UNIVERSITI PUTRA MALAYSIA

METABOLIC PROFILING OF SMALL RUMINANTS AFFECTED WITH LAMENESS AND HOOF DISORDERS BASED ON SUBSTANCE P AND B-HYDROXY BUTYRATE ASSESSMENT

ALYA AFIDA BINTI AHMAD TAJUDDIN

METABOLIC PROFILING OF SMALL RUMINANTS AFFECTED WITH LAMENESS AND HOOF DISORDERS BASED ON SUBSTANCE P AND B-HYDROXY BUTYRATE ASSESSMENT

by

ALYA AFIDA BINTI AHMAD TAJUDDIN

Project paper submitted to the Faculty of Veterinary Medicine, Universiti Putra Malaysia, in fulfilment of the requirements for the Degree of Doctor of Veterinary Medicine

DECEMBER 2023

CERTIFICATION

It is hereby certified that Dr Mohammad Babatunde Sadiq read this project paper entitled "Metabolic Profiling of Small Ruminants Affected with Lameness and Hoof Disorder" by Alya Afida Ahmad Tajuddin and, in my opinion, is satisfactory in terms of the quality and presentation as partial fulfilment of the requirement for the course VPD4999 – Final Year Project.

DR. MOHAMMED BABATUNDE SADIQ
Faculty of Veterinary Medicine,
Department of Farm and Exotic Animal Medicine and Surgery,
Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia

DR. AZALEA HANI OTHMAN
Faculty of Veterinary Medicine,
Department of Veterinary Laboratory Diagnostics,
Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia

ACKNOWLEDGEMENT

First and foremost, I would like to express my gratitude to Allah SWT, the Almighty God, for the strength and blessings He has given me throughout my research. I would like to thank my parents and family for their unwavering support and encouragement throughout my studies.

Then, I would also like to thank my supervisor, Dr. Mohammed Babatunde Sadiq, and my co-supervisor, Dr. Azalea Hani Othman, for their invaluable guidance and knowledge on the farm and in the laboratory. Their expertise and insights have been instrumental in shaping my project.

I am grateful to my friends, especially Sathishwaran, Nadia, Atiqah and to those who have provided me with a supportive and stimulating research environment. Their love and encouragement have been a constant source of inspiration and motivation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Profil Metabolik Ruminan Kecik yang Terkesan dengan Ketempangan dan Gangguan Kuku Berdasarkan Penilaian Substance P dan B-Hydroxybutyrate

ABSTRAK

Ketempangan adalah masalah kesihatan, kebajikan dan produktiviti utama dalam ruminan kecil (biri-biri dan kambing). Kebanyakan kes ketempangan timbul daripada gangguan kuku, yang dianggap menimbulkan kesakitan dan perubahan metabolik pada haiwan. Namun begitu, terdapat maklumat terhad tentang profil metabolik ruminan kecil yang terjejas dengan ketempangan dan gangguan kuku. Bahan P dan B-hydroxybutyrate telah ditunjukkan sebagai penunjuk yang boleh dipercayai bagi profil metabolik haiwan lumpuh. Kajian keratan rentas ini menyiasat profil metabolik biri-biri dan kambing tempang dengan menilai tahap bahan P dan B-hydroxybutyrate. Sebanyak 48 ekor ruminan kecil (24 ekor biri-biri dan 24 ekor kambing) telah diambil dengan mudah daripada 5 Ladang Angkat Ladang Universiti Putra Malaysia. Ketempangan dinilai menggunakan pemarkahan pergerakan lima mata dan haiwan dengan skor 3 dan ke atas dianggap tempang. Gangguan kuku dan ciri haiwan direkodkan dengan sewajarnya. Sampel darah dikumpul dan dianalisis untuk tahap serum ß-hydroxybutyrate (BHB) dan plasma Substance P (SP) menggunakan kit ELISA komersial. Data dianalisis menggunakan statistik deskriptif dan ujian-t bebas. Tiada perbezaan ketara dikesan dalam tahap SP antara kumpulan tempang dengan bukan tempang, serta antara kehadiran atau ketiadaan gangguan kuku. Sementara itu, walaupun kumpulan tempang dan mereka yang mengalami

gangguan kuku mempunyai tahap BHB yang lebih tinggi berbanding yang tidak tempang dan mereka yang tidak mengalami gangguan kuku, perbezaannya tidak ketara secara statistik (*p* > .05). Saiz sampel yang agak kecil dan reka bentuk keratan rentas mungkin menyumbang kepada penemuan ini. Walau bagaimanapun, kajian ini boleh digunakan sebagai kerja awal untuk analisis lanjut untuk menjelaskan perubahan dalam profil metabolik ketempangan dan gangguan kuku dalam ruminan kecil.

Metabolic Profiling of Small Ruminants Affected with Lameness and Hoof Disorders Based on Substance P and Β-Hydroxy Butyrate Assessment

ABSTRACT

Lameness is a major health, welfare, and productivity concern in small ruminants (sheep and goats). Most cases of lameness arise from hoof disorders, which are considered to elicit pain and metabolic alterations in animals. Nevertheless, there is limited information on the metabolic profile of small ruminants affected with lameness and hoof disorders. Substance P and Β-hydroxybutyrate have been demonstrated as reliable indicators of the metabolic profile of lame animals. This cross-sectional study investigated the metabolic profile of lame sheep and goats by assessing the levels of substance P and Β-hydroxybutyrate. A total of 48 small ruminants (24 sheep and 24 goats) were conveniently sampled from 5 Ladang Angkat Farms of Universiti Putra Malaysia. Lameness was assessed using a five-point locomotion scoring and animals with a score of 3 and above were considered lame. Hoof disorders and animal characteristics were recorded accordingly. Blood samples were collected and analysed for serum ß-hydroxybutyrate (BHB) and plasma Substance P (SP) levels using commercial ELISA kits. Data were analysed using descriptive statistics and independent t-tests. No significant difference was detected in SP levels between lame and non-lame groups, as well as between the presence or absence of hoof disorders. Meanwhile, although the lame group and those with hoof disorders had higher BHB levels compared to the non-lame and those without hoof disorders, the difference was not statistically significant ($p > .05$). The relatively small sample size and cross-sectional design might contribute to these

findings. Nevertheless, this study can be used as a preliminary work for further analysis to elucidate the alterations in the metabolic profile of lameness and hoof diseases in small ruminants.

CHAPTER 1

INTRODUCTION

1.1 Introduction

This chapter discusses the background of the study, research objectives, research questions, research hypotheses, and justification of the study.

1.2 Background of Study

According to The Food and Fertilizer Technology Center (FFTC), the livestock industry is an essential component of the agricultural sector in Malaysia as it provides the largest source of protein to the population, job opportunities and contributes to the country's economic development. Malaysia produced approximately 4,026.4 tons of mutton in 2020. However, the number of small ruminants to produce mutton has dropped significantly from year to year due to the closing operation of small farms. As the self-sufficiency level for mutton is only 11.3%, Malaysia needs to import live small ruminants, and frozen and processed mutton from various countries.

Lameness is a painful clinical manifestation associated with an animal's feet which affects its normal walking gait (Van Nuffel et al., 2015). Lameness remains a major welfare issue and cause of economic losses to ruminant farmers (Sadiq et al., 2017). In goats, lameness may arise from several causes such as overuse, trauma, and hoof disorders (Silva et al., 2021). These health issues are associated with inflammatory conditions that affect the production of horn tissues, which are responsible for the proper functioning of the hoof (Langova et al., 2020).

β-Hydroxybutyrate (BHB) stands out as the predominant ketone body among mammals that provide energy to tissues when glucose is limited, such as during fasting or prolonged exercise. Recent findings suggest that BHB serves not just as an energy transporter but also possesses numerous signalling roles that can impact processes like neural activity and metabolic rate within cells (Newman & Verdin, 2017). It has shown excessive negative energy balance can be characterized by elevations of BHB, as lower feed intake is associated with lameness in high-producing ruminants this might suggest that there will be elevations of BHB when small ruminants are affected with lameness (Hut et al., 2021).

Substance P (SP) is a neuropeptide belonging to the tachykinin family, it acts as a neurotransmitter and neuromodulator. It is expressed by the central nervous system, the peripheral nervous system, and immune cells (Zieglgänsberger, 2018). SP is most studied for its role in pain perception, but it is also involved in the neuroinflammatory process (Graefe et al., 2022). In the last ten years, SP has been used as a variable for pain assessment during procedures such as castration or dehorning, results from these studies implied that SP might be a promising biomarker associated with pain in ruminants (Tschoner & Feist, 2022).

1.3 Problem Statement

Lameness is a significant animal welfare issue in small ruminants, with a high prevalence reported between studies. Although lameness is accompanied by pain in the animal's feet, farmers' decision to treat lameness depends on various factors, and the effects of lameness on small ruminants have been largely overlooked. If left untreated, lameness can compromise the animal's health and welfare (Nalon & Stevenson, 2019).

Lameness has economic consequences for farmers as lameness management is costly for the farmers. Even though, it is not yet concluded how lameness in small ruminants is linked to a reduction in milk yield, fertility, and longevity as the literature on the effects of lameness in small ruminants is limited. Jaques et al., (2023) reported that when the prevalence of lameness was between 5 and 20% of the herd, the estimated daily milk income lost was between NZD 19.5 and 104 per day.

Most studies use the VLS (visual lameness scoring) system to evaluate lameness in small ruminants by identifying changes in the gait and the posture of the animal. this method is not effective in detecting lameness as it is very subjective and requires trained observers to evaluate. Although this method is quick to apply, inexpensive, and easy to perform in farm practice, lameness is often difficult to detect before showing clinical signs, and it is difficult to treat by the time lameness is diagnosed, leading to premature culling (Van Nuffel et al., 2015; He et al., 2022).

Study that assessed metabolic alterations of ruminants affected with lameness only involves cows (He et al., 2022). However, there is no study in Malaysia that has reported the metabolic alterations in small ruminants (sheep and goat) affected with lameness and hoof disorders. Therefore, the purpose of this study is to identify potential biomarkers for lameness in the metabolic profile by Substance P and B-hydroxybutyrate assessment as an early detection of lameness and hoof disorders.

1.4 Research Objectives

1. To determine the differences in β-hydroxybutyrate (BHB) concentration between lame and non-lame small ruminants (sheep and goats).
2. To determine the differences in Substance P concentration between lame and non-lame small ruminants (sheep and goats).
3. To determine the differences in β-hydroxybutyrate (BHB) concentration between overgrown hoofs and normal hoofs in small ruminants (sheep and goats).
4. To determine the differences in Substance P concentration between overgrown hoofs and normal hoofs in small ruminants (sheep and goats).

1.5 Research Questions

RQ1 Is there any significant difference in β-hydroxybutyrate (BHB) concentration between lame and non-lame small ruminants?

RQ2 Is there any significant difference in Substance P concentration between lame and non-lame small ruminants?

RQ3 Is there any significant difference in β-hydroxybutyrate (BHB) concentration between overgrown hoofs and normal hoofs in small ruminants?

RQ4 Is there any significant difference in Substance P concentration between overgrown hoofs and normal hoofs in small ruminants (sheep and goats)?

1.6 Research Hypotheses

$H_0$1: There is no significant difference in β-hydroxybutyrate (BHB) concentration between lame and non-lame small ruminants (sheep and goat).

$H_0$2: There is no significant difference in Substance P concentration between lame and non-lame small ruminants (sheep and goat).

$H_0$3: There is no significant difference in Substance P concentration between lame and non-lame small ruminants (sheep and goat).

$H_0$4: There is no significant difference in β-hydroxybutyrate (BHB) concentration between overgrown hoofs and normal hoofs in small ruminants (sheep and goat).

1.7 Justification of the Study

Lameness is difficult to be diagnosed in sheep and goats because they are well known to mask signs of pain and lameness. Therefore, a proper understanding of pain related to lameness needs to be evaluated other than a visual approach. For effective management of lameness and hoof diseases, early detection is important (He et al. 2022). Thus, this study will give a better understanding on the change in metabolic profile in small ruminants affected with lameness and hoof disorder.

1.8 Summary

This chapter reviews the introduction of B-hydroxybutyrate and substance P, the importance of lameness on animal welfare issues and the economic consequences to the farmers, subjective methods to evaluate lameness, lack of metabolic profile studies that associated with lameness and the purpose of this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter will focus on the literature review of this study, where the overview of lameness in small ruminants, lameness among small ruminants in Malaysia, the causes for lameness in small ruminants, the risk factors for lameness in goats and sheep, physiological changes in lame goats and sheep, pain assessment in lame goats and sheep and alterations in the metabolic profile; Substance P and B-hydroxybutyrate, in ruminants affected with lameness will be discussed.

2.2 Overview of lameness in Small Ruminants

According to Bustamante et al., (2015), Animal welfare studies have highlighted pain as one of the most important aspects to be addressed and lameness in ruminants is included in the animal's welfare issues. Over the past two decades, the occurrence of lameness within dairy goat herds on certain European commercial farms has varied, with prevalence rates ranging from 1.7% to as high as 67%. Although there is significant variation in prevalence across different studies, lameness remains a widespread issue on commercial farms worldwide (Jaques et al., 2023).

Lameness resulted by hoof disorders are not only a welfare issues but it has a major economic impact on farmers and has been studied extensively in dairy cattle and sheep but there are scarce studies on lameness in goats. The reason lameness in small ruminants is less commonly reported compared to large ruminants is because lameness is the cause of premature culling by the farmers (Langova et al., 2020; Jaques et al., 2023).

According to Jaques et al. (2023), farmers may not understand the importance of lameness because they generally underestimate the impact of lameness on productivity and tend to normalise mild lameness within their farm as it is an unavoidable phenomenon. However, it is concluded that there are negative effects of lameness on the milk production in dairy goats in commercial farms, where severe lameness resulted in a 7.05-8.67% decline in milk production for seasonal and extended lactating goats.

2.3 Lameness among Small Ruminants in Malaysia

There is no recent study on the prevalence of lameness among small ruminants in Malaysia. Nevertheless, there is a study that has been conducted to give an idea of the prevalence of lameness among small ruminants in Malaysia. A retrospective study conducted by Nor'Nasuha (2019) to determine the distribution of lameness cases in small ruminants at Universiti Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM), from January 2013 to July 2019 reported that of all 2 519 cases at UVH, 5.6% were lameness cases. Based on the lameness cases reported, 12.6% were in sheep, and 87.3% were in goats.

