ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 11 (6): 400-404, 2012
ISSN 1682-8356

The Hummoral Immune Response of Heat Stressed Broiler Chicks Fed Different Levels of Energy and Methionine

Hind A.A. Elagib and E.A. Elzubeir
Department of Poultry Production, University of Khartoum, Khartoum, Sudan

Abstract: The present study was conducted to investigate the effect of feeding different levels of energy and methionine on the total antibody, IgG, IgM immunoglobulin titer of broiler chicks. Thirty chicks were reared in an open sided house with temperature range from 29-42°C. The birds were divided into six groups of five replicate with individual bird. Six experimental diets were used with two levels of energy 3050, 2850 kcal/kg and three levels of methionine: control (0%, 0.2% and 0.4%) above the recommended level. At 14 day of age the birds were immunized with 0.2% Sheep Red Blood Cell (SRBC) suspension. The total antibody titer against SRBC was evaluated at 5 and 10 days post immunization using haemagglutination test. 0.1M 2-meracaptoethanol was used to determine IgG, IgM immunoglobulin. The total antibody titer response to SRBC was significantly ($p<0.05$) increased in birds fed 0.2 and 0.4% methionine supplemented diets. The different levels of energy 3050 kcal/kg and 2850 kcal/kg had no significant ($p>0.05$) effect on total antibody titer. At 5 and 10 days post immunization IgG increased significantly ($p<0.05$) with 0.2 and 0.4% methionine above the recommended level. However, no change was observed in the titer of IgM. The different levels of energy had no effect on IgG and IgM titer.

Key words: Immune response, immunoglobulin, methionine, broiler, energy

INTRODUCTION

The humoral immune and cellular immune system of chicken was affected by caloric-protein deficiency Glick *et al*. (1981). Glick *et al*. (1983) found that feeding diet one third deficient in calories, amino acids or both through 5 week of age cause depression in primary immune response to Sheep Red Blood Cell (SRBC) and secondary immune response was suppressed. However, cellular immunity was not affected by nutritional deficiency. Moreover, protein deficiency negatively affected the cellular immunity by decreasing lymphocyte number and overall white blood cell numbers as demonstrated by Payne *et al*. (1990).

Benson *et al*. (1993) examined the effect of energy density and energy source on broilers under immunological stress. It was found that feed intake was depressed by immunologic stress but the birds receiving high diet show high energy intake. The same authors demonstrated that increasing metabolizable energy by using corn-starch carbohydrate not corn oil reduce negative effect of immunological stressed birds on feed intake and weight gain. Primary immune response to SRBC and immune organ development (spleen and bursa) was not affected by feeding broiler chick diets differing in energy (2500, 2650, 2800 kcal/kg of ME) from 21 to 42 day of age.

The effects of heat stress (44.4-47.8°C) in the development of immune response in young chicks were studied earlier by Thaxton *et al*. (1968). These effects include the suppression of circulating White Blood Cell (WBC) (Heller *et al*., 1979) and increase in the Heterophil Lymphocyte ratio (H/L ratio) which is an indicator stress (Gross and Siegel, 1983). Also heat stress causes a reduction in antibody titer in young chickens as reported by Zulkifli *et al*. (2000). High temperature increase activity of adrenal gland and level of serum corticosteroids causing decreased in performance of poultry (Siegel and Latimer, 1984) and inhibits antibody production (Gross, 1992).

Bhargava *et al*. (1971) found that the antibody level is increased by methionine deficiencies, while Tsiagbe *et al*. (1987) suggested that the requirement for methionine for maximum antibody titers was greater than for growth, which give indication that the synthesis for IgG antibodies or the thymus requirement for derived T cell helper function needs extra methionine.

Klasing and Barnes (1988) reported that during immunological stress the chicks fed deficient methionine had suboptimal interleukin production indicating the need of methionine for immunity. Zulkifli *et al*. (1994) stated that the immune system maturation and the development of primary lymphoid organ is negatively affected by nutrient deficiency. It is well known that high temperature affect feed intake and hence the essential nutrients needed for growth and health. The objective of the present study is to evaluate the immune response of broiler chick fed different levels of methionine and energy under high environmental temperature.

