Replacement of Normal Maize with Quality Protein Maize on Performance, Immune Response and Carcass Characteristics of Broiler Chickens

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ABSTRACT: An experiment was conducted to evaluate the effect of dietary replacement of normal maize (NM) with quality protein maize (QPM) on performance, immune response and carcass characteristics of broiler (Krishibro) chickens. Six experimental diets were prepared separately for starter and finisher phases. Diet 1 was a control diet formulated with NM and soybean meal. In diets 2-5, the NM was replaced with QPM at 25, 50, 75 and 100%, respectively. Diet 6 was the same as the control diet, but supplemented with synthetic lysine similar to the industry standard. Each test diet was fed to 8 replicates, each of 5 chicks, reared in stainless steel battery brooders. The AME content of QPM (3382 kcal/kg) was similar to that of NM (3,352 kcal/kg), but protein (9.91 vs. 8.94%), lysine (0.40 vs. 0.26%) and tryptophan (0.09 vs. 0.07%) contents of QPM were higher than NM. Dietary replacement of NM with 50% QPM significantly (p<0.05) improved body weight gain, feed conversion ratio, humoral immune response, relative bursa weight, and breast muscle yield and lowered abdominal fat content. No further improvement in these parameters was recorded by increasing the level of replacement of NM with QPM to either 75% or 100%. Further, the improvement noticed in the 50% QPM group was similar to the group fed the NM diet with lysine supplementation, and thus dietary replacement of NM with QPM at 50% did not need extra synthetic lysine supplementation. It is concluded that dietary replacement of NM with QPM at the 50% level resulted in optimum performance, higher breast muscle yield and higher immune response in broiler chickens. (Key Words: Normal Maize, Quality Protein Maize, Performance, Immune Response, Carcass Characteristics, Broiler Chickens)

INTRODUCTION

Cereal grains are the main source of energy in poultry diet and maize is the popular cereal used in combination with soybean meal, to formulate compounded poultry feed. Maize is the most preferred energy source due to its high energy, low fibre, better palatability, presence of pigments and essential fatty acids. However, the protein content of the traditional maize grain is low compared to other cereals (NRC, 1994). Further, normal maize contains high zein fraction, which is practically devoid of lysine and low in tryptophan (Prasanna et al., 2001). Though maize is being used primarily as an energy source it contributes about 25-30% of dietary protein in broiler diets. The maize protein has lower nutritional value which is attributed to high concentration of prolamin storage protein fraction (50-60% of protein) which is deficient in lysine and tryptophan (Salamini and Soave, 1982).

In poultry, methionine is the first limiting amino acid in maize-soybean meal based diets, followed by lysine, threonine, and tryptophan. Therefore, while formulating the diets, all these amino acids are supplemented in synthetic form to meet the requirement, which are quite expensive. Alternatively, if the amino acid content in the maize is increased through breeding techniques, the need for external supplementation of amino acids will be reduced. Breeding for improved protein quality in maize began in the mid-1960 with the discovery of mutants, such as opaque-2 (Mertz et al., 1964) and floury-2 (Nelson et al., 1965) which produced higher levels of lysine and tryptophan, the two amino acids deficient in maize endosperm proteins. Lysine deficient zein or prolamine fraction reduced dramatically by about 50% whiles other fractions such as albumins, globulins and glutelins rich in lysine show a marked increase. However, pleiotropic effects of this mutation,
namely a soft endosperm that results in damaged kernels, as increased susceptibility to pests and fungal diseases, inferior food processing and reduced yields imposed constraints on successful exploitation of these mutants. The search then continued for new mutants that could alter the amino acid profile of maize endosperm protein, by increasing the concentration of lysine and tryptophan. Interdisciplinary and concerted research efforts led to amelioration of the negative features of opaque phenotypes and rebirth of quality protein maize (QPM) took place. It is hypothesized that the use of such quality maize will enable the feed manufacturer to produce balanced poultry feeds with minimal/low additional supplementation of amino acids. Only limited information is available with respect to the utilization of QPM in broiler chickens. Lysine is one of the amino acids which influences the magnitude of antibody response (Praharaj et al., 2002; Kidd et al., 2004) and to our knowledge no information is available on the effect of QPM (rich in lysine) on the immune response. Therefore, the present study was conducted to study the effect of QPM replacing normal maize on performance, immune response and carcass characteristics of broiler chickens.

