Genotype by Dietary Lysine Interaction for Growth and Response to Sheep Red Blood Cells and *Escherichia coli* Inoculation in Commercial Broiler Chicks

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ABSTRACT : An experiment was conducted to assess the interaction between genotypes and dietary lysine content in commercial broiler chicks by measuring growth, and response to sheep red blood cells (SRBC) and *Escherichia coli (E.coli)* inoculation. Female chicks from four genotypes (A=Anak 2000; B=Hubbard; C=Cobb and D=Synthetic broiler) were fed four levels of lysine in diet from d old till the end of experiment. The lysine content of the diet was 9.61, 10.51, 11.41 and 12.31 g/kg. Body weights at 0, 14, 28 and 42 d of age and pen-wise feed intake till 14, 28 and 42 d of age were recorded. Production of antibody against SRBC and resistance to *E.coli* were measured at 5 d of post inoculation (PI) at 43 d of age. Also, response to phytohemaglutinin-P (PHA-P) was measured at 12 and 24 h of PI at 48 d of age. Genotype by dietary lysine interaction was significant for body weights at 14 and 28 d of age, but not at 42 d of age. Genotype by dietary lysine interaction was not significant for feed efficiency, for antibody titers against SRBC, and for air sac lesion score, relative bodyweight change, and relative weights of bursa and spleen in response to *E.coli* inoculation. However, a significant interaction was observed between the levels of lysine and dosage of SRBC for antibody titers. There was significant genotype by dietary lysine interaction for cutaneous basophilic hypersensitivity (CBH) response to PHA-P at 12 and 24 h of PI. It may be concluded that to obtain optimum body weight and immunity in commercial broilers the dietary lysine requirement may be recommended specific to the genotype. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 8 : 1170-1177)***

Key Words : Genotype By Dietary Lysine Interaction, Commercial Broiler, Growth, Feed Efficiency, *Escherichia Coli*, Immunity

INTRODUCTION

Researches on various aspects of poultry production have made a significant growth in broiler industry during the last two decades. To achieve the same, the breeders have imposed high selection intensity for juvenile body weight to attain the marketable weight at an early age. Although there was significant improvement in juvenile weight, the chicks are not able to withstand the environmental stress because of negative correlation between body weight and immune potent traits (Siegel and Gross, 1980; Siegel and Dunnington, 1987; Kreukniet et al., 1994). This may be the result of selection for increased growth at juvenile stage, resulted in a conflict with maturation of immune system and magnitude of the immune response (Siegel et al., 1982; Maatman et al., 1993) and limited availability of biological resources to accommodate all the physiological demands (Dunnington, 1990; Solkner and James, 1994). This competition for finite resources can result in a negative correlation between the physiological traits (Rendel, 1963) such as growth and immunity.

Certain non-genetic factors like dietary nutrient concentration can influence growth and modify and/or alter the expression of the genes responsible for immunoresponsiveness (Klasing and Barnes, 1988; Katanbaf et al., 1988; Rama Rao et al., 1999) as the dietary protein quality is an essential factor in the synthesis of immunoglobulins and their function. These nutrients influence the maturity of immune system and magnitude of antibody response in poultry (Cook, 1991; Latshaw, 1991). Little information is available on the effect of essential nutrient density on the threshold and magnitude of immune response in young broilers (Dunnington et al., 1994). Again, animals with different genetic background may respond differently to the dietary nutrient concentration (Rama Rao et al., 1999). However, information on immuno-responsiveness in various genetic stocks that differ in growth potentialities when fed various levels of limiting amino acids is scanty (Maatman et al., 1993). An experiment was conducted to assess the interaction between genotype and the dietary lysine content in terms of growth, immune response to a non-pathogenic antigen and response to *E.coli* inoculation in commercial broiler chicks.

MATERIALS AND METHODS

Stocks, husbandry and diets

Commercial broiler female chicks from four genetic backgrounds (A=Anak 2000; B=Hubbard; C=Cobb and D=Synthetic broiler from the Indian Council of Agricultural Research. Higher juvenile body weight has been the criteria for 22 generations while developing the synthetic broiler strain) were used in this experiment. In each genotype 100 d old female chicks were distributed randomly into four groups of five replicates in each. Each replicate contained five chicks. On d-one chicks were wing banded,

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vaccinated against Marek's disease, and were housed in wire-floored battery brooders. The brooder temperature was maintained at 34±1°C up to 7 d of age and was gradually decreased to 26±1°C by 21 d of age, after which, chicks were maintained at room temperature. Chicks were vaccinated against New Castle (7th and 28th d) and infectious bursal diseases (14th and 28th d of age).

