ORIGINAL ARTICLE

Effect of dexamethasone on differential white blood cell counts and heterophil / lymphocyte ratio in Japanese quails (*Coturnix coturnix* japonica)

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Abstract

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Laying Japanese quails (n = 60), 30 weeks of age reared at the experimental laboratory unit of the Faculty of Technology, Maha Sarakham University, Maha Sarakham province, Thailand. Birds were in cages with wire floors. Randomized Complete Block was the design of the experiment. During the first 4 days of experimental period, quails were fed on a standard commercial diet with four treatments: supplemented with dexamethasone at 4 levels namely 0 (control group), 1.25, 2.50 and 5.00 mg/kg diet. On days 1, 3, 7, 10 and 14 of the experimental period, percentage of heterophil, lymphocyte, monocyte, basophil, eosinophil, and heterophil/ lymphocyte ratio values of laying quails were examined. The results revealed that percentage of heterophil of laying quail with added dexamethasone at 1.25 and 2.50 mg/kg in diets were significantly higher than others (P<0.05). Percentage of lymphocyte of Japanese quails receiving dexamethasone at 1.25 and 2.50 mg/kg was significantly lower than birds receiving dexamethasone at 0 and 5.00 mg/kg (P<0.05). Heterophil: lymphocyte ratio of laying birds supplemented with dexamethasone at 1.25

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and 2.50 mg/kg in diets was significantly higher than that of laying quail that added dexamethasone at 0 and 5.00 mg/kg in diets (P<0.05). The percentage of monocyte, basophil and eosinophil after receiving dexamethasone each levels were not significantly different (P>0.05). After discontinuing dexamethasone administration on day 4 of the experimental period, percentage of heterophil, lymphocyte and eosinophil, monocyte, and heterophil: lymphocyte ratio, of quails recovered to normal within 10 days.

Key words : dexamethasone, differential white blood cell counts, heterophil / lymphocyte ratio, Japanese quail (*Coturnix coturnix* japonica)

บทคัดย่อ

วรพล เองวานิช และ อรวรรณ ชินราศรี

ผลของเด็กซาเมทาโซนต่อค่าเปอร์เซ็นต์เม็ดเลือดขาวชนิดต่าง ๆ และอัตราส่วนระหว่างเปอร์เซ็นต์เม็ดเลือดขาวชนิดเฮทเทอโรฟิลต่อลิมโฟไซต์ในนกกระทาญี่ปุ่น