MATERIALS AND METHODS

Analysis of feed ingredients

The quality protein maize used in the present study was procured from Zonal Agricultural Research centre, Mandya (UAS, Bangalore, India) and normal maize and soybean meal were procured from the local (Hyderabad, India) feed suppliers. All the feed ingredients were analyzed for proximate composition, calcium and phosphorus (AOAC, 1990). The amino acid compositions of feed ingredients were measured at Degussa, Germany (Llames and Fontaine, 1994). The apparent metabolizable energy (AME) contents in NM, QPM and soybean meal were estimated following the European Reference Method (Bourdillon et al., 1990) by feeding each ingredient to six, 28 week old White Leghorn cockerels. During the preliminary feeding of 14 d, the birds were maintained on a commercial grower diet containing 18% protein and 2,600 kcal ME/kg diet. Subsequently, each ingredient (QPM, NM and soybean meal) was fed at 97% with 3% vitamin and mineral premix to 6 White Leghorn cockerels for 3 day, 7 hours after 7 days adaptation period on the same diets. The total excreta collection method was used. Dry matter intake of feed and total excreta voided was recorded and samples were analyzed for gross energy. The AME content was calculated as the difference between energy intake and energy voided.

Birds and management

Day-old commercial broiler chicks (Krishibro, a multicoloured variety developed by the Project Directorate on Poultry, Hyderabad, India) of either sex were distributed equally to 6 test groups with 8 replicates of 5 chicks each. The chicks were individually wing banded and reared in stainless steel battery brooders with raised wire floor in open sided house. 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 after which, chicks were maintained at room temperature. Uniform management and vaccination schedules were followed for all the birds. The experiment was conducted following the guidelines of the Institute (Project Directorate on Poultry, Hyderabad, India) Animal Ethics Committee.

Diets

Six experimental diets were formulated separately for starter and finisher phase (Table 1). Diet 1 was a control diet formulated with NM and soybean meal. In diets 2-5, the NM was replaced with QPM at 25, 50, 75 and 100%, respectively. Diet 6 was same as control diet, but supplemented with synthetic lysine similar to the standard prescribed for the stock (Krishibro, PDP, ICAR, India). The starter (0-3 wk) and finisher (4-5 wk) diets were offered ad libitum to the respective groups.

Traits measured

Individual body weights and feed intake of replicates were recorded at weekly intervals and the feed per gain was calculated as the ratio between feed consumed and weight gained. One bird representing the mean body weight of each replicate (eight birds per treatment) was selected and sacrificed by cervical dislocation on 43 d of age. The data on weight of edible carcass, liver, gizzard, abdominal fat and breast meat were recorded and all the data were expressed as percentage of the pre-slaughter weight of the same bird. The humoral immune response in broilers was measured by injecting sheep red blood cells (SRBC), a non-pathogenic antigen. Eight birds from each dietary group were injected with 0.1 ml of 0.5% SRBC suspension into the brachial vein on the 29th day, and blood samples were collected on the 5th day of post-inoculation. Subsequently, micro-haemagglutination activity of serum was estimated and the antibody titers (log2) were measured following the standard procedure (Wegmann and Smithies, 1966).

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 test (Duncan, 1955). Significance was considered at p<0.05 levels.
RESULTS

The apparent metabolizable energy (AME), proximate and amino acid composition of NM and QPM are given in Table 2. The AME content of QPM (3,382 kcal/kg) was similar to that of NM (3,352 kcal/kg), but protein, lysine and tryptophan contents of QPM were higher than NM.

