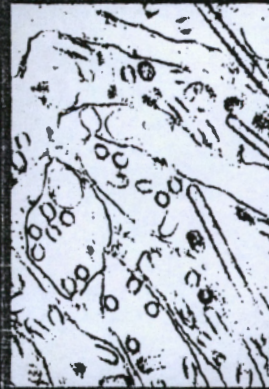




**FACULTY OF VETERINARY MEDICINE
UNIVERSITAS GADJAH MADA**

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International Seminar on Zoonotic and Tropical Disease

**ONE WORLD ONE HEALTH CHALLENGE:
GLOBAL MOVEMENT
ON ZOOONOTIC DISEASE**

**Friday - Saturday, 26 - 27th June 2009
Hotel Saphir Yogyakarta**

P R O C E E D I N G S

**PROCEEDING
INTERNATIONAL SEMINAR ON ZOO NOTIC AND TROPICAL DISEASE**

One World One Health™ Challenge: Global Movement on Zoonotic Disease

Yogyakarta, 26-27th June 2009

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FACULTY OF VETERINARY MEDICINE
UNIVERSITAS GADJAH MADA
2009

PROCEEDING
INTERNATIONAL SEMINAR ON ZONOTIC AND TROPICAL DISEASE
**One World One Health™ Challenge:
Global Movement on Zoonotic Disease**

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Comparison Level of Cortisol and Ratio of Neutrophil/Lymphocytes as Acute Stress Marker to Long Road Transportation of Bligon Bucks

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ABSTRACT

Transportation of animal is generally recognized as a stressful event and related to immune system. The purpose of this study is to compare the pattern of cortisol secretion during transportation and ratio Neutrophil/Lymphocyte (N/L) as a stress marker. Six adult bligon buck ranging in body weight 26-30 Kg were used in this study. Two weeks prior to the experiment, the animals had been given anthelmintic Albendazole to eliminate egg worm. All of animals were fed standard ration in their pen at 10% from their body weight per head daily and commercial concentrate also was given everyday. All of the animals were transported around village for 16 hours starting from 18.00 pm until 10.00 am in open small truck (3 x 2 m); eye contact with each others was provided. Blood samples were withdrawn from jugular vein using vacutainer tubes containing heparin, transferred into 1.5 mL glass tubes in aliquot, and centrifuged at 500 xg for 15 minutes. Plasma was collected and stored at -20° C. The blood samples were collected every 4 hours from - 8 hours before until 8 hours after transportation at 10.00 am. Separate whole blood samples were used to measure number of Neutrophil and Lymphocyte. Plasma was harvested and stored at -20° C until cortisol concentrations were measured using ELISA. The result showed that transportation of Bligon bucks for 16 hours affects both level of cortisol ($P < 0.05$) and the ratio of N/L ($P < 0.05$). The pattern of cortisol and ratio of N/L is very similar. Furthermore, the peak of cortisol was obtained at 4 hours of transportation whereas ratio N/L was reached later namely 8 hours after transportation. It is concluded that ratio of N/L can be used as stress marker for Bligon goat even less sensitive than the use of level of cortisol.

Keywords: Cortisol, ratio Neutrophil/Lymphocyte, stress, transportation, Bligon

Introduction

Routine transportation of goats between farm and other cities moreover export to other countries for both slaughtering and breeder has been running almost everyday. The distance of transportation is vary start from 2 until 16 hours. Transported animals may be exposed to a variety of physical and psychology stimuli including crowding, noise, handling, isolation and extreme temperatures

(Al-Kindi et al. 2005). Usually transportation of animal is generally recognized as a stressful event and related to immune system. Even tremendous study on the effects of handling and transportation of cattle, pigs and poultry, little work has been carried out to assess the effect of transported local goat on cortisol as indicator of stress.

Up to now, measurement level of cortisol is the best way to determine both acute and

chronic stress. Unfortunately this process is very expensive and not practical, it would be looked for another methods using ratio of neutrophil/lymphocyte (N/L) which is much cheaper and practical. The aim of this study are to determine 1) level of cortisol during long transportation (16 hours) in Bligon goat; 2) ratio of neutrophil/lymphocyte; and 3) to compare pattern of cortisol secretion and ratio of N/L. This physiological parameters have been proposed as sensitive indices of physiological stress response in animals that encountered long-term welfare problems such as handling and transportation.

Materials and Methods

Animals: Six adult bligon bucks ranging in body weight 26-30 Kg were used in this study. Two weeks prior to the experiment, the animals had been given anthelmintic Albendazole to eliminate egg worm. All of animals were fed standard ransom in their pen at 10% from their body weight per head daily and commercial concentrate also given everyday. Fresh water was provided as *ad libitum*.

Treatment: All of animals were transported around village during 16 hours starting from 18.00 pm until 10.00 am in open small truck (3 x 2 m); eye contact each others would be provided. Blood samples were withdrawn from jugular vein using vacutainer tubes containing heparin was transferred into 1.5 mL glass, then centrifuged at 500 g for 15 minutes. Plasma was collected to be stored at -20° C until cortisol concentrations were measured using commercial ELISA (Enzyme Linked Immunosorbent Assay) kit product DRG, Germany. The blood was collected every 4 hours from - 8 hours before transportation (at 10.00 am, 14.00 pm and

18.00 pm) until the time arriving after transportation at 10.00 am.

