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Improved Immune Responses of Broiler Chicken (Hubbard JV Breed) Supplemented with L-Lysine and DL-Methionine to Infectious Bursal Disease Vaccination at Debre- Zeit Agricultural Research Center, Ethiopia

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Abstract: Effective prevention and control of diseases such as Newcastle and infectious bursal diseases require multiple vaccinations in which immuno supplements are needed. This study was aimed at evaluating the effect of L-lysine and DL-methionine supplementation on immune response of broiler chicken against infectious bursal disease vaccine at Debre Zeit Agricultural Research Centre from January to April, 2013. Randomized controlled design was used to allocate the experimental chicken into different treatment groups. Two hundred and twenty five chickens were randomized into 5 treatment groups of chicken comprising 45 birds per group. One of the groups served as negative control and did not receive any amino acid supplementation and one group was provided with the recommended dose of the amino acids (100%) and served as positive control. The remaining three groups were provided with 80%, 120% and 140% of the recommended doses by Hubbard JV broilers management guide of L-lysine and DL-methionine. The chickens were vaccinated against infectious bursal disease at day 7 and day 28. The anti-body titer of the chickens in each treatment group was assayed using IBD anti-body ELISA. Besides, the live body weight, weight of lymphoid organs and liver and differential leukocyte count were carried out. All the chickens were followed for 47 days. The results showed that the geometric mean anti-body titre was statistically significantly different among treatment groups after booster vaccination was given ($F = 23.83$, $P = 0.000$). The highest mean titer was observed in chicken provided with highest dose of L-lysine and DL-methionine. No statistically significant difference was observed among treatment groups in live body weight but statistically significant ($P < 0.05$) difference was observed in the weight of lymphoid organs and liver except that of thymus among the different treatment groups. Higher weight was observed in chicken provided with higher doses of L-lysine and DL-methionine. Statistically significant difference ($P = 0.000$) was observed in differential leukocyte counts among the treatment groups except for basophils. In general higher counts were observed in chicken provided with higher doses of L-lysine and DL-methionine. There was no statistically significant difference in median survial time and the results of linear regression of the mean anti-body titer aganist various predictors included but the weight of spleen was shown to cause significant mean rise in anti-body titre after booster vaccination was given. The present work showed higher dose of L-lysine and DL-methionine improved immune response of chicken against infectious bursal disease vaccination. Further study under different management systems, using different breeds and challenge with wild virus is recommended.

Key words: DL-Methionine · L-Lysine · Infectious Bursal Disease · Immune Response · Broiler Chicken · Debre Zeit · Ethiopia

INTRODUCTION

As agricultural sector remains the main stay of Ethiopia's economy [1] poultry production has a peculiar advantage to contribute to the family livelihood and food security due to their small size and fast reproduction rate compared to other livestock species [2, 3]. Almost every family in Ethiopia has poultry with dominantly indigenous

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chicken of various ecotypes raised under free range system. Such management exposes the poultry to malnutrition and various infectious agents [4,5]. The wide spread occurrence of infectious agents under such conditions has been the main obstacle to the attempts to improve the productivity of small scale poultry farms through introduction of high yielding exotic breeds which are at higher risk of acquiring diseases than the indigenous ones [6]. Infectious bursal disease (IBD) or commonly called Gumboro's disease, is one the infectious diseases of poultry known to hinder poultry industries in the country [7-10].

Infectious bursal disease (IBD) is an acute and highly contagious viral infection of immature chickens caused by a *Birnavirus* belonging to the family *Birnaviridae* with tropism to the bursa of Fabricius and to a lesser extent other lymphoid organs [11]. The disease has been known to negatively affect the antibody response of chicken to vaccinations, cause strong post vaccinal reactions and increased susceptibility of chicken to concurrent or secondary infections [12- 14]. So far two serotypes (serotype 1 and 2) of IBD virus have been recognized with wide spread clinical disease associated with serotype 1 [12]. Epidemiological investigations carried out revealed that it is distributed worldwide [13]. Few studies carried out in Ethiopia showed that IBD is prevalent in poultry farms in several regions [10, 15, 16]. Vaccination has been a common place against IBD and other infectious diseases throughout the country but the level of protection achieved is lower due to various reasons including lack of nutrient supplementation. For instance a deficiency of amino acids has long been demonstrated to impair immune function and increase the sensitivity of chicken to infectious challenges [17].