2.4 Causes of Lameness in Sheep and Goats

Lameness is defined as a clinical manifestation rather than the disease itself, therefore, lameness in small ruminants is associated with multifactorial causes. Causes of lameness can be classified as infectious and non-infectious, majority of the lameness cases are associated with bacterial infections, lesions and diseases located at the foot. The common infectious agents causing lameness include *Fusobacterium necrophorum* and *Dichelobacter nodosus*, these agents cause interdigital dermatitis, foot rot and contagious ovine digital dermatitis in small ruminants (Gelasakis et al., 2019). In the United Kingdom (UK), it is confirmed that the most common infectious cause of lameness in sheep is foot rot by *Dichelobacter nodosus*. However, only one out of four dairy goat herds investigated to be affected by foot rot and there is scarce published evidence for high levels of foot rot in UK dairy goat farms (Groenevelt et al., 2015).

Non-infectious causes of lameness also vary from many factors. For example, farm management, hoof care management, and nutritional deficiency have been reported to increase the risk of WLD. Laminitis is a hoof disorder involving the inflammation of the laminar corium resulting from the disruption in the blood circulation on the claw after ruminal acidosis, toxaemia, or severe infection. Even though there is scarce study on the correlation between laminitis due to ruminal acidosis in small ruminants, there is evidence of a correlation between laminitis and rumen acidosis in cattle (Gelasakis et al., 2019).

2.5 Risk factors for Lameness in Sheep and Goats

Environmental could be one of the risk factors for lameness in small ruminants. It is concluded that most lameness cases were observed during wet seasons, especially in sheep. Small ruminants are predisposed to the proliferation and transmission of pathogens when there is increased moisture in relatively warm weather and extended housing periods. Elevated moisture in the floor also results in the reduction of claw horn hardness and, an increase in claw size and weight. Softer and swollen claws wear out rapidly due to friction (Gelasakis et al., 2019).

Nutrition plays a fundamental role in preventing hoof diseases. It also influences the quality and growth of hoof horn. The composition of the animal's diet directly impacts the strength and structure of the hoof horn (Langova et al., 2020). In dairy cattle, there is a correlation between ruminal acidosis due to a high carbohydrate diet and claw horn lesions. Although there is still no conclusive evidence of the pathogenesis, this correlation is well documented. However, in goats, no literature has established the relationship between nutrition and lameness (Groenevelt et al., 2015).

Farm-level characteristics is also a risk factor for lameness in small ruminants. Those characteristics includes the herd size, management system, housing conditions, floor types and hoof care management. There is evidence that inappropriate floor material with the absence of floor stratification for appropriate drainage, poor ventilation can lead to increase of moisture on the ground. Furthermore, increase stocking density is also a significant risk factor for lameness as overcrowding of animals are usually followed by increased moisture, urine, and faeces accumulation.

2.6 Physiological changes in lame Sheep and Goats

Lameness also has been reported to be the cause of loss of reproductive efficiency in small ruminants as lame male animals are not able to mount the female. Lame female animals are also unable to withstand the weight of the male during mating (Eze et al., 2002). Furthermore, the difference in the incidence of lameness in lactating goats and young goats was due to a parturition effect (Groenevelt et al., 2015). This reason may be due to the elevated B-hydroxybutyrate during the early lactation as reported by Zamuner et al., (2020), the elevation of BHB associating with negative energy balance are more pronounced in the early lactation in goat than in late pregnancy. Additionally, it is reported that a relatively strong relationship between BHB concentration have been observed in dairy cows (Zamuner et al., 2020)

2.7 Pain assessment in lame Sheep and Goats

It is said that the signs of pain in sheep and goats are generally similar in cattle, only that goats are more vocalize during painful procedures compared to sheep and cattle (Goldberg, 2018). Tschoner (2021) reported that the numerical rating scale (NRS) is mostly used in evaluating pain assessment in cattle calves and the problem with NRS are lack of sensitivity as the categories for an activity level are often simplified.

Furthermore, Weeder et al., (2023) used several methods to assess pain in goats induced with lameness. These method includes facial grimace score; a scale that measure the extent of facial expressions associated with pain, cortisol concentration;

a hormone that is released in response to stress and pain, substance p concentrations; a neuropeptide that is involved in the transmission of pain signals, visual lameness scoring; a scoring system that assesses the degree of lameness in animal and visual analogue scale; a scale that measures the intensity of pain experience by an animal.

2.8 Alterations in Substance P and B-hydroxybutyrate in Lame Ruminants

There is scare information on the alterations in Substance P and B-hydroxybutyrate of metabolic profile in small ruminants affected with lameness. However, it is proven that Substance P did differ by timepoint and treatment over time interactions in pain assessment of post-induction lameness in goats (Weeder et al., 2023).

There is no recent study on the alteration of B-hydroxybutyrate in small ruminants affected with lameness and hoof disorder. However, it is reported that the excessive energy balance is related to body condition score (Hut et al., 2021). According to Hut et al., (2021), body condition score loss in early lactation was associated with lameness and it is resulted by less feeding time.

2.9 Summary

This chapter reviews all the literature related to this study. It is concluded that there is lack of evidence on the metabolic profile in small ruminants affected with lameness and hoof disorders despite the animal welfare issues, the economic loss and the risk factors of lameness are often overlooked by farmers.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter introduces various methodologies used in this study in gathering data and analysis which are required to fulfil the research objective. This study is conducted to determine the BHB and substance P levels in small ruminants affected with lameness and hoof disorders.

3.2 Study Location

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia (UPM), with the reference number UPM/IACUC/AUP-U022/2023. This study is a cross-sectional study and was conducted at 5 Ladang Angkat Farms of UPM in Selangor. These farms were selected conveniently as it is chosen based on their availability of small ruminants.

Table 3.1

Description of the 5 Ladang Angkat Farms of UPM

Farm	Management	Herd Size	Hoof Care Management
A	Intensive	<50	None
B	Semi-Intensive	>50	None
C	Intensive	>50	None
D	Intensive	<50	None
E	Semi-Intensive	.>50	None

	Farm	Location
A	Asrar Bangi Farm	Lot 753, Kampung Rinching Hilir, 43000 Kajang, Selangor
B	Nurfarm Agro	650, Jalan Kuari, 56100 Hulu Langat, Selangor
C	Tropika Ustaz Farm	Lot 6157, Jalan Parit 10 Sungai Panjang, 45300 Sungai Besar, Selangor
D	Arumugam Farm	No. 16, Jln Meranti 10, P. Meranti, 47700 Puchong, Selangor
E	Putrajaya Farm	32, Jalan Desa Pinggiran Putra 3/7, 43000 kajang, Selangor.

3.3 Study Population

Sample size calculation for this study is determined using G*Power Software where the number of samples required per group is 25 with an effect size of 0.50, power of 0.80 and precision level of 0.05. The samples were selected conveniently based on the locomotion scoring. The study population utilized 48 adult small ruminants, (2-4 years old) which consist of 24 sheep and 24 goats. For each species there is an equal number of small ruminants affected by lameness and not affected with lameness.

3.4 Animal Assessment

Physical examination was conducted for each animal following the lameness evaluation. Physical examination comprised the general inspection of the animal including the head and the integument. The purpose of this physical examination was to identify the animals age as only adult animals are selected in this study and to ensure that the animals were healthy. Then, the hoofs are examined to detect any indications of hoof diseases including overgrown hoofs. The characteristics of each animal: Species, Breed, Age, Sex, Pregnancy status and Parity, are recorded during the physical examinations.