Corresponding Author: Hind A.A. Elagib, Department of Poultry Production, University of Khartoum, Sudan

Table 1: Formulation of the experimental diet used in the experiment

	Diet composition					
Ingredient %	1	2	3	4	5	6
Sorghum	57.83	57.83	57.89	46.28	46.45	46.43
Groundnut meal	16.50	16.50	16.50	14.55	14.55	15.00
Sesame meal	15.50	15.50	15.50	14.80	14.80	14.95
Wheat bran	4.00	4.00	3.72	18.28	17.94	17.12
Superconcentrate*	5.00	5.00	5.00	5.00	5.00	5.00
Dicalcium phosphate	0.67	0.47	0.45	0.59	0.59	0.56
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50
Lysine	0.00	0.00	0.00	0.00	0.00	0.00
Methionine	0.00	0.20	0.40	0.00	0.20	0.40
Total	100.00	100.00	100.00	100.00	100.00	100.00

*Super concentrate contains the following/kilogram: CP 40%, ME 2100 kcal/kg, EE 2%, CF 2%, Calcium 10%, Phosphorus 4%, Lysine 12%, Methionine 3%, Methionine + cystine 3.2%

Table 2: Calculated composition

	Diets					
Item	1	2	3	4	5	6
ME	3050.85	3050.58	3050.08	2850.73	2850.14	2850.00
Crude protein	23.90	23.96	23.69	23.69	23.65	23.78
Calcium	1.05	1.09	1.07	1.07	1.06	1.07
Phosphorus	0.46	0.45	0.45	0.45	0.45	0.46
Lysine	1.10	1.10	1.13	1.13	1.12	1.13
Methionine	0.50	0.70	0.90	0.50	0.70	0.90
Meth + Cystine	0.82	0.82	0.91	0.91	0.92	0.913

Table 3: Proximate analysis of the experimental diet

	Diets					
Item %	1	2	3	4	5	6
Dry matter	95.00	69.70	95.40	95.91	94.60	94.50
Crude protein	24.97	25.01	24.90	24.36	25.02	25.50
Ether extract	4.03	4.00	4.50	4.30	4.80	4.12
Crude fiber	5.10	5.98	5.70	7.20	6.81	7.00
Ash	7.68	7.00	6.99	7.99	7.80	6.89
NFE	53.22	54.71	53.31	52.06	50.17	50.99
ME kcal/kg	3.6823	3.6935	3.809	3.726	3.8434	3.673

MATERIALS AND METHODS

Experimental birds: Day old unsexed Ross broilers chicks were reared in an open sided deep litter poultry house. The temperature of the poultry house was measured and it was ranged from 29-42°C. Thirty chicks were weighed and randomly distributed in the experimental pens with approximately the same initial weight into six groups. Each group was further divided into five replicate with one bird each.

There were six dietary treatments, two level of Metabolizable Energy (ME), high energy level (3050 kcal/kg), low energy level (2850 kcal/kg) and three levels of methioine (NRC level, 0.2% and 0.4% above the NRC level). The composition of the experimental diets was presented in Table 1. Table 2, 3 represent calculated composition and proximate analysis of the experimental diet, respectively. Feed and water were provided *ad libitum*. Continuous light was provided.

Immunization of the birds: At 14 day of age, broiler chicks were injected intravenously via wing vein, using 1 ml disposables syringe, with 0.2 ml of 10% suspension of packed Sheep Red Blood Cell (SRBC) in Phosphate Buffer Saline (PBS). After 10 days interval post first injection a booster injection of SRBC was given.

Collection of blood samples: After 5 and 10 days post the immunization blood was drown from the wing vein using 1 ml disposal syringe. The skin was first dampened with 70% alcohol to disinfect the area and to make vein visible. Immediately the blood was poured into labeled test tubes which were placed horizontally a rack at room temperature.

Separation of serum samples: Test tubes containing clotted blood sample were centrifuged at 1500 rpm for 10 min. The serum samples were then carefully collected using a pipette into sterile appendorff tube and stored at -20°C until used.

Preparation of reagents

0.1M 2-mercaptoethanol: The solution was prepared by dissolving 652 µL of 2 mercaptoethanol in 100 ml of Phosphate Buffer Saline (PBS).