A corn-soya-deoiled rice bran basal diet was prepared to contained 11.90 MJ ME/kg and 204.2 g crude protein (CP)/kg (table 1). The CP level was fixed at 200 g/kg because the performance of the same four commercial broiler stocks was not influenced by increasing the dietary protein concentration beyond 200 g/kg when fed different levels of CP from 1 to 42 d of age (Rama Rao et al., 1999). Feed grade L-lysine HCl (98%) was supplemented to the basal diet to derive four levels (9.61, 10.51, 11.41 and 12.31 g/kg diet) of lysine. The levels of other nutrients were maintained according to the NRC (1994) recommendation for commercial broilers. Five replicates from each genetic group were fed with one of the experimental diets. Lighting was continuous and water and feed were available *ad libitum* through 1 to 48 d of age.

Experimental procedure

Individual chick weights and pen-wise feed intakes were recorded at 0, 14, 28 and 42 d of age. Feed efficiencies for the respective periods were calculated as the ratio between the body weight and feed intake. On d 43, two chicks from each replicate in each genotype-diet were inoculated intravenously with 0.1 ml of 0.5 or 2.5% suspension of sheep red blood cells (SRBC) in normal saline. On the same d, two chicks from each replicate in each genotype-diet were weighed individually and injected in the posterior thoracic air sac with 0.1 ml of a 10^{-4} dilution of *E. coli* (Serotype O2) culture incubated for 18 h in nutrient broth. Mortality, if any, were recorded at 24 h interval up to 120 h, after which, the survived chicks were weighed and killed by cervical dislocation. The lesions at postmortem were scored according to O'Sullivan et al. (1991) with slight modifications as: (1) no lesions in air sac, (2) mild air sac lesions, (3) moderate air sac lesions, (4) extensive heart and air sac lesions and (5) dead. The relative weights of bursa and spleen in relation to pre-slaughter body weight (g/kg) were recorded. The relative body weight change of individual bird was calculated as {(body wt. after inoculation-body wt. before inoculation/body wt. before inoculation)×100}.

On d 48, 0.5 ml of blood was collected in EDTA from the brachial vein of the chicks injected with SRBC and also from non-inoculated chicks and the antibody titers (log2) were measured employing the microtiter haemagglutination procedure of Wegmann and Smithies (1966). On d 47, one chick from each replicate in each genetic-diet were injected intradermally in right wattle with 100 µg of PHA-P in 0.1 ml of normal saline solution (NSS) and left wattle with 0.1 ml NSS to measure cutaneous basophilic hypersensitivity (CBH) response as thickness index (TI). The thickness of the wattles was measured using a micrometer gauge before inoculation and at 12 and 24 h post inoculation (PI). The TI was expressed as percentage increase wattle thickness i.e. (right wattle thickness due to PHA-P at 12 or 24 h PI/corresponding right wattle thickness before inoculation-left wattle thickness due to NSS at 12 or 24 h PI/corresponding left wattle thickness before inoculation). On d 48, these chicks were weighed and killed by cervical dislocation and blood smears were made to determine heterophill/lymphocyte (H/L) ratios. The relative weight (g/kg) of bursa and spleen were recorded.

Statistical analysis

Data were subjected to analysis of variance (General Linear Model, SAS® 1995) with genotype and diets as main effects. When interactions were significant, separate analyses were conducted within each main effect. Comparisons of multiple means were made by Duncan's multiple range test (Duncan, 1955) and significance was considered at $p \leq 0.05$.

