UNIVERSITI PUTRA MALAYSIA

CLINICO-PATHOLOGICAL AND HAEMATO-BIOCHEMICAL RESPONSES IN SHEEP FED WITH LOW AND HIGH LEVELS OF Brachiaria decumbens (Stapf) DIET

KALAI VAANI A/P MUNIANDY

IPTSM 2021 11

CLINICO-PATHOLOGICAL AND HAEMATO-BIOCHEMICAL RESPONSES IN SHEEP FED WITH LOW AND HIGH LEVELS OF *Brachiaria decumbens (*Stapf) DIET

By

KALAI VAANI A/P MUNIANDY

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

July 2021

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

CLINICO-PATHOLOGICAL AND HAEMATO-BIOCHEMICAL RESPONSES IN SHEEP FED WITH LOW AND HIGH LEVELS OF *Brachiaria decumbens* (Stapf) DIET

By

KALAI VAANI A/P MUNIANDY

July 2021

Chairman : Eric Lim Teik Chung, PhD
Institute : Tropical Agriculture and Food Security

Brachiaria decumbens is a highly productive tropical grass for ruminant production that present abundantly in Malaysia. Nevertheless, there were many reports of sporadic outbreaks of general ill-thrift and deaths in ruminants attributable to the presence of steroidal saponins. As a result, farmers would not be able to utilise this grass as a feed source for ruminants. The present study aims to determine the clinico- pathology, haemato-biochemistry, and acute phase proteins (APPs) responses of sheep fed with low and high levels of *B. decumbens* diets at different time phases. A total of 30 six-month-old male Dorper cross sheep were randomly divided into three treatment groups consisted of 10 sheep each. Treatment 1 (control) sheep were fed with *Pennisetum purpureum* and concentrates as the basal diet, whereas Treatment 2 and 3 sheep were fed with low (10%) and high (60%) level of *B. decumbents*, respectively. The low and high levels were determined by evaluating the saponins concentration of *B. decumbens* at different percentages mixed with *P. purpureum*. This study was conducted in two phases consisted of the acute (7 days) and chronic (90 days) stages. Throughout the experiment, the clinical responses such as rectal temperature, respiratory rate, heart rate, and mucous membrane colour were evaluated and recorded. At day 0, 7, and 90, blood samples were collected via jugular venipuncture for complete blood count and acute phase proteins analysis, while cerebrospinal fluid (CSF) were collected via lumbar puncture at the lumbosacral site for haptoglobin (Hp) and serum amyloid A (SAA) analyses. At the end of 7th day (acute stage) and 90th day (chronic stage) of experiment period, five animals from each treatment group were slaughtered for post mortem examination and histopathology evaluation. There were no significant changes ($p > 0.05$) in the rectal temperature, pulse, and respiration rate throughout the study period except for the mucous membrane colour. T3 sheep demonstrated pale mucous membrane starting from day 60 until the end of experiment on day 90, whereas T2 sheep only showed pale mucous membrane at the chronic phase of the study which was on day 90. In addition, the haematology results revealed significant differences ($p < 0.05$) in the red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration

(MCHC), white blood cells (WBC), neutrophils, monocytes, eosinophils, basophils, platelets, and plasma proteins between groups. Except for packed cell volume (PCV), there were also significant differences in all haematology parameters at different time phases. All biochemistry parameters except creatinine revealed significant differences ($p < 0.05$) among treatment groups. There were significant differences in all parameters between phases. On the other hand, APPs results also showed significant differences ($p < 0.05$) in the serum Hp, serum SAA, and CSF SAA between groups and time. At necropsy, no apparent lesions were observed in all organs as well as no significant differences in the organ morphometric analysis. Histologically, both T2 and T3 sheep exhibited mild and moderate necrosis & degeneration; haemorrhages & congestion; and oedema of the liver and brain respectively. In summary, this study has established the effects of the low and high levels of *B. decumbens* diets and at different time phases in sheep which could provide valuable information to future *B. decumbens* research.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

RESPON KLINIKO-PATALOGI DAN HEMATO-BIOKIMIA PADA BIRI-BIRI YANG DIBERI MAKAN *Brachiaria decumbens* (Stapf) PADA TAHAP RENDAH DAN TINGGI

Oleh

KALAI VAANI A/P MUNIANDY

Julai 2021

Pengerusi : Eric Lim Teik Chung, PhD
Institut : Pertanian Tropika dan Sekuriti Makanan

Brachiaria decumbens adalah rumput tropika yang sangat produktif untuk produksi ruminan. Walaubagaimana pun, terdapat banyak kes mengenai wabak penyakit dan kematian pada ruminan disebabkan oleh saponin bersteroid yang terdapat dalam rumput ini. Oleh itu, peladang tidak dapat menggunakan rumput ini sebagai sumber makanan untuk ternakan ruminan. Kajian ini bertujuan untuk menyiasat perubahan klinikal-patologi, haemato-biokimia, dan kepekatan akut protein fasa (APP) dalam bebiri selepas diberi rumput *B. decumbens* yang mengandungi tahap saponin rendah dan tinggi pada fasa masa yang berbeza. Sebanyak 30 ekor bebiri (Dorper cross) berusia enam bulan dibahagikan sama rata kepada tiga kumpulan rawatan yang masing-masing terdiri daripada 10 ekor bebiri. Kumpulan 1 (kawalan) diberi makan dengan *Pennisetum purpureum* dan pellet sebagai diet basal, sedangkan kumpulan 2 dan 3 diberi masing-masing diberi *B. decumbens* tahap saponin rendah (10%) dan tinggi (60%). Tahap saponin rendah dan tinggi ditentukan dengan menilai kepekatan saponin *B. decumbens* pada peratusan berbeza yang dicampurkan dengan *P. purpureum*. Kajian ini dilakukan dalam dua fasa yang terdiri daripada tahap akut (7 hari) dan kronik (90 hari). Sepanjang eksperimen ini, perubahan klinikal seperti suhu, kadar pernafasan, kadar denyutan jantung (TPR), dan perubahan warna membran mukus dinilai dan direkodkan. Sampel darah dan cecair serebrospinal (CSF) diambil pada hari 0, 7, dan 90 bagi analisis untuk menentukan kepekatan akut protein fasa. Pada akhir hari ke-7 dan hari ke-90, