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SUPPRESSION OF CELLULAR IMMUNE RESPONSE OF CHICKENS FOLLOWING *IN VIVO* AND *IN VITRO* HEAT STRESS

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SUMMARY

Two experiments were conducted to study the effects of heat stress *in vivo* and *in vitro* on lymphocyte proliferation of 6-weeks-old Arbor Acres chicks. In the first experiment, the heat stressed (HS) birds were exposed to 42°C for 2 hrs vs. 24°C for control (C) group. The lymphocytes were collected from both groups at 0, 3, and 24 hrs post heat stress to measure mitogenic activity. In general, heat stress caused decline in proliferation of T and B lymphocytes at all times post heat exposure. At 3 hrs post exposure, the proliferation of T and B lymphocytes declined significantly ($P<0.01$). On the other hand, at 24 hrs,the T-cells and not B-cells had significantly ($P<0.05$) lower activity than those of C group. In second experiment, the splenic lymphocytes were collected and divided into four groups, the first was the control group and was exposed to 41°C, the other groups were exposed to 45°C for 1, 3 or 6 hrs. The proliferation of lymphocytes decreased as the period of *in vitro* heat exposure increased. The proliferation of male T-lymphocytes and female B-lymphocytes were reduced significantly by exposure to 45°C for 6 hrs when compared to controls.

Keywords: Chicken, heat stress, Con. A, pokweed, lymphocyte

INTRODUCTION

The high environmental temperature is one of the critical stressful conditions when the high density-open side poultry houses are exposed during the summer months, this unfavorable condition can impedes the performance and immune response of poultry. Environmentally stressed poultry generally have depressed humeral immune response (Thaxton *et al.*, 1968; Atta, 1990, Siegel and Latimer, 1984). Furthermore phagocytic potential of chicken macrophages is decreased during heat stress condition *in vitro* (Miller and Qureshi, 1992). Profiles of circulating leukocytes are also affected (Gross and Siegel, 1973; Trout and Mashaly, 1994).

In chickens, environmental or behavioral stressors causes an increase in pituitary-adrenal gland activity, and, as a consequence, are increase in plasma corticosteroid levels is responsible for producing immunosuppression (Thaxton and Siegel, 1970; Gross and Sirgel, 1973; and Regnier and Kelley, 1981). These immunosuppression in vivo has been attributed to an increase in the incorporation of endogenously produced corticosterids into lymphoid cells (Gould and Siegel, 1981, Siegel and Gould, 1982), which in turn, causes the suppression of cell proliferation factors or

Interleukin II (Gillis *et al.*, 1979; Farrar *et al.*, 1980). The present study was conducted to examined spleen lymphocytes proliferation following *in vivo* and *in vitro* heat stress.

MATERIALS AND METHODS

Experimental animals:

Two experiments were conducted in this study using Arbor Acres chicks, commercial starter ration and water were provided *ad libitum*. The birds received 24 hrs of light for the first three days of age thereafter they were exposed to 23 hrs of light and one hr of darkness. All birds were vaccinated at seven days of age against Newcastle disease with B1 strain vaccine, and were revaccinated at 21 days of age with Lasota strain vaccine, and against infection bursal disease at 15 days of age.

***In vitro* lymphocyte proliferation assay:**

The spleen were removed, processed by removing the capsule and expressing the tissue through a 60-mesh screen and than 300-mesh screen with 8 ml RPMI 1640 media, the suspension were then layered onto histopaque and centerifugat at 1000 rpm for 30 min. Following centrifugation, the leukocyte cells were collected, washed twice with large-volume of RPMI 1640. Splenic lymphocyte were plated $5*10^5$ cells per well in 96 well microtiter plates. Mitogen stimulation was carried out by incubating the cells for 48 hours at 41°C under 5% Co_2 with concanavalin A (con .A), T-lymphocyte mitogen (2.5µg/well) or pokweed mitogen (PWM), a B-lymphocyte mitogen (a dilution of 1:10 of reconstituted stock prepared according to package instructions). Following incubation, 50 µl of H^3-thymidine (1 µCi) was added to each wells and plates were incubated for an additional 18 hrs. The cells were then harvest onto glass fiber filter papers. The filters were placed in scintillation fluid and radio activity was counted using a scintillation counter.