Sterilization of the equipments: Glass graduated pipettes, test tubes, Appendorff tube, appendorff pipette tips, were sterilized in the autoclave at 121°C for 20 min.

Preparation of Sheep Red Blood Cell (SRBC): Blood was drown from sheep jugular vein, in sterile disposable syringe containing heparin. The blood was transferred slowly into plastic centrifuge tubes for washing. The blood was centrifuges 1500 rpm for 10 min. Then the plasma from the condensed cells was removed using a pipette.

Then equal amount of Phosphate Buffer Saline (PBS) was added to each tube and the suspension was centrifuged at 1500 rpm for 10 min. The supernatant fluids were poured off and the packed cells were resuspended in PBS and washed 3 times.

The packed cells were then used to prepare 10% suspension of RBCs in PBs solution and held at 4°C.

Haemagglutination test: The test was performed in 96 well U bottomed plastic plates (Microtitre plate) as described by (Hudson and Hay, 1989).The antibody titer was expressed as the $\log_2$ of reciprocal of the highest dilution of serum that gave 50% agglutination of SRBCs. The test was read by a titre tech magnifying mirror.

Positive agglutination is seen when cells form continuous carpet on the base of the cup. If no agglutination occur, the cells fall down as a tight bottom of the U shape. 0.1M 2-mercaptoethanol in each right rows of dilutions shows the 1gG immunoglobulin (2-mercatpo ethanol resistant antibody). 1gM 2-mercaptoethanol sensitive antibody were determined by subtracting the 2-mercatpoethanol resistant antibody from the total antibody titer. The data was subjected to analysis of variance according to SPSS using computer program. Means were compared using Duncan' s multiple range test.

RESULTS

The effect of feeding broiler chicks with different levels of methionine and metabolizable energy on antibody response to Sheep Red Blood Cells (SRBC) during summer was shown in Table 4.

Total antibody response to SRBC was increased significantly ($p<0.05$) by feeding birds methionine above the NRC recommended level (0.2, 0.4%) at both energy levels 3050 kcal\kg (high level), 2850 kcal\kg (low level). The different groups fed different levels of energy did not show significant difference ($p>0.05$) in antibody response to SRBC. However, when the birds was fed diet supplemented with 0.2 or 0.4% methionine above the NRC level a significant ($p<0.05$) increase in the antibody response was observed. The immunoglobulin IgG (2M-mercaptoethanol resistant antibody) titer showed a significant improvement ($p<0.05$) with dietary increment of methionine, higher value of IgG was attained in the groups fed 0.2, 0.4% methionine above the NRC level. The different levels of energy appeared to have no significant effect on IgG antibody titer. IgM (2M-mercaptoethanol sensitive antibody) titer was not significantly ($p>0.05$) affected by the different levels of methionine or energy.

Table 5 shows the antibody response of broiler chicks after second immunization with SRBC. The booster dose of SRBC resulted in higher values of antibody titer, there was a significant ($p<0.05$) increase in antibody titer as a result of feeding high levels of methionine (0.2 and 0.4%) above the NRC recommended level.

However, there was no significant ($p<0.05$) difference between the groups fed high energy diet and those fed low energy diet on total antibody titer, IgG (2 mercaptoethanol resistant antibody) and IgM (2M-mercaptoethanol sensitive antibody) titer.

At 5 and 10 days post immunization IgG response increased significantly ($p<0.05$) in birds fed diets

Table 4: Immune response of broiler chicks after primary immunization with SRBC fed different levels of methionine and energy during summer