Previously attaining crude protein requirements of chicken have been considered important activity to maximize their immune response following vaccination and natural infection. The growth of the synthetic amino acids and their inclusion in the poultry feed, however, is thought to reduce the dependence of the poultry industry on the crude protein level. This has the advantage of attaining specific requirements of essential amino acids [18]. In this regard, mass production of two most limiting amino acids (L-lysine and DL-methionine) in crystalline form have been achieved and used to replace natural protein sources [19]. These two amino acids are thought to be required for protein synthesis by simple-stomached mammals and avian species for optimal growth and immune response [20]. The effects of dietary L-lysine and DL-methionine supplementation on the growth performance and carcass characteristics of broiler chicken were well documented [21, 22]. They have also been shown to improve the immune response of broilers through T-cell proliferation, IgG secretion, leucocyte migration [23, 24] and by raising anti-body titre [25- 27]. L-Lysine has also been demonstrated to be involved in the synthesis of cytokines, proliferation of lymphocytes and thus in the optimum functioning of immune system in response to infection [28].

The beneficial role of synthetic amino acids in the poultry industry has been proven. However, most of the previous investigations have focused on the role of amino acids in improving feed conversion efficiency and the immune response in general. They did not study the impact of these amino acids on the immune response of poultry when vaccinated with infectious diseases such as IBD which is known for its immunosuppression. We thought that supplementation with L-lysine and DL-methionine could be a way for overcoming immunosupression and enhancing the immune response of chicken through nutritional manipulation. This may have crucial economic advantage as the supplementation can reduce mortality and morbidity and enhance weight gain. Therefore, the objective of this study was to evaluate the effect of different doses of synthetic L-lysine and DL-methionine supplementation on the immune response of broiler chicken against IBD vaccine.

MATERIALS AND METHODS

Study Area: The study was carried out from January to April, 2013 at Debre Zeit Agricultural Research Centre situated in Bishoftu town under the aupsis of the Ethiopian Institute of Agricultural Research. The research centre is located 47 km south east of the capital, Addis Ababa. Bishoftu is geographically situated at a latitude of 8°44' N and longitude of 38° 38' E with average altitude of about 1900 meters above sea level. The area is featured by its unimodal rainfall of about 1100mm per annum. The temperature of the area ranges from a minimum of 8.9°C to a maximum of 28.3°C. Mixed crop-livestock production is the main economic activity of Bishoftu area. Bishoftu area is known for its dairy and poultry production [29].

Experimental Animals and Their Management: The study animals were homogenous day old broiler chicken of Hubbard JV breed that were hatched at Debre Zeit Agricultural Research Centre. Fifteen chickens were bled at day old to estimate the baseline anti-body titer while 225 chickens were used for the experiment. The chickens were kept in clean disinfected brooder house equipped with infrared bulbs. The house was made of a concrete

floor with open sides and divided into 15 pens of 2 m^2. The floor was bedded with straw that was disinfected using recommended disinfectants. The temperature of the experimental house was maintained between 24°C and 34°C with relative humidity ranging from 80 to 90%. The ration provided to the experimental chicken was formulated using feed formulation computer program (Feed-win) according to the Hubbard broiler management guideline [30]. The chickens were initially provided with a starter ration that was followed by finisher1 and finisher 2 rations while water was provided *ad libitum.* The experiment was completed in 47 days. The chickens were vaccinated against diseases such as Newcastle disease and standard bio-security measures were employed throughout the experimental period. Live weight of the experimental chicken was recorded weekly. All chicken that died during the experiment were recorded and subjected to post-mortem and laboratory examination.