The dataset of this study was split into two groups by its locomotion score. A visual locomotion scoring (VLS) which is the 5-point gait scoring developed by Deeming (2018) as shown in Table 3.1, is used in this study to evaluate lameness in the study population. Briefly, the scores were defined as 1-normal, 2-uneven, 3-mildly lame, 4-moderately lame, and 5-severly lame. Animals are classified as clinically lame if their locomotion scoring is 3 and above. If the animals' locomotion scoring is 1 or 2, the animals are classified as non-lame.

Table 3.2

Description of the 5-Point Gait Scoring System (Deeming et al., 2018)

Gait Scoring System		Assessment Criteria					
Category	5-point	Limp	Moving forward	Weight-bearing	Head nod	Identify affected leg (s)	Other descriptors
Normal gait	1	No	Yes	Yes	No	-	Even stride on all four legs, tracking up, walks with a fluid motion.
Uneven gait	2	No	Yes	Yes	No	No	Shorter stride, not tracking up, joints slightly stiff, inward or outward swinging of a hoof at each stride.
Mildly lame	3	Yes	Yes	Yes	No	Possibly	One or more legs may be affected. The observer may not be able to determine the affected leg(s). Mild limp.
Moderately lame	4	Yes	Reluctant	Reluctant	Possibly	Yes	One or more legs may be affected. Moderate limp or slight goose-stepping.
Severely lame	5	Yes	Unwilling/ unable	Unable	Yes	Yes	One or more legs may be affected. Severe limping or walking on knees or pronounced high goose-stepping.

3.5 Sample Collection

Blood samples were obtained through the jugular vein using a vacutainer and needle and collected in both EDTA tubes and serum tubes. Subsequently, the blood tubes underwent centrifugation at 5000rpm for 5 minutes to separate and collect the plasma and serum of each animal. All samples were then stored in a freezer at -4°C.

3.6 ELISA Analysis

Laboratory procedures are carried out following the completion of all farm visits. Two ELISA kits are employed in this study; the Enzyme-Linked Immunosorbent Assay for Substance P (ELISA SP) and the Beta Hydroxybutyrate Metabolism Assay kit (BHB MA).

3.5.1 BHB ELISA Analysis

The BHB MA involves a total of 48 serum samples, comprising 24 from non-lame animals and 24 from lame animals. Pre-assay protocol involves the preparation of the standard dilution and required amounts of reagents while all the serum samples are thawed at room temperature. ELISA assay protocol was conducted following the detailed operating steps from the lab manual provided with the kit. The concentration of BHB from each sample was obtained from the standard curve.

3.5.2 Substance P ELISA Analysis

In the case of ELISA SP, only 16 plasma samples are processed due to the restricted availability of kits coated with Substance P. The set of 16 plasma samples consists of 8 samples from non-lame animals and 8 samples from lame animals. Each of these samples are duplicated in the assay. Pre-assay protocol involves the preparation of the standard dilution and required amounts of reagents while all the plasma samples are thawed at room temperature. ELISA assay protocol was conducted following the detailed operating steps from the lab manual provided with the kit. The average concentration of substance P from each sample was obtained from the standard curve.

3.6 Statistical Analysis

In this study, both data sets of Substance P and BHB concentration of the samples shows a normal distribution with a p value>0.05 by using Shapiro-wilk test. All statistical analyses were conducted using IBM SPSS version 24.0 software, to determine the differences between the serum level of BHB and plasma level of SP in small ruminants affected with lameness and hoof disorders.

3.7 Summary

Various methods conducted in this study are elaborated in this chapter. Farm selection and samples were selected conveniently. Both ELISA assay was conducted by following the detailed operation steps from the lab manual provided with the ELISA kit. Lastly, independent t-test was employed in this study as the data set is normally distributed.

CHAPTER 4

RESULTS

4.1 Introduction

In this chapter, the animal characteristics of the sample collected, the data analysed by descriptive analysis will be presented. The analysis involves comparing the means of BHB concentrations and Substance P concentrations between the groups. Microsoft Excel and Statistical Package for the Social Sciences (IBM SPSS) software were used in this data analysis.

4.2 Animal Characteristics

Table 4.1

Animal Characteristics of 5 Ladang Angkat Farms of UPM

Farm	Total	Species		Age		Gender		BCS	
		Sheep	Goat	<2.5	>3	F	M	1-3	3.5-5
A	8	0	8	4	4	2	6	3	5
B	17	17	0	6	11	12	5	11	6
C	14	7	7	9	5	8	6	12	2
D	5	0	5	5	0	0	5	5	0
E	4	0	4	0	4	3	1	3	1
	48	**24**	**24**	**24**	**24**	**25**	**23**	**34**	**14**

Table 4.1 shows the animal's characteristics obtained from each Ladang Angkat Farms. The table includes information on the total number of animals, species, age, gender, and body condition score. Total number of 48 animals with equal number of goats and sheep were utilized for sample collection in this study. The table shows 25 of the animals are female and 23 of the animals are male. Table also shows that most of the animals selected in this study are having low body condition score which is less than 3.

4.3 Descriptive Analysis

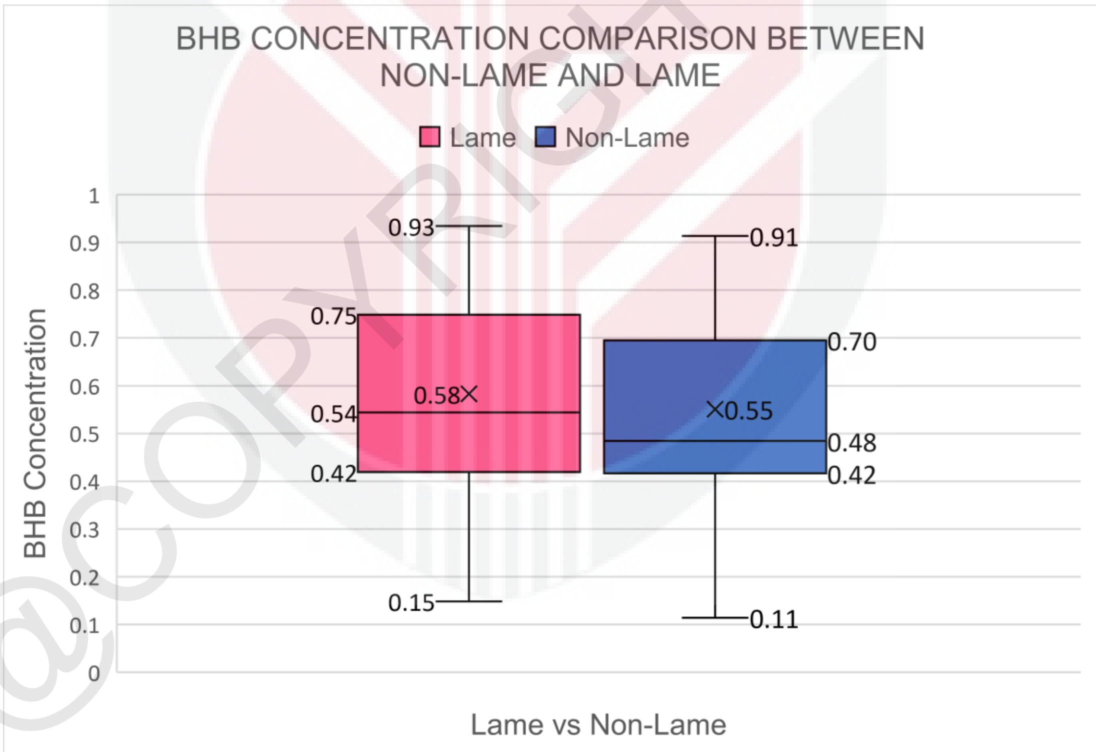

Figure 4.1 *Comparison of Beta-Hydroxybutyrate (BHB) Concentrations between Lame and Non-Lame group of Small Ruminants (Sheep and Goats)*

Figure 4.1 is a box plot graph that illustrates the comparison of beta-hydroxybutyrate (BHB) concentrations between two groups of small ruminants (sheep and goats); lame and non-lame group. The mean of BHB concentration for the lame group is 0.58mmol/L while the mean of BHB concentration for the non-lame group is 0.55mmol/L. Then, BHB concentrations range from 0.15mmol/L to 0.93mmol/L in the lame group, whereas the non-lame group exhibits concentrations ranging from 0.11mmol/L to 0.91mmol/L. Approximately 50% of the non-lame group animals have BHB concentrations exceeding 0.48mmol/L, while around 50% of the lame group animals surpass 0.54mmol/L. According to the findings represented in Figure 4.1, it can be deduced that animals in the lame group demonstrate higher BHB concentrations compared to those in the non-lame group.

