Effect of Feed Restriction on Performance and Immune Response of Two Commercial Broiler Strains

Khajavi, M.,[1] Rahimi,*[1§] S., Hassan, Z.,[1] Kamali, M.A.,[2] Mousavi, T.[3] and Lotfolahian, H.[2]

§: Rahimi_s80@yahoo.com

1. Poultry Raising Management Dept., Agriculture Faculty, Tarbiat Modarres University, P.O.Box 14115-111, Tehran, Iran

2. State Animal Science Research Institute, P.O.Box 1483, Karaj, Iran

3. Biotechnology Dept., Razi Vaccine & Serum Rresearch Institute, P.O.Box 11365-1558, Tehran, Iran

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Summary

This experiment was conducted to determine the effects of feed restriction (FR) on performance and the immune system of Arian and Ross strains. Feed efficiency was significantly better for restricted birds. Body weight (BW) of the restricted chickens was significantly lower than those of the *ad libitum* group at day 42. Arian strain had lower BW at day 21 but higher BW at day 42 than Ross strain. Birds in FR group showed a significant increase in percentage of CD4+ (helper T cell) and decreased CD8+ (cytotoxic T cell) at day 21 and 42. T cell subsets of Ross and Arian birds was not significantly different on day 21, but in day 42 the Arian chickens had higher CD4+ and lower CD8+ than those of Ross chickens. Antibody response to sheep red blood cells was significantly higher in the birds on the FR group at day 21. Antibody titer was not affected by strain at day 21 but Ross chickens had higher antibody titer than Arian chickens at day 42. The results indicate that the feed restriction may have some adavantages, mainly by increasing feed efficiency and immune system competence.

Key words: feed restriction, Ross, Arian, performance, immune system

Introduction

Over the last few decades, selection for greater body weight gain resulted in considerable improvements in broiler performance. These genetic manipulations accompanied by increase some disorders (Katanbaf *et al* 1988a, 1988b, 1989). Several researchers have suggested a negative genetics correlation between growth rate and immunocompetence in chickens (Bacon 1992, Klasing 1988, Latashaw 1991, McMorty *et al* 1988, Qureshi & Havenstine 1994).

Several management strategies have been used to reduce these unfavorable disorders. One of these procedures involves early growth rate reduced by food restriction in broiler chickens. Even though the immunocompetence was affected by various nutrient deficiencies such as vitamins and minerals (Ferket & Qureshi 1999, Latashaw 1991), many researchers have reported that immune system was not affected by food restriction program (Ballay *et al* 1992, Dagass & Flores 1998, Zulkifli *et al* 1993). Ballay *et al* (1992) found no significant effect of long term food restriction, from day 1 to 39, on the antibody titer against SRBC injection. Also their experiment show that food restriction has no effect on lesion scores of chickens challenged with *Escherichia coli*. Glick *et al* (1981) reported that a diet low in all amino acids severely reduced growth of chickens, but this diet did not decrease antibody response to SRBC injection. Praharaj *et al* (1999) demonstrated that reduced metabolizable energy in broiler chickens had no effect on antibody titer.

We have not found information about the effects of food restriction on T-lymphocyte subsets. T lymphocytes are divided in two main populations: helper and cytotoxic. The main function of helper T-cells is stimulating the proliferation of the B-cells specific for antigen. Cytotoxic T-cells are important in killing infected cells (Ferket & Qureshi 1999). Helper and cytotoxic T-cells are recognized by CD4 and CD8 protein molecules on the their surface, respectively. For this reason, helper and cytotoxic T-cells were termed CD4+ and CD8+, respectively. These two T-cells subset can be recognized with fluorescent monoclonal antibodies, which have been produced against CD4 and CD8 surface protein molecules (Chen *et al* 1991, 1994).

The present experiment was designed to research the effect of feed restriction on CD4+ and CD8+ T-cell subsets in peripheral blood of male and female of two common broiler chicken strains in Iran. In addition, other features of immune system and broiler performance have been measured.

Materials and Methods

Experimental design. A 3-factorial experiment (2×2×2) was set up to investigate the effect of two feed intake pattern (restricted feeding and *ad libitum*), on performance and immune system of male and female broiler chickens of Ross and Arian strains. Each treatment had three replicates consisting of seven chickens in each.

