Global Science Research Journals

ISSN: 2229-3239 Vol. 2 (5), pp. 135-142, June, 2017

http://www.globalscienceresearchjournals.org/

Global Journal of Virology and Immunology

Full Length Research Paper

Effect of concentration levels of dietary lysine on growth performance and immune response to Newcastle disease (ND) vaccinations

Ayodedji B. Christian

Department of Animal Production and Health, Federal University of Technology, Akure, Ondo State, Nigeria.

Accepted 06 May 2017

The effect of supplementing varying concentration levels of dietary lysine on growth performance and immune response to Newcastle disease (ND) vaccinations of broiler chickens was assessed. A total number of 180 day-old broiler chicks of mixed sex were used for the experiment. The birds were divided into 4 treatments (Treatment A, B, C and D), in which each treatment was replicated 3 times with 15 birds per replicate. Diet A was the control diet that was not supplemented with dietary lysine and diet B contained the recommended level of lysine by National Research Council (NRC) standards of 1.12%, while diets C and D both contained 1.13 and 1.14% lysine which is 10 and 15% increment of the NRC requirements, respectively. The result of the statistical analysis showed that there was significant difference ($P \leq 0.05$) among the various dietary treatments for feed intake, weight gain, final body weight and feed conversion ratio. Birds fed diet C had the highest final body weight (2.16 kg), total weight gain (2.12 kg) and feed intake (3.11 kg) while birds fed diet A had the best feed conversion ratio (1.37). The immunological response to ND vaccinations showed that birds fed diet C had the highest mean antibody titre values while those fed diet A with no lysine supplemented in their diets had the lowest antibody titre values. The haematological parameter was only significantly ($P \leq 0.05$) different for erythrocyte sedimentation rate (ESR), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC). It was concluded that supplementing lysine above NRC requirement at the inclusion rates used in this study had significant effect on performance characteristics and immune response in broiler chickens.

Key words: Lysine, immune response, growth performance, antibody titre.

INTRODUCTION

Disease challenge is one of the many factors that will have an effect on the nutrient requirements of poultry. Insufficient nutrient consumption will reduce the effectiveness of the bird's defense mechanisms. Therefore, poultry

*Corresponding author: E-mail : chrisbee1018@yahoo.co.uk

must be supplied enough dietary nutrients and energy to allow the bird to express desired growth and feed efficiency. Poultry have been produced commercially since the early 1900's, and research has been conducted for years to improve production efficiency. Poultry nutrition experts employed by the industry have access to a great amount of information allowing them to optimize the particular production parameters most important to the producers (such as breast meat yield, feed conversion, weight gain). A good example of some of this information is that supplied by the National Research Council for Poultry (NRC).

The protein and essential amino acids (EAA) requirements for broilers proposed by NRC (1994) are unable to accommodate the terms of production for modern strains of birds. In order to catch up the additional growth, levels of commercially available amino acids are generally increased (Corzo et al., 2002). Most of these amino acids, particularly lysine, are now being supplemented in free form, enabling dietary crude protein to decrease below NRC (1994) levels (Corzo et al., 2002). The development of amino acid supplementation allows meeting the EAA needs at low protein levels (Dirain and Waldroup, 2002). The use of synthetic amino acids to meet the amino acid needs of broilers leads to production of cost effective diets. Lysine is one of the limiting amino acids common in broiler diets. Lysine requirements of broilers are higher in low protein diets for maximum weight gain and feed efficiency (Labadan et al., 2001). Even at normal crude protein (CP) level, high lysine content has been reported to increase the growth rate in broilers (Holsheimer and Veerkamp, 1992). Increasing lysine over and above NRC (1994) recommendations has been reported to improve weight gain, feed efficiency and breast meat yield (Si et al., 2004) and reduce the deposition of extra fat in the carcass (Moran and Bilgili, 1990). As widely described, increasing dietary lysine generally results in improved feed intake, feed conversion, and body weight gain (Holsheimer and Veerkamp, 1992; Sterling et al., 2008).

Kidd et al. (2001) reported that dietary amino acid lysine in poultry diet in concentration recommended by the NRC (1994) support proper immune system functions in healthy chicks. Improvements in immunity, as affected by dietary lysine in animals include improved thymic weight and function, enhanced lymphocyte mitogenesis, improved immunity against tumours and enhanced wound healing (Efron and Barbul, 1998; Evoy et al.,1998).

Newcastle disease is one of the most rampant viral diseases of poultry with a prevalence rate of 28.9% (Adene, 2004). The major tools that can be used to provoke immunity in birds for both the prevention and control of the spread of the disease are vaccination, good nutrition and immunomodulation (Pangasa and Singla, 2007; Pangasa et al., 2007). Information as to the effect of lysine supplementation on growth and immunological responses to Newcastle disease in broiler chickens are rare.

This study was therefore designed to determine the effect of supplementing lysine above the minimum NRC requirement in feed of broiler chickens on their growth performance and immunological response to Newcastle disease vaccinations with a view to improving animal welfare.

MATERIALS AND METHODS

Experimental chickens

A total of 180 day-old broiler chicks (Arbor acre) purchased from a commercial hatchery in Ibadan, Oyo State were used for this study. The approval to conduct this study was given by the Research Committee of the Department of Animal Production and Health, The Federal University of Technology, Akure (FUTA) Nigeria. Thus, the feeding trial was conducted at the Poultry unit of the Teaching and Research Farm of FUTA, Nigeria where adequate biosecurity measures were put in place. Brooding was done in a conventional manner with temperature ranging from 35°C at day old to 29°C at 3 weeks of age and then kept stable at approximately 25°C thereafter. Feed and water were provided *ad libitum*. They were vaccinated at stipulated times against Newcastle disease.

Experimental layout

The completely randomized design was used. The birds were divided into 4 treatment groups A, B, C, and D during the experimental period. There were 3 replicates per treatment with 15 birds per replicate. The chicks were fed *ad-libilium* on their respective experimental diets. The live weight, weight gain and feed intake of the birds were recorded weekly.

Experimental diets

The experimental birds were fed four different diets which were prepared at the feed mill of the teaching and research farm of the Federal University of Technology, Akure.

Diet A: was not supplemented with any dietary lysine (0%).
Diet B: contained 1.12% dietary lysine, which is the NRC requirement for lysine supplementation in broiler diets.
Diet C: contained 1.13% of lysine and this concentration was 10% increment over NRC requirement.
Diet D: contained 1.14% of lysine which was 15% increment of NRC requirement.

The gross composition of the diets is shown in Table 1.

Vaccination

The experimental chickens were vaccinated with Newcastle disease vaccines (NDV) - NDV intra-ocular (Hithner B1 strain), NDV LaSota and NDV Komarov using a stipulated vaccination regime as shown in Table 2. The Newcastle disease vaccines used were produced by the National Veterinary Research Institute (NVRI), Vom, Jos,

Table 1. Gross composition of experimental diets (g/kg).

Ingredient	Treatment			
	A	B	C	D
Maize	58.63	58.50	58.49	58.48
Soya bean meal	18.50	18.50	18.50	18.50
Fishmeal	4.00	4.00	4.00	4.00
Groundnut cake	12.50	12.50	12.50	12.50
Vegetable oil	2.00	2.00	2.00	2.00
Bone meal	2.75	2.75	2.75	2.75
Oyster shell	1.00	1.00	1.00	1.00
Salt	0.30	0.30	0.30	0.30
Premix	0.20	0.20	0.20	0.20
Lysine	0.02	0.10	0.11	0.12
Methionine	0.15	0.15	0.15	0.15
Total	100	100	100	100
Calculated composition				
Crude protein (%)	23.09	23.08	23.07	23.07
ME (MJ/Kg)	13.15	13.13	13.13	13.12
Calcium (%)	1.73	1.73	1.73	1.73
Average Phosphorus (%)	0.77	0.77	0.77	0.77
Lysine (%)	1.06	1.12	1.13	1.14
Methionine (%)	0.49	0.49	0.49	0.49

ME= metabolizable energy.

Table 2. Vaccination schedule for broiler chickens fed diets supplemented with varying levels of lysine.

Age of birds	Vaccination	Route of administration
3 days	NDV Hitchner B1 strain	Intra-occular
21 days	NDV LaSota	Oral
42 days	NDV Komarov	Intra-muscular

NDV- Newcastle disease vaccine.

Plateau state, Nigeria. The vaccines were reconstituted and administered according to the manufacturer's recommendation.

Data and sample collection

Performance characteristics

Feed intake: This was taken by measuring daily feed consumption of birds in each replicate for the different treatment groups on a 24 h interval base.

Weight taking: The weight of experimental birds was taken before they were fed in the morning. The initial weight of birds was measured at day old and thereafter weight changes on a weekly basis over the trial period were measured as the difference between the initial weight and the final weight.

Feed conversion ratio: Feed conversion ratio (FCR) was calculated as feed intake per unit of weight gain per replicate. All these parameters were used to evaluate the performance characteristics of experimental chickens.

Blood and sera collection

Samples of blood for the purpose of serum analysis were collected from 3 birds per replicate in each treatment group before the trial commenced via the heart to determine baseline maternal antibody titre levels against Newcastle disease. The birds were sedated using chloroform before the bleeding exercise. Thereafter, in each treatment 9 birds (3 per replicate) were randomly selected and blood was collected 10 days after administering each of the ND vaccines through the jugular vein for serological analysis to determine the antibody titre values. At the end of the 8 weeks experimental period blood was also collected for haematological and serum protein biochemistry analysis from 9 birds in each treatment.

Table 3. Performance characteristics of broiler chickens fed Lysine supplemented diets.

Parameter	Diet A	Diet B	Diet C	Diet D	±SEM
Initial weight (g/bird)	41.19	41.21	41.26	41.20	0.22
Final weight (kg/bird)	1.90[b]	2.02[a]	2.16[a]	1.88[b]	0.50
Total weight gain (kg/bird)	1.87[b]	1.98[a]	2.12[a]	1.84[b]	0.54
Weight gain(g/bird/day)	33.24[b]	35.33[a]	37.81[a]	32.89[b]	0.57
Total feed intake (kg/bird)	2.56[b]	2.94[a]	3.11[a]	2.58[b]	0.30
Feed intake (g/bird/day)	45.76[b]	52.5[a]	55.47[a]	46.09[b]	0.46
Feed conversion ratio	1.37[a]	1.48[b]	1.46[b]	1.40[b]	0.43

Means on the same column with different superscripts are statistically significant (p≤0.05). Diet A- 1.06% lysine. Diet B- 1.12% lysine. Diet C- 1.13% lysine. Diet D- 1.14% lysine.

Laboratory analysis

Haemagglutination and haemagglutination inhibition test (HA/HI test)

Serum samples were analysed using beta (β) micro haemagglutination inhibition technique (Thayer and Beard, 1998) to determine the antibody titre levels as a measure of the immuno-logical response elicited in the vaccinated experimental birds.

Haemagglutination (HA) titration

The aim of the HA titration was to determine the viability or the potency of the vaccine used. The Newcastle disease vaccine (LaSota strain) used as the antigen for the HA titration was locally produced by the National Veterinary Research Institute (NVRI), Vom, Jos, Plateau State. Clean, dry, micro-titre plates used were labelled as required, and 0.2 ml of normal saline was dispensed into each of a pair of wells using a micro-pipette. A drop of the antigen was added into the first pair of wells and mixed thoroughly using a pair of inoculating loops and serial dilution was carried out. Finally, 0.02 ml of the guinea pig red blood cell (RBC) indicator previously diluted with normal saline was added to each well. The plates were then incubated on the laboratory bench for about 30 min at room temperature. After precisely 30 min, the end point of the titre was determined as the pair of wells where haemagglutination was clearly observed.

Haemagglutination inhibition (HI) titration

The beta haemagglutination inhibition technique was used and the stock antigen was diluted according to the HA titre obtained; thus for an antigen with a titre 1:256, the 4HAµ will be equal to 1:64 dilution of test stock. The micro-titre plates were labelled as required and 0.2 ml of the test stock antigen was then dropped into each pair of the wells on a row of the micro-titre plates. After this, a drop of the serum sample was added into the first pair of wells, thoroughly mixed and serially diluted. Lastly, a drop of the prepared guinea pig RBC indicator was added to each well. The micro-titre plates were incubated at room temperature on the bench for 30 min. The end point of the titre was determined as the pair of wells where haemagglutination inhibition is clearly observed.

Haematological parameters

The erythrocyte sedimentation rate (ESR), packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (HB) and white blood cell differentials were analysed. The mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and the mean corpuscular volume (MCV) were calculated as described by Lamb (1981).

Serum protein biochemical analysis

The protein content- albumin, globulin and the total protein of serum samples were estimated using diagnostic kits (Randox Laboratories Limited, UK test kits).

Statistical analysis

Data on immunological responses, haematological variables, serum protein biochemistry and performance characteristics were subjected to one-way analysis of variance (ANOVA). Where significant differences were found, the mean were separated using the statistical analysis system (SAS).

RESULTS

Performance characteristics

In Table 3, final body weight (FBW), total weight gain (TWG), total feed intake (TFI) and feed conversion ratio (FCR) of experimental birds was seen to be significantly influenced by the different diets. The FBW of birds fed diet C (2.16 kg) was significantly ($p < 0.05$) higher than birds on diet D (1.88 kg), although it was not significantly ($p > 0.05$) different from birds on diet B (2.02 kg). The total weight gain (TWG) also followed the same trend as the FBW. The TFI was lowest for birds fed diet A (2.56 kg) which was significantly ($p < 0.05$) different from that of birds on diet C (3.11 kg) that consumed the highest amount of feed. Although birds fed diet A consumed the least amount of feed they had the best FCR (1.37) which was significantly ($p < 0.05$) different from that of birds on the rest test diets.

Immunological response to ND vaccinations

The results of the HA/HI tests in Table 4 shows the mean

Table 4. Average antibody titre values of chickens fed lysine supplemented diets after Newcastle disease vaccinations

Parameter	Diet A	Diet B	Diet C	Diet D
Baseline titres	$Log_2 5$	$Log_2 5$	$Log_2 5$	$Log_2 5$
Titre After NDV Hitchner B1strain	$Log_2 6$	$Log_2 7$	$Log_2 8$	$Log_2 6$
Titre After NDV Lasota	$Log_2 7$	$Log_2 8$	$Log_2 9$	$Log_2 7$
Titre After NDV Komarov	$Log_2 8$	$Log_2 9$	$Log_2 11$	$Log_2 9$

NDV-Newcastle disease vaccine. Diet A- 1.06% lysine. Diet B- 1.12% lysine. Diet C- 1.13% lysine. Diet D- 1.14% lysine.

Table 5. Haematological parameters of experimental birds fed lysine supplemented diets.