The body weights of the chicks at day one were comparable (varied between 41.89 to 42.65 g) among the dietary treatments (Table 3). Dietary replacement of NM with QPM at 50% significantly improved the body weight gain of the broilers compared to those fed the control or 25% QPM diet both at 3rd and 6th week of age. No further improvement in the weight gain was noticed by higher level of replacement of NM with QPM (75 or 100%). The weight gain of broilers fed 50% QPM diet was comparable with the diet containing NM supplemented with synthetic lysine (NM+Lys). The feed conversion ratio (FCR) improved progressively with increase in the level of QPM upto 50% and further improvement was not observed at higher levels of QPM in diet at 3rd week. At 6 weeks of age, the improvement in FCR was observed at ≥25% QPM. The levels of QPM in the diet did not influence the FCR. Lysine fortification to control NM diet also significantly improved the feed efficiency compared to those fed the QPM diets.

The humoral immune response as measured by antibody titre to SRBC injection was significantly higher in the diet in which NM was replaced with QPM at 50% compared to either control or 25% replacement group (Table 4). The SRBC titre of 50% replacement group was comparable with the diet containing NM supplemented with synthetic lysine (NM+Lys).

Table 1. Ingredient and calculated nutrient composition (%) of starter and finisher diets

| Ingredient                  | Percent replacement of normal maize with quality protein maize |
|-----------------------------|---------------------------------------------------------------|
| D1 (0)                      | D2 (25)           | D3 (50)           | D4 (75)           | D5 (100)         | D6 (0+lys)       |
| Starter (0-3 wk)            |                  |                  |                  |                  |                  |
| Normal maize                | 56.22             | 42.17             | 28.11             | 14.05             | 0.0              | 56.12             |
| Quality protein maize       | 0.0               | 14.05             | 28.11             | 42.17             | 56.22            | 0.0               |
| Soybean meal                | 38.60             | 38.60             | 38.60             | 38.60             | 38.60            | 38.60             |
| Lysine                      | -                 | -                 | -                 | -                 | -                | 0.10              |
| Vegetable oil              | 1.5               | 1.5               | 1.5               | 1.5               | 1.5              | 1.5               |
| Constant                     | 3.68              | 3.68              | 3.68              | 3.68              | 3.68             | 3.68              |
| Nutrient composition        |                  |                  |                  |                  |                  |                  |
| ME (kcal/kg)                | 2,932             | 2,936             | 2,940             | 2,942             | 2,944            | 2,932             |
| Protein (%)                 | 22.02             | 22.24             | 22.30             | 22.36             | 22.40            | 22.02             |
| Lysine (%)                  | 1.20              | 1.22              | 1.24              | 1.26              | 1.28             | 1.30              |
| Methionine (%)              | 0.35              | 0.35              | 0.35              | 0.35              | 0.35             | 0.35              |
| Phosphorous (%)             | 0.45              | 0.45              | 0.45              | 0.45              | 0.45             | 0.45              |
| Calcium (%)                 | 1.01              | 1.01              | 1.00              | 1.00              | 1.00             | 1.01              |
| Finisher (4-6 wk)           |                  |                  |                  |                  |                  |                  |
| Normal maize                | 58.34             | 43.76             | 29.17             | 14.58             | 0.0              | 58.29             |
| Quality protein maize       | 0.0               | 14.58             | 29.17             | 43.76             | 58.34            | 0.0               |
| Soybean meal                | 35.80             | 35.80             | 35.80             | 35.80             | 35.80            | 35.80             |
| Lysine                      | -                 | -                 | -                 | -                 | -                | 0.05              |
| Vegetable oil              | 2.50              | 2.50              | 2.50              | 2.50              | 2.50             | 2.50              |
| Constant                     | 3.36              | 3.36              | 3.36              | 3.36              | 3.36             | 3.36              |
| Nutrient composition        |                  |                  |                  |                  |                  |                  |
| ME (kcal/kg)                | 3,030             | 3,034             | 3,038             | 3,042             | 3,046            | 3,042             |
| Protein (%)                 | 20.58             | 20.64             | 20.72             | 20.80             | 20.88            | 20.58             |
| Lysine (%)                  | 1.10              | 1.12              | 1.14              | 1.16              | 1.18             | 1.15              |
| Methionine (%)              | 0.35              | 0.35              | 0.35              | 0.35              | 0.35             | 0.35              |
| Phosphorous (%)             | 0.40              | 0.40              | 0.40              | 0.40              | 0.40             | 0.40              |
| Calcium (%)                 | 0.90              | 0.90              | 0.90              | 0.90              | 0.90             | 0.90              |