Birds. Five hundred Ross and five hundred Arian chicks were obtained from a local hatchery. Male and female chicks were separated 24h after hatching. The chicks were kept in floor pens on chopped straw, from day one to day nine. At 10 day of age, 168 uniformed chickens consisted of 42 male and 42 female of each strain were selected. They were randomly distributed into eight treatments of three replicates and were moved to batteries.

Diets and feeding regimens. Except for the duration of the feed restriction, the chickens were fed *ad libitum*. Birds in restriction group were restricted to alternate-day feeding from 11 to 20 day of experiment. A starter diet was given until 21 day of age, and from day 22 to day 42 a grower diet offered. The diets were formulated to meet NRC (1994) nutrient requirement.

Chickens performance. All chickens were weighed individually at day 11, 20, and 42 to determine body weight, weight gain and feed efficiency. Feed consumption was recorded daily for all replicates.

Flow cytometry analysis. Briefly, the following procedure was used to measure the CD4+ and CD8+ T cells. On day 21 and 42, 0.5ml of heparinized blood was collected from one chicken in each replicate. Thirty µl of blood sample was incubated with 0.3µg monoclonal phycoerythrin conjugated antibodies against chicken CD8 antigen (Southern Biotechnology, USA) for 5min in darkness. Samples were incubated with 1.5µg mouse monoclonal fluorescein isothiocyanate conjugated antibodies against

chicken CD4 antigen (Southern Biotechnology, USA) for 15min in darkness. Labeled cells were subsequently washed twice in phosphate buffered saline (PBS) and suspended in 1ml PBS. Per each sample, 10,000 cells were counted using a flow cytometer (FACSort, Becton Dikinson, USA). The percentage of T-cell subset was determined by PC-LYSIS II package (Becton Dickinson, USA).

Antibody response. On day 16 and 37, one chicken randomly chosen from each replicate was injected intraperitoneumly with 0.1ml suspension of SRBC (Razi Institute, Karaj). Five days later blood samples were collected and antibody measured by microagglutination (Wegmann & Smithies 1966). Antibody titer expressed as $\log_2$ of the reciprocal of the last dilution in which there was agglutination (Ambrose & Donner 1973).

Heterophil/Lymphocyte ratios. On day 21 and 42, one chick from each replicate was chosen at random and blood samples were obtained from wing vein using heparin as the anticoagulant. Blood smears were prepared using May-Grunwald-Giemsa stain and herterophil (H) and lymphocyte (L) were counted to a total of 100-cell (Gross & Sigel 1983).

Statically analysis. All results were expressed as means$\pm$standard error of the mean (SEM). The data obtained from variable measured, analyzed with analysis of variance, using MSTATC computer package. A full factorial design was used, containing feeding regimens, strains and sex. Comparisons among multiple means were made by Duncan's multiple range test.

Results

1. Chickens performance. Results of body weight and food intake are shown in table 1. Body weight and feed intake of chickens submitted to the early feed restriction at the end of the restriction had decreased 210 and 255g, respectively ($P<0.01$). On the 42nd day of age, body weight and feed intake of restricted birds was significantly lower compared to that of the *ad libitum* fed controls. During the period after restriction, up to day 42, feed intake of restricted birds was significantly higher than the *ad libitum* fed birds. At the age of 21, the body weight of Arian chicks was

significantly lower than that of Ross chicks. At the slaughter age, Arian chicks were significantly heavier than Ross chicks. Body weight and feed intake of male broiler at all time was significantly higher than female chicks. Feed efficiency (data are not presented in table) was significantly better for restricted birds (P<0.01). Feed efficiency was not affected by strain throughout the duration of the study. Sex differences in feed efficiency were observed during the period of study.

Table 1. *Effects of feeding regimens, strains and sex on body weight and consume at different days and duration of experiments*

Variables	Body Weight (g)		Feed Consumption (g)		
	21 day of age	42 day of age	10-21 day of age	22-42 day of age	1-42 day of age
Feeding Regimens					
Feed Restriction	385	1810	489	2185	3077
ad Libitum	595	1982	744	2247	3488
SEM[1]	6.02	8.32	7.50	6.82	9.23
MC[2]	**	**	**	**	**
Strains					
Ross	512	1912	645	2226	3365
Arian	472	1950	585	2309	3450
SEM	5.15	6.55	4.50	7.02	14.33
MC	*-	**	*-	**	*-
Sex					
Male	511	2085	639	2480	3649
Female	465	1870	586	2290	3347
SEM	5.03	6.14	7.21	9.03	12.80
MC	*-	**	*-	**	**

1:Standard Error of Means. 2:Means Comparison: *: significant at P<0.05 **:significant at P<0.01.