Parameter	A	B	C	D ±SEM
ESR (mm/h)	3.33^c	4.67^b	2.67^d	5.67^a 0.14
PCV (%)	26.00	25.00	26.67	27.00 0.53
RBC ($\times 10^6/mm^3$)	2.18	2.12	2.08	2.04 0.08
Hb (g/100ml)	8.57	8.24	8.77	8.67 0.11
MCHC (%)	32.96	32.96	32.88	32.11 0.29
MCH (pg)	39.35^b	38.82^b	42.13^a	42.51^a 2.15
MCV (μ^3)	119.13^b	117.26^b	128.02^a	132.14^a 1.17
Lymphocyte (%)	59.00	60.00	60.42	59.20 0.86
Heterophils (%)	23.00	23.33	24.35	24.00 0.43
Monocytes (%)	14.00	13.95	13.67	14.00 0.64
Basophils (%)	2.67	2.33	2.67	2.10 0.05
Eosinophils (%)	0.93	1.00	0.97	0.90 0.03

ESR = Erythrocyte sedimentation rate, PCV = Packed cell volume, RBC= Red Blood cell. Hb = Haemoglobin, MCHC = Mean cell haemoglobin concentration, MCH = Mean cell Haemoglobin, MCV = Mean cell volume. Diet A- 1.06% lysine. Diet B- 1.12% lysine. Diet C- 1.13% lysine. Diet D- 1.14% lysine.

antibody titre values of experimental birds after the ND vaccinations. The table reveals that antibody titre values of experimental birds were influenced by the dietary treatments. Birds on diet C supplemented with 10% lysine above the NRC requirement had the highest mean titre values of $\log_2 8$ after NDV intraocular, $\log_2 9$ after NDV LaSota and $\log_2 11$ after NDV komarov during the study. The lowest antibody production was seen in birds fed diet A which had no supplementation with dietary lysine having antibody titre values of $\log_2 6$, $\log_2 7$ and $\log_2 8$ after each ND vaccination.

Haematological parameters

Table 5 shows that only the ESR, MCH and MCV values were significantly ($p < 0.05$) different among treatments. Birds on diet D had significantly ($p < 0.05$) higher ESR (5.67 mm/h) than those fed on the other test diets, followed by those on diets B, A and C. The MCH of birds on diet D (42.51 pg) was significantly ($p < 0.05$) different from those on diet B (38.82 pg) but not significantly different ($p > 0.05$) from those on diet C (42.13 pg). The MCV of birds on diet D (132.14 μ^3) was not significantly different from that of birds fed diet C (128.02 μ^3) but was significantly different from that of birds on diet A (119.13 μ^3) and diet B (117.26 μ^3).

Serum protein biochemical analysis

Table 6 shows that the albumin and total protein content of serum of experimental birds were significantly influenced ($p < 0.05$) by the dietary treatments. The albumin value of birds on diet C (1.69 g/dl) was significantly ($p < 0.05$) lower than those on other test diets. The total protein value of birds on diets B (3.96 g/dl) and D (3.78 g/dl) were not significantly ($p > 0.05$) different from one another but significantly ($p < 0.05$) different from those fed diets A (3.12 g/dl) and C (3.14 g/dl).

DISCUSSION

Since the possibility of disease challenge is always

Table 6. Serum biochemical protein values of broilers fed lysine supplemented diets (g/dl)**.**

Treatment	Total protein	Albumin	Globulin
Diet A	3.12[b]	2.08[a]	1.04
Diet B	3.96[a]	2.36[a]	1.33
Diet C	3.14[b]	1.69[b]	1.45
Diet D	3.78[a]	2.60[a]	1.18
±SEM	0.17	0.08	0.04

Means on the same column with different superscripts are significantly different ($p \leq 0.05$).