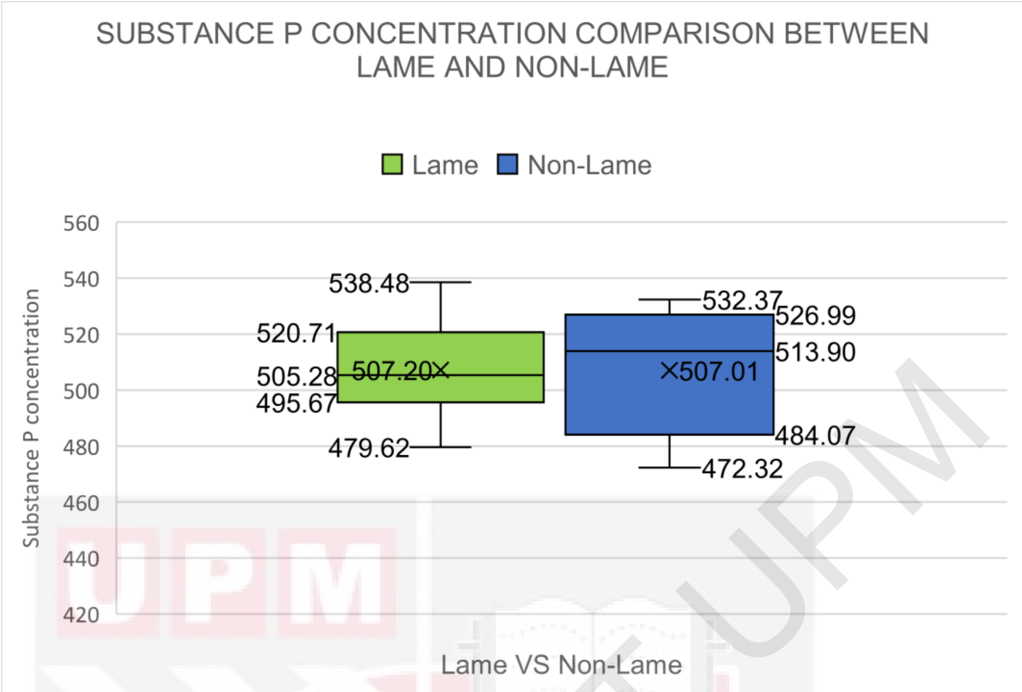

Figure 4.2 *Comparison of Substance P Concentrations between Lame and Non-Lame group of Small Ruminants (Sheep and Goats)*

Figure 4.2 presents a box plot graph comparing Substance P concentrations between two groups of small ruminants (sheep and goats); lame and non-lame groups. The mean of Substance P concentration for the lame group is 507.20pg/ml while the mean of substance P concentration for the non-lame group is 507.01pg/ml. Approximately 50% of the animals affected with lameness have a range of 495.7pg/ml to 520.71pg/ml Substance P concentration, while animals that are not affected by lameness have a range of 484.07pg/ml to 526.00pg/ml Substance P concentration. Based on the findings shown in Figure 4.2, it can be concluded that substance P concentration in animals affected with lameness and animals not affected with lameness exhibit similar results.

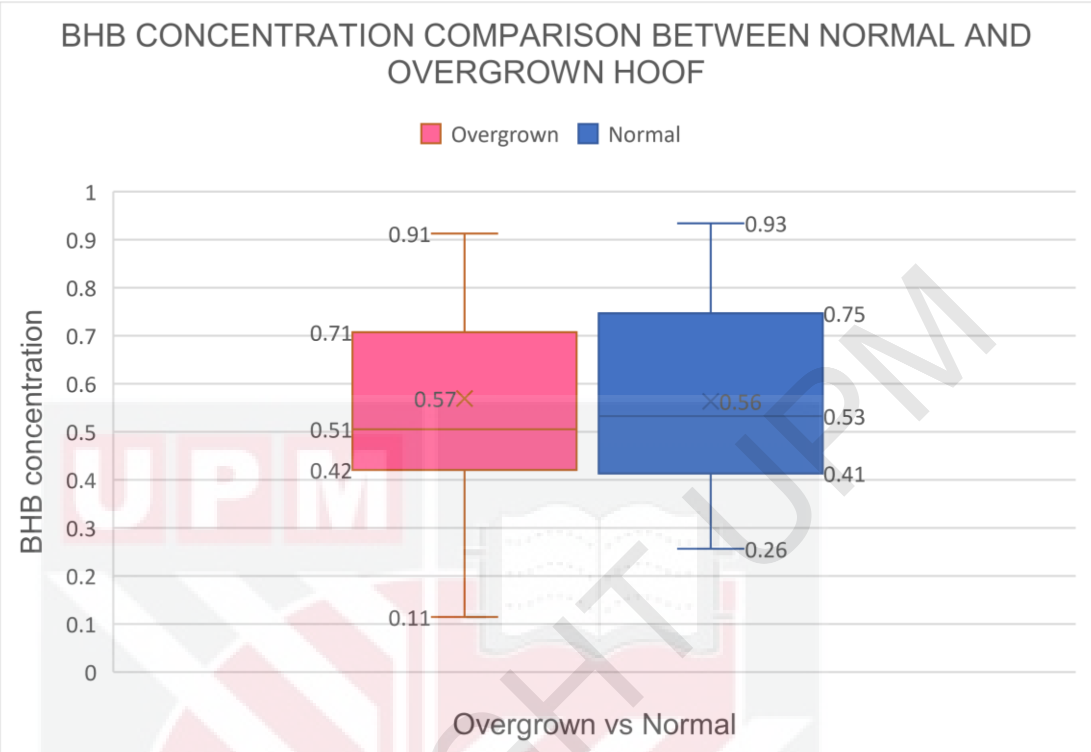

Figure 4.3 *Comparison of Beta-Hydroxybutyrate (BHB) Concentrations between Overgrown and Normal hoof length group of Small Ruminants (Sheep and Goats)*

Figure 4.3 depicts a box plot graph illustrating the comparison of beta-hydroxybutyrate (BHB) concentrations between two groups of small ruminants (sheep and goats): those with overgrown and normal hoof lengths. The mean BHB concentration for the overgrown hoof length group is 0.57mmol/L, while the mean for the normal hoof length group is 0.56mmol/L. BHB concentrations range from 0.11mmol/L to 0.91mmol/L in the overgrown hoof length group, whereas the normal hoof length group exhibits concentrations ranging from 0.26mmol/L to 0.93mmol/L. About 50% of the overgrown hoof length group has BHB concentrations exceeding

0.51mmol/L, while approximately 50% of the normal hoof length group surpasses 0.53mmol/L. Based on the observations in Figure 4.3, it can be inferred that animals with overgrown hoof length demonstrate similar findings of BHB concentrations in the normal hoof length group.