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เลี้ยงนกกระทาญี่ปุ่นอายุ 30 สัปดาห์จำนวน 60 ตัวที่กำลังให้ไข่ ในกรงพื้นลวดในห้องปฏิบัติการทดลองของคณะเทคโนโลยี มหาวิทยาลัยมหาสารคาม วางแผนการทดลองแบบบล็อคสมบูรณ์ ในวันที่ 1-4 ของการทดลองให้นกกระทากินอาหารสูตรมาตรฐานที่ผลิตในเชิงการค้า แล้วเติม dexamethasone 4 ระดับได้แก่ 0 (กลุ่มควบคุม) 1.25 2.50 และ 5.00 มก./กก. ของอาหาร ทำการตรวจหาค่าเปอร์เซ็นต์เม็ดเลือดขาวชนิดเฮทเทอโรฟิล ลิมโฟไซต์ โมโนไซต์ แบโซฟิล อีโอซิโนฟิล และอัตราส่วนระหว่างเปอร์เซ็นต์เม็ดเลือดขาวชนิดเฮทเทอโรฟิลต่อลิมโฟไซต์ ในวันที่ 1 3 7 10 และ 14 ของการทดลอง ผลการศึกษาพบว่าค่าเปอร์เซ็นต์เม็ดเลือดขาวชนิดเฮทเทอโรฟิลของนกกระทาที่เติม dexamethasone ที่ระดับ 1.25 และ 2.50 มก./กก. ของอาหาร มีค่าสูงกว่านกกระทาที่ได้รับ dexamethasone ในกลุ่มควบคุมและกลุ่มที่ได้รับที่ระดับ 5.00 มก./กก. ของอาหาร (P<0.05) ค่าเปอร์เซ็นต์เม็ดเลือดขาวชนิดลิมโฟไซต์ของนกกระทาที่เติม dexamethasone ที่ระดับ 1.25 และ 2.50 มก./กก. ของอาหาร มีค่าต่ำกว่านกกระทาที่ได้รับ dexamethasone ในกลุ่มควบคุมและกลุ่มที่ได้รับที่ระดับ 5.00 มก./กก. ของอาหาร (P<0.05) ค่าอัตราส่วนระหว่างเปอร์เซ็นต์เม็ดเลือดขาวชนิดเฮทเทอโรฟิลต่อลิมโฟไซต์ของนกกระทาที่เติม dexamethasone ที่ระดับ 1.25 และ 2.50 มก./กก. ของอาหาร มีค่าสูงกว่านกกระทาที่ได้รับ dexamethasone ในกลุ่มควบคุมและกลุ่มที่ได้รับที่ระดับ 5.00 มก./กก. ของอาหาร (P<0.05) ส่วนค่าเปอร์เซ็นต์เม็ดเลือดขาวชนิดโมโนไซต์ แบโซฟิล และอีโอซิโนฟิลของนกกระทาที่ได้รับ dexamethasone ทุกระดับมีค่าไม่แตกต่างกัน (P>0.05) และหลังจากหยุดเติม dexamethasone ในอาหารในวันที่ 4 ของการทดลอง พบว่าค่าเปอร์เซ็นต์เม็ดเลือดขาวชนิดเฮทเทอโรฟิล ลิมโฟไซต์ โมโนไซต์ แบโซฟิล อีโอซิโนฟิล และอัตราส่วนระหว่างเปอร์เซ็นต์เม็ดเลือดขาวชนิดเฮทเทอโรฟิลต่อลิมโฟไซต์จะกลับคืนมาสู่ระดับปกติภายในเวลา 10 วัน

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Stress is based in agricultural animals quantified probably by several variables together. Performance alone does not reflect stressful conditions completely. Plasma glucocorticoid concentration may be one indicator of stress, but circadian rhythms, age, and reproductive and emotional status can influence values (Widowski *et al*., 1989). Forty different stressors caused poultry to stress (Puvadolpirod and Thaxton, 2000) including transportation (Mitchell and

Kettlewell, 1998), overcrowing (Patterson and Siegel, 1998), beak trimming and coccidiosis (Mckee and Harrison, 1995), and temperature (Arjona *et al*. 1998; May and Lott, 1992; Mckee and Harrison, 1995; Mckee *et al*., 1997; Yahav *et al*., 1997 and Sandercock *et al*., 2001) and infection.

When birds exposed to a stressor they become excited, the corticosterone located is in the adrenal cortex is released into the blood circulation to help them increase metabolism (Richard, 1998). Harmon (1998) reported that glucocorticoid administration results in a lymphopenia and an increase in circulating heterophils in the chicken. Therefore, it appears that chickens have a "stress leukogram" similar to that of mammals. Heterophil/ lymphocyte ratio and concentration of plasma corticosteroides do not correlate well. The heterophil/ lymphocyte ratio measures a physiological change in organs such as an atrophy of the bursa of fabricius and thymus that is influenced by the effect of corticosteroids (Daghir, 1995), while corticosteroids cause the release of heterophils from hemopoeitic system through the blood circulation (Jain, 1993) Thus, the heterophil/ lymphocyte ratio was reported to be a better measure for long-term change, and the concentration of glucocorticoids a better measure for short-term change (Gross and Siegel, 1983).