present in today's poultry operations, the bird's metabolism and immune system are constantly adjusting to the disease condition or stress of the environment and thus nutrient requirements may need to be increased at certain times. In recent times, this has lead to much research attention in the area of improving the nutritional quality of poultry feed by supplementing various micro-nutrients in a bid to improve immunity against diseases. In this present study, the results is similar to findings of several researchers which stipulated that increasing dietary lysine at low levels above NRC requirement in the feed of broilers can produce highly productive and healthy poultry birds. The results showed that birds fed 1.13% of lysine (10% above NRC requirement) had the best performance characteristics as seen in their final body weight, weight gain and feed intake which is in line with Azman and Yilmaz (2005) that recorded satisfactory body weight and feed conversion ratio in chickens fed 1.5% lysine supplemented diets. Morris et al. (1987), Morris and Abebe (1990) and Surisdiarto and Farrell (1991) confirmed the positive linearity of the relationship between lysine requirement and dietary protein level which support the view that lysine requirement for maximum productivity (maximum weight gain and feed efficiency) is in the range of 1.26 to 1.33% and this is above the NRC (1994) requirement of 1.00%. According to Han and Baker (1994), the lysine requirement for broilers at 2 to 4 weeks of age was also slightly above the NRC (1994) recommendation of 1.10% for 0 to 3 week old broilers. Since the final body weight and average weight gain of birds fed 1.13% of lysine appeared to be highest in this present study, the result can therefore be said to be in line with the recommendation that lysine requirement for maximum growth is slightly above that of NRC (1994). Corzo et al. (2005) also found that lysine supplementation significantly improved the live body weight and feed conversion efficiency.

The trend of the results showed that final body weight, weight gain and feed intake was lowest in birds fed diets deficient in lysine which may be attributed to limiting supplies of the essential amino acids. This result support the general principle by Sklan and Plavnik (2002) that chick diets should be formulated to provide sufficient amounts of all amino acids corresponding to requirement for protein synthesis. Evidence has been provided by Sakomura and Coon (2003) and by Nonis and Gous (2008) that lysine is involved in the release of the growth hormone, insulin like growth factor I (IGF-I) and modulation of bone growth by differentiation of osteoblasts and collagen synthesis and this explains why birds fed lysine supplemented diets had better growth performance. This study showed a decrease in performance and efficiency of birds at the highest lysine supplemental level of 1.14% as compared to the other test diets with lysine supplementation. This may be due to less efficient use of amino acids above the requirements for protein synthesis. In contrast, the lower growth in birds fed lysine deficient diets may be attributed to limiting supplies of the essential amino acids. Recently, the results of Nasr and Kheiri (2011) suggested that additional lysine at a level of 12% of NRC in starter and grower diets optimized body weight gain, carcass and breast percentage in Arian broiler chickens, whereas reductions in lysine level reduced growth and live weight (Kerd et al., 1998).

The birds fed diets supplemented with 1.13% of lysine which is 10% above NRC requirement elicited the highest immune response to ND vaccinations as shown in their mean HI antibody titre values. This is similar to report by Mehrdad (2012) that reported increasing lysine in diets of today's broiler in excess of NRC recommendations can improve immune system functions, FCR, abdominal fat deposition, breast meat yield and carcass efficiency. Eduardo et al. (2009) also suggested that addition of lysine to poultry diets improve immunity of the birds against different diseases. The least immune response to ND vaccinations was recorded in birds fed diets not supplemented with any dietary lysine. Similarly, Chen et al. (2003) reported that humoral immune response evaluated from antibody response to NDV vaccination was reduced in broiler chickens fed a lysine-deficient diet and the cell-mediated immune response was also reduced due to lysine deficiency.

Also judging from this study, the lysine content of the control diet is lower than the ideal requirement while the concentration in diets containing 1.14% lysine is higher than the ideal requirement of broiler chickens currently under study. Therefore, by implication the amino acid fed to birds in the control group and those fed 1.14% lysine supplemented diets could lead to imbalance in amino acids metabolism which consequently lead to negative effects on both the weight gain and immune response as evident in this study.

In general, the requirement of lysine is higher for increased immunity than for growth. The competition for limited resources may contribute to a negative relationship between growth and immunity. However, the present study has negated this hypothesis, because the highest immune response to ND vaccinations was recorded in birds that had the best growth performance which were birds fed 1.13% of lysine. In contrast to this result, some authors (Liu et al., 1995; Parmentier et al., 1996) found that body weight and antibody titers are negatively correlated. Also, more immune competent birds have poor nutrient utilization ability. In another study, results indicated that the lysine requirement for maximum antibody response was greater than for maximum growth for broilers (Klasing, 2007).