1 Constant (%): Salt, 0.4; dl-calcium phosphate, 1.89; shell grit, 0.71; DL-methionine, 0.22; AB2D3K, 0.015; B-complex, 0.012; choline chloride, 0.06; trace mineral, 0.12; toxin binder, 0.2; antibiotic, 0.05.

2 Constant (%): Salt, 0.4; dl-calcium phosphate, 1.62; shell grit, 0.68; DL-methionine, 0.20; AB2D3K, 0.015; B-complex, 0.012; choline chloride, 0.06; trace mineral, 0.12; toxin binder, 0.2; antibiotic, 0.05.

3 Supplies per kg diet: Vitamin A, 16,500 IU; vitamin D3, 3,200 ICU; vitamin E, 12 mg; vitamin K, 2 mg; vitamin B12, 12 μg; niacin, 18 mg; pantothenic acid, 12 mg; Mn, 90 mg; Zn, 72 mg; Fe, 60 mg; Cu, 10 mg; I, 1.2 mg.
Table 2. Analyzed apparent metabolizable energy (AME), proximate and amino acid* composition of feed ingredients

| Nutrients     | Normal maize | Quality protein maize | Soybean meal |
|---------------|--------------|-----------------------|--------------|
| AME (kcal/kg) | 3,352        | 3,382                 | 2,254        |
| Crude protein (%) | 8.94        | 9.91                  | 45.8         |
| Crude fibre (%) | 3.51         | 3.01                  | 6.42         |
| Ether extract (%) | 4.66         | 4.82                  | 1.04         |
| Calcium (%)   | 0.22         | 0.19                  | 0.30         |
| Phosphorus (%)| 0.28         | 0.33                  | 0.61         |
| Amino acids (%) |
| Methionine    | 0.18         | 0.19                  | 0.64         |
| Cystine       | 0.19         | 0.29                  | 0.68         |
| Lysine        | 0.26         | 0.40                  | 2.74         |
| Threonine     | 0.31         | 0.38                  | 1.74         |
| Tryptophan    | 0.07         | 0.09                  | 0.69         |
| Arginine      | 0.40         | 0.66                  | 3.18         |
| Isoleucine    | 0.29         | 0.32                  | 2.02         |
| Leucine       | 1.09         | 0.92                  | 3.38         |
| Valine        | 0.41         | 0.53                  | 2.28         |
| Histidine     | 0.25         | 0.39                  | 1.24         |
| Phenyl alanine| 0.44         | 0.43                  | 2.27         |

* Amino acid analyzed by Degussa, Germany.

