



UNIVERSITI PUTRA MALAYSIA

***EVALUATION OF SERUM CORTISOL CONCENTRATION AND
NEUTROPHIL TO LYMPHOCYTE RATIO BEFORE AND AFTER
RECTAL PALPATION IN A SWAMP BUFFALO COWS
(*Bubalus bubalis carabanensis*)***

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A project paper submitted to the
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CERTIFICATION

It is hereby certified that we have read this project paper entitled “Evaluation of Serum Cortisol Level and Neutrophil to Lymphocyte Ratio Before and After Rectal Palpation in a Swamp Buffalo Cows (*Bubalus bubalis carabanensis*), by WeiweiCierra and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4901 – Project.

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DEDICATIONS

To Lord God Almighty my creator

My beloved family,

Daddy

Mummy,

Sisters,

Brother

And Pets,

Pipo, Snowy, Hunny, Bubu and Nana

Thank you for the unconditional love and endless support

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ABSTRAK

PENILAIAN KEPEKATAN SERUM KORTISOL DAN NISBAH NEUTROFIL KE LIMFOSIT SEBELUM DAN SELEPAS PALPASIPEREKTAL DALAM KERBAU

(Bubalus bubalis carabanensis)

by

Weiwei Cierra

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Palpasiperektal merupakan salah satu prosedur veterinar yang digunakan untuk diagnosis kebuntingan dalam kerbau. Walaubagaimanapun, prosedur ini mungkin menyakitkan dan mengakibatkan tekanan kepada haiwan. Tekanan adalah salah satu factor penting yang perlu dipertimbangkan apabila menilai kebajikan haiwan. Objektif kajian ini adalah menilai tindak balas tekanan semasa palpasiperektal dengan membandingkan kepekatan serum kortisol dan nisbah neutrophil kepada limfosit sebelum dan selepas palpasiperektal. Darah dikumpulkan dari urat koksigeal sebelum dan terus selepas palpitasirektum dari 3 tidak hamil dan 12 hamil kerbau berumur antara 5-8 tahun dengan berat 300 hingga 400 kg. Kepekatan serum kortisol dan nisbah neutrophil kepada limfosit telah ditentukan dengan menggunakan radio imun ocerakinan dan pengkamiran kiraan untuk menilai tindakbalas tekanan kepada proses urini. Hasilnya

menunjukkan bahawa terdapat peningkatan yang ketara dalam kepekatan serum kortisol berikutan palpasi perrektal dalam kumpulan kerbau hamil dan tidak hamil. Walaubagaimanapun, tiada perbezaan yang ketara antara kumpulan hamil dan tidak hamil dalam kerbau dari segi responsive kepada palpasi perrektal. Selain itu, tidak ada peningkatan yang ketara dalam nisbah neutrophil kepada limfosit dalam kedua-dua kumpulan berikutan palpasi perrektal. Oleh itu, kajian ini menunjukkan bahawa palpasi perrektal dalam kerbau menyebabkan tekanan dan status kehamilan tidak mempengaruhi tindakbalas kepada palpasi perrektal.

Kata kunci: Palpasi perrektal kerbau, tekanan, nisbah neutrophil kepada limfosit, tahap kortisol

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4901 – Project.

**EVALUATION OF SERUM CORTISOL CONCENTRATION AND
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(*Bubalus bubalis carabanensis*)**

by

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Rectal palpation is one of the veterinary procedures that is used to diagnose pregnancy in buffalo cows. However, this procedure might be painful and stressful to the animal. Stress is one of the important factors that need to be considered when assessing the welfare of the animal. The objective of this study is to evaluate stress response to rectal palpation by comparing serum cortisol concentration and neutrophil to lymphocyte ratio before and after rectal palpation in a buffalo cows. Blood was collected from the coccygeal vein before and directly after rectal

palpation from 3 non-pregnant and 12 pregnant swamp buffalo cows aged between five to eight years old and weighing 300 to 400 kg. The serum cortisol concentration and neutrophil to lymphocyte ratio was determined using radioimmunoassay and differential count respectively to evaluate stress response to this procedure. The result revealed that there is significant increase in the serum cortisol concentration following a rectal palpation in pregnant and non-pregnant buffalo cows. However, there is no significant different between pregnant and non-pregnant buffalo cow in terms of responsiveness to rectal palpation. There is also no significant increase in neutrophil to lymphocyte ratio in both groups following a rectal palpation. Thus, this study show that rectal palpation in buffalo cows causes stress and the pregnancy status does not seem to influence the response to rectal palpation.