The haematological indices aside from the ESR, MCH and MCV values recorded in the present study were not significantly affected by the different dietary treatments and this is in line with the reports of Sahir et al. (2006) and Corzo et al. (2005) which stated that lysine supplementation in feed of poultry had no significant effect on haematological parameters. Though the birds fed 1.13% lysine supplemented diets recorded the highest antibody titre levels, the percentage of lymphocytes of experimental birds in the different treatment groups was similar. This may be explained by the fact that there are many types of lymphocytes and only lymphocytes specific to ND produced antibodies and that percentage of specific lymphocytes is very small in relation to the total number of lymphocytes.

The results of serum biochemical study revealed that the varying supplementation rates of lysine had significant effect on total protein and albumin values though the birds fed the NRC requirement diet had the highest values. This can be said to be similar to the work of Sahir et al. (2006) which reported that serum total protein concentration increased with increasing dietary lysine content. The haematological and serum biochemical parameters obtained from this study suggest that dietary lysine supplementation has no deleterious effects on some physiological indices of broilers chickens since the haematological values and serum protein values fell within the normal range.

Conclusion

The result obtained in this study shows that lysine supplementation above NRC requirement in broiler diets improved final body weight, total weight gain and feed intake and that 1.13% supplementary rate could be the most suitable inclusion level for enhanced productivity. Also, lysine supplementation at this inclusion level elicited the best immunological response to Newcastle disease vaccinations in broiler chickens. It can therefore be concluded that lysine supplementation is of benefit to high productive performance and boosting of the immune system in broiler chickens.

Conflict of interests

The authors declare that they have no competing interests

REFERENCES

Adene DF (2004). Poultry Health and Production: Principles and Practices. 1st Edition, Stirring Holden Publishers, Nigeria. 296p.

Azman MA, Yilmaz Y (2005). The growth performance of broiler chicks fed with diets containing cottonseed meal supplemented with lysine. Rev. Med. Vet. 156(2):104-106.

Chen C, Sander JE, Dale NM (2003). The effect of dietary lysine deficiency on the immune response to Newcastle disease vaccination in chickens. Avian Dis. 47 (4):1346-1351.

Corzo A, Moran ET Jr., Hoohler D (2002). Lysine need of heavy broiler males applying the ideal protein concept. Poult. Sci. 81:1863.

Corzo A, William D, Kidd D (2005). Dietary lysine needs of late developing heavy broilers. Arch. Anim. Nutr. Poult. Sci. 85:457-461.

Dirain CP, Waldroup PW (2002). Protein and amino acid needs of broilers in warm weather: A Review. Int. J. Poult. Sci. 1:40-46.

Eduardo GA, Gisele CF, Daniela C, Fabiana G, Jose CD (2009). The effect of lysine and methionine in poultry and fish diets. J. Arabian Aquacult. Soc. 295:266-270.

Efron DT, Barbul A (1998). Modulation of inflammation and immunity by arginine supplements. Curr. Opin. Nutr. Metab. Care 1:531-538.

Evoy D, Liberman MD, Fashey III TJ, Delay JM (1998). Immunonutrition: The role of arginine. Nutrition. 14:611-617.

Han V, Baker DH (1994). Digestible lysine requirement of male and female broilers chicks during the period three to six weeks post hatching. Poult. Sci. 73:1739-1745.

Holsheimer JP, Veerkamp CH (1992). Effect of dietary energy, protein and lysine contents on performance and yields of two strains of male broiler chicks. Poult. Sci. 71:872-879.

Kerd BJ, Kidd MT, Halpin KM, McWard GW, Quarles CL (1998). Lysine level increases live performance and breast meat yield in male broilers. J. Appl. Poult. Res. 8:381-390.

