Pak. J. Agri. Sci., Vol. 30, No. 3, 1993

EFFECT OF FOOD-RESTRICTION ON MACROPHAGE FUNCTIONS IN COMMERCIAL CHICKEN BROILERS

Hameed Afzal, Nadeem Iqbal & M. Tauseef Ahmad
Department of Veterinary Microbiology,
University of Agriculture, Faisalabad

Four commercial chicken broiler breeds were compared after 7 days of food-restriction for the competence of mononuclear phagocytic system cells. Peritoneal exudate cells collected from food-restricted birds after single intraperitoneal injection of 3% Sephadex G-50 were assayed for incidence, phagocytic activity against opsonised and unopsonised sheep red blood cells (SRBC) and bactericidal potential against opsonised *Escherichia coli.* Macrophages from food-restricted birds exhibited reduced phagocytic activity against opsonised and unopsonised SRBC and diminished bactericidal potential against opsonised *E. coli* compared with controls. The results of the present study suggest that food-restriction adversely affects the immune response and makes the birds susceptible to different pathogens.

INTRODUCTION

Food restriction in poultry has been studied comprehensively (Pearson & Shannon, 1979) and it is now an accepted procedure in commercial practice for improving biological and economic performance. Haematological studies on food-restricted birds have shown that these birds do not only acquire a chronic microcytic normochromic anaemia but they may also experience acute stress, indicated by significantly higher basophil counts (Maxwell *et al.,* 1990 a, b).

Macrophages provide at least two distinct functions in the generation of humoral immune response (Weaver & Unanue, 1990). Initially, they are required for antigen uptake and processing into immunogenic peptides. These peptides are then displayed on the cell surface in conjunction with Class II (Ia) molecules for presentation to $CD4^+$ T helper cells. Phagocytosis of antigen by macrophages also induces the production of numerous cytokines. Some of the cytokines [e.g., interleukine-1 (IL-1), IL-10], tumour necrosis factor mediate the early localised inflammatory response at the site of antigen clearance. Second, the production of IL-1 functions as a second signal to type 2 T helper cells for their clonal expression and production of B cell growth and differentiating factors (Durum *et al.,* 1985).

Suppression of the macrophage- and T cell-dependent antibody response to sheep red blood cells (SRBC) is one of the most sensitive measures of immunosuppression (Davies & Safe, 1988). Deprivation of food and water for 48 hours resulted in decreased haemagglutinating antibody titre and decreased antibody producing cells in the spleens of chicks immunised with SRBC and also showed slowed clearance of bacteria *(Staphylococcus aureus)* from the circulation (Ben-Nathan *et al.,* 1981). The present study was designed to assess and investigate the specific macrophage functions *in vitro* in response to food-restriction in an effort to determine whether this procedure reduces the degree of non-specific immunity.

MATERIALS AND METHODS

Eight day-old broiler chicks were procured from four different commercial broiler breeders and reared up to 2 weeks of age in open floor pens. Feed and water were provided *ad libitum*. Birds from each breed were randomly divided into two groups, designated as group I and group II; each group having 10 birds. Food was restricted in group I from day 15 to day 22 whereas group II, fed *ad libitum* was kept as control. Both the groups were inoculated intraperitoneally with 3% Sephadex G-50 solution and the birds were slaughtered 42 hours post-Sephadex injection.

Peritoneal exudate cells (PEC) were harvested using a Sephadex stimulation method modified from Sabet *et al.* (1977) as previously described by Trembicki *et al.* (1984). The phagocytic activity of macrophages from pooled PEC of each group was determined using an *in vitro* SRBC phagocytosis assay (Qureshi *et al.*, 1986). The bactericidal assay was performed as previously described for bovine (Desiderio and Campbell, 1983) and chicken (Qureshi *et al.*, 1988) macrophages. A highly pathogenic Congo Red positive (CR+) phenotypic variant derived from *Escherichia coli* strain (Akhtar *et al.*, 1991) was used in this assay.

RESULTS

The mean percentage of macrophages of broiler chicks after food-restriction is given in Table 1. After restricting food for 7 days, the incidence of macrophages ranged from 24.10 to 39.93% compared with 62.00 to 77.01% in the *ad libitum*-fed control birds. There was a concomitant decrease in macrophages in the food-restricted birds compared with controls (Table 1). This difference was statistically significant ($P<0.05$).

Phagocytic ability of macrophages from four different breeds was determined using opsonised and unopsonised SRBC (Table 2). Macrophages from group A control birds exhibited significantly reduced and group B enhanced phagocytic activity for opsonised SRBC. The phagocytic potential from groups C and D was not significantly different. However, diminished phagocytic activity for unopsonised SRBC was observed in macrophages from all the food-restricted groups.

The bactericidal ability of macrophages from the food-restricted and control birds was also examined against opsonised *E. coli*. Macrophages from all the control groups internalised *E. coli* quite efficiently within 15 minutes of feeding time (Table 3). However, macrophages from control birds of groups

Table 1. Incidence of macrophages in food-restricted Sephadex-stimulated birds

	Percentage of macrophages			
Treatment	Group A	Group B	Group C	Group D
Food-restriction	26.75 d	39.93 c	24.10 d	31.16 cd
Control	63.21 b	76.96 a	77.01 a	62.00 b

Values sharing at least a letter in common are statistically non-significant ($P<0.05$).

B, C and D exhibited maximum killing of internalised bacteria than macrophages from group A. Macrophages from group A control birds killed fewer bacteria as compared with other groups. However, in food-restricted birds, this difference could not be seen. Macrophages from groups A and B food-restricted birds were found statistically non-significant when compared with *ad libitum* fed control birds in terms of their bactericidal activity.

and the influence of exposure to food-restricted immunosuppression on these responses.

The current study demonstrates evidence of food-restricted versus control differences in macrophage functions. As previously reported for 5-week-old White Leghorn x Rhode Island Red chicks (Ben-Nathan *et al.*, 1981), major differences in macrophage functional competence were evident in food-restricted and control birds.

Table 2. Phagocytic potential of macrophages from food-restricted Sephadex-stimulated birds

Treatment	Phagocytic macrophages (%)			
	Group A	Group B	Group C	Group D
	Opsonised			
Food-restriction	15.18 e	17.29 e	14.55 e	14.27 e
Control	55.02 c	81.35 a	67.35 a	70.29 b
	Unopsonised			
Food-restriction	17.16 e	14.51 e	16.99 e	12.61 e
Controls	31.05 d	67.73 b	65.19 b	51.16 c

Values sharing at least a letter in common are statistically non-significant ($P<0.05$).

DISCUSSION

The purpose of the present study was to evaluate the effects of food-restriction on macrophage functions in order to test the hypothesis that food-restriction alters the macrophage functions that play a role in non-specific immunity. Macrophages provide several important functions in response to T-cell dependent, particulate antigen like SRBC (Weaver & Unanue, 1990). The present study describes the cellular inflammatory response and macrophage activation that occurs in the peritoneal cavity following the injection of 3% Sephadex G-50

Statistically, significant differences were observed in the incidence of macrophages in the inflammatory exudate as well as their functional competence. Birds from all food-restricted groups produced relatively fewer macrophages in the inflammatory exudate cells (Table 1) with significantly reduced phagocytic activity against opsonised and unopsonised SRBC as compared with control groups (Table 2). This suppression may be due to existence of stress (Maxwell *et al.*, 1990 a, b) or to macrophage- and T cell antibody response to SRBC (Davies & Safe, 1988).

Table 3. Bactericidal potential of food-restricted Sephadex-stimulated birds

Treatments	Bacteria killed within 15 minutes (%)			
	Group A	Group B	Group C	Group D
Food-restriction	14.66 efghi	25.33 bcde	17.33 efgh	18.66 efg
Control	21.33 def	32.00 abc	34.667 ab	38.66 a

Values sharing at least a letter in common are statistically non-significant ($P<0.05$).