Individual treatment diets	Added meth. %	5 days PPI Total Ab	5 days PPI IgM	5 days PPI IgG	10 days PSI Total Ab	10 days PSI IgM	10 days PSI IgG
3050 kcal\kg	None	3.50^b	2.50	1.00^b	2.23^b	1.40	0.83^b
	0.2	5.40^a	2.90	2.50^a	4.00^b	2.00	2.00^a
	0.4	5.20^a	2.90	2.30^a	3.70^b	1.70	2.00^b
2850 kcal\kg	None	3.20^b	2.20	1.00^b	2.30^b	1.30	1.00^b
	0.2	5.00^a	2.60	2.40^a	3.80^a	1.60	2.20^a
	0.4	4.80^a	2.30	2.50^a	3.70^a	1.40	2.30^a
±SE		0.21	0.33	0.48	0.35	0.25	0.23
Individual factor							
3050 kcal\kg		4.70	2.77	1.93	3.64	1.71	1.61
2850 kcal\kg		4.33	2.37	1.96	3.27	1.43	1.83
	±SE	0.20	0.27	0.17	0.21	0.15	0.13
	None	3.35^b	2.35	1.00^b	2.27^b	1.35	0.92^b
	0.2	5.20^a	2.75	2.45^a	3.90^a	1.80	2.10^a
	0.4	5.05^a	2.65	2.40^a	3.70^a	1.55	2.15^a
	±SE	0.30	0.21	0.34	0.25	0.18	0.17

^{a,b}Means with different superscript in the same column differ significantly ($p<0.05$)

Table 5: Immune response of broiler chicks after second immunization with SRBC fed different levels of methionine and energy during summer

Individual treatment diets	Added meth. %	5 days PPI Total Ab	IgM	IgG	10 days PSI Total Ab	IgM	IgG
3050 kcal\kg	None	5.00[b]	3.90	1.10[b]	3.00[b]	2.00	1.00[b]
	0.2	6.90[a]	3.80	3.10[a]	4.60[ab]	2.30	2.30[a]
	0.4	6.70[a]	4.00	2.70[a]	4.40[a]	2.00	2.40[a]
2850 kcal\kg	None	4.60[b]	3.60	1.00[b]	3.10[b]	2.10	1.00[b]
	0.2	6.80[a]	3.60	3.20[a]	4.50[a]	2.20	2.30[a]
	0.4	6.50[a]	3.50	3.00[a]	4.30[a]	2.10	2.20[a]
±SE		0.34	0.51	0.24	0.48	0.57	0.26
Individual factor							
3050 kcal\kg		6.20	3.90	2.20	4.77	2.10	1.90
2850 kcal\kg		5.97	3.57	2.47	4.73	2.13	1.83
	±SE	0.20	0.28	0.14	0.19	034	0.15
	None	4.80[b]	3.75	1.05[b]	3.05[b]	2.05	1.00[b]
	0.2	6.85[a]	3.70	3.15[a]	5.05[a]	2.15	2.90[a]
	0.4	6.60[a]	3.75	3.85[a]	5.15[a]	2.15	3.00[a]
	±SE	0.24	0.35	0.17	0.34	0.48	0.18

[a,b]Means with different superscript in the same column differ significantly (p<0.05)

supplemented with 0.2 and 0.4% methionine above the NRC (1994) level. But no significant difference (p<0.05) in the IgG titer was found between the two level of methionine (0.2 and 0.4%). IgM titer was not significantly affected by the different methionine level supplemented.

DISCUSSION

It appeared that broiler chicks fed the diet supplemented with methionine had improved antibody titer. In this study it was observed that total antibody titer response to SRBC was significantly increased in birds fed 0.2 and 0.4% methionine supplemented diets. Klasing and Barnes (1988) reported that during immunological stress the chicks fed deficient methionine had suboptimal interleukin production indicating the need of methionne for immunity. This indicates that methionine is important for the antibody production due to its role as a precursor of cytokines. Cytokines are thought of as the hormones of the immune system and their main function and their main function is to promote and sustain an immunological response that is appropriate for the pathogen or antigen towards which the response is directed (NRC, 1994).

This result agrees with the findings of Tsiagbe *et al.* (1987) that total antibody titer increased when methionine was added to the diet. It is also in agreement with the results of (Fasuyl and Aletor, 2005) who confirmed that methionine affected antibody titer positively and this caused lymphocyte repletion of lymphoid organ resulting in greater weight of thymus, spleen and bursa weight. Al-Mayah (2006) reported better immune response obtained by methionine supplementation. The immunoglobin antibody titer revealed high titer of IgG when methionine was added to the diet. However, no change was observed in the titer of IgM. This result agrees with Tsiagbe *et al.* (1987) who concluded that methionine is important for the synthesis of IgG antibody or perhaps required for thymus-derived T-cell helper function.