First experiment: (Heat stress *in vivo*)

At six weeks of age, 42 male chicks were divided into two groups, the first was heat stressed group (HS) and were subjected to 42°C for two hrs, while the second was control group and were subjected to 24°C. The spleen were removed and lymphocyte were collected from both group at 0, 3 and 24 hrs post stress to study the effect on *in vivo* heat stress on the lymphocyte activity.

Second experiment: (Heat stress *in vitro*)

Ten males and ten females chicks were slaughtered at 6 weeks of age and splenic lymphocytes were collected and divided into four groups, the first is the control group and was exposed to 41°C, the other groups were exposed to 45°C for 1, 3 or 6 hrs. After the *in vitro* heat stress was done, the lymphocyte proliferation assay was carried out.

Statistical analysis of data:

The data were analyzed using General Linear Models Procedure adapted by SAS (1988) for P.C. users, with a one-way ANOVA model was used in the first experiment, and two-ways ANOVA model was used in the second experiment using heat stress and sex as main effects. Where appropriate, means were separated using Duncan's multiple range test.

RESULTS

First experiment:

The effect of heat exposure (*in vivo*) on mitogenic activity (proliferation) of lymphocytes, as measured by H^3-thymidine uptake (cpm) are shown in Table 1. Generally, heat stress caused a decline in proliferation of both T and B lymphocytes at all times post heat exposure. There were no significant differences between splenic lymphocyte proliferation of HS group that were collected at 0 hr post heat exposure and those of C group, but after three hrs post exposure the proliferation of T and B lymphocytes declined sharply with highly significant differences ($P<0.01$). On the other hand, after 24 hrs post exposure, the lymphocyte proliferation of HS group increased to more than three folds as in their counterparts at three hrs. But T cells still significantly had lower activity than those of C group, whereas the activity of B-cells didn't significantly differ between the two groups.

Table 1. Effect of in vivo heat stress on proliferation of con.A and PWM stimulated splenic lymphocytes of 6-wk-old Arbor Acres broiler male chicks

Hrs. post heat	Con. A		PWM	
	Heated	Control	Heated	Control
0	10877±1656a	13186±1912a	4094±817a	5043±944a
3	2317±1205A	13290±1520B	1441±611A	5155±723B
24	7811±1369a	13331±1836b	4855±518a	5143±656a

a,b,A,B values are means (cpm)±SE within time and within mitogen stimulant with different superscripts are significantly different
a,b ($P<0.05$); A,B ($P<0.01$).

Second experiment:

The effect of *in vitro* heat exposure on lymphocyte proliferation of both male and female chicks are shown in Figure 1. The results indicate that the proliferation of both T and B splenic lymphocytes is inversely proportional to the length of *in vitro* heat exposure period. The proliferation of male T-lymphocytes and female B-lymphocytes were reduced significantly ($P<0.05$) when were exposed to 45°C for 6 hrs than their counterparts that were not exposed to high temperature (control group). On the other hand, the male had more active T and B lymphocyte than those of females.

DISCUSSION

The results of the first experiment confirm the observation of Thaxton and Siegel (1970); Regnier and Kelly (1981), and Siegel and Latimer (1984), that heat stress is one of the environmental stressor having an immunesupperssive effect. This phenomena attributed to the increase in plasma corticosteroid hormone in response to heat stress exposure (Ben Nathan *et al.*, 1976; Siegel and Latimer, 1984). The mechanisms through which corticosteroid suppresses immune activities appear to

function on at least two levels. The first of these is that heat stress increase the incorporation of endogenously produced corticosterone onto lymphoid cells (Gould and Siegel, 1981; and Siegel and Gould, 1982), which in turn, causes the suppression of Interleukin II (Gillis *et al.*, 1979, Farrar *et al.*, 1980). A second mechanism that has been studied in mammalian system is that glucocorticoid initiates programmed thymocyte death, which involves a calcium mediated endonuclease activation resulting in the fragmentation of the cellular DNA leading to cell death (Cohen and Duke, 1984). In chicken this process is also induced in the bursa by elevated glucocorticoid levels (Compton *et al.*, 1990).