Table 1. Ingredients and nutrient composition of basal diet fed to different genotypes (1 to 42 d of age)

Ingredient	g/kg
Yellow maize	620.0
Soyabean meal	235.0
Deoiled rice bran	99.6
Dicalcium phosphate	17.2
Oyster shell grit	16.4
Common salt	4.0
DL-methionine	1.50
Vitamin premix[a]	0.40
Coccidiostat	0.50
Choline chloride, 50%	2.30
Antibiotic	1.00
Trace mineral mixture[b]	1.50
Nutrient composition, g/kg	
Crude protein	204.2
ME MJ / kg	11.90
Calcium	10.03
Non phytin phosphorus	4.51
Lysine	9.20
Methionine	4.46

[a] Supplies per kg of diet : vitamin A, 8,250 IU; vitamin D_3, 1,200 ICU; riboflavin, 5 mg; vitamin K, 1 mg; vitamin B_1, 1 mg; vitamin B_6, 2 mg; vitamin B_{12}, 10 mcg; vitamin E, 10 mg; pantothenic acid, 10 mg; niacin, 12 mg.

[b] Supplies per 100 kg of diet: $MnSO_4$, 45 g; $ZnSO_4$, 28 g; $FeSO_4$, 40 g; $CuSO_4$, 4 g; KI, 0.3 g; selenium premix, 50 g.

RESULTS

Body weight

Genotype by dietary lysine interaction was not significant for d-old body weight. The weights for the chicks of the genotypes B (42.2±0.70 g) and D (42.5±0.42 g) did not differ from each other but were significantly heavier than those of genotypes A (39.3±0.39 g) and C (40.5±0.31 g), which also did not differ from each other. There were no significant differences in d-old body weights among the chicks of various lysine levels, which varied from 40.4±0.50 g to 41.1±0.42 g. This indicated that the randomization of chicks in different diet groups were uniform. Body weights at 14 and 28 d of age were significantly influenced by the interaction between genotype and dietary lysine content. At 14 d of age the body weight of genotype A was significantly higher compared to other genotypes when fed the diet containing 9.61 g of lysine/kg (table 2). At 10.51 g lysine/kg diet, however, the weight of genotype C was similar to genotype A. Further increase in lysine content did not elicit any advantage on body weight in genotypes A and D. In genotypes B and C maximum body weight was observed at 11.41 g lysine/kg diet and further increase in lysine content showed no response. At 28 d of age, the level of dietary lysine did not influence the body weight of genotype A and D, while in genotype B and C, the body weight increased significantly with the increase in level of dietary lysine up to 11.41 g/kg (table 3). Among the chicks fed 10.51 and 11.41 g lysine/kg diets the rankings for the genotypes were C=A>B=D and A=C=B>D, respectively. The rankings of genotypes fed 12.31 g lysine was similar to those fed 10.51 g lysine/kg diet. In general, at 28 d of age, genotype D weighed significantly lower compared to other genotypes at all levels of lysine tested (table 3). Genotype by dietary lysine interaction was not significant for body weight at 42 d of age. Birds in genotype D, however, were significantly lighter compared to other genotypes (table 4). Whereas, dietary level of lysine did not elicit any influence on 42 d body weight.

Feed efficiency

Genotype by dietary lysine interaction was not significant for feed efficiency between 0-14, 0-28 and 0-42 d of age. The cumulative feed efficiency between 0-14 and 0-28 d of age was not influenced among various genotypes. At 0-42 d of age, genotype C utilized feed more efficiently followed by genotypes A and D, while the feed efficiency for genotype B did not differ from those of genotypes A and C (table 4). The levels of lysine did not significantly influence the feed efficiency at 0-14 and 0-42 d of age. At 0-28 d of age, the feed utilization was better in chicks fed 12.31 g compared to those fed 10.51 g lysine/kg diet, while the efficiency of feed utilization in chicks fed 9.61, and 11.41 g lysine/kg diet did not differ from each other.

Response to sheep red blood cells (SRBC) inoculation

On hemaglutination test it was observed that there was absence of antibodies against SRBC in the serum collected from the SRBC non-inoculated birds. Among the SRBC inoculated birds, three way interaction among genotypes, levels of lysine and dosage of SRBC and two way interactions between genotypes and levels of lysine, and genotype and dosage of SRBC were not significant for antibody titers. The interaction was significant between the levels of lysine and dosage of SRBC (table 5). In general, both the dose of SRBC injected and the level of lysine in the diet significantly influenced the mean antibody titer resulting in an interaction. In general the antibody titer was higher in groups injected with 2.5% SRBC compared to those injected with 0.5% SRBC, irrespective of the level of dietary lysine. Similarly, the serum antibody titers progressively and significantly increased with the dietary concentration of lysine up to 11.41 g/kg at both 0.5 and 2.5% SRBC concentration (table 5). Further increase in lysine level did not elicit any positive response in producing more antibodies. Among the genotypes, genotype D (7.95±0.45) had significantly higher antibody titer compared to genotypes A (7.05±0.59), B (6.50±0.41) and C (6.78±0.48), which did not differ from each other.

Response to *Escherichia coli* (*E.coli*) inoculation

There was no significant genotype by dietary lysine

Table 2. Body weights (g) of commercial broilers at 14 d of age where genotype by dietary lysine interaction was significant

Genotype	Lysine, g/kg			
	9.61	10.51	11.41	12.31
A	260.65^{ay}	282.65^{axy}	295.60^{ax}	296.80^{ax}
B	235.95^{by}	218.40^{cy}	260.15^{bx}	269.85^{bx}
C	238.75^{bz}	275.80^{ay}	298.95^{ax}	312.75^{ax}
D	230.80^{by}	256.15^{bx}	249.85^{bxy}	248.15^{bxy}
SEM±	3.93	4.10	4.05	4.96

$^{a-c}$ Means within a column with no common superscript differ significantly ($p<0.05$).

$^{x-z}$ Means within a row with no common superscript differ significantly ($p<0.05$).

Table 3. Body weights (g) of commercial broilers at 28 d of age where genotype by dietary lysine interaction was significant

Genotype	Lysine, g/kg			
	9.61	10.51	11.41	12.31
A	847.90^{ax}	845.95^{ax}	892.75^{ax}	897.15^{ax}
B	794.75^{aby}	760.45^{by}	871.00^{ax}	883.55^{ax}
C	761.10^{bcz}	848.05^{ay}	879.35^{axy}	924.75^{ax}
D	718.05^{cx}	727.95^{bx}	730.10^{bx}	725.85^{bx}
SEM±	11.33	10.27	12.37	12.17

$^{a-c}$ Means within a column with no common super script differ significantly ($p<0.05$).
$^{x-z}$ Means within a row with no common super script differ significantly ($p<0.05$).

Table 4. Body weight at 42 d of age and feed efficiency of various genotype of commercial broilers at 0-14, 0-28 and 0-42 d of age fed diets containing different lysine levels

	Body weight, g	Feed efficiency		
	42-d	0-14 d	0-28 d	0-42 d
Genotype				
A	1,528^{a}	0.758^{a}	0.548^{a}	0.464^{b}
B	1,477^{a}	0.760^{a}	0.568^{a}	0.472^{ab}
C	1,499^{a}	0.771^{a}	0.563^{a}	0.479^{a}
D	1,212^{b}	0.745^{a}	0.541^{a}	0.438^{c}
Lysine, g/kg				
9.61	1,394^{a}	0.754^{a}	0.551^{ab}	0.465^{a}
10.51	1,427^{a}	0.744^{a}	0.548^{b}	0.463^{a}
11.41	1,444^{a}	0.768^{a}	0.558^{ab}	0.461^{a}
12.31	1,450^{a}	0.770^{a}	0.564^{a}	0.463^{a}
SEM±	11.9	0.004	0.002	0.002

$^{a-c}$ Means within a sub column, with no common superscript differ significantly ($p<0.05$).

Table 5. Antibody titers (log 2) to SRBC in commercial broilers where dietary lysine level and concentration of SRBC inoculation interaction was significant

SRBC dosage	Lysine, g/kg			
	9.61	10.51	11.41	12.31
0.5%	4.05^{bz}	4.95^{byz}	5.45^{bxy}	6.25^{bx}
2.5%	7.65^{ay}	7.50^{ay}	10.15^{ax}	11.45^{ax}
SEM±	0.42	0.34	0.52	0.53

$^{a-b}$ Means within a column with no common superscript differ significantly ($p<0.05$).
$^{x-z}$ Means within a row with no common superscript differ significantly ($p<0.05$).

level interaction for air sac lesion score, relative change in body weight during the course of infection, and relative weights of bursa and spleen. The lesion score was significantly influenced due to variation in genotype and dietary lysine content (table 6). Among the genotypes, the lesion score for genotype D was significantly lower than those of other genotypes. The lesion score was not influenced by the dietary lysine content. By inoculating *E. coli*, growth depression was observed in genotypes A. Birds fed lowest dietary lysine (9.61 g/kg) lost more weight compared to those of other lysine levels, which did not differ from each other. Neither genotypes nor the levels of lysine did affect the relative weight of lymphoid organs (spleen and bursa).

Heterophill to lymphocyte (H/L) ratio

There was no significant genotype by lysine interaction for H/L ratio. The level of lysine in the diet did not influence the H/L ratio. The H/L ratio was significantly low in genotype D compared to other genotypes.

Response to PHA-P inoculation

There was significant genotype by dietary lysine interactions for TI at 12 and 24 h PI in response to intra dermal injection of PHA-P in wattle. At both 12 and 24 h of PI there was no significant difference in TI among various levels of lysine in genotypes C and D except at 12.31 g lysine/kg, where the TI was significantly higher in genotype D compared to genotype C. In genotype B the TI was highest at 11.41 g lysine/kg diet (tables 7 and 8) and a further increase in lysine levels did not elicit any positive response in TI in these genotypes. Although similar finding was observed in genotype A, the TI at 11.41 g lysine/kg diet did not differ from those at lower levels of lysine (tables 7 and 8). In general, the TI was higher in genotypes C and D at all the levels of lysine tested except at 12.31 g lysine/kg diet.

Weight of lymphoid organs

Genotype by dietary lysine interaction was not significant for relative weight of bursa. Also, neither the genotype nor the level of dietary lysine influenced the relative weight of bursa. The bursa weight varied from 0.55 ±0.02 to 0.62±0.06 and 0.55±0.04 to 0.71±0.07 g/kg among the genotypes and among the levels of lysine, respectively. The spleen weight was significantly influenced by the interaction between genotype and dietary lysine content (table 9). At lower dietary lysine content (9.61 g/kg) the

Table 6. Air sac lesion score, relative body weight change, relative bursa and spleen weight of *E.coli* injected commercial broilers fed different dietary lysine contents

	Lesion score	Weight change	H/L ratio	Relative weight, g/kg live weight	
				Bursa	Spleen
Genotype					
A	3.900[a]	-0.22[b]	0.496 [a]	0.67[a]	2.80[a]
B	4.300[a]	-1.23[c]	0.515 [a]	0.53[a]	2.47[a]
C	3.800[a]	-1.08[bc]	0.513 [a]	0.65[a]	3.07[a]
D	2.850[b]	0.79[a]	0.456 [b]	0.74[a]	3.01[a]
Lysine, g/kg					
9.61	4.200[a]	-1.10[b]	0.502[a]	0.66[a]	3.07[a]
10.51	3.200[a]	-0.03[a]	0.505[a]	0.64[a]	2.70[a]
11.41	3.800[a]	-0.30[a]	0.493[a]	0.61[a]	2.69[a]
12.31	3.650[a]	-0.30[a]	0.480[a]	0.67[a]	2.89[a]
SEM±	0.156	0.18	0.007	0.02	0.13

[a,b] Means within a column, with no common superscript differ significantly ($p<0.05$).

Table 7. Wattle thickness index in commercial broilers at 12 h post inoculation where genotype by dietary lysine interaction was significant

Genotype	Lysine, g/kg			
	9.61	10.51	11.41	12.31
A	2.14[bxy]	2.28[bcxy]	2.59[bx]	1.75[cy]
B	1.29[cy]	1.79[cy]	2.41[bx]	2.48[bx]
C	2.60[abx]	3.12[ax]	2.98[abx]	2.48[bx]
D	3.32[ax]	2.79[abx]	3.80[ax]	3.12[ax]
SEM±	0.21	0.15	0.20	0.14

[a-c] Means within a column with no common superscript differ significant ($p<0.05$).

[x-y] Means within a row with no common superscript differ significantly ($p<0.05$).

Table 8. Wattle thickness index in commercial broilers at 24 h post inoculation where genotype by dietary lysine interaction was significant

Genotype	Lysine, g/kg			
	9.61	10.51	11.41	12.31
A	2.10[abxy]	2.01[bxy]	2.57[abx]	1.61[cy]
B	1.317[by]	1.64[by]	2.29[bx]	2.45[abx]
C	2.78[ax]	2.97[ax]	2.70[abx]	2.19[bx]
D	2.98[ax]	2.92[ax]	3.44[ax]	2.91[ax]
SEM±	0.23	0.16	0.17	0.13

[a-c] Means within a column with no common superscript differ significantly ($p<0.05$).