Kidd MT, Peebles ED, Whitmarsh SK, Yeatman JB, Wideman Jr. RF (2001). Growth and immunity of broiler chicks as affected by dietary arginine. Poult. Sci. 80:1535-1542.

Klasing KC (1998) . Avian macrophages: Regulators of local and systemic immune responses. Poult. Sci. 77:983-989.

Labadan MC Jr., Hsu KN, Austic RE (2001). Lysine and arginine requirements of broiler chickens at two to three week intervals to eight weeks of age. Poult. Sci. 80:599-606.

Lamb GN (1981). Manual of veterinary laboratory techniques. CIBA-GEIGY, Kenya. pp 70-110.

Liu G, Dunnington EA, Siegel PB (1995). Correlated responses to long-term divergent selection for eight -week body weight in chickens: Growth, sexual maturity, and egg production. Poult. Sci. 74:1259-1268.

Mehrdad B (2012). Effect of Excess Lysine and Methionine on Immune system and Performance of Broilers. Ann. Biol. Res. 3(7):3218-3224.

Moran ET Jr., Bilgili SF (1990). Processing losses, carcass quality and meat yield of broiler chickens receiving diets marginally deficient to adequate in lysine prior to marketing. Poult. Sci. 69:702-710.

Morris TR, Abebe S (1990). A note on the effect of protein concentration on response to dietary lysine by chicks. Br. Poult. Sci. 31:267-272.

Morris LC (1987) . The significant advance of the past fifty years in nutrition. Poult. Sci. J. 37:265-270.

Nasr J, Kheiri F (2011). Effect of different lysine levels on Arian broiler performances. Italian J. Ani. Sci. 10:32.

National Research Council (NRC) (1994). Nutrient requirement of Poultry. 9th Rev. Ed. National Academy Press, Washington, DC.

Nonis MK, Gous RM (2008). Threonine and lysine requirements for maintenance in chickens. South Afr. J. Ani. Sci. 38:75-82.

Pangasa A, Singla LD (2007). Effect of coccidiostats and immunomodulators on haematology of *Eimeria tenella* infected brolers. Indian Vet. J. 84:1131-1134.

Pangasa A, Singla LD, Ashuma (2007). Biochemical alterations in chicken during *Eimeria tenella* infection medicated with coccidiostats and immunomodulator. Indian J. Field Vet. 3(2): 06-10.

Parmentier HK, Nieuwland MGB, Rijke E, de Vries Reilingh G, Schrama JW (1996). Divergent antibody responses to vaccines and divergent body weights of chicken lines selected for high and low humoral responsiveness. Avian Dis. 40:634-644.

Sahir MH, Shariatmadari F, Mirhadi SA, Chwalibog A (2006).The effect of lysine supplements on haematology and serum biochemical indices of broiler breeders. Arch. Geflugelk. 70(2):S74-79.

Sakomura NK, Coon NC (2003). Amino acid requirements for maintenance of broiler breeder pullets. Proceedings of 14th European Symposium on Poultry Nutrition, Lillehammer, Norway. pp. 280-281

Si J, Fritts CA, Burnham DJ, Waldroup PW (2004). Extent to which crude proteins may be reduced in corn-soybean meal broiler diets through amino acid supplementation. Int. J. Poult. Sci. 3:46-50.

Sklan D, Plavnik I (2002). Interactions between dietary crude protein and essential amino acid intake on performance in broilers. Br. Poult. Sci. 43:442-449.

Sterlıng KG, Pest G, Bakall MRI (2008). Performance of different broiler genotypes fed diets with varying levels of dietary crude protein and lysine. Poult. Sci. 85(6):1045-1054.

Thayer SG, Beard CW (1998). Serological Procedure In: A Laboratory Manual for the Isolation and Identification of Avian Pathogens, 4th Ed. Ameican Association of Avian Pathologist, Pennsylvania, USA. pp. 255-258.