Table 3. Dietary replacement of NM with QPM on body weight gain and feed per gain of broiler chickens

| Diets (NM:QPM) | Body weight gain (g/bird) | Feed/gain | Body weight gain (g/bird) | Feed/gain |
|----------------|----------------------------|-----------|---------------------------|-----------|
| D1 (100:0)    | 634<sup>b</sup>           | 1.454<sup>a</sup> | 1,464<sup>b</sup>       | 2.046<sup>c</sup> |
| D2 (75:25)    | 641<sup>b</sup>           | 1.382<sup>b</sup> | 1,461<sup>b</sup>       | 1.9912<sup>b</sup> |
| D3 (50:50)    | 675<sup>a</sup>           | 1.341<sup>c</sup> | 1,510<sup>a</sup>       | 1.99<sup>b</sup> |
| D4 (25:75)    | 686<sup>a</sup>           | 1.3522<sup>c</sup> | 1,516<sup>c</sup>      | 1.991<sup>b</sup> |
| D5 (0:100)    | 673<sup>a</sup>           | 1.35<sup>c</sup>  | 1,522<sup>a</sup>      | 1.984<sup>b</sup> |
| D6 (100:0+lys) | 676<sup>a</sup>           | 1.346<sup>c</sup> | 1,514<sup>a</sup>      | 1.973<sup>b</sup> |
| SEM           | 4.84                      | 0.015      | 6.79                     | 0.009     |
| p-value       | 0.049                     | 0.053      | 0.016                    | 0.051     |

Means with different superscripts in column differ significantly (p≤0.05).

Table 4. Dietary replacement of NM with QPM on carcass and organ weights (% live weight) and humoral immune response of broiler chickens

| Diets (NM:QPM) | Dressed yield | Breast | Giblet | Abdominal fat | Spleen | Bursa | Antibody titre (log2) |
|----------------|---------------|--------|--------|---------------|--------|-------|-----------------------|
| D1 (100:0)    | 72.1          | 14.55<sup>b</sup> | 5.35   | 2.04<sup>a</sup> | 0.09   | 0.18<sup>b</sup> | 6.0<sup>a</sup> |
| D2 (75:25)    | 72.7          | 14.65<sup>b</sup> | 5.76   | 1.76<sup>b</sup> | 0.08   | 0.19<sup>b</sup> | 6.2<sup>b</sup> |
| D3 (50:50)    | 72.1          | 15.24<sup>a</sup> | 5.97   | 1.08<sup>c</sup> | 0.09   | 0.21<sup>b</sup> | 6.9<sup>a</sup> |
| D4 (25:75)    | 72.7          | 15.21<sup>a</sup> | 5.53   | 1.01<sup>c</sup> | 0.09   | 0.29<sup>a</sup> | 7.2<sup>a</sup> |
| D5 (0:100)    | 72.5          | 15.30<sup>a</sup> | 5.76   | 1.06<sup>c</sup> | 0.09   | 0.28<sup>a</sup> | 7.1<sup>a</sup> |
| D6 (100:0+Lys)| 72.6          | 15.26<sup>a</sup> | 5.82   | 1.68<sup>b</sup> | 0.10   | 0.30<sup>a</sup> | 7.2<sup>a</sup> |
| SEM           | 0.23          | 0.18   | 0.78   | 0.10          | 0.01   | 0.02     | 0.01             |
| p-value       | 0.179         | 0.015  | 0.186  | 0.021         | 0.928  | 0.014    | 0.009            |

Means with different superscripts in column differ significantly (p≤0.05).
concentrations of lysine and tryptophan compared to NM contained not only higher protein but also higher mention here that, the QPM that was used in this study attributed to variation in nutrient composition among the diet. These variations noticed on the performance could be QPM diet to broiler chickens compared to those fed the NM conversion and nutrient utilization efficiencies by feeding significant difference with respect to body weight gain, feed efficiency of QPM. However, Tyagi et al. (2008) did not find any affected by feeding the control diet containing NM to the birds of this group were similar to those of synthetic lysine supplemented group. The relative weight of bursa improved significantly by dietary replacement of NM with QPM at 75% level compared to the lower levels of replacement and was at par with the 100% replacement and NM+lysine supplemented groups.