ELISA for plasma hormone

concentration: The cortisol kit is a solid phase enzyme-linked immunosorbent assay based on the principle of competitive binding. The microtiter wells are coated with monoclonal antibody directed towards an antigenic site on the cortisol molecules. Endogenous hormone of sample competes with cortisol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation, the unbound conjugate was washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of hormone in sample. After addition of the substrate and stop solution, the intensity of color would be inversely to concentration of hormone. Concentrations of cortisol were determined in duplicate samples by EIA.

Statistical analysis: The differences in level of cortisol and ratio N/L were contrasted using ANOVA and continued by Tukey HSD test.

Results and Discussions

Plasma cortisol levels of bucks are given in Table 1 and Figure 1. until reached the peak at 161,9±78,9 ng/mL. Cortisol exhibited significant fluctuation ever time subject to pulsatile regulation by the episodic release of ACTH from the anterior pituitary and CRF (Corticotropin Releasing Factor) from hypothalamus (Liota *et al.* 1990; Litwack *et al.* 2003). Statistically, there was significant differences which found before and after transportation (P<0.05). Stress has been demonstrated to increase the number of CD4+ lymphocytes while decreasing CD8+ lymphocytes (Degabriele and Fell, 2001).

Table 1. Average±SD level of cortisol before and after transportation. Symbol O indicate the timing of transportation.

	Period of Transportation								
	-8	-4	0	4	8	12	16	20	24
Cortisol (ng/mL)	70,7±43,69	66,23±33,8	95,96±2,9	161,9±78,9	123,7±7,05	87,01±2,81	99,31±25,7	77,5±38,51	72,9±33,77

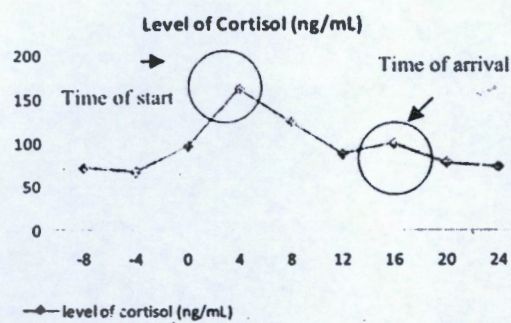


Figure 1. Level of cortisol (ng/mL) before and after transportation. Symbol 0 indicate the timing of transportation whereas 20 indicate timing of arrival.

Based on hormone profile, cortisol is fluctuated (figure 1) by time to time, start from the lowest at 4 hours before transportation namely $66,23 \pm 33,8$ ng/mL. Changes in gene expression due to glucocorticoids and catecholamines disregulate the immune system (Padgett and Glaser, 2003).

Table 2. Average \pm SD of ratio of Neutrophil/Lymphocyte before and after transportation. Symbol O indicate the timing of transportation.

Period of Transportation									
	-8	-4	0	4	8	12	16	20	24
Ratio of N/L	$0,87 \pm 0,40$	$0,85 \pm 0,30$	$0,74 \pm 0,51$	$1,43 \pm 1,13$	$1,67 \pm 0,57$	$1,43 \pm 0,92$	$1,12 \pm 0,33$	$1,4 \pm 0,68$	$0,79 \pm 0,38$

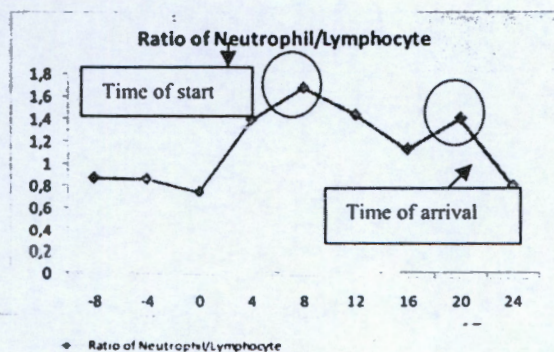


Figure 2. Ratio of N/L before and after transportation. Symbol 0 indicate the timing of transportation whereas 20 indicate timing of arrival

In this experiment, level of cortisol starting to rise at timing of transportation (0 hour) and reaching a peak at 4 hours after transportation ($P < 0.05$). It has been demonstrated that excessively high stocking

In this study, number of lymphocyte (data not shown) was getting decrease and the lowest number has been occurred at the peak of ratio N/L namely 8 hours after transportation, probably that CD8+ was dominated than CD4+ lymphocytes. In acute stress of boars due to transportation, ACTH rose quickly in response to a 5-min nose snare stressor reaching a peak at 5 min after the application of stress, while the peak of cortisol levels was found 15 min later (Weaver et al., 2000).

The initial period of transport is the most stressful time for animals (Knowles et al., 1995; Broom et al., 1996). On the other hand, transportation of Bligon bucks for 16 hours were decreased level of tetraiodothyronine (T4). Decreasing of T4 levels indicated conversion of T4 to T3 to form active hormone (Astuti et al, 2009 in press). Many factors contribute to stress during transport and each species has a different stress response to transport (Manteca and de la Torre, 1996).

density during transport, handling, leads to increased injury and stress (Tarrant, 1990; Cockram et al., 1996). Even many factors contribute to stress transportation, it would be predicted that handling is the most contributor to this experiment because of no high stocking density (is only 6 bucks in trucks), no heat stress (transportation was occurred evening to night) while increasing of cortisol level and ratio N/L has been done when the time of transportation and the time of arriving.

The pattern of cortisol and ratio of N/L is very similar. Furthermore, the peak of cortisol was got at 4 hours of transportation whereas ratio N/L was reached later namely 8 hours after transportation. It would be concluded that ratio of N/L can be used as stress marker for Bligon goat even less sensitive than level of cortisol.

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