Experimental Design: The experimental design employed was randomized controlled trial. Two hundred and twenty five unsexed chickens were randomly assigned into 5 treatment groups and each treatment group has 15 chickens with 3 replicates which were kept in separate pens. The treatment groups were as follows:

- Treatment1: 0% L- Lysine and DL- Methionine (negative control)
- Treatment2: 100% of the recommended dose of L-Lysine and DL- Methionine of the Hubbard JV breeders manual (positive control)
- Treatment3: 80% of the recommended dose of L-Lysine and DL- Methionine of the Hubbard JV breeders manual
- Treatment4: 120% of the recommended dose of L-Lysine and DL- Methionine of the Hubbard JV breeders manual
- Treatment5: 140% of the recommended dose of L-Lysine and DL- Methionine of the Hubbard JV breeders manual

The L- Lysine (Lysine 99%, Lot number 110312, Ailnomonto Do, Brasil Industria E. Commercio De Alimentos LTDA, Brasil) and DL- Methionine (Metamino® feed grade 99%, Evonik Degussa Antwerpen N.V, α D E H 1000004, Beligium) were used in this experiment. All chicken in all treatment groups were vaccinated against IBD at day 7 and booster dose was given at day 28.

Evaluation of the Effect of Different Doses of L-Lysine and DL- Methionine: The effects of the supplementation of the chicken feed with L- Lysine and DL- Methionine was carried out by monitoring their antibody titer, comparison of live body weight, the weights of lymphoid organs including liver, differential leukocyte counts and median survival time among different groups.

Serological Monitoring

Blood Collection: Blood samples (1 ml per chick) were collected using sterile syringe without any anticoagulant from wing vein according to the method described by Alders and Spradbrow [31]. The pre vaccination blood samples were collected at day old and the post vaccination blood samples were collected at day 20 and day 35. The blood samples were allowed to clot overnight at room temperature and serum was separated. The sera collected were then stored at -20°C until analyzed. The mean anti-body titer in the sera samples were analyzed to compare the mean titer after IBD vaccinations with the baseline titer and to compare the mean titer among the experimental groups.

ELISA to Determine Antibody Titre against IBD Vaccine: The antibody response of chicken against IBD vaccines was carried out using by ELISA described by Howie and Thorson [32] and the kit was used X-OVO FLOCK SCREEN™ IBD Anti-body ELISA Kit (X-OvO limited, Burnside Business Court, Inverkeithing KY11 1NZ, UK, Cat. No. V090) following the manufacturer's instructions. The procedure is briefly described below.

The pre-coated plates were used to carry out the test. The positive and negative controls were run in duplicate. Fifty µL of the undiluted controls were dispended into their respective wells. Similarly 50 µL of diluted samples were added to sample wells. The plates were covered with cover plate provided with the kit and incubated at +37°C for 30 minutes and the plates were then gently taped on the side to mix the contents. The plates were washed 4 times with wash buffer (phosphate buffer with Pro Clin 0.63% V/V; 300 µL per well), inverted and taped firmly. Fifty µL of enzyme conjugate (alkaline phosphatase labelled rabbit anti-chicken IgG) was added to each well, mixed by gentle tapping, covered with cover plate and incubated at +37°C for 30 minutes. The plates were then washed 4 times with wash buffer (300 µL per well), inverted and taped firmly. Fifty µL of substrate (phenolphthalein monophosphate) was added to each well and mixed by gentle tapping, covered with cover

plate and incubated at +37°C for 15 minutes followed by addition of 50 µL of stop solution (sodium hydroxide) to each well and mixed well using orbital shaker. Finally, reading of the results was carried out using ELISA reader at 550 nm immediately after the addition of stop solution.

Interpretation of Results: According to the manufacturer's guide, the assay procedure is valid when the mean absorbance of the negative control is less than 0.2 and that of the positive control is greater than or equal to 0.2. The results of the samples tested were interpreted based on the guideline given by the manufacturer, that is, the sample is considered positive when the S/P ratio is greater than 0.306. The S/P (sample absorbance value relative to absorbance of positive control) was determined using the formula:

$$S/P = \frac{\text{Sample absorbance - negative control absorbance}}{\text{Positive control absorbance - negative control absorbance}}$$

As described by the manufacturer anti-body level with S/P ratio of greater than 0.306 is considered protective to IBD virus infection.

Measurement of Live Body Weight and Wight of Lymphoid Organs: At the end of the study period 9 chickens were randomly selected from each treatment groups and their live weights were taken individually and scarified by decapitation. The weights of lymphoid organs (bursa of Fabricius, spleen, liver and thymus) were taken using sensitive digital balance.

Differential Leukocyte Count: For differential leukocyte count blood was collected using sterile syringe and immediately transferred to tubes containing EDTA. Thin blood smears were prepared from the blood samples for microscopic scanning and counting but when smear preparation and counting were not accomplished as scheduled, the blood samples were stored at +4°C until analyzed. Diff-Quik staining was used for differential staining of blood cells as described by Antony and Sirois [33]. The monolayer area of the smear was scanned initially under low power (10x) magnification to have an impression of the cellular distribution while leukocyte counting was done under oil immersion (100x) magnification using battlement method. A total of 100 leukocytes were counted and the proportion of each cell type determined. The total leukocyte count was estimated using the formula described by Antony and Sirois [33] and Fudge [34], that is:

$$\text{Total leukocyte count} = \frac{\text{Total leukocyte counted x 2000}}{\text{Number of fields counted}}$$

While the absolute differentaial leukocyte count is obtained by multiplying the estimated total leukocyte count by the proportion of each leukocyte type.

Data Analysis: The data collected in this study was stored in MS excel and analyzed using SPSS version 20 and EPI Info version 3.5.1. The variation in continuous variables such as the antibody titers and weights measured among treatment groups were analyzed using one way analysis of variance (ANOVA). When significant difference was observed, Bonferroni multiple pair wise comparison was used to identify the group (s) that differ from the rest of the treatment groups. The effect of predictor variables such as body weight and weight of lymphoid organs and liver and leukocyte count the anti-body titer was analyzed using linear regression. The variation in leukocyte count among the treatment groups was analyzed with poisson regression but the variation in basophile count was analyzed using negative binomial regression since it was zero inflated. The survival time of each treatment groups was compared using the Kaplan-Meier survival analysis. The difference in median survival time and median death time among the treatment groups was tested with Log Rank test.

RESULTS

Anti-body Titer Against IBD Using ELISA: The baseline geometric mean anti-body titer against IBD was shown to be lower than the protective level in all the treatment groups (Figure 1). Similarly the geometric mean antibody titer of the experimental chicken was lower than the protective level in all treatment gropus two weeks after the primary vaccinations (at day 20). However, higher mean titer was observed in chicken which received 140% of the recommended doses of L- Lysine and DL- Methionine even though the difference in mean anti-body titer among the different treatment groups was not statistically insignificant. After the booster vaccination was given statistically significant ($F = 23.83$, $P = 0.000$) variation was observed in geometric mean anti-body titer among the different treatment groups. The geometric mean anti-body titer was found to be highest (0.705) in chicken exposed to the highest dose (140%) of L- Lysine and DL- Methionine (Table 1). This was almost twice the geumetric mean anti-body titer required for protection against the virulent challenge. The lowest geometric mean anti-body titer was

Table 1: Geometric mean anti-body titer ±2SE of experimental chicken after vaccinations against IBD

Treatment group	No. of chicken	Anti-body titer At day 20	Anti-body titer At dat 35
No amino acid	15	0.015±0.01	0.13±0.07
100 % of recommended dose	15	0.013±0.007	0.435±0.1
80% 0f recommended dose	15	0.009±0.008	0.361±0.1
120% of recommended dose	15	0.025±0.008	0.538±0.1
F-value	-	1.4	23.83
P – value	-	0.25	0.000

Note: anti-body titer at day 20 = two weeks after the first vaccination; antibody titer at day 35 = two weeks after the booster vaccination.

Table 2: Mean ±2SE of live body weight of chicken, weight of lymphoid organs and liver in gram at the end of the study period (day 47)

Treatment group	No. of chicken	Live body weight	Weight of spleen	Weight of liver	Weight of thymus	Weight of bursa
No amino acid given	9	1860.2±156	1.9±0.4	35.8±5.4	5.2±1.8	1.7±0.3
100 % of recommended dose	9	1844.0± 196	2.7±0.4	41.6±6.6	5.7±2.0	2±1.0
80% of recommended dose	9	1728.3±140	2.0±0.4	42.0±3	4.4±1.0	1.8±0.8
120% of recommended dose	9	1861.1±100	3.2±0.4	46.3±2.8	3.9±0.8	1.8±0.6
140% of recommended dose	9	1998.3±178	4.2±.06	48.3±4	6.3±1.0	3.6±1.7
F-vale	-	1.50	16.01	4.29	1.90	3.37
P-value	-	0.22	0.000	0.006	0.13	0.019