**DISCUSSION**

The findings of the study revealed that the AME content of QPM and NM was similar and the values obtained were with in the range as reported by Osei et al. (1999) and Tyagi et al. (2008). However, protein, lysine and tryptophan contents of QPM were 10.85, 53.84 and 28.57% higher than NM, respectively. Several other researchers have also reported higher protein quality of QPM over NM (Paes and Bicudo, 1995; Osei et al., 1999; Onimisi et al., 2009).

In the present study, body weight gain and FCR were affected by feeding the control diet containing NM to the broilers. However, dietary replacement of NM with QPM significantly improved the performance and the best result was obtained in group fed 50:50 NM and QPM, respectively. The performance of the birds of this group was similar to those of synthetic lysine supplemented group. Similar to the findings of the present study, Onimisi et al. (2009) reported significantly higher weight gain and feed efficiency of broiler chickens due to dietary replacement of NM with QPM. However, Tyagi et al. (2008) did not find any significant difference with respect to body weight gain, feed conversion and nutrient utilization efficiencies by feeding QPM diet to broiler chickens compared to those fed the NM diet. These variations noticed on the performance could be attributed to variation in nutrient composition among the QPM cultivars used in different studies. It is worthy to mention here that, the QPM that was used in this study contained not only higher protein but also higher concentrations of lysine and tryptophan compared to NM (Table 2). It is well known that lysine is crucial in protein synthesis for growth of tissues. Lysine is also involved in the cross linking process of bone collagen and in the biosynthesis of carnitine and elastin (Civitelli et al., 1992; Flodin, 1997). Similarly, tryptophan is an essential amino acid and the biological precursor of niacin. It is further reported that biological value of NM protein is 45% compared to 80% in high lysine maize (Graham et al., 1980). Thus, the improved performance noticed in the present study could be attributed not only to higher amino acids content but higher bioavailability resulting in higher protein synthesis (Onimisi et al., 2009).

Breast muscle growth has become a variable of interest in recent years because of high economic value (Tang et al., 2007). Dietary composition and nutrient content are potent regulators of muscle metabolism and development (Grizard, 1995). Dietary lysine concentrations have a large influence on breast muscle development (Kerr et al., 1999). It has been reported that low lysine diet not only leads to poor performance but also reduces breast muscle yield (Kidd et al., 1998; Bastianelli et al., 2007). A similar finding was observed in the present study. The breast muscle yield of the bird fed the control diet without synthetic lysine supplementation was significantly lower compared to those fed NM with lysine supplementation. However, significantly higher breast meat yield and lower abdominal fat content was noticed in the diet in which NM was replaced with QPM at 50% level. Replacement of NM with QPM at 50% increased the lysine level in starter (1.20 to 1.24%) and finisher (1.10 to 1.14%) diet compared to those fed the control diet. Similarly, Renden et al. (1994) reported improved performance and breast muscle yield, and reduced abdominal fat content due to elevating lysine concentration in the diet.

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 (Prahajaraj et al., 2002; Kidd et al., 2004). This could be the reason that lowest immune response was observed in the control diet where synthetic lysine was not supplemented. Probably, 1.2 and 1.10% lysine in starter and finisher diet respectively 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).

The control diet contained 1.2 and 1.1% lysine in the starter and finisher phase, respectively. Birds fed the control diet resulted in relatively poor performance, reduced breast muscle yield and low immune response. However, all the above responses were improved in broilers fed the diet which contained 50% QPM in place of NM and the same was comparable with the synthetic lysine supplemented diet. The 50% QPM diet contained 1.24% and 1.14% lysine during starter and finisher phase, respectively and was adequate for eliciting optimum response. Though, the levels were marginally higher, the better performance noticed in this group can be attributed to higher bioavailability of protein
and amino acid from QPM (Onimisi et al., 2009). Further, it is also observed that dietary replacement of NM with QPM did not need extra synthetic lysine supplementation for producing optimum performance. Therefore, it is concluded that, dietary replacement of NM with QPM at 50% level resulted in higher performance, higher breast muscle yield and humoral immune response compared to broilers fed the NM diet.

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