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Immune Response of Broiler Chicks to DL-Methionine Supplementation at Different Ages

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Abstract: The study was conducted to evaluate the effect of methionine (ME) supplementation on immune response of broiler chicks during the period of 1^{st} -7^{th} and 29^{th} - 36^{th} day of age. The amino acid powder was supplemented as 1gm/L of drinking water or as 1gm/kg of feed during these early and late period of supplementation. The mean value of bursa of Fabricius, spleen and thymus weights of chicks supplemented DL-ME during the early period of age showed significant increment ($P<0.05$). Whereas chicks which were supplemented during the late period of the maturation of the immune system did not show significant increasing in lymphoid organ weights. Of all the blood parameters examined, the red blood cell counts and mean cell volume showed significant increment ($P<0.05$) from the control. Other haematological values were more than that of the normal values. The values of serum and liver total protein, albumin and globulin were highest but did not vary significantly from one another and from the control. We concluded that better immune response could be obtained with adequate supplementation of ME which have been identified to be in marginal quantities in poultry feed.

Key words: Methionine, broiler, Immune system, Basrah, haematology

Introduction

There are extremely important interactions, synergisms, and antagonisms between nutrition and immunity that markedly affect productivity of poultry. Sever chronic deficiencies of most nutrients impair the immune response and increase susceptibility to infectious diseases. Sever nutrient deficiencies are particularly deleterious to the immune system when they occur early in life during the development of the primary lymphoid organs and the maturation of immune system (Kirk, 1997).

The amino acids are often referred to as the "building stones" from which the proteins are made. Synthetic amino acids are being used in increasing quantities to supplement the natural proteins. Methionine can be added economically to many practical diets. Typical poultry feeds, in which much of the protein is furnished by soybean meal, may contain less than the desired quantity of methionine. In such cases, the first limiting amino acids is methionine and it is preferable to add methionine rather than to raise the total protein content (Harry, 1971).

Chicks fed diets low in essential amino acid had decreased delayed hypersensitivity and secondary 1gG responses relative to chicks fed adequate diets, although this effect may have been due to amino acid imbalances rather than a deficiency *per se* (Cook, 1991).

Specific amino acids in general tend to decrease humeral response while having a lesser effect on cellular immunity. Generally, results of studies in which deficiencies in amino acids were caused have had mixed results, some researches found that, for example methionine deficiency resulted in increased antibody level whereas others found that deficiency might lead to decrease in the antibody titer (Doug and Kirk, 2004).

Our objective was to examine the interactions between certain amino acid, methionine, supplementation on the immune response of broiler chicks.

Materials and Methods

Chicks from a commercial broiler strain were purchased from a local hatchery. They had been vaccinated via drinking water for Newcastle disease (ND) at 7th and 22nd day and at 14th day of age for infectious bursal disease (IBD). They were randomly assigned in compartments on litter floor and reared for 56 days of age. Ten chicks were placed in each of 5 groups. The test amino acid powder which was consisted of DL – methionine (DL-ME) [DL- Methionine, Degussa Huls, Germany] was provided either with the diet or with the drinking water and was given from 1st – 7th or from 29th – 36th day of age (before and after maturation of immure system). The AA was given for 7 continues days as 1gm / L of water or 1gm/Kg of feed according to the direction of manufacturer. Three birds were sacrificed from each group at 28th or 50th day of age by decapitation (Karaoglu and Durdage, 2005) as shown in Table 1.

Immediately after slaughtering blood samples were collected and bursa of Fabricius, spleen and thymus weights were measured. Blood was allowed to flow freely into sterile test tubes, some of which contained E

Table 1: Experimental design

Group	Age of administration	Route of administration	Age of slaughtering
A	1^{st}-7^{th}	Drinking water	28^{th}
B	1^{st}-7^{th}	Feed additive	28^{th}
C	29^{th}-36^{th}	Drinking water	50^{th}
D	29^{th}-36^{th}	Feed additive	50^{th}-
E	control	-	28^{th}- and 50^{th}

Table 2: Lymphoid organ weights in mg/100g BW of broiler chicks at 28^{th} and 50^{th}-days of age after supplementation with DL-ME powder

Group	Time of administration	Route of administration	Time of examination	Organs		
				Bursa	Thymus	Spleen
A	1^{st}-7^{th}	Drinking water	28^{th} day	*218±8[a]	416±5[a]	137±4[a]
B	1^{st}-7^{th}	Feed additive	28^{th} day	217±9[a]	416±6[a]	136±7[a]
C	29^{th}-36^{th}	Drinking water	50^{th} day	232±3	531±4	153±5
D	29^{th}-36^{th}	Feed additive	50^{th} day	231±2	532±4	152±7
E	Control		28^{th}	212±9[b]	411±12[b]	132±11[b]
			50^{th}	231±4	530±10	152±9

Figures are mean values (±SEM) for 3 birds in each group with different treatment. a,b Means in vertical row with different superscripts were statistically differed at P< 0.05.