Japanese quail (*Coturnix coturnix* japonica) populations have increased appreciably in Thailand in the past decade, because these quail can provide high egg yield. Besides, this bird is appropriate for rearing in many parts of Thailand. At present, we have a little knowledge about the physiological stress in Japanese quails. Therefore, the purpose of this study was to determine the effect of ingesting diets having various dexamethasone concentrations on percentage of heterophil, lymphocyte, monocyte, basophil, eosinophil and heterophil: lymphocyte ratio changes in this birds. The knowledge of this effect may be useful for diagnosis, be fundamental for solving problems, and provide believable information for other studies on stresses in Japanese quail.

Materials and Methods

Animals: Sixty healthy laying Japanese quails from commercial flock, 30 weeks of age, were reared at the experimental laboratory unit of the Faculty of Technology, Maha Sarakham University, Maha Sarakham province, Thailand. Birds placed in cages with a wire floor (three floors).

Experimental design: Randomized complete block was the design of the experiment. Floor was a block and treatment randomized into within block. Experiments began after 7 days adaptation. On day 1 of the experimental period, birds received a standard feeding of commercial diets (22% CP, 3,200 Kcal/Kg ME) with four treatments. Group one, group two, group three and group four of laying quail received dexamethasone at 0 (control group), 1.25, 2.50, and 5.00 mg/kg in their diets, respectively. Continuous light and water were provided ad libitum throughout the experiment. Dexamethasone was withdrawn from their diets on day 4 of the experimental period.

Differential white blood cell counts: On days 1 (before added dexamethasone in their diets), 3, 7, 10 and 14 of the experimental period, blood samples (via wing vein: 0.10 ml) of five birds per treatment was collected and transferred to tubes containing EDTA as an anticoagulant. Blood films were made, air dried then stained with Giemsa - Wright's stain. Differential WBC counts were performed by using standard avian guidelines of Ritchie *et al*. (1994). Heterophil/lymphocyte ratios were calculated.

Statistical analysis: Percentage of heterophils, lymphocytes, monocytes, basophils, and eosinophils, and heterophil/ lymphocyte ratio values of laying quails were analyzed by using repeated measurement procedure of Statistical Analysis System (SAS, 1990). Differences among means were identified by Duncan's multiple range tests (Duncan, 1955). The level of significance was determined at $P < 0.05$.

Result and Discussion

The effects of dexamethasone on differential white blood cell counts are presented in Table 1. The pattern of white blood cell parameters after receiving dexamethasone on day 1 and discontinuing on day 4 of the experimental period are show in Table 2 and Figure 1.

Heterophilia and lymphopenia were occurred in this study were similar to the neutrophilia and lymphopenia of mammals receiving glucocorticoid in the report of Jain (1993). He suggested that glucocorticoid cause neutrophilia primarily by inducing the increased release of neutrophils from the bone marrow reserve through the circulation. Associated changes in the differential leukocyte count include lymphopenia. Glucocorticoid-induced lymphopenia is attributed to lympholysis in blood and lymphoid tissue, and increased shift of lymphocytes from blood to other body compartments, or both. However, the eosinopenia and monocytopenia of quails in this study were not clear by using total mean values, because these values were not significantly different. Both

Table 1. Percentage of white blood cell counts in experimental laying quail supplemented with dexamethasone of 4 levels.

Parameter	Dexamethasone levels				Mean	SEM
	0mg/kg	1.25mg/kg	2.5mg/kg	5mg/kg		
Heterophil (%)	47.39[b]	52.67[a]	51.36[a]	45.75[b]	49.29	2.63
Lymphocyte (%)	48.21[a]	41.27[b]	42.50[b]	47.83[a]	44.95	3.13
H/ l ratio	1.07[b]	1.40[a]	1.35[a]	1.09[b]	1.23	0.13
Monocyte (%)[ns]	2.43	2.03	2.70	2.70	2.46	0.70
Basophil (%)[ns]	1.79	2.41	2.44	1.93	2.15	0.68
Eosinophil (%)[ns]	1.81	1.66	1.66	1.58	1.68	0.75

[a and b] **within row, means with no common superscript differ significantly ($P \leq 0.05$) ;**
[ns] **not significantly different ($P > 0.05$) ; SEM = standard error of the mean**

Table 2. Differential white blood cell counts and heterophil/ lymphocyte ratio of Japanese quails change after receiving dexamethasone on day 1 and discontinuing on day 4 of the experimental period.