Table 3: Post vaccination mean ±2SE of differential leukocyte count (x 10^3/ µL) of chicken exposed to different doses of L- Lysine and DL- Methionine

Treatment groups	No. of chickens	Hetrophil count	Lymphocyte count	Monocyte count	Eosinophil count	Basophil count
No amino acid given	9	70.2±2.6	82.1±4.2	4.4±4.2	4.7±2.2	0.2±0.4
100 % of recommended dose	9	63.8±8.0	102.3±33.4	8.2±4.2	8.7±2.0	0
80% 0f recommended dose	9	67.6±10.8	120.2±11.0	7.4±1.64	5.2±1.0	0.4±0.64
120% of recommended dose	9	123.2±6.6	100.7±7.2	10.4±0.58	7.6±1.0	1.6±1.42
140% of recommended dose	9	98.1±4.5	180.1±4.4	16.3±1.3	9.7±1.1	1.6±0.87

Table 4: Results of poisson regression of leukocyte counts among treatment groups

Treatment	Hetrophils	Coefficient for lymphocytes	Monocytes	Eosinophils	Basophils
Intercept	4.593	180.111	-11.889	9.667	.442
No amino acid	-0.341	-98.000	-11.889	-5.000	-1.946
Recommended dose	-0.437	-77.778	-8.111	-1.000	-30.737
80 % of amino acid	-0.380	-59.889	-8.889	-4.444	-1.253
120% of amino acid	0.221	-79.444	-5.889	-2.111	-1.283E-016
140% of amino acid	Ref	Ref	Ref	Ref	Ref
P value	0.000	0.000	0.000	0.000	0.112

Table 5: Results of linear regression analysis of anti IBD anti-body titer against body weight, weights of lymphoid organs and liver and differential leukocyte count

Variables	Constant(a)	Coefficient(ß)	Standard error	F-test	R2	P- value
Over all live body weight Vs antibody titer day 20 titer	0.029	0.000	0.000	0.27	0.01	0.61
Over all live body weight Vs antibody titer day 35 titer	0.07	0.000	0.000	1.55	0.03	0.22
Spleen weight Vs antibody titer at day 20 titer	0.008	0.003	0.004	0.75	0.02	0.39
Liver weightVs antibody titer at day 20 titer		0.000	0.001	0.005		0.94
Thymus weightVs antibody titer at day 20 titer		0.000	0.002	0.07		0.79
Bursa weightVs antibody titer at day 20 titer		-0.001	0.002	0.06		0.80
Spleen weightVs antibody titer at day 35 titer	-0.14	0.11	0.04	0.005	0.32	0.005
Liver weightVs antibody titer at day 35 titer		0.005	0.005	0.35		30.35
Thymus weightVs antibody titer at day 35 titer		0.008	0.02	0.62		0.62
Bursa weightVs antibody titer at day 35 titer		0.02	0.02	0.51		0.52
HetrophilVs ELISA IBD day 20 titer		0.000	0.000	4.69		
Lymphocyte Vs ELISA IBD day 20 titer		0.000	0.000	0.38		
Monocyte Vs ELISA IBD day 20 titer		-0.001	0.001	0.84		
Eosinophil Vs ELISA IBD day 20 titerBasophil		0.002	0.001	3.41		
Vs ELISA IBD day 20 titer		-0.04	0.002	2.68		
Lymphocyte Vs ELISA IBD day 35 titer		0.002	0.001	3.29		
Monocyte Vs ELSA IBD day 35 titer		0.010	0.009	1.13		
Eosinophil Vs ELISA IBD day 35 titerBasophil Vs		0.021	0.010	4.33		
ELISA IBD day 35 titer		-0.016	0.019	0.72		

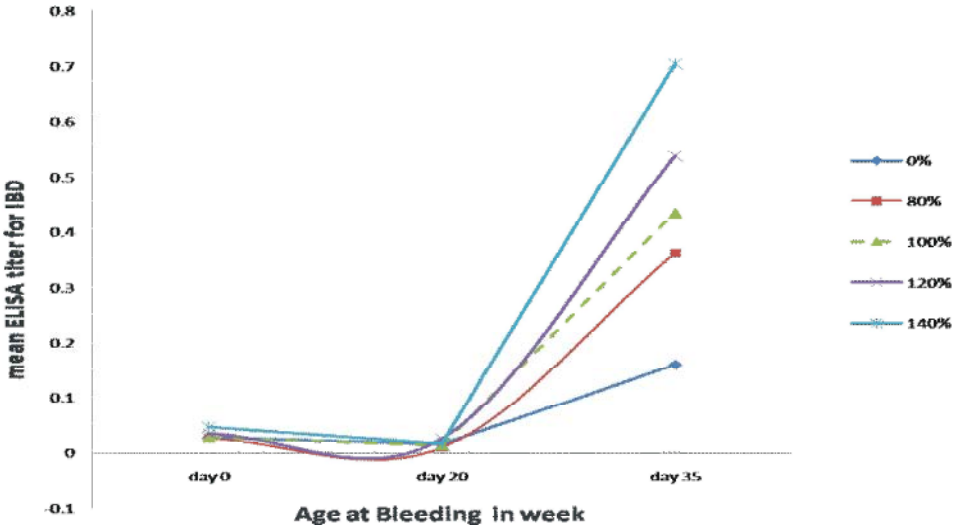

Fig. 1: Geometric mean anti-body titre of chicken exposed to different doses of L- Lysine and DL- Methionine

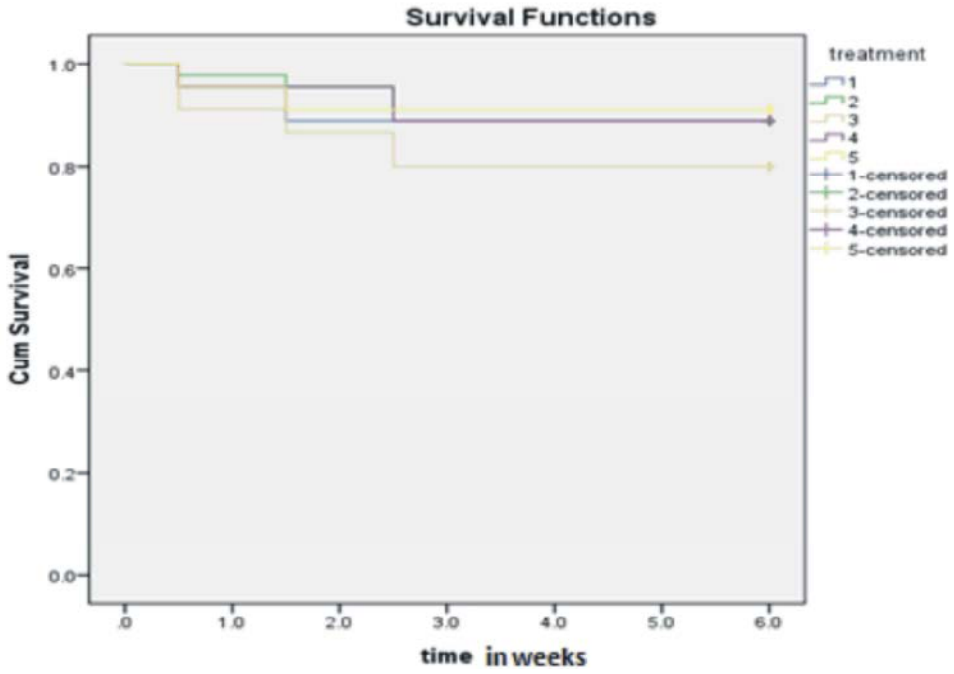

Fig. 2: Kaplan-Meier survival graph of experimental chicken receiving different doses of L- Lysine and DL- Methionine

observed in chicken in the negative control (0.159) in which the mean titer was below the level required to ensure protection against IBD virus. The geometric mean anti-body titer increased with increasing the doses of L- Lysine and DL- Methionine. The Bonferroni multiple comparisons showed that the mean antibody titer in chicken in the negative control was significantly lower than that of the other groups while the mean titer of chicken in treatment 5 (140% of recommended dose) was significantly higher than that of all other groups.

Live Body Weight and Weight of Lymphoid Organs and Liver: The live body weight and the weight of lymphoid organs of chicken in each treatment group are presented in Table 2. Higher mean live body weight was recorded in chicken exposed to higher doses of L- Lysine and DL-Methionine (140%) and it was observed to increase with increasing level of the amino acids tested but the difference observed was not statistically significant. Similarly the mean weights of the spleen, liver and bursa of Fabricius were statistically significant increases with

increasing level of amino acids in the feed and more significantly higher in chicken provided with 140% of the doses of amino acids than all other groups. The difference in mean weight of thymus was, however, not statistically significant.