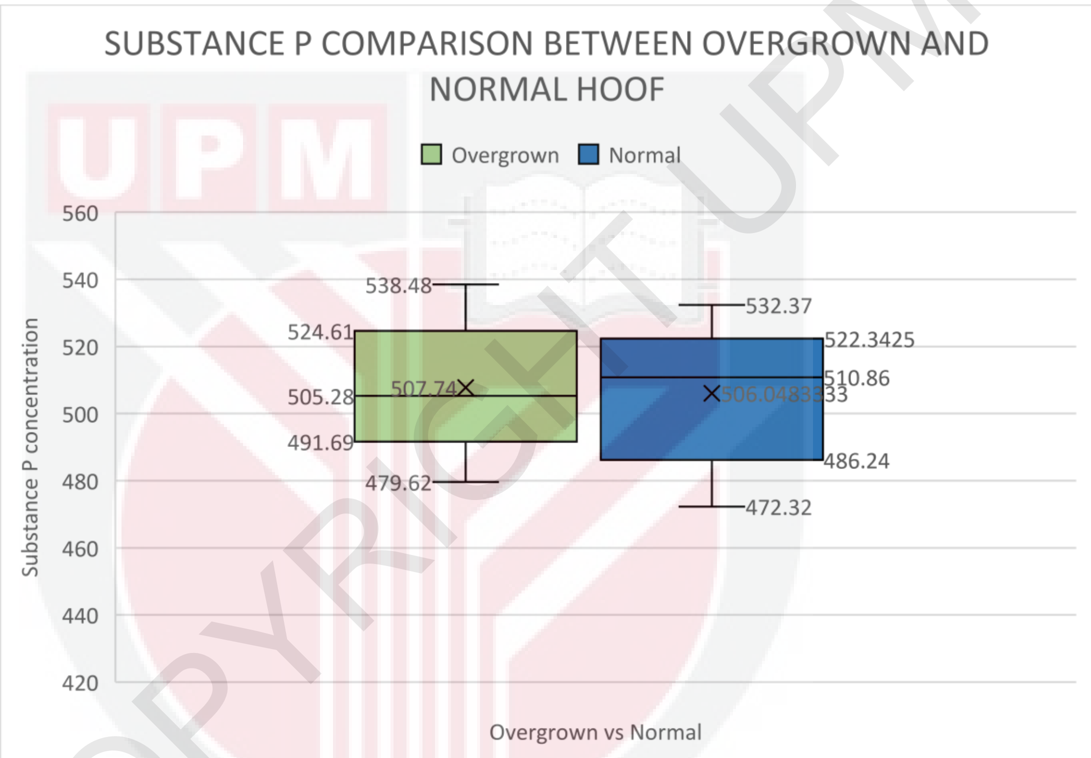

Figure 4.4 *Comparison of Substance P Concentration between Overgrown and Normal hoof length of Small Ruminants (Sheep and Goats)*

Figure 4.4 shows a box plot graph that compares the substance P concentration between two groups of small ruminants (sheep and goats): those with overgrown and normal hoof lengths. The mean substance P concentration for the overgrown hoof length group is 507.74pg/ml, while the mean substance P concentration for the normal hoof length is 506.05pg/ml. Based on the figure, approximately 50% of the animals with overgrown hoofs have substance P concentrations ranging from 491.69pg/ml to 524.61pg/ml whereas the animals with normal hoof length have substance P concentrations ranging from 486.24pg/ml to 522.34pg/ml. Therefore, it can be concluded that animals with overgrown hoof length have a higher range of substance P concentration compared to animals with normal hoof length.

4.4 Results of T-test

Table 4.2

The comparison of mean BHB Serum between Lame and Non-Lame Small Ruminants

Groups	*n*	*M*	*SD*	*t*	*df*	*p*	Result
Ruminants							
Lame	24	0.58	0.24	- 0.462	46	.65	$H_0$1 Not
Non-Lame	24	0.55	0.23				Rejected
Sheep							
Lame	12	0.66	0.27	-1.018	22	.32	$H_0$1a Not
Non-Lame	12	0.57	.17				Rejected
Goats							
Lame	12	0.50	0.19	0.312	22	.76	$H_0$1b Not
Non-Lame	12	0.53	0.29				Rejected

Table 4.2 shows the t-test results for BHB Serum between Lame (n = 24) and non-lame (n = 24) small ruminants. The p-value for this t-test results is .65 which is

more than .05. This shows that there is no significant difference in serum level of BHB between lame and non-lame small ruminants (sheep and goats). The null hypothesis ($H_0$1) failed to be rejected.

Table 4.3

The comparison of mean Substance P between Lame and Non-Lame Small Ruminants

Group	*n*	*M*	*SD*	*t*	*df*	*p*	Result
Ruminants							
Lame	8	507.20	18.32	-0.018	14	.99	H_0*2* Not
Non-Lame	8	507.01	22.64				Rejected
Sheep							
Lame	4	515.09	17.01	0.566	6	.592	$H_0$2a Not
Non-Lame	4	520.63	9.72				Rejected
Goats							
Lame	4	499.31	18.09	0.388	6	.712	$H_0$2b Not
Non-Lame	4	499.38	24.63				Rejected

Table 4.3 shows the t-test results for Substance P concentrations between Lame (n = 8) and non-lame (n = 8) small ruminants. The p-value for this t-test results is .99 which is more than .05, this shows that there is no significant difference in the plasma level of Substance P between lame and non-lame small ruminants (sheep and goats). The null hypothesis ($H_0$2) failed to be rejected.

Table 4.4

The comparison of mean BHB between Overgrown and Normal Hoof Length in Small Ruminants

Group	*n*	*M*	*SD*	*t*	*df*	*p*	Result
Ruminants							
Overgrown	29	0.57	0.26	0.81	46	.936	H_0*2* Not
Normal	19	0.56	0.20				Rejected
Sheep							
Overgrown	13	0.59	0.27	-0.536	22	.597	$H_0$2a Not
Normal	11	0.64	0.18				Rejected
Goats							
Overgrown	16	0.55	0.26	0.93	22	.361	$H_0$2b Not
Normal	8	0.45	0.17				Rejected

Table 4.4 shows the t-test results for BHB concentration between overgrown hoofs (n = 29) and normal hoofs (n = 19) small ruminants. The p-value for this t-test results is .936 which is more than 0.05, this shows that there is no significant difference in BHB level between overgrown and normal hoof length in small ruminants (sheep and goats). The null hypothesis ($H_0$3) failed to be rejected.

Table 4.5

The comparison of mean Substance P between Overgrown and Normal Hoof Length in Small Ruminants

Group	*n*	*M*	*SD*	*t*	*df*	*p*	Result
Ruminants							
Overgrown	10	507.74	20.11	0.159	14	.876	H_0*3* Not
Normal	6	506.05	21.37				Rejected
Sheep							
Overgrown	4	517.45	17.26	-0.082	6	.938	$H_0$3a Not
Normal	4	518.27	10.29				Rejected
Goats							
Overgrown	6	501.26	20.59	1.323	6	.264	$H_0$3b Not
Normal	2	481.60	13.12				Rejected

Table 4.5 shows the t-test results for Substance P concentrations between Overgrown (n = 10) and normal (n = 6) small ruminants. The *p*-value for this t-test results is .876 which is more than .05, this shows that there is no significant difference in plasma level of Substance P between overgrown and normal hoof length in small ruminants (sheep and goats). The null hypothesis ($H_0$4) failed to be rejected.