2. T-lymphocyte population. The influence of feeding programs, strain and sex on percentage of the CD4+ and CD8+ T cells are presented in table 2. Data analysis revealed that feed restriction affects mean percentage of CD4+ and CD8+ T-cells in peripheral blood of broiler chickens. The proportion of CD4+ and CD4+/CD8+ ratio were increased and the proportion of CD8+ were decreased at 21 and 42 day of age by feed restriction. Strain had no effect on percentage of CD4+ and CD8+ T cells at 21 day of experiment. However, at the age of 42 d the percentage of CD4+ was increased and the percentage of CD8+ was decreased in Arian chickens. The percentage of CD4+ T-cell and CD4+/CD8+ ratio in male broiler was higher than female at 21 and 42 day of age.

Table 2. *Effects of feeding regimens, strains and sex on peripheral blood CD4+ and CD8+ percentage and CD4+/CD8+ proportion at different days of age*

Variables	21 day of age			42 day of age		
	CD4+	CD8+	CD4+/ CD8+	CD4+	CD8+	CD4+/ CD8+
Feeding Regimens						
Feed Restriction	22.3	8.7	2.52	17.3	10.1	1.73
ad Libitum	19.8	11.2	1.77	16.9	12.3	1.39
SEM	0.6	0.71	0.09	0.52	0.57	0.09
MC	*	*	**	*	*	**
Strains						
Ross	21.5	9.9	2.16	16.8	13.5	1.25
Arian	21.8	10.5	2.15	18.5	11.8	1.56
SEM	0.54	0.31	0.03	0.6	0.8	0.08
MC	NS[1]	NS	NS	*	*	**
Sex						
Male	22.6	8.1	2.78	20.5	7.1	2.87
Female	18.8	10.8	1.79	15.3	10.3	1.47
SEM	0.7	0.6	0.07	0.4	0.7	0.05
MC	**	*	**	**	**	**

NS: not significant

3. Antibody titer. On day 21, at the end of the restriction period, restricted birds showed a higher antibody titer than the *ad libitum* group (Table 3). There were, however, no significant differences among the feeding regimens for antibody response at day 42 of age.

Table 3. *Antibody titer response to SRBC injection among different feeding regimens, strains and sex at 21 and 42 day of age*

Variables	21 day of age	42 day of age
Feeding regimens		
Feed restriction	5.48	8.79
ad libitum	4.35	8.17
SEM	0.14	0.21
MC	*	NS
Strains		
Ross	5.13	8.85
Arian	4.89	8.11
SEM	0.23	0.19
MC	NS	**
Sex		
Male	5.76	8.64
Female	4.93	7.12
SEM	0.14	0.33
MC	**	**

Table 4. *Effects of feeding regimens, strains and sex on heterophil per lymphocyte ratio of broiler chickens at 21 and 42 day of age*

Variables	21 day of age	42 day of age
Feeding regimens		
Feed restriction	0.44	0.49
ad libitum	0.35	0.55
SEM	0.032	0.043
MC	**	*
Strains		
Ross	0.40	0.53
Arian	0.38	0.51
SEM	0.06	0.031
MC	NS	NS
Sex		
Male	0.45	0.52
Female	0.37	0.58
SEM	0.087	0.05
MC	**	**

Antibody production was similar for Ross and Arian chickens on day 21. On day 42, Ross birds showed significantly higher humoral immune response to SRBC. There were significant differences in antibody response between male and female chicks at 21 and 42 day of age.

4. Heterophil/lymphocyte ratio. The effects of feeding regimens, strains and sex on heterophil per lymphocyte ratio are given in table 4. Results showed that proportion of heterophyl per lymphocyte cells were increased at the end of restriction period, on day 21, by restriction. There were, however, no significant differences among Ross and Arian birds at 21 and 42 day of age. Significant differences between male and female chickens were observed at 21 and 42 day of age.

Discussion

The present study confirmed earlier evidence that feed restriction decreased body weight and feed intake at the end of restriction period. Our finding demonstrated that after 10 days alternate-day feed restriction, body weight and feed intake at the end of restriction period had decreased by 210 and 255g, respectively. During the period after restriction, up to day 42, restricted birds grew faster than the *ad libitum* group. However this was not sufficient to catch up with the final body weight of the *ad libitum* chicks. This is in contrast with the statement of Plavnik and Hurwitz (1985, 1988, and 1991) who found compensatory growth with a better food conversion after a period of food restriction in broiler chicken. In current trial, even though the catch-up growth did not occur in the restricted group, their food efficiency was significantly better than the *ad libitum* fed chickens. Current results agree with those reported by Pinchasov and Jensen (1989) and Cristofori *et al* (1997), who found that the improved food conversion was at the expense of final body weight. In reviewing the literature, Yu and Robinson (1992) reported the factors such as severity, timing, duration of food restriction, food intake during the periods of refeeding, sex and strain may affect the subsequent ability of broiler chickens to recover from a growth deficit state.

The current results show that food restriction increased peripheral blood CD4+ cells and decreased peripheral blood CD8+ in broiler chickens. Several researches have reported the effects of various nutrients on T-lymphocyte subset. The effect of food restriction on T-cells subset has not been reported. Our finding may be attributed to the endocrinological changes induced by feed restriction. This theory, however, is supported by the finding of Johonson *et al* (1993), who reported that growth hormone elevated CD4+ populations and decreased the proportion of CD8+ cells in birds. Also, Marsh and Scanes (1994) observed that growth hormone increased ratio of peripheral CD4+ to CD8+ T-cells in chickens. More research is needed to fully understand the mechanism of food restriction effects on T cells subsets.

Our finding show significant differences between Ross and Arian chicks in peripheral T cells subsets at the 42 day of age. Several studies show the variation affects of genetics on immune system (Erf & Smyth 1996, Lamont1994, Zulkifli *et al* 2000, 1993). Current results show significant differences between sex in peripheral percentage of T cells subsets. This finding may be resulted due to sex steroid hormones. This hypothesis is supported by the Dayens *et al* (1990), Who reported that dehydroepiandrosterone (a weak androgen) affect immune system development and function. Our results confirm previous finding (Klasing 1988), that feed restriction enhances antibody response to SRBC injection. These studies show that food restriction increase the ability of humoral immune system against foreign antigens. However, Ballay *et al* (1992) found no significant effects of some form of feed restriction on humoral immune system.

The present study, confirm earlier evidence that food restriction can induce stress. As measured by H/L ratio, stress response to food restriction occurred at the end of restriction period. These results agree with those reported by Zulkifli *et al* (1993) who found that food restriction enhanced H/L ratio in broiler chickens. It may be concluded from the results of this investigation that feed restriction offer some economic advantages over an *ad libitum* feeding regimen by reducing feed conversion ratio and increasing the immune system competence.

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References

Ambrose, C.T., Donner, A (1973). Application of the analysis of variance to hemagglutination titration. *Journal of Immunological Methods* 3:165-210.

Bacon, L.D. (1992). Measurement of immune competence in chicken. *Poultry Science* (Review) 4:187-195.

Ballay, M.E., Dunnigton, E.A., Gross, W.B. and Siegel, P.B. (1992). Restricted feeding and broiler performance: age at initiation and length of restriction. *Poultry Science* 71: 440-447.

Chen, C.H., Gobel, T.W.F., Kubota, T. and Cooper, M.D. (1994). T cell development in the chicken. *Poultry Science* 73:1012-1018.

Chen, C.H., Pickel, J.M., Lathi, J.M. and Cooper, M.D. (1991). Surface markers on avian Immune cells. In: J.M. Sharma (Ed.), *Avian cellular immunology*. Pp:10-22. CRC Press, Boca, Raton, FL.

Cristofori, C., Melozi, A., Giordani, G. and Sirri, F. (1997). Early and late quantitative feed restriction of broiler: effects on productive traits and carcass fatness. *Archive Fur Geflugelkunde* 61:162-166.

Dagass, C.T., Flores, D.N. (1998). Compensatory growth in broiler subjected to different levels of feed restriction during the second week of age. In: *Proceeding of the Philippine Society of Animal Science, VPLB-CVM.* Pp:180-186.

Daynes, R.A., Dudley, D.J. and Araneo, B.A. (1990). Regulation of marine lymphokine production in vivo 2.Dehydropiandrostron is a natural enhances of interleukin-2 synthesis by helper T cell. *European Journal of Immunology* 20:793-802.

Erf, G.F., Smyth, J.R. (1996). Alternation in blood leukocyte populations in Smyth line chickens with autoimmune vitiligo. *Poultry Science* 75:351-356.