D T A and the others without anticoagulant for serum analysis. The serum was kept deep frozen prior to analysis (Fasuyl and Aletor, 2005).

Some hematological indices were measured, included packed cell volume (PCV), total red blood cell (RBC) count, hemoglobin concentration (HBC), the mean corpuscular hemoglobin concentration (MCHC), the mean corpuscular hemoglobin (MCH), the mean corpuscular volume (MCV), erythrocyte sedimentation rate as well as the total white blood cell (WBC) count (Ross *et al.*, 1976).

After removal of liver from each carcass of killed chicks 0.5g of each liver was taken and homogenized in 5ml of sterile distilled water using porcelain mortar and pestle. Then 1ml was taken from this homogenate and diluted with 19ml of distilled water. Thus making the concentration of homogenate to be 0.5g /100ml or 0.5%. The homogenate was then kept in test tubes and left frozen prior to analysis. The total serum and liver protein as well as the albumin and globulin were determined (Florencio *et al.*, 1990) .

Results and Discussion

The relative weight of the organs measured were presented in Table 2 .The mean value of lymphoid organ weights of chicks fed ME at 1st -7th day of age were significantly different (P< 0.05) from the control. The weight of bursa Fabricius, thymus and spleen showed that a significant difference was not existed in their treatment mean values between group A and B. This means that the route of administration has no important effect on lymphoid organ weights during the experiment. Supplementation of this AA at 29th -36th day of age was not significantly increased the lymphoid organ weights. Thus the relative weights of bursa of Fabricius, thymus and spleen in group C and D were not significantly different from one another and control. These results were in the same line with those of Zulkifli *et al.* (1994) who stated that nutrient deficiencies are particularly deleterious to the immune system when they occur early in life during the development of the primary lymphoid organs and the maturation of immune system.

Fasuyl and Alertor (2005) reported that better performance can still be obtained with adequate supplementation of essential amino acids especially ME which has been identified to be in marginal quantities in most poultry feeds. However, the lymphoid organ weights increment might be due to the general improvement of the body weight of birds supplemented with ME which might caused lymphocytes repletion of lymphoid organs resulting in greater thymus, splenic and bursal weights .

Research evaluating the impact of amino acid density in commercial broilers demonstrated the positive impact of high amino acid density diets both balanced and unbalanced, in addition to accentuating the importance of amino acid nutrition from day 0 to 14. High amino acid nutrition early in a birds life is economically desirable (Kidd *et al.*, 2005.

The effect of ME supplementation on hematological indices of the broiler chicken is shown in Table 3. There were significant differences (P< 0.05) in more or less, all of the parameters investigated. Of all the blood parameters examined, especially the red blood cell. (RBC) counts and mean cell volume (MCV) showed significant variations from the control. This was in agreement with reports of Ologhobo *et al.* (1986) who stated that RBC counts is one variable that is most

Table 3: Haematological indices of birds supplemented with DL- ME powder

Parameters	Group				
	A 1^{st}-7^{th}-	B 1^{st}-7^{th}-	C 29^{th}-36^{th}-	D 29^{th}-36^{th}-	**E -
PCV%	*30.9±5[a]	30.8±6[a]	30.6±5[a]	30.5±3[a]	28.5±2[b]
RBC (Million/mm^3)	3.2±0.1[a]	3.1±0.2[a]	2.9±0.6[a]	3.0±0.3[a]	2.2±0.4[b]
WBC (Thousand/mm^a)	30.1±7[a]	30±9[a]	30.2±4[a]	30.1±6[a]	25.0±5[b]
Hbc (g/dl)	6.5±0.3	6.4±0.5	6.2±0.1	6.2±0.2	6.0±0.3
MCHC %	23.1±4[a]	23.0±2[a]	22.9±5[a]	22.8±4[a]	21.2±6[b]
MCH (Pg)	27.1±6[a]	27.0±5[a]	27.2 ± 8 [a]	26.9±6 [a]	25.0±3[b]
MCV (mm^3)	124.9±5[a]	125.0±8[a]	124.5±4 [a]	124.8±5 [a]	122.3±7[b]
ESR (mm)	4.4± 0.7	4.5±0.4	4.3±0.6	4.2±0.7	4.1±0.2

*Figures are mean values (± SEM) for 3 birds in each group. a,b, Means in horizontal row with different superscripts were significantly different (P< 0.05). ** Figures at both periods were nearly similar.

Table 4: Serum total protean, albumin and globulin of DL-ME supplemented birds (mg/100ml)

Group	Total protein	Albumin	Globulin
A	*5.99±0.2	1.77±0.3	4.22±0.1
B	5.97±0.3	1.76±0.2	4.21±0.3
C	5,96±0.2	1.74±0.1	4.22±0.4
D	5.95±0.5	1.75±0.4	4.20±0.6
E	4.89±0.7	1.20±0.02	3.69±0.3

*Figures are mean values (± SEM) for serum of 3 birds in each group at 28^{th} day only because there was no important numerical difference between both time of examination

Table 5: Liver total protein, albumin and globulin (mg/100g) of DL- ME supplemented birds

Group	Total protein	Albumin	Globulin
A	*10.2±0.3	2.5±0.2	7.7±0.6
B	10.0±0.4	2.4±0.1	7.6±0.4
C	9.9±0.6	2.5±0.3	7.4±0.3
D	10.1±0.5	2.6±0.6	7.5±0.5
E	8.5±0.7	2.2±0.9	6.3±0.3

*Figures are mean values (±SEM) for livers of 3 birds in each group at 50^{th} day only because there was no important numerical difference between both time of examination.

consistently affected by dietary influence. The Hbc was highest but not significantly different from the value for control. However, the range 6.5 in group A was some what departure from value obtained for control. Values for the mean cell volume (MCV) for supplemented birds also showed a significant differences from the control. Other haematological values were, in general, more than the normal values previously reported by Bell and Sturkie (1965). On a similar note, the MCHC, MCH and HBC were clearly affected by the dietary treatment suggesting higher hemoglobin contents.

The ESR of the test AA supplemented birds were similar to that of control group. This indicated that the test AA did not predispose the birds to any abnormal blood condition of any type. Franson (1986) reported that ESR is increased in cases of acute general infection and malignant tumors. The higher population of WBC (P< 0.05) in the peripheral blood in this study was in agreement with that of Bhargava *et al.* (1970) who stated that dietary manipulations of some nutrients result in immunoregulatory consequences due to the participation of the nutrient or its products in communication within and between leukocytes .

The haematological values in the experimental broiler birds were analogous with normal ranges, this suggest adequate and healthy nutrition. However, the preponderant values in the DL-ME supplemented groups justifies the established positive relationship between haematological parameters and health of bird. The total serum protein, albumin and globulin (Table 4) were highest but did not vary significantly from one another and from control group. Also the total liver protein albumin and globulin (Table 5) did not significantly affected by the AA supplementation. These findings correlate with increased numbers of both WBC and lymphoid organ weights in treated birds. All these findings indicated that ME administration enhance the immune system of growing chickens. Development of normal immune competence is reported to be completed at 4 wk of age in chicken (Florencio *et al.*, 1990). Therefore expression of immunological effects in this study were assumed to be the result of enhancement during development period. The study also revealed that there were no important numerical differences between early and late period of treatment on serum and liver total protein. Eggum (1987). Tewe (1985) stated that the total serum protein and total liver protein are indirect indices for measuring the nutritional protein adequacy.

Specific amino acids, in general tend to decrease humeral response, while having a lesser effect on cellular immunity (Doug and Kirk, 2004). Results of studies about amino acids have had mixed results. Bhargave *et al.* (1970) found that a methionine deficiency resulted in increased antibody levels, while Tsiagbe *et al.* (1987) suggested that the requirement for methionine

for maximum antibody titers was greater than that for growth. This means that the IgG levels did increase in birds fed increasing percent of ME. This may indicate that extra ME is important for the synthesis of the IgG antibodies or perhaps required for thymus- derived (T) – cell helper function. Discrepancies in these results may be the result of differing experimental designs as well as other experimental conditions.

We could concluded that better immune response can still be obtained with adequate supplementation of ME which has been identified to be in marginal quantities in poultry feeds. Typical poultry feeds in which much of the protein is furnished by soybean meal, may contain less than the desired quantity of methionine. In such cases, it is preferable to add ME rather than to raise the total protein content .

Further study is needed to explore whether the improvement of immunocompetence of birds was incidental or it has the ability to protect the birds along life, as well as if the bird can resist challenge against different pathogens .

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