4.5 Summary

Results reviewed in this chapter have proven that the null hypotheses of this study failed to be rejected as there is no significant difference in BHB and SP concentration between all the variable group, p-value>0.05.

CHAPTER 5

DISCUSSIONS

5.1 Introduction

This study reported that there is no significant difference in the serum level of β-Hydroxybutyrate (BHB) and plasma level of Substance P between the examined groups—non-lame versus lame and overgrown hoofs versus normal hoofs. The factors influencing the null hypotheses that failed to be rejected are discussed in this chapter, which includes the subjective lameness scoring system, animal characteristics, and the duration of lameness affecting the animals.

5.2 Subjective Lameness Scoring System

A 5-point system of lameness scoring was used in this study instead of a 4-point system because it was found that a 5-point system is more sensitive. However, the lameness scoring system used in this study was developed in a controlled environment (Deeming et al., 2015). Additionally, the farm-level characteristics of all 5 Ladang Angkat Farms varies and due to the challenges in on-farm settings, for example, the weather of the day, the types of flooring system. It should be noted that the lameness scoring system can cause a misevaluation of the animal's gait. Hence, the lameness event scoring might misclassify the animal into lame and non-lame group.

5.3 Animal-level Characteristics

Animal characteristics can affect the B-hydroxybutyrate and substance P-level assessment. This includes the pregnancy stages, parity status and the body condition score (BCS) changes of the animal. According to Hut's (2021) research, ruminants in the late pregnancy stage have a high risk of ketosis and pregnancy toxaemia occurrence. Subclinical ketosis and pregnancy toxaemia in ruminants show increased serum BHB concentrations. Excessive negative energy balance can be characterized by elevations of Beta-hydroxybutyrate. Body condition score (BCS) change is an indirect measure of energy balance, those with higher BCS difference after a time had higher concentration of BHB.

In this study, the pregnancy stages, the parity and the BCS change of the animal are unknown as there is a lack of data record keeping in these farms. The limitation of this study is the availability of an ultrasound machine, pregnancy diagnosis was not conducted on the animal and the pregnancy stages of the animal were not identified.

5.4 Duration of animal affected with Lameness.

Study by Bustamante (2015) has shown that the level of substance P concentrations will start to decrease after 48 hours of lameness induction. In this study, the lameness scoring event and physical examination including the hoof examination were only conducted once on the farm as this is a cross-sectional study. The duration of lameness affecting the animals is unknown. Therefore, the SP concentrations during the later stage of lameness might not be as high as the SP concentrations during the early stage of lameness.

5.5 Summary

This chapter discusses the various factors that might affect the result of this study. This includes the misevaluation of lameness scoring, the change of body condition score, the parity and pregnancy status of the animal, and the duration of the animal affected with lameness is unknown.

CHAPTER 6

CONCLUSION

6.1 Introduction

This chapter will review the conclusion of this study based on the results and discussion in the previous chapter.

6.2 Conclusion

There is no significant difference in B-hydroxybutyrate (BHB) concentration and Substance P concentration in Small Ruminants Lameness and Hoof Disorders. Although, there is no significant difference, this study can still be used as a preliminary work to increase our understanding on the metabolic profile in small ruminants affected with lameness and hoof disorders in effective management and prevention of lameness by early detection assessment.

6.3 Summary

This chapter concludes that effective management and prevention of lameness needs to be by early detection. The purpose is to care for the livestock welfare and reduce production loss to the farmers. Although, there is no significant difference, this study can be used as a preliminary work for better understanding on metabolic profiles in small ruminants affected with lameness.

CHAPTER 7

RECOMMENDATIONS

7.1 Introduction

This chapter will discuss few recommendations on the study design, sample size and animal-level characteristics selection for future studies on understanding the metabolic profile in small ruminants affected with lameness and hoof disorder.

7.2 Study Design

Prospective study design is highly recommended to reach the objective of this study due to the challenges faced in this cross-sectional study such as the varieties of management system which can contribute to cofounding factors affecting the results. Furthermore, a prospective study can provide the duration of lameness affecting the animals. Blood samples can be collected when lameness is detected and when the animals is treated, giving the understanding of the change in the metabolic profile when the animal is affected with lameness and when the animal is sound.

There is experimental study design to assess Substance P concentration on induced lameness conducted by Bustamante (2015). However, there is still no studies that investigate substance P concentration in natural occurring lameness.

7.3 Sample Size

In Chapter 3, it is recommended to have at least 25 samples for each classified group, larger sample sizes are needed to obtain more accurate average values for comparing variable groups. However, due to the limitations of lame animals on the farm, this study was unable to achieve that sample size.

7.4 Summary

This chapter reviews the suggested recommendations to improve for further studies in understanding the metabolic profile in small ruminants affected with lameness.

REFERENCES

Bustamante, H., Rodríguez, A. R., Herzberg, D., & Werner, M. (2015). Stress and pain response after oligofructose induced-lameness in dairy heifers. *Journal of Veterinary Science*, *16*(4), 405–405. https://doi.org/10.4142/jvs.2015.16.4.405

Calamari, L., Ferrari, A., Minuti, A., & Trevisi, E. (2016). Assessment of the main plasma parameters included in a metabolic profile of dairy cow based on Fourier Transform mid-infrared spectroscopy: Preliminary results. *BMC Veterinary Research*, *12*(1), Article 4. https://doi.org/10.1186/s12917-015-0621-4

Deeming, L., Beausoleil, N. J., Stafford, K. J., Webster, J., & Zobel, G. (2018). Technical note: The development of a reliable 5-point gait scoring system for use in dairy goats. *Journal of Dairy Science*, *101*(5), 4491–4497. https://doi.org/10.3168/jds.2017-13950

Eze, C. A. (2002). Lameness and reproductive performance in small ruminants in Nsukka Area of the Enugu State, Nigeria. *Small Ruminant Research*, *44*(3), 263–267. https://doi.org/10.1016/s0921-4488(02)00030-5

Gelasakis, A. I., Kalogianni. A., I., & Bossis, I. (2019). Aetiology, risk factors, diagnosis and control of foot-related lameness in dairy sheep. *Animals, 9*, 509. https://doi.org/10.3390/ani9080509

Goldberg ME. Pain recognition and scales for livestock patients. J Dairy Vet Anim Res. 2018;7(5):236–239. DOI: 10.15406/10.15406/jdvar.2018.07.00218

González, L. A., Tolkamp, B. J., Coffey, M. P., Ferret, A., & Kyriazakis, I. (2008). Changes in feeding behavior as possible indicators for the automatic monitoring of health disorders in dairy cows. *Journal of Dairy Science*, *91*(3), 1017-1028. https://doi.org/10.3168/jds.2007-0530

Graefe, S. B., Rahimi, N., & Mohiuddin, S. S. (2022). *Biochemistry, Substance P.* State Pearls Publishing.