[x-y] Means within a row with no common superscript differ significantly ($p<0.05$).

relative spleen weights in genotypes A and D was significantly higher compared to other genotypes. In both genotypes the relative weight of spleen showed a decline trend with the level of lysine. While in genotype B the relative weight of spleen increased progressively with the levels of lysine in the diet. In genotype C, the levels of lysine did not influence the spleen weight.

Table 9. Relative weight (g/kg) of spleen in commercial broilers where genotype by dietary lysine interaction was significant

Genotype	Lysine, g/kg			
	9.61	10.51	11.41	12.31
A	2.6[abx]	1.7[aby]	1.8[bcy]	1.7[by]
B	1.1[cy]	1.5[bxy]	1.6[cxy]	1.9[abx]
C	2.0[bcx]	2.7[ax]	2.5[abx]	2.4[ax]
D	3.6[ax]	2.4[aby]	3.1[axy]	2.4[ay]
SEM±	0.3	0.2	0.2	0.1

[a-c] Means within a column with no common superscript differ significantly ($p<0.05$).

[x-y] Means within a row with no common superscript differ significantly ($p<0.05$).

DISCUSSION

Significant differences in d-old weight of the chicks from different genotypes might be due to variation in performance of respective parent stocks used in this experiment. This is in consistent with the earlier findings of Raju et al. (1997), in which they reported the differences in chick weight in both male and female lines at various ages of production with variation in size of the eggs. The weight of d-old chick is dependent on weight of egg which depends on several other factors such as variation in genetic architecture and stage of production of the parents, size of the eggs and the hatching management of the chicks etc. However, the influence of initial chick weight was not observed in subsequent body weights in the present study. Genotype by level of lysine interaction for body weight at 14 and 28 d of age observed in this experiment may be attributed to differential expression of the genes associated with physiological function such as growth and feed

utilization efficiency, which are influenced by various environmental factors and are also age dependent (Praharaj et al., 1996, 1998). The interaction at initial stage of growth, but not at later stages, may be due to significant changes during the first few weeks of post hatch in the development and function of various vital organs in a chick's life. These include development and function of demand and supply organs (Lilja, 1983; Katanbaf et al., 1988), the immune system (Heller et al., 1992; Nelson et al., 1995), and thermoregulatory system (Whittow, 1974).

Growth depression was observed in all genotypes by feeding low lysine diets (9.61 and 10.51 g/kg) at 14 d of age. At 28 d of age, birds in genotypes A and D were not influenced by dietary variation in the lysine content, while growth depression was observed in genotypes B and C at 9.61 and 10.51 g lysine/kg compared to higher levels of lysine. Although the birds fed lower levels of lysine gained significantly low weight at both 14 and 28 d of age in some genotypes, they could be able to compensate the weight loss by 42 d of age. Similarly, growth compensation was also observed by Noble et al. (1993) by feeding amino acid (lysine, methionine and tryptophan) deficient diet at early growth phase. Similarly, Rama Rao et al. (1990) reported growth depression in commercial broilers fed low density diets during the initial phase of growth and subsequent compensatory growth at 42 d of age when fed normal density diet during finisher phase. These observations indicate the existence of compensatory growth mechanism in certain genotypes (e.g. genotype A and D) when fed sub-optimal levels of nutrients during initial phase of growth. The body weight at 28 d of age indicated that the genotypes A and D require 9.61 g lysine/kg diet while, genotypes B and C require about 11.41 g lysine/kg diet. Again, at later age (42 d of age), there was no significant influence of dietary lysine content on body weight. From this it may be concluded that a large variation in lysine requirements existed among different commercial broiler genotypes. Therefore, it is essential to provide the accurate nutrient requirement considering the genetic make up of the stock in order to minimize the cost of feeding and to have optimum performance. In general, from this experiment, it may be concluded that the lysine requirement of commercial birds for optimum body weight may not be greater than 9.61 g/kg diet, which is lower than the recommendation of NRC (1994). However, it is essential to note that these broilers may need more than 9.61 g lysine/kg diet for optimum functionality of the immune system.