Differential Leukocyte Count: Significantly higher counts of lymphocytes, monocytes and eosinophils were observed in chicken provided with 140% of the recommended dose of L- Lysine and DL- Methionine than chicken in other treatment groups. Tables 3 and 4 present the results of absolute differential leukocyte counts of experimental chicken in each treatment group. The counts were on average found to be different among the treatment groups except for basophils (P = 0.000). The hetrophil count was not consistent but, in general, the leukocyte counts tend to be higher with higher doses of L- Lysine and DL- Methionine.

Survival Analysis: The median survival time in weeks of chicken in all treatment groups was similar (Figure 2). There was no statistically significant difference (log Rank $X^2 = 3.66$; P = 0.45) in survival time of chicken among the various treatment groups even though some variation was observed among the different groups. The highest median death time was recorded for chicken in the positive control and in those chickens exposed to 120% of the recommended dose of the amino acids while the lowest median death time was observed for chicken receiving 140% of the recommended dose of amino acids.

Effect of Chicken Body Weight, Weight of Lymphoid Organs and Liver and leukocyte on Anti-body Titer: The results of linear regression of antibody titer on body weight and the weight of lymphoid organs and liver showed that the mean rise in anti-body titer associated with increase in weights was not statistically significant (Table 5). However, after booster vaccination was given, the increase in weight of spleen was shown to signifcantly inrease the anti-body titer (P = 0.0053). The effect of the weights of lymphoid organs on anti-body titer was minimal.

DISCUSSION

The anti-IBD anti-body titer using ELISA technique is considered protective when its absorbance is > 0.306 as recommended by the manufacturer. Hence, we used this cut off values to interpret our results. The baseline and the mean anti-body titer after the first vaccination were lower than the minimum protective level of 0.306 in all treatment groups. Even though it was not statistically significant, higher mean titer was observed in chicken in the treatment group5 (those which received highest dose of L-lysine and DL-methionine). The lower baseline anti-body titer clearly shows that the level of maternal anti-body was minimal. The lower mean anti-body titer following the primary vaccination could be due to the younger age of the chicken and the low anti-body level at baseline. In younger animals the immune function is not well developed [35]. This observation is in consent with the previous work of Rubin *et al.* [20] who reported that vaccination against IBD was shown to induce minimal anti-body response and impair the immune response of chicken on the first administration.

After the booster vaccination was given chicken in all treatment groups had significantly higher mean anti-body titer than the minimum titer (0.306) required for protection against the virulent challenge except chicken in the negative control in which the mean titer was < 0.306. This shows that inclusion of L-lysine and DL-methionine in the feed is beneficial in enhancing the immune response of chicken to IBD vaccine. The mean anti-body titer was shown to be different among different treatment groups and the difference observed was statistically significant. It was clearly shown that as the dose of the L-lysine and DL-methionine increase the mean anti-body titer was shown to increase. This is particularly important to counterbalance the immunosuppressive effect of IBD viruses. The broiler industry can benefit from higher doses of L-lysine and DL-methionine in areas where IBD is common and in areas where IBD vaccine is regularly given to chicken. Previously Jahanian [36] and Zhang and Guo [37] have shown that amino acids (arginine and DL-2-Hydroxy-4-methylthio butanoic acid) supplementation has enhanced the immune responses of broilers and increased growth of lymphoid organs. Our results agree with these earlier findings.

