 <sup>a</sup> | 139.79 <sup>b</sup> | 108.46 <sup>a</sup> | 26.42 <sup>a</sup> | 4.38 <sup>a</sup> |
| 17                            | 363.75 <sup>c</sup> | 55.75 <sup>c</sup> | 64.08 <sup>b</sup> | 101.54 <sup>c</sup> | 86.63 <sup>b</sup>  | 26.17 <sup>a</sup> | 3.33 <sup>c</sup> |
| 14                            | 206.67 <sup>d</sup> | 29.33 <sup>d</sup> | 36.04 <sup>c</sup> | 52.29 <sup>d</sup>  | 50.42 <sup>c</sup>  | 16.63 <sup>b</sup> | 2.63 <sup>c</sup> |
| SEM                           | 17.29               | 3.10               | 3.12               | 4.90                | 5.11                | 1.80               | 0.31              |
| <b>Factorial Effect</b>       |                     |                    |                    |                     |                     |                    |                   |
| Lysine                        | 0.0001              | 0.0001             | 0.0001             | 0.0001              | 0.0011              | 0.0480             | 0.0411            |
| Crude Protein                 | 0.0001              | 0.0001             | 0.0001             | 0.0001              | 0.0001              | 0.0001             | 0.0001            |
| Lys x CP                      | 0.0380              | 0.0599             | 0.0611             | 0.0041              | 0.0566              | 0.7719             | 0.0118            |

**Table 12:** Effect of crude protein, lysine and their interaction on Antibody titre

| Treatment                     | Antibody titre       |
|-------------------------------|----------------------|
| <b>Lysine (g/100g)</b>        |                      |
| 1.2                           | 3856.7               |
| 1.6                           | 3163.1               |
| 2.0                           | 3150.5               |
| SEM                           | 269.71               |
| <b>Crude Protein (g/100g)</b> |                      |
| 23                            | 2467.2 <sup>b</sup>  |
| 20                            | 3512.6 <sup>ab</sup> |
| 17                            | 3903.0 <sup>a</sup>  |
| 14                            | 2980.7 <sup>ab</sup> |
| SEM                           | 345.02               |
| Factorial Effect              | P (ANOVA)            |
| Lysine                        | 0.2146               |
| Crude Protein                 | 0.0615               |
| Lys x CP                      | 0.5312               |

<sup>a,b</sup>Means within the row with different superscripts are significantly different ( $p < 0.05$ )  
SEM-Standard error of mean.

methionine for maximum antibody titers was greater than that for growth. This means that the IgG levels did increase in birds fed increasing percent of methionine. This may indicate that extra methionine is important for the synthesis of the IgG antibodies or perhaps required for thymus-derived T-cell helper function. Also Swain and Johri (2000) and Shini et al. (2005) reported that methionine requirements are higher when the purpose is to maintain optimal immunity levels, as compared to growth and that lower supply of sulphur amino acids like methionine and cysteine results in a severe lymphocyte depletion in the intestinal tissues (Peyer's patches) and in the lamina propria. Deficiency or excesses of dietary protein or amino acids has previously been said to alter immune response in chickens (Konashi et al., 2000, Chen et al., 2003), but among the various experimental diets fed in study two, birds on 20% CP and 1.6% lysine diet produced the highest antibody titre against infectious bursal disease vaccine. This is in support of the work of Konashi et al. (2000), which reported that lysine, one of the key amino acids for protein synthesis and muscle deposition is involved

in the synthesis of cytokines, proliferation of lymphocytes and thus in the optimum functioning of immune system in response to infection. According to Chen et al. (2003), inadequate supply of lysine would reduce antibody response and cell-mediated immunity in chicken.

## Conclusions

Results of the current study showed that the growth performance and carcass measures of broilers on 20% CP diet with 1.0% methionine or 1.6% lysine was similar to those on a control diet containing 23% CP with 0.6% methionine or 1.2% lysine. Antibody titre values in birds on the 20% CP with 1.0% methionine or 1.6% lysine were higher than those on 23% CP and 0.6% methionine or lysine. Better immune response in broilers can be obtained with adequate supplementation of methionine and lysine, which have been identified to be in marginal quantities in most poultry diets. Typical poultry feeds, in which much of the protein may contain less than the desired quantity of methionine and lysine necessitates their supplementation of such diets for improved immune response against Infectious Bursal Disease virus.

## Impact

The results of the research will educate poultry farmers that they may not need to include up to 23%CP in broiler diets in accordance to NRC (1994) recommendation, but that a lower inclusion rate of 20% CP supplemented with 1.0% methionine and 1.6% lysine will produce growth performance and carcass characteristics as a 23% CP, 0.6% methionine and 1.2% lysine diet. Furthermore, such lower CP diet supplemented with methionine and has no adverse effect on immune response against IBD. The results point to the fact that methionine and lysine in excess of NRC recommendations are required for optimum antibody formation.

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