Among all, the genotype D had significantly higher antibody production to SRBC at 48 d of age. This was expected because this genotype gained less body weight and had poor nutrient utilization (feed efficiency) ability compared to other genotypes. The inverse relation between body weight at 42 d of age and antibody titers to SRBC inoculation indicates that the better growth in broilers is associated with reduced immunity (Siegel and Dunnington, 1987; Gross and Siegel, 1988; Boa-Amponsem et al., 1991b). The competition for allocation of resources may contribute to a negative relationship between rapid growth and immunity (Martin et al., 1990; Miller et al., 1992; Liu et al., 1995 and Parmentier et al., 1996). This may be due to poor nutrient utilization ability in the birds having high immune competence (Gross and Siegel, 1988). Also, this agrees with the findings that birds selected for high growth allocate majority of their resources towards muscle development and less for other physiological functions such as defense mechanism against pathogens and other stress factors (Cook, 1991; Boa-Amponsem et al., 1991a and Praharaj et al., 1995). Competition for finite resources results in a negative correlation between the body weight and general fitness in broilers (Boa-Amponsem et al., 1991b; Solkner and James, 1994).

The antibody titers were significantly higher at 2.5% SRBC inoculation compared to 0.5% inoculation at all dietary lysine levels. At lower level of SRBC inoculation the antibody titers were gradually increased with dietary level of lysine. At higher level of inoculation the increase in antibody production was not proportional to the level of lysine in the diet. The magnitude of antibody response to SRBC antigen is both dose and host dependent (Tsiagbe et al., 1987 and Leitner et al., 1992). The dose related antibody titers to SRBC inoculation in broilers fed different levels of lysine and methionine reported by Glich et al. (1981). Similarly, Dunnington et al. (1994) also observed significantly higher immune response at higher dose of SRBC inoculation (25%) than that of the low dose (0.25%). The low antibody titers observed at lower dietary levels of lysine could be due to impaired immune response because of less critical amino acid content in the diet (Klasing and Barnes, 1988).

In general *E.coli* challenge had negative effect on growth in broilers. This may be due to the diversion of biological resources towards growth rather than defensive mechanism against bacterial diseases during the process of selection for higher juvenile body weight (Boa-Amponsem et al., 1991b and Praharaj et al., 1997). In such case it has been observed that in certain stocks when the genetic make up is directed to the maximum limit for growth traits using various breeding methods, the inherent mechanism of re-appropriation of resources is lost (Gross, 1983; Gross and Siegel, 1988). However, the birds in genotype D did not loose weight even after injection of *E. coli*. This might be due to utilization of excess nutrients for maintenance of the optimum immunity that reduced the susceptibility to *E.coli* infection by a poor growth potential bird. Also, selection methods adopted to increase the juvenile body weight in this genotype (genotype D) may be a moderate one and might have changed the genetic make-up in such a manner

that it drains substantial amount of resources to satisfy the demand for growth even at the face of challenge. The lower susceptibility of genotype D for *E. coli* inoculation as evidenced by air sac lesion score indicated that this genotype is having higher general immune status compared to other genotypes. This is also evidenced by significantly higher antibody titers against SRBC in this genotype compared to other genotypes.

The level of lysine in the diet did not influence the air sac lesion score. Our earlier study (Rama Rao et al., 1999) has shown that the severity of *E. coli* infection had progressively decreased with increase in dietary protein. The lower susceptibility to *E. coli* at higher dietary protein but not at higher dietary lysine content implies that factors other than lysine in the total protein might be responsible for higher resistance to *E. coli* in broiler chicks.

Based on the 42 d body weight, lysine requirement for all genotypes may be about 9.61 g/kg diet, whereas, the requirement of lysine for optimum CBH response may differ from genotype. Genotype A (Anak 2000) and D (Hubbard) require more lysine (11.41 g/kg diet) compared to genotype C (Cobb) and D (synthetic broiler) (9.61 g/kg diet) for optimum CBH response. The higher CBH response to PHA-P and humoral antibody response to SRBC might be an indicator to have better resistance against *E.coli* infection in synthetic broiler compared to other fast growing genotypes. Concomitant to this result the chicks of this genotype had lower H/L ratio (an indicator of stress), which indicate that these chicks were less stressed compared to others. Based on the result it may be concluded that majority of the commercial broilers (0-42 d of age) do not require more than 9.61 g lysine/kg diet for optimum body weight. However, when both growth and immunity are considered the requirement need to be recommended specific to the genotype.

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