Ferket, P.R., Qureshi, M.A. (1999). The turkey immune system and nutritional immunomodulatory. *European Symposium on Poultry Nutrition*. Neldhoven, The Netherlands. Pp:17-30.

Glick, B., Day, E.J. and Thompson, D. (1981). Calorie-protein deficiencies and the immune response of the chicken 1.Humoral immunity. *Poultry Science* 60:2494-2500.

Gross, W.B., Sigel, H.S. (1983). Evaluation of hetrophil/lymphocyte ratio as a measure of stress in chickens. *Avian Diseases* 27:972-979.

Johnson, B.E., Scans, C.G., King, D.B. and Marsh, A. (1993). Effect of hypophysoctomy and growth hormone on immune development in the domestic fowl. *Devlopmental and Comparative Immunology* 17: 331-339.

Katanbaf, M.N., Dunnington, E.A. and Siegel, P.B. (1988a). Allomorphic relationship from hatching to 56 days in parental lines and F1 crosses of chickens selected 27 generations for high or low body weight. *Growth and Development Aging* 52:11-22.

Katanbaf, M.N., Dunnington, E.A. and Siegel, P.B. (1989). Restricted feeding in early and late feathering chickens. 3: Organ size and carcass composition. *Poultry Science* 68: 359-368.

Katanbaf, M.N., Jones, D.E., Dunnington, E.A., Gross, W.B. and Siegel, P.B. (1988b). Anatomical and physiological responses of early and late feathering broiler chickens to various feeding regimens. *Archive Fur Geflugelkunde* 52:119-126.

Klasing, K.C. (1988). Influence of acute feed deprivation or excess feed intake on immune competence of broiler chickens. *Poultry Science* 67:626-631.

Latashaw, J.D.M. (1991). Nutrition-mechanism of immunosuppression. *Veterinary Immunology and Immunopathology* 30:111-120.

Lamont, S.J. (1994). Poultry immunogentics: which way do we go? *Poultry Science* 73:1044-1048.

Marsh, J.A., Scanes, C.G. (1994). Neuroendocrine-immune interactions. *Poultry Science* 73:1049-1061.

McMorty, J.P., Plavnik, I., Rosbrough, R.W., Steel, N.C. and Proudman, A. (1988). Effect of early feed restriction in male broiler chickens on plasma metabolic hormones

during feed restriction and accelerated growth. *Comparative Biochemistry and Physiology* 91A: 67-70.

National Research Council. (1994) *Nutrient Requirement of Poultry*. (9th Revised edn). Washington, DC, National Academic Press.

Pinchasov, Y., Jensen, L.S. (1989). Comparison of physical and chemical means of feed restriction in broiler chickens. *Poultry Science* 68:61-69.

Plavnik, I., Hurwitz, S. (1985). The performance of broiler chickens during and flowing a sever feed restriction on early age. *Poultry Science* 64:348-355.

Plavnik, I., Hurwitz, S. (1991). Response of broiler chickens and turkey poults to feed restriction of varied severity during early life. *British Poultry Science* 32:343-352.

Plavnik, I., McMurty, J.P. and Rosebrough (1988). Effect of early feed restriction in broiler. I: Growth performance and carcass composition. *Poultry Science* 77:870-872

Praharaj, N., Reddy, M.R., Ramma, S.V. and Sridevi, S. (1999). Growth, immune competence and disease resistance in diverse stocks of broiler parents reared under various energy levels. *Archive Fur Geflugelkunde* 63:270-276.

Qureshi, M.A., Havenstine, G.B. (1994). A comparison of the immune performance of a 1991 commercial broiler with a 1957 random bred strain when fed "typical" 1957 and 1991 broiler diets. *Poultry Science* 73:1805-1812.

Wegmann, T.G., Smithies, O. (1966). A simple hemagglutination system requiring small amount of red cells and antibodies. *Transfusion* 6:67-73.

Yu, M.W., Robinson, F.E. (1992). The application of short term feed restriction to broiler chicken production: a review. *Journal of Applied Poultry Research* 1:147-153.

Zulkifli, I., Abdollah, N., Azarin, M.D., and Ho, Y.W. (2000). Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress condition. *British Poultry Science* 41:593-597.

Zulkifli, I., Dunnigton, W.B., Larsen, A.S., Martin, A. and Siegel, P.B. (1993). Responses of dwarf and normal chickens to feed restriction, *Eimeria tenella* infection and sheep red blood cell antigen. *Poultry Science* 72:1630 1640.