Keywords: Rectal palpation, buffalo cows, stress, neutrophil to lymphocyte ratio, cortisol level

CHAPTER 1

INTRODUCTION

The scientific name of domesticated water buffalo is *Bubalus bubalis* (Abd El-Salam and El-Shibiny, 2011). There are two sub-species of water buffalo that can be found in Asia which are the river type and the swamp type (Yue *et al.*, 2013). Buffaloes are valuable species as their meat, milk and horns can be exploited (de la Cruz-Cruz, 2014). Moreover, buffaloes are hardy animal and can be used as work animals such as for draught power purposes (Spanghero *et al.*, 2004). Due to its multiple purposes, there is a high demand for buffaloes and therefore breeding of water buffaloes is important. Water buffaloes require little demand in terms of food and shelter. Thus, breeding of the animal does not need a high capital expenditure (Czerniawska-Piatkowska *et al.*, 2010). For breeding purposes, the reproductive management of the animal is important to ensure the increase of buffalo's number. Rectal palpation is one of the methods that is used in the farm to determine the success of the reproductive management.

Rectal palpation is the palpation of the reproductive tract through the rectal wall and one of the methods that is frequently used by veterinarians to diagnose pregnancy and manipulation for artificial insemination (AI). It is the fastest and most convenient way to detect early pregnancy in the field (Cingiet *et al.*, 2012). Rectal palpation can be used to detect pregnancy in cows as early as day 30 of pregnancy.

In a non-pregnant animal, pregnancy diagnosis should never be made until uterus has been retracted (Momont, 1990).

Although rectal palpation is a non-traumatic procedure but it can be painful and cause stress to the animal (Cingiet *al.*, 2012). The procedure can be a fast and only induce an acute stress but the process of handling the animals can also be stressful. The nature of water buffalo is semi-wild type of behaviour (Das *et al.*, 2007). They are often extensively reared and according to Probstet *al.* (2012), extensively reared animals usually have an anxious and excited temperament that makes handling difficult. Thus, there is an impact in human-animal interactions during veterinary procedures on welfare, productivity as well as behaviour (Cavallinaet *al.*, 2008). This is because there will be induction of pain and stress during these procedures (stressor). The performance of the animal can be affected when being subjected to repeated stressor.

Stress in animals can be reflected through its behaviour such as kicking and struggling as well as physiological changes such as increase in the cortisol level in the blood. Stress is a condition that affects the body system of the animals which originates from one or more sources either from inside of the body or come from the surroundings (Christiansen *et al.*, 2007). According to Alam and Dobson (1986), rectal palpation causes increase in the cortisol concentration in the plasma in cows. Rectal palpation can cause an acute stress which can affect conception rate (Unshelm, 1990) as well as estrus cycle (Nakaoet *al.*, 1994).

The stress hormone or cortisol in the circulation is widely used by researchers to assess stress in various animals. Cortisol is the major glucocorticoid product of adrenal cortex (Turpeinen *et al.*, 2013). Plasma cortisol concentrations can increase in response to acute stress through stimulation of hypothalamic-pituitary-adrenal axis and this can be used to evaluate pain (Stafford and Mellor, 2011). Both ACTH (adrenocorticotrophic hormone) and glucocorticoids have negative feedback effects on the pituitary and the hypothalamus to maintain normal resting blood levels of ACTH and glucocorticoids but stressful stimuli can override these effects.

Besides using stress hormone in the circulation to evaluate stress, other measurement of stress response can be used. For example, physiological stress can also be assessed through haematological parameters such as white blood cell count through blood smear (Davis *et al.*, 2008). Neutrophil to lymphocyte ratio can be used as stress indicator (Maheshwari *et al.*, 2013). This is because glucocorticoids hormone affect the function ability and migration pattern of the leukocyte. Increase level of cortisol level during stress will lead to increase life span of neutrophil and thus raise the number of neutrophil in the circulation (Neutrophilia) (Kim *et al.*, 2005). The increase level of cortisol also caused reduced number of lymphocyte in the circulation (Lymphopenia) (Dhabhar, 2002). Cortisol which increase during stress response affect the numbers of neutrophils and lymphocytes in opposite directions. Therefore, the increase in neutrophil to lymphocyte ratio can be used to determine stress level which is corresponding to the increased of cortisol (Davis *et al.*, 2008). The combination of both parameter (cortisol level and neutrophil to lymphocyte ratio) has been used to assess stress in transportation in buffalo (Maheshwari, 2013).

Rectal palpation may induce pain and cause stress to the buffalo cows and this might be a concern to the welfare of the animal. There is no study has been conducted to measure the stress response in buffalo cows following a rectal palpation. Therefore, this study was conducted on selected pregnant and non-pregnant swamp buffalo cows whereby rectal palpation was done for pregnancy diagnosis.

Hypothesis

The serum cortisol level and neutrophil to lymphocyte ratio in buffalo cows are increased following rectal palpation as an indicator of stress response.

Objectives

This study is conducted to evaluate serum cortisol concentration and the neutrophil to lymphocyte ratio as stress indicator before and after rectal palpation in swamp buffalo cows (*Bubalus bubalis carabanensis*).

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