Parameter	Days of differential WBC counts change					Mean	SEM
	Day1[nv]	Day3	Day7	Day10	Day14		
Heterophil (%)	41.26[c]	63.18[a]	49.38[b]	51.72[b]	40.92[c]	49.29	2.63
Lymphocyte (%)	50.87[a]	34.82[c]	43.65[b]	42.00[b]	53.42[a]	44.95	3.13
H/ l ratio	0.83[c]	2.06[a]	1.16[b]	1.26[b]	0.79[c]	1.23	0.13
Monocyte (%)	3.49[a]	0.88[c]	3.23[a]	1.93[b]	2.78[ab]	2.46	0.70
Basophil (%)[ns]	1.99	2.72	2.26	2.07	1.68	2.15	0.68
Eosinophil (%)	2.41[a]	1.00[b]	1.24[ab]	2.42[a]	1.33[ab]	1.68	0.75

[a, b and c] **within row, mean with no common superscript differ significantly ($P \leq 0.05$);** [ns] **not significantly different ($P > 0.05$);** [nv] **normal value of laying quail before administering dexamethasone; SEM = standard error of the mean**

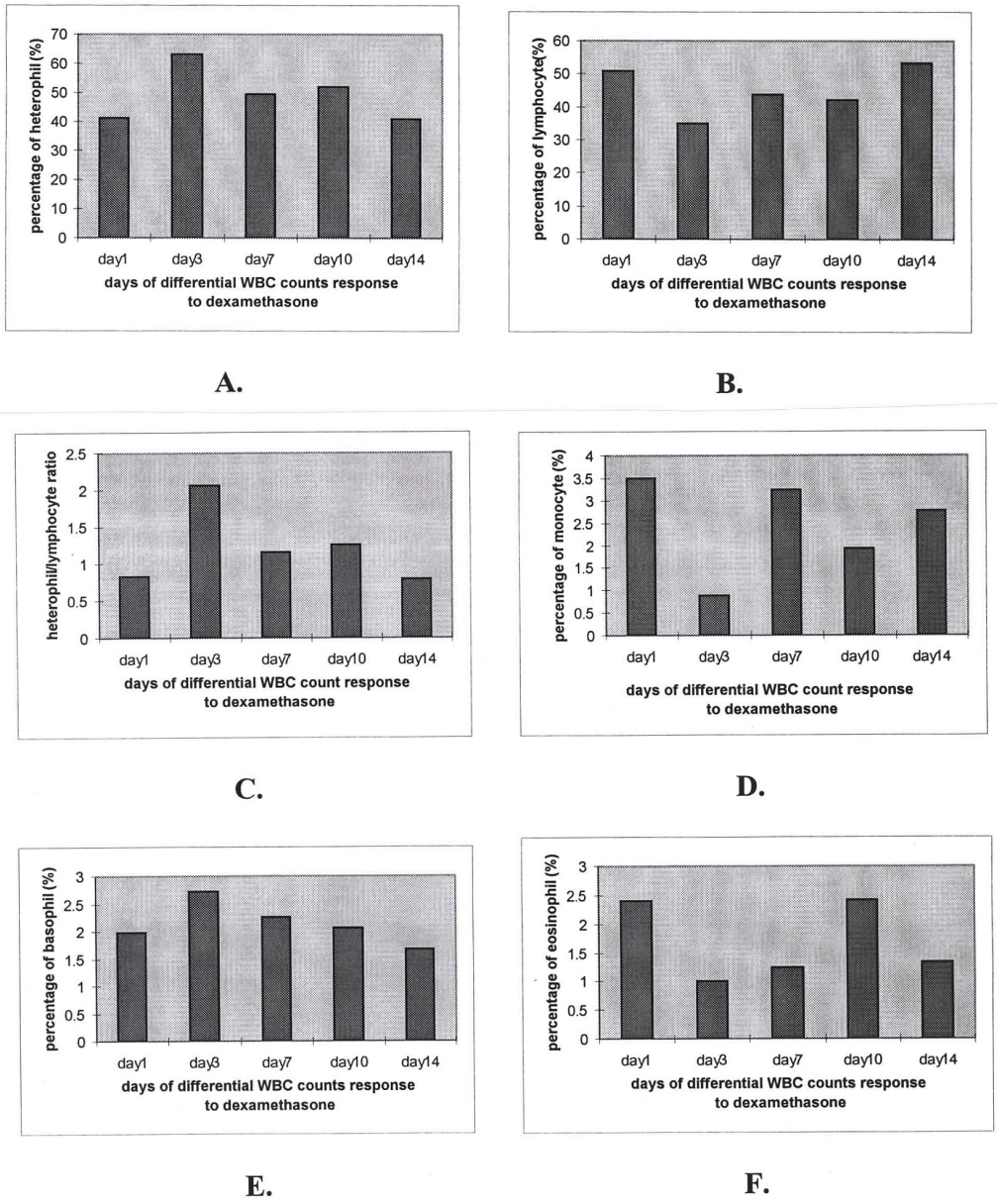

Figure 1. Differential white blood cell counts and heterophil/lymphocyte ratio pattern of Japanese quails after receiving dexamethasone on day 1 and discontinuing on day 4 of the experimental period.

leukocytopenia could be explained by differential white blood cell count pattern in Table 2.

The heterophil/lymphocyte ratio of quails after receiving dexamethasone increased. This was in accordance with the report of Siegel (1995). He suggested that elevation of plasma glucocorticoid and increasing of circulating heterophil/lymphocyte ratio, are the two most accepted indicators of the stress condition in birds. Besides, heterophil/lymphocyte ratio of quails that receiving various dexamethasone concentrations was significantly different. However, dexamethasone

had no effect on heterophil/ lymphocyte ratio in quail after adding this glucocorticoid over 5.00 mg/kg in the diet (Table 1). This occurrence could not be explained.

