



Study of the relationship between performance and immune responses of Arian chicks following use of different patterns of expression and supply of energy and amino acids requirements

Pouya Yari¹, Akbar Yaghobfar^{2*}, Habib Aghdam Shahryar¹, Yahya Ebrahimnezhad¹ and Sara MirzaieGoudarzi³

¹Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, Iran

²Animal Science Research Institute, Karaj, Iran

³Department of Animal Science, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran

(Received on: 30 October, 2014; accepted on: 26 December, 2014)

ABSTRACT

A total of 720 one-day-old Arian broiler chicks were used to determination of effects of different feed formulation patterns on productive and immune responses. Birds were randomly allocated to 4 group with 6 replicates containing 30 bird (15 males + 15 females). Measurements were subjected to analysis of variance for completely randomized 2×2 factorial design that including 2 dietary energy expression patterns (Apparent (AMEn) and true (TMEn) metabolizable energy corrected to nitrogen equilibrium) and 2 amino acid requirement patterns (Total (TAA) and digestible (DAA)). The results showed that when the feed formulation was based on TMEn and, the productive parameters increased compared with AMEn ($p < 0.05$). However the most suitable of immune responses were belonging to treatments were fed diets which were set based on AMEn and DAA. There is a negative correlation between immune responses and productive parameters. It is recommended that in normal conditions use of TMEn and DAA system and in stress conditions use of AMEn and DAA to feed formulation.

Key Words: Feed formulation pattern, performance and immune responses, Arian broilers.

INTRODUCTION

During 50-40 years ago, the poultry industry had the major progress and improvement. This is a result of advances in genetics, nutrition and control of growing environment (Chambers et al., 1981 and Havenstein et al., 1994). Genetic selection for increased production potential in the poultry also causes some negative consequences (Paraharaj et al., 1997). That can be pointed to an increased susceptibility to diseases of birds (Parmentier et al., 1996). For example, found that body weight is negatively correlated with antibody responses against to sheep red blood cell (SRBC) (Miller et al., 1992 and Parmentier et al., 1996). The humoral immune response of new

commercial broilers decreased compared to strains that were breeding last years (Cheema et al., 2003). The fast-growing poultry compared with those which have a slower growth incur more damage from disease and peripheral and have lower antibody (Roa et al., 1999). Diets formulation in poultry industry are mainly done based on productive parameters such as growth rate, rate of egg production, feed intake and feed conversion ratio, so does not consider the physiological responses. Energy and protein as an essential nutrient, can effect on the growth rate and immune system of broilers (Golian et al., 2010). Also according to the protonic structure of cells and immunogenic agents of immune system, so that value, suitability and availability of essential amino acids have vital role in the

*Corresponding author: yaghobfar@yahoo.com

success of this system (Sturkie, 1986; Humphrey et al., 2006 and Klasing, 2007). Lack of dietary nutrient imbalance would compromise the process of protein synthesis and so immune system; it will also affect the performance (Dasgupta et al., 2005). In addition to the genetic selection, some non-genetic factors such as concentration of amino acids in the diet can alter the expression of genes related to immune response through a change in the rate of maturation of the immune system and the antibody level in response to infections (Klasing, 2007). Total energy and amino acids content in diet are not fully utilized by birds, their availability depend on the species of bird, feed intake, anti-nutritional factors, feed processing, systems of feeding, etc. It has been suggested that proper nutrients is supplied through regulation of diets based on digestible amino acid (DAA) method compared to total amino acid (TAA). Formulation of diets based on Apparent (AMEn) and true (TMEn) metabolizable energy corrected to nitrogen equilibrium pattern, provide different levels of energy for broilers so make different productive and metabolic responses (Farrell et al., 1999; Leeson, 2011 and Sibbald, 1989). The purpose of this study was to determine the effects of diet formulation patterns (AMEn, TMEn, TAA and DAA) on productive parameters and immune system of Arian broiler chicks.

MATERIALS AND METHODS

Experimental Design

This study was carried out at Animal Science Research Institute of Iran. A total of 720 one-day-old Arian broiler chicks were randomly allocated to 4 groups with 6 replicates containing 30 bird (15 males + 15 females). The experimental diets were formulated with 2 methods of energy expression of diets (Apparent and true metabolizable energy corrected to nitrogen equilibrium) and 2 methods of amino acid requirement expression (Total and digestible amino acid). Formulation and composition of experimental diets are given in table 1.