The result of the present study revealed that feeding different levels of energy 3050 kcal/kg and 2850 kcal/kg had no effect on immune response of broiler chicks. This result agrees with the result obtained by Benson *et al.* (1993) who found that feeding broiler chicks different energy had no effect on primary antibody response to SRBC and immune organ development. However Glick *et al.* (1981, 1983) found suppression in the immune response to SRBC in birds fed diet deficient in calories and amino acid.

Conclusion: It can be concluded that supplementation of methionine to broiler diet at high temperature had a positive effect in the immune response as it increase total antibody titer, IgG, however, the energy level did not affect immune response.

REFERENCES

Al-Mayah, A.A.S., 2006. Immune response of broiler chicks to DL methionine supplementation of different ages. Int. J. Poult. Sci., 5: 169-172.

Benson, B.N., C.C. Calvert, E. Roura and K.C. Klasing, 1993. Dietary energy source and density modulate the expression of immunologie stress in chicks. J. Nutr., 123: 1714-1723.

Bhargava, K.K., R.P. Hanson and M.L. Sunde, 1971. Effects of methionine and valine on growth and antibody production in chicks infected with live or killed newcastle disease virus. Poult. Sci., 50: 614-619.

Fasuyl, A.O. and V.A. Aletor, 2005. Protein replacement value of Cassava (Manihot esculenta, Crantz) Leaf Protein Concentrate (CLPC) in broiler starter: Effect on performance, muscle growth, haematology and serum metabolites. Int. J. Poult. Sci., 4: 339-349.

Glick, B., E.J. Day and D. Thompson, 1981. Calorie-protein deficiencies and the immune response of the chicken I. Humoral immunity. Poult. Sci., 60: 2494-2500.

Glick, B., R.L. Taylor Jr., D.E. Martin, M. Watabe, E.J. Day and D. Thompson, 1983. Calorie-protein deficiencies and the immune response of chicken. II. Cell mediated immunity. Poult. Sci., 62: 1889-1893.

Gross, W.B. and H.S. Siegel, 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis., 27: 972-978.

Gross, W.B., 1992. Effect of short-term exposure of chickens to corticosterone on resistance to challenge exposure with *Escherichia coli* and antibody response to sheep erythrocytes. Am. J. Vet. Res., 53: 291-293.

Heller, E.D., D.B. Nathan and M. Perek, 1979. Short heat stress as immunostimulant in chick. Avian Pathol., 8: 195-203.

Hudson, L. and F.C. Hay, 1989. Practical Immunology. 3rd Edn., Blackwell Scientific Publications, Oxford London.

Klasing, K.C. and D.M. Barnes, 1988. Decreased amino acid requirements of growing chicks due to immunologic stress. J. Nutr., 118: 1158-1164.

NRC, 1994. National Research Council. Nutrient requirements of domestic animals. Nutrient. Nutrient requirements of domestics poultry. 9th Rev. Edn., Nat. Acad. Sci. Washington, D.C.

Payne, C.J., T.R. Scott, J.W. Dick and B. Glick, 1990. Immunity to Pasteurella multocida in protein-deficient chickens. Poult. Sci., 69: 2134-2142.

Siegel, H.S. and J.W. Latimer, 1984. Interaction of high temperature and salmonella pullorum antigen concentration on serum agglutinin and corticosteroid response in white rock chicken. Poult. Sci., 63: 2483-2491.

Tsiagbe, V.K., M.E. Cook, A.E. Harper and M.L. Sunde, 1987. Enhanced immune responses in broiler chicks fed methionine-supplemented diets. Poult. Sci., 66: 1147-1154.

Thaxton, P., C.R. Sadler and B. Glick, 1968. Immune response of chicken following heat exposure or injections with ACTH. Poult. Sci., 47: 264-266.

Zulkifli, I., E.A. Dunnington, W.B. Gross and P.B. Siegel, 1994. Food restriction early or later in life and its effect on adaptability, disease resistance and immunocompetence of heat-stressed dwarf and nondwarf chickens. Br. Poult. Sci., 35: 203-214.

Zulkifli, I., M.T. Che Norma, D.A. Israf and A.R. Omart, 2000. The effect of early age feed restricition on subsequent response to high environmental temperature in female broiler chickens. Poult. Sci., 79: 1401-1407.