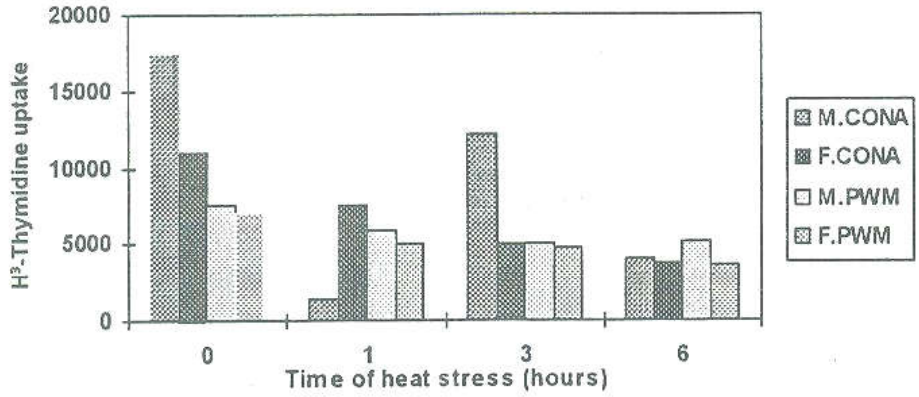

Fig. 1. Interaction of sex and *in vitro* heat stress time on proliferation of Con.A and PWM stimulated splenic lymphocytes of 6-wk-old *Arbor acres* broiler chicks.

A sharp decline in lymphocyte proliferation after 3 hrs post exposure, whereas after 24 hrs increased to almost normal rate is attributed to the high level of endogenous corticosteron that according to Ben Nathan *et al.* (1976), increased markedly following exposing the birds to 42°C for 1 hr and remained high for 5 hrs, then decreased gradually to almost normal values after 20 hrs.

The results from the *in vitro* heat stress indicate that the high level of endogenous corticosterone is not the only cause for immune suppression but there is also a direct effect of heat stress on lymphocyte activity. This phenomena may be attributed to the decline in Interleukin II production (Reginier and Kelly, 1981), which is needed for lymphocytes proliferation. Furthermore, heat stress could be killing lymphocytes by activating a synthesize the stress proteins all the way up to the point of cell death (cellular suicide) (Welch, 1992). The results of the *in vitro* heat stress are consistent with those reported by Miller and Qureshi (1992) who reported that phagocytic potential of chicken macrophages is decreased during heat stress condition *in vitro*. The present results indicate that heat stress suppress the activity of T and B

lymphocytes which responsible for the cell mediated and humeral immune response which in turn explain the reasons of outbreak and spread of the epidemic diseases in high density-open side poultry houses during the summer months.

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كبت نشاط الخلايا المناعية فى الدجاج عقب التعرض للإجهاد الحرارى (دراسات داخل وخارج الجسم)

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أجريت تجربتان لدراسة تأثير الإجهاد الحرارى (داخل وخارج الجسم) على نشاط وتوالد كرات الدم الليمفاوية لكتاكيت الأربوراكرز عمر ٦ أسابيع. فى التجربة الأولى تم تعريض كتاكيت مجموعة الإجهاد لـ ٤٢°م لمدة ساعتين مقابل ٢٤°م لمجموعة المقارنة. وتم جمع كرات الدم الليمفاوية من الطحال فى كلا المجموعتين عند صفر، ٣، ٢٤ ساعة عقب التعرض للإجهاد الحرارى بغرض دراسة نشاطها المناعى. وقد وجد أن الإجهاد الحرارى بصفة عامة أحدث إنخفاض فى توالد كل من كرات الدم الليمفاوية B,T . وقد لوحظ أن الإنخفاض كان معنوياً بعد ٣ ساعات من التعرض للإجهاد الحرارى لكلا النوعين من الخلايا الليمفاوية ، ولكن بعد ٢٤ ساعة من التعرض لهذا الاجهاد كان نشاط كرات الدم الليمفاوية T فقط منخفض معنويا عن مثيلاتها فى المجموعة المقارنة .

فى التجربة الثانية جمعت كرات الدم الليمفاوية من الطحال وقسمت الى ٤ مجاميع وكانت الأولى هى محموعة المقارنة حيث عرضت لدرجة ٤١°م ، أما المجاميع الاخرى فلقد تم تعريضها لدرجة ٤٥°م لمدة ساعة واحدة او ٣ أو ٦ ساعات. فلوحظ فى هذه التجربة أن نشاط وتوالد كرات الدم اليمفاوية يقل كلما ازدادت مدة التعريض للاجهاد الحرارى (خارج الجسم).

وكان هذا الانخفاض معنويا فى نشاط كرات الدم اللميفاوية T الخاصة بالذكور وأخرى B الخاصة بالإناث وذلك بعد تعرضها لدرجة ٤٥°م لمدة ٦ ساعات مقارنة لمجموعة المقارنة.