Productive parameters and immune system determination: Body weight (BW) and feed consumption were obtained weekly then daily feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were calculated from

these data. One bird in each replicate unit was vein injected with 1.5ml sheep red blood cell (SRBC) 15% suspension at 28 days of age. Blood samples were collected from brachial vein 5 days after ejection. The serum from each sample was collected; heat inactivated at 56°C for 30min and then analysed for total IgG (Mercaptoethanol-resistant) and IgM (Mercaptoethanol-sensitive) anti-SRBC antibodies as described by Cheema et al (2003). At 35 days of age, direct blood samplings were taken from 1 chick by a wing vein of each replicate unit. The blood samples were stained using May-Grünwald-Gieamsa stain than heterophils and lymphocytes were counted to a total of 60 cells (Siegel and Gross, 1983).

Statistical analysis: Measurements of productive and serum biological parameters were subjected to analysis of variance for completely randomized 2×2 factorial design that including 2 dietary energy expression patterns (AMEn and TMEn) and 2 amino acid requirement patterns (TAA and DAA), using ANOVA-General linear method (SAS User's Guide: Statistics Version 7.0). Significant differences between treatment means were identified by Duncan's multiple range with 5% probably.

RESULTS AND DISCUSSION

The results of productive parameters and immune response are presented in table 2 and 3 respectively. The final BW, BWG and FI were affected significantly by both diet formulation methods ($P<0.05$). The diets that regulated based on TMEn and DAA patterns have greater final BW, BWG and FI. The FCR decreased significantly when used the DAA pattern to diet regulation compared to TAA. There were significant interactions between diet formulation methods on BW and BWG but not on FI and FCR. Lower BW and BWG observed in treatment that fed diet regulated based on AMEn × TAA pattern ($P<0.05$). Studies have shown that broilers are capable of adaptation to diets containing low-energy, if they have enough time to match with these diets, can reach to optimal weight (Lesson et al., 1996). In the present experiment, any negative effect on growth was observed during using the TMEn method (lower energy diets), even the growth rate was significantly increased in comparison to AMEn. The broilers often adjust their feed intake to get

Table 1. Composition of experimental diets

<i>Ingredients (%)</i>	Starter (1-21 day old)				Grower (22-42 day old)			
	AMEn		TMEn		AMEn		TMEn	
	TAA	DAA	TAA	DAA	TAA	DAA	TAA	DAA
Corn	54.34	54.80	54.56	54.37	56.47	56.85	57.86	58.55
Soybean meal	37.55	36.78	37.34	36.20	33.27	32.54	32.49	31.53
Wheat	-	-	-	-	2.50	2.50	4.00	4.00
Wheat meal	-	-	2.21	2.96	-	-	-	-
Fish meal	1.75	2.36	1.25	2.05	0.75	1.25	0.85	1.55
Vegetable oil	2.94	2.80	1.12	1.10	3.86	3.74	1.25	1.00
DL-Methionine	0.24	0.16	0.25	0.17	0.13	0.15	0.13	0.13
L-Lysine	0.12	0.14	0.13	0.15	0.12	0.13	0.12	0.11
Oyster shell	0.89	0.90	0.92	0.93	0.90	0.91	0.97	0.98
Dicalcium phosphate	1.35	1.25	1.39	1.25	1.22	1.13	1.43	1.25
Salt	0.32	0.31	0.33	0.32	0.28	0.30	0.40	0.40
Vitamin mix ¹	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral mix ²	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
<i>Calculated composition of diets (%)</i>								
AMEn(kcal/kg)	3050	3050	-	-	3150	3150	-	-
TMEn(kcal/kg)	-	-	3050	3050	-	-	3150	3150
Crude Protein	22	22	22	22	20	20	20	20
Methionine	0.46	0.40	0.46	0.40	0.38	0.33	0.38	0.33
Methionine+Cystine	0.85	0.73	0.85	0.73	0.81	0.70	0.81	0.70
Lysine	1.25	1.07	1.25	1.07	1.15	1.00	1.15	1.00
Threonine	0.79	0.67	0.79	0.67	0.74	0.64	0.74	0.64
Tryptophan	0.21	0.18	0.21	0.18	0.17	0.15	0.17	0.15
Arginine	1.31	1.12	1.31	1.12	1.15	1.00	1.15	1.00
Valine	0.76	0.65	0.76	0.65	0.55	0.48	0.55	0.48
Leucine	1.21	1.04	1.21	1.04	0.87	0.76	0.87	0.76
Isoleucine	0.68	0.58	0.68	0.58	0.52	0.45	0.52	0.45
Calcium	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Available Phosphorus	0.50	0.50	0.50	0.50	0.45	0.45	0.45	0.45
DCAB ³ (meq/kg)	250	205	250	250	225	225	225	225

¹ Vitamin mix provided the following (per kg of diet): thiamin-mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B12 (cobalamin), 12.0 mg; pyridoxine HCL, 4.7 mg; D-biotin, 0.11 mg; folic acid, 5.5 mg; menadione sodium bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 27.5 mg; transretinyl acetate, 1892 mg; all-rac α tocopheryl acetate, 11 mg; ethoxyquin, 125 mg.

² Trace mineral mix provided the following (per kg of diet): manganese (MnSO₄-H₂O), 60 mg; iron (FeSO₄-7H₂O), 30 mg; zinc (ZnO), 50 mg; copper (CuSO₄-5H₂O), 5 mg; iodine (ethylene diamine dihydroiodide), 0.15 mg; selenium (NaSeO₃), 0.3 mg

³ Dietary cation-anion balance

Table 2. Effects of feed formulation methods on productive parameters of broiler chicks (1-42d)

<i>Main Effects¹</i>	BW (g)	BWG (g/bird/day)	FI (g/bird/day)	FCR
AMEn	2088 ^b	48.8 b	96.0 b	1.97 ^b
TMEEn	2245 ^a	52.5 a	107.4 a	2.05 ^a
P.value	<0.001	<0.001	<0.001	0.009
TAA	2145 ^b	50.1	100.7 b	2.01
DAA	2333 ^a	52.2	105.2 a	2.02
P.value	0.044	0.095	0.038	0.966
<i>Interaction Effects</i>				
AMEn × TAA	1955 ^b	45.6 b	93.1 b	2.04 ^{ab}
AMEn × DAA	2085 ^{ab}	48.7 b	103.8 a	1.95 ^b
TMEEn × TAA	2191 ^a	51.2 a	108.2 a	2.06 ^{ab}
TMEEn × DAA	2176 ^a	50.8 a	106.6 a	2.10 ^a
P.value	<0.001	<0.001	0.022	0.040
SEM	47.82	0.57	2.52	0.02

Means within Colum with different superscripts are significantly different (p<0.05)

Table 3. Effects of feed formulation methods on productive parameters of broiler chicks (1-42d)

<i>Main Effects¹</i>	Antibody against SRBC			Leukocyte (%)		
	SRBC	IgG	IgM	Lymphocyte (L)	Heterophile (H)	H/L
AMEn	8.31 ^a	5.31	3.00	76.63	18.00	0.241
TMEEn	7.31 ^b	4.62	2.69	78.29	16.67	0.217
P.value	0.009	0.165	0.352	0.290	0.220	0.224
TAA	7.37	4.75	2.62	78.42	16.58	0.216
DAA	7.37	4.62	2.75	78.58	16.67	0.216
P.value	0.170	0.591	0.075	0.413	0.390	0.533
<i>Interaction Effects</i>						
AMEn × TAA	7.25 ^b	4.50 ^{ab}	2.75	78.83	16.00	0.210
AMEn × DAA	9.00 ^a	6.00 ^a	3.00	74.00	18.50	0.224
TMEEn × TAA	7.50 ^b	5.00 ^{ab}	2.50	78.00	17.17	0.221
TMEEn × DAA	5.75 ^c	3.25 ^b	2.50	80.83	14.83	0.188
P.value	0.005	0.040	0.746	0.058	0.057	0.077
SEM	0.41	0.33	0.20	1.03	0.73	0.01

Means within Colum with different superscripts are significantly different (p<0.05)

the enough energy; it is known that this adjusting is more accurate during the consuming low-energy diets (NRC, 1994). In the present study increasing of growth rate during use TMEEn system may be due to increasing feed intake. The results of FI in this study were agreement with results of Dozier et al (2007) and Kamran et al (2008), they found that FI decreased during consuming the high-energy diets. In various reports, such as Smith and Pesti (1998) stated that reducing energy of diet will cause increasing FI to access more energy. Khaksar and Golian

(2009) reported that diet regulation based on DAA pattern, significantly increased body weight and use of TAA pattern leads to reduced feed intake. These results are similar to the results of the present study. Although Maiorka et al (2004) reported that diet formulation based on total amino acid has no effect on feed intake and weight gain. Similar to this trial, Zaghari (2006) reported that diet formulation based on DAA method compared to TAA can be accurately supply the amino acid requirements and improved FCR of broilers. When used the AMEn pattern to