Differences were also observed in the bactericidal activity of macrophage. Macrophages from all the food-restricted birds displayed diminished bactericidal activity compared with their respective *ad libitum* fed control birds (Table 3). This decrease in microbicidal activity of macrophages from food-restricted birds may be attributed to decrease in their lysosomal contents, however, the mechanism(s) whereby food-restriction decreases the cellular responses is(are) not known. This suggests that stress induced by food-restriction may reduce the production or potency of chemoattractants that are responsible for the influx of phagocytic cells to the antigen.

Since the rapid influx of phagocytic cells to the site of pathogen invasion is an important factor in resistance to infection, the results of this study, therefore, suggest that food-restriction affects birds resistance. However, since food-restriction at the same time, is immunosuppressive, and also decreases the specific immune responses, the overall input of food-restriction on disease susceptibility is likely to vary with different pathogens and their mode of clearance. Thus, the results of the present study may help to explain some of the disparate effects of food-restriction in different host-resistant mechanisms.

REFERENCES

Akhtar, M., M. Ashfaque, H. Afzal and M. Afaq. 1991. Comparison of Congo Red and biological tests in differentiating enteropathogenic *Escherichia coli*. J. Anim. Plant Sci. 1: 63-66.

Ben-Nathan, B., N. Drabkin and D. Heller. 1981. The effect of starvation on the immune response of chickens. Avian Dis. 25: 214-217.

Davies, D. and S. Safe. 1988. Immunosuppressive activities of polychlorinated dibenzofuran congeners: Quantitative structure-activity relationships and interactive effects. Toxicol. App. Pharmacol. 94: 141-149.

Desiderio, J.V. and S.G. Campbell. 1983. Intraphagocytic killing of *Salmonella typhimurium* by liposome-encapsulated cephalothin. J. Infect. Dis. 148: 563-570.

Durum, S.K., J.A. Schmidt and J.J. Oppenheim. 1985. Interleukin 1: An immunological perspective. Ann. Rev. Immunol. 3: 263-287.

Maxwell, M.H., G.W. Robertson, S. Spence and C.C. McCorquodale. 1990 a. Comparison of haematological values in restricted and *ad libitum*-fed domestic fowls. I. White blood cells and thrombocytes. Brit. Poult. Sci. 31: 399-405.

Maxwell, M.H., G.W. Robertson, S. Spence and C.C. McCorquodale. 1990 b. Comparison of haematological values in restricted and *ad libitum*-fed domestic fowls. II. Red blood cell characteristics. Brit. Poult. Sci. 31: 407-413.

Pearson, A.R. and D.W.F. Shannon. 1979. Controlled Feeding Systems. Food Intake Regulation in Poultry. (Boorman, K.N. & B.M. Freeman, eds.), Edinburgh, Brit. Poult. Sci., pp: 365-390.

Qureshi, M.A., R.R. Dietert and L.D. Bacon. 1986. Genetic variation in recruitment and activation of chicken peritoneal macrophages. Proc. Soc. Exp. Biol. Med. 181: 560-568.

Qureshi, M.A., R.R. Dietert and L.D. Bacon. 1988. Chemotactic activity of chicken blood mononuclear leukocytes from $15I_5$-*B*-congenic lines to bacterially derived chemoattractants. Vet. Immunol. Immunopathol. 19: 351-360.

Sabet, T., W.C. Hsia, M. Stanisz, A. El-Domeiri and P.V. Alten. 1977. A simple method for obtaining peritoneal macrophages from chickens. J. Immunol. Methods, 14: 103-110.

Trembicki, K.A., M.A. Qureshi and R.R. Dietert. 1984. Avian peritoneal exudate cells: A comparison of stimulation protocols. Dev. Comp. Immunol. 8: 395-402.

Weaver, C.T. and E.R. Unanue. 1990. The costimulatory function of antigen presenting cells. Immunol. Today, 11: 49-55.