Jain (1993) suggested that eosinopenia and monocytopenia were developed after stress that attributed to evaluate levels of catecholamines, or administration of corticosteroids. Mechanism of corticosteroid-induced eosinopenia remains to be established unequivocally. Various mechanisms have been proposed, including decreased bone marrow release, intravascular lysis, reversible sequestration in organs rich in the mononuclear phagocyte system, and increased migration in tissues. In this study, after discontinuing dexamethasone administration on day 4 of the experimental period it was found that both heterophil and lymphocyte count of Japanese quails recovered to the normal levels within 10 days.

Puvadolpirod and Thaxton (2000) reviewing research articles found that a variety of stressors has been used to study stress responses in poultry species. These stressors include mediation of the adrenal gland directly by exogenous administration of adrenocorticotropin (ACTH) and exogenous administration of steroid moieties, including corticosterone, cortisone, cortisol, deoxycorticosterone, and dexamethasone. The present study was conducted to determine the minimum effect dose of dexamethasone that will evoke physiological stress in Japanese quail. Our results showed that laying quails receiving dexamethasone at 1.25 mg/kg in the diet should a marked response including increased percentage of heterophil and heterophil/ lymphocyte ratio; and decrease percentage of lymphocyte. This suggests that, in control situations, an increase in percentage of heterophil, heterophil: lymphocyte ratio and decrease of percentage of lymphocyte are reliable indicators of the quail's response to stress.

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