The weight of spleen, bursa of Fabricius and liver was found to vary significantly among different treatment groups. The weight of these organs was shown to increase as the doses of L-lysine and DL-methionine increases. But the difference in live body weight of chicken was not statistically significant. This is good indication of the effect of L-lysine and DL-methionine on the immune response than body weight. The higher weight of the lymphoid organs could be due to increased cellular proliferation, secretory function and tissue building stimulated by the L-lysine and DL-methionine. The improvement in the mean weight of lymphoid organs

was highest in chickens exposed to highest dose (140%) of L-lysine and DL-methionine than chicken in the rest of the treatment groups. Remarkable variation was observed especially for the weight of spleen. The mean weight of spleen from chicken in treatment 5 was twice the weight of spleen in the positive control group. The bursa of Fabricius also had the highest mean weight in chicken exposed to 140% of the recommended dose of L-lysine and DL-methionine. Similar finding was seen for mean weight of liver and thymus even if the difference in mean weight of thymus was not statistically significant. The variation in the mean weight of lymphoid organs is interesting finding and reflect that L-lysine and DL-methionine have beneficial effect on the immune response of chicken. It has been established that lymphoid organs are main sites for immune cells production, maturation, storage and release. Cells involved in both humoral and cellular responses are produced and primed in these organs [35]. This supports the variations observed in mean anti-body titer among different treatment groups. As there was no significant difference among treatment groups in live body weight, this finding implies that the improvement in the weight of lymphoid organs was not simply due to improvement in growth or body weight gain but is rather due to proliferation and production of defence cellular components. Previous reports have also shown that there was negative correlation between live body weight and anti-body response in broilers [38, 39].

In consent to our observation a study conducted in Iraq by Maroufyan *et al*. [40] showed that chicken feed with higher doses of methionine had highest mean weight of lymphoid organs. But in contrary to our finding the pervious observation reported the existence of difference in the weight of thymus. This disagreement may be due to difference in the age of chicken studied since they recorded the weight of the chicken at early age (between 1 and 39 days). We took measurements on weight of lymphoid organs at day 47. Since thymus is lymphoid organs of very young chicken, its size and then weight diminishes as the chicken get older. Similarly the findings of Cengiz *et al*. [41] showed that as the dose of Methionine and Lysine increased the weight of liver and bursa of Fabricius was shown to increase. Our findings, however, differ from the observation of Al-Mayah [23].

The differential leukocyte count was found to differ significantly among the treatment groups except for basophils. Leukocytes have been known to involve in defending the body of animals against various infections. Lymphocytes and monocytes are particularly important cells in defending the body systems against viral infections [35]. The increasing counts of lymphocytes and monocytes with increasing level of L-lysine and DL-methionine is a good evidence for improvement in the immune response of the chicken as a result of supplementation. Lymphocytes were known to play central role in regulating immune response, antibody production and in effecting cell-mediated immune response [42] while monocytes were known for their role in presenting antigens to lymphocytes and for their final effecter function in clearing intracellular infections [43,44]. Therefore, the higher lymphocytes and monocytes count in chicken supplemented L-lysine and DL-methionine is in consent with the observation of higher antibody titer and higher weights of lymphoid organs. The higher counts of these cells could also be beneficial in conferring protection against other concurrent infections as the amino acids are not specific in their effect. Our finding agrees with previous reports made by Al-Mayah [23] and Adeyemo *et al*. [24] elsewhere that have shown that L-lysine and DL-methionine supplementation has caused significantly higher leukocyte count especially at the finisher phase.

The weights of lymphoid organs had a positive effect on ELISA antibody titer of IBD after the first vaccination but it was not statistically significant. This observation supports the earlier findings of Rubin *et al.* [20]. This lack of effect of weight of lymphoid organs on the antibody titer might be due to timing of the measurement. Previously a study conducted by Cengiz *et al.* [41] revealed that evaluation of bursal and spleenic responses need to be carried out at early and delayed time after challenge. After booster vaccination, the weight of spleen had significant effect on the anti-IBD antibody titer. Since the weight of lymphoid organs was higher in chicken provided with higher doses of amino acids and since the weight of spleen is found to significantly increase the antibody titer, it is important to note that AAs have positive impact on this organ and enhance the immune response of chicken. The findings of Jahanian [36] revealed that AAs supplementation caused significant effect on the weights of lymphoid organs and immune responses.

In conclusion, the results of our experiment showed that there was significant difference in the anti-IBD antibody titer, weights of lymphoid organs and leukocyte counts among the different treatment groups depending on the level of L-lysine and DL-methionine provided. Higher dose (140% of recommended dose) of L-lysine and DL-methionine seemed better in improving antibody titer,

lymphoid organ growth and leukocyte counts but virus challenge is needed to definitely elucidate the protective of chicken under supplementation. Evaluation of the effect of these amino acids under various management systems and in different breeds is recommended.

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