Grimm, K., Haidn, B., Erhard, M., Tremblay, M., & Döpfer, D. (2019). New insights into the association between lameness, behavior, and performance in Simmental cows, *Journal of Dairy Science*, *102*(3), 2453-2468. https://doi.org/10.3168/jds.2018-15035

Groenevelt, M., Anzuino, K., Smith, S. J., Michael, & Grogono-Thomas, R. (2015). A case report of lameness in two dairy goat herds; a suspected combination of nutritional factors concurrent with treponeme infection. *BMC Research Notes*, *8*(1). Article 791. https://doi.org/10.1186/s13104-015-1734-3

He, W., Cardoso, A. S., Hyde, R., Green, M. J., Scurr, D. J., Griffiths, R. L., Randall, L.V., & Kim, D.-H. (2022). Metabolic alterations in dairy cattle with lameness revealed by untargeted metabolomics of dried milk spots using direct infusion-tandem mass spectrometry and the triangulation of multiple machine learning models. *Analyst*, *147*(23), 5537–5545. https://doi.org/10.1039/d2an01520j

Herzberg, D., Strobel, P., Ramírez-Reveco, A., Werner, M., & Bustamante, H. (2020). Chronic inflammatory lameness increases cytokine concentration in the spinal cord of dairy cows. *Frontiers in Veterinary Science*, *7*:125. https://doi.org/10.3389/fvets.2020.00125

Hut, P.R, Hostens, M., Beijaard, M.J., Van Eerdenburg, F., Hulsen, J., Hooijer, G.A., Stassen, E., Nielen, M. (2021). Associations between body condition score, locomotion score, and sensor-based time budgets of dairy cattle during the dry period and early lactation. *Journal of Dairy Science*, *104*(4), 4746–4763. https://doi.org/10.3168/jds.2020-19200

Jaques, N., Turner, S.-A., Émilie Vallée, Heuer, C., & N. López-Villalobos. (2023). The effect of lameness on milk production of dairy goats. *Animals*, *13*(11), 1728–1728. https://doi.org/10.3390/ani13111728

Landinger, A., Yury Zablotski, Knubben-Schweizer, G., & Tschoner, T. (2023). Evaluation of Substance P concentrations in the blood plasma of jugular and tail vein of healthy German Simmental cows. *BMC Veterinary Research*, *19*(1). Article 218. https://doi.org/10.1186/s12917-023-03768-0

Langova, L., Novotna, I., Nemcova, P., Machacek, M., Havlicek, Z., Zemanova, M., & Chrast, V. (2020). Impact of nutrients on the hoof health in cattle. *Animals (Basel), 10*(10), Article 1824. https://doi.org/10.3390/ani10101824

Nalon, E., & Stevenson, P. (2019). Addressing lameness in farmed animals: an urgent need to achieve compliance with EU animal welfare law. *Animals, 9*, 576. http://doi.org/10.3390/ani9080576

Newman, J. C., & Verdin, E. (2017). β-hydroxybutyrate: A signalling metabolite. *Annual Review of Nutrition*, *37*(1), 51–76. https://doi.org/10.1146/annurev-nutr-071816-064916

Nor'Nasuha, M. H. (2019). *Lameness in small ruminants reported to the University Veterinary Hospital, Universiti Putra Malaysia from 2013 to July 2019* (DVM final year project thesis, University Putra Malaysia). http://discoverylib.upm.edu.my/discovery/Record/585433

Noronha Filho, A. D. F., Freitas, S. L. R., Rodrigues, D. F., Mendes, F. F., Miguel, M. P., Cunha, P. H. J., Fioravanti, M. C. S., & Silva, L. A. F. (2019). Characterization of ruminal acidosis and initial phase of laminitis inducted by oligofructose in crossbred calves. *Pesquisa Veterinária Brasileira*, *39*(2), 99–106. https://doi.org/10.1590/1678-5150-pvb-5619

Sadiq, M. B., Ramanoon, S. Z., Shaik Mossadeq, W. M., Mansor, R., & Salmah, S. (2017). Association between lameness and indicators of dairy cow welfare based on locomotion scoring, body and hock condition, leg hygiene and lying behavior. *Animals*, *7*(11), 79. https://doi.org/10.3390/ani7110079

Silva, S., Araújo, J. P., Guedes, C., Silva, F., Almeida, M., & Cerqueira, L. (2021). Precision technologies to address dairy cattle welfare: Focus on lameness, mastitis and body condition. *Animals*, *11*(8), 2253–2253. https://doi.org/10.3390/ani11082253

Steagall, P. V., Bustamante, H., Johnson, C. B., & Turner, P. V. (2021). Pain management in farm animals: Focus on cattle, sheep and pigs. *Animals, 11,* 1483. https://doi.org/ 10.3390/ani11061483

Tarlton, J. F., Holah, D. E., Evans, K. M., Jones, S., Pearson, G. R., & Webster, A. J. F. (2002). Biomechanical and histopathological changes in the support structures of bovine hooves around the time of first calving. *The Veterinary Journal, 163,* 196-204 https://doi.org/10.1053/tvjl.2001.0651

Tschoner, T. (2021). Methods for pain assessment in calves and their use for the evaluation of pain during different procedures: A review. *Animals*, *11*(5), 1235–1235. https://doi.org/10.3390/ani11051235

Tschoner, T., & Feist, M. (2022). Substance P concentrations in the blood plasma and serum of adult cattle and calves during different painful procedures and conditions: A systematic review. *BMC Veterinary Research, 18*(1), Article 232. https://doi.org/10.1186/s12917-022-03304-6.

Van Nuffel, A., Zwertvaegher, I., Van Weyenberg, S., Pastell, M., Thorup, V. M., Bahr, C., Sonck, B., Saeys W. (2015). Lameness detection in dairy cows: Part 2. Use of sensors to automatically register changes in locomotion or behavior. *Animals (Basel), 5*(3), 861-85. https://doi.org/10.3390/ani5030388.

Vieira, A., Oliveira, M. D., Nunes, T., & Stilwell, G. (2015). Making the case for developing alternative lameness scoring systems for dairy goats. *Applied Animal Behaviour Science*, *171,* 94-100. https://doi.org/10.1016/j.applanim.2015.08.015

Webster, A. J. F. (2002). Effects of housing practices on the development of foot lesions in dairy heifers in early lactation. *The Veterinary Record, 151*(1), 9-12. https://doi.org/10.1136/vr.151.1.9

Weeder, M.M., Kleinhenz, M.D., Reppert, E.J., Fritz, B.R., Viscardi, A.V., Montgomery, S.R., Martin, M.S., Curtis, A.K., Leslie, A.A., Lou, M.E., Hall, M.G., Coetzee, J.F. Optimal lameness induction model development using amphotericin B in meat goats. Translational Anim Sci. 2023 Sep 2;7(1):txad105. doi: 10.1093/tas/txad105. PMID: 37720823; PMCID: PMC10503652.

Winter, A. (2008). Lameness in sheep. *Small Ruminant Research*, *76*(1-2), 149–153. https://doi.org/10.1016/j.smallrumres.2007.12.008

Zamuner, F., DiGiacomo, K., Cameron, A.W.N., Leury, B.J. Short communication: Associations between nonesterified fatty acids, β-hydroxybutyrate, and glucose in periparturient dairy goats, Journal of Dairy Science,Volume 103, Issue 7, 2020, Pages 6672-6678, ISSN 0022-0302, https://doi.org/10.3168/jds.2019-17163.

Zieglgänsberger, W. (2018). Substance P and pain chronicity. *Cell and Tissue Research*, *375*(1), 227–241. https://doi.org/10.1007/s00441-018-2922-y