diet formulation, SRBC titer increased but lipase activity and triglyceride level decreased ($P<0.05$). IgM and IgG were not affected by energy expression pattern ($P<0.05$). Amino acid requirement patterns had not significant effect on SRBC, IgM and IgG. There were significant interactions between diet formulation pattern on SRBC and IgG titer but not on IgM titer. Highest SRBC and IgG level observed in treatments that were fed diet regulated based on AMEn×DAA pattern ($P<0.05$). The amount of Heterophil and lymphocyte and Ratio of these two white blood cells, were not significantly affected by main and interaction effects of feed formulation methods. Specified that, increase of antibody against sheep red blood cells (SRBC) and a high level of lymphocytes will cause a powerful immune system (Sturkie, 1986). In the present study demonstrated that increasing levels of dietary energy (using the AMEn pattern) enhances the immunity system, while this pattern reduced performance compared to TMEn pattern. According to results, the Amount of energy required to Creating an appropriate response via immune system is higher than the energy needs for optimal growth. However, in contradiction with the results it was announced that Immune response of broiler that are fed diet that is deficient in calories and amino acids was not differ with broilers that fed diets Contains adequate nutrients (Cook, 1997). Golian et al (2010) reported that during stepwise increasing dietary energy from Kcal 2900 Kcal 3200 reduced antibody against SRBC linearly. Korver et al (1998) stated that feeding of low-energy diets (2900 kcal/kg) reduced the production compared with high energy diets (3200 kcal/kg), while no effect on the efficiency of the immune system. Paraharaj et al (1997) established that the Low concentrations of nutrients in the diet can cause similar or more immune response than normal levels of nutrients. While most studies have noted that Immune response of chickens that grow faster is lower than those grow slower (Boa et al., 1999; Emara et al., 2002 and Roa et al., 1999). This indicates that there was a negative correlation between the growth rate and immune response (Kreukniet et al., 1994 and Marsteller., 1980). In according of this study Sklan et al (1994) stated that if want to create a strong and efficient immune system in poultry, must be diet containing higher levels of energy and amino acids.

CONCLUSION

There is a direct relationship between immune tissues such as lymph nodes, thymus, spleen and central nervous system. After meal, the immune system receives physiological and metabolic changes through the nervous system than responds to them (Klasing, 2007). Also some hormones are affected by feeding, which can enhance (growth and thyroid hormones) or weakened (cortisol) of immune systems (Davison et al., 2008). So rations through effects on the nervous and hormonal system affected immune response. It is recommended that under normal conditions of breeding, use of TMEn and DAA pattern to regulation of diets. But In cases that there is likelihood of stress, to ensure achieving of enhance and improve immune system, used of higher levels of energy and amino acids in diet.

REFERENCES

- Boa-Amponsem K, Dunnington EA, Baker KS and Siegel PB. 1999. Diet and immunological memory of lines of white Leghorn chickens divergently selected for antibody response to sheep red blood cells. *Poult Sci* 78: 165-170.
- Chambers JR, Gavora JS and Forin A. 1981. Genetic change in meat type chickens in the past twenty years. *Can J. Amin. Sci.*, 61: 555-563.
- Cheema MA, Qureshi MA and Havenstein GB. 2003. A comparison of the immune response of 2001 commercial broiler with a 1957 random bred broiler strain when fed representative 1957 and 2001 broiler diets. *Poult Sci* 82: 1519-1529.
- Cook ME. 1991. Nutrition and the immune response of the domestic fowl. *Crit. Rev. Poultry Biol* 3:167-189.
- Dasgupta M, Shaekey JR and Wu G. 2005. Inadequate intakes of indispensable amino acids among homebound older adults. *J Nut Elderly* 24: 85-99.
- Davison F, Kaspers B and Schat KA. 2008. *Avian Immunology*. 1 th ed. Published by Elsevier Ltd. San Diego, USA.
- Dozier WA, Corzo A, Kidd MT and Branton SL. 2007. Dietary apparent metabolizable energy and amino acid density effects on growth and carcass traits of heavy broilers. *Poult Sci* 16: 192-205.

- Duncan DB. 1995. Multiple range and multiple F Tests. *Biometrics*, 11, pp. 1-42.
- Emara MG, Lapiere RR, Greene GM, Knieriem M, Rosenberger JK, Pollock DL, Sadjadi M, Kim CD and Lillehoj HS. 2002. Phenotypic variation among three broiler pure lines for Marek's disease, coccidiosis and antibody response to sheep red blood cells. *Poult Sci* 81: 642-648.
- Farrell DJ, Mannion PF and Perez-Maldonado RA. 1999. A comparison of total and digestible amino acid in diets for broilers and layers. *Anim Feed Sci Technol* 82(1): 131-142.
- Golian A, Zaghari M, and Pilevar M. 2010. Influence of various levels of energy and protein on performance and humoral immune responses in broiler chicks. *Global veterinaria* 4(5): 434-440
- Han PFS and Smyth JR. 1972. The influence of growth rate on the development of Marek's disease in chickens. *Poult Sc* 51:975-985.
- Havenstein GB, Ferket PR, Scheideler SE and Larson BT. 1994. Growth livability and feed conversion of 1957 vs. 1991 broiler when fed typical 1957 and 1991 broiler diets. *Poul. Sci* 73: 1785-1794.
- Humphrey BD, Stephensen CB, Calvert CC and Klasing, KC. 2006. Lysine deficiency and feed restriction independently alter cationic amino acid transporter expression in chickens. *Comparative Biochemistry and Physiology. Part A*. 143: 218- 227.
- Isakov N, Feldmann M and Segel S. 2005. The mechanism of modulation of humoral immune responses after injection of mice with SRBC. *J Immunology* 128: 969-975.
- Kamran Z, Sarwar M, Nisa M, Nadeem MA, Mahmood S, Babar ME and Ahmed S. 2008. Effect of low-protein diets having constant energy-to-protein ratio on performance and carcass characteristics of broiler chickens from one to thirty-five days of age. *Poult Sci* 87:468-474.
- Khaksar V and Golian A. 2009. Comparison of ileal digestible versus total amino acid feed formulation on broiler performance. *J Anim Vet Adv* 8 (7): 1308-1311.
- Klasing KC. 2007. Nutrition and the immune system. Gordon Memorial Lecture. *Br Poult Sci* 48: 525-537.
- Korver DR, Roura E, Klasing KC. 1998. Effect of dietary energy level and oil source on broiler performance and response to an inflammatory challenge. *Poult Sci* 77: 1217-1227.
- Kreukniet MB, Nieuwland MGB and Van Der Zijpp AJ. 1994. Phagocytic activity of two lines of chickens divergently selected for antibody production. *Vet. Immunol Immunopathol* 44: 377-387.
- Leeson L. 2011. Feed stuffs and reference issue and buyer guide 2012: *Nutr Heal poult P*: 52-60
- Maiorka A, Dahlke F, Santin E, Kessle, AM and Penz JRAM. 2004. Effect of energy levels of diets formulated on total digestible amino acid basis on broiler performance. *Braz J Poult Sci* 6 (2): 87-91.
- Marsteller FA, Gross WB, Siegel PB. 1980. Antibody production and *Escherichia coli* resistant in socially stable flocks of dwarf and non-dwarf chickens. *Poult Sci* 59: 1947-1948.
- Miller LL, Siegel PB, Dunnington EA. 1992. Inheritance of antibody response to sheep erythrocytes in line of chickens divergently selected for fifty six days body weight and their crosses. *Poult Sci* 71: 47-52.
- National Research Council. 1994. *Nutrient Requirements of Poultry*. 9th. Rev. Edn. National Academy Press, Washington, DC.
- Paraharaj NK, Dunnington EA and Gross Siegel WB. 1997. Dietary effects on immune response of fat fast growing chicks to inoculation of sheep erythrocytes and *Escherichia coli*. *Poult Sci* 76: 244-247.
- Parmentier HK, Nieuwland MGB, Rijke E, De Vries Reilingh G and Schrama JW. 1996. Divergent antibody responses to vaccines and divergent body weight of chicken line selected for high and low humoral responsiveness to sheep red blood cells. *Avian Dis* 40: 634-644.
- Roa SV, Praharaj NK, Reddy MR and Sridevi B. 1999. Immune competence resistant to *Escherichia coli* and growth in male broiler parent chicks fed different levels of crude protein. *Vet Res Commun* 23: 323-326.
- SAS Institute 2003. *SAS User's Guide: Statistics Version 7.0*. SAS Institute Inc., Cary, NC.
- Sibbald IR. 1989. Metabolizable energy evaluation of poultry diets. In: *Recent Development in Poultry Nutrition*. Edit. Cole, D. J. A., W. Haresign Butterworths. London.
- Siegel HS and Gross WG. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chicken. *Avian Dis* 27: 972-979.

Sklan D, Melamed D and Friedman A. 1994. The effect of varying levels of dietary vitamin A on immune response of the chick. *Poult Sc.* 73:843-847.

Smith ER and Pesti GM. 1998. Influence of broiler strain cross and dietary protein on performancr of broiler. *Poul Sic* 77: 276-281.

Sturkie PD. 1986. *Physiology*. 4 th ed. Springer verlag. New york.

Zaghari M. 2006. Formulation of broiler diets on a total amino acid versus a digestible amino acid basis. *First Congress on Animal and Aquatic Science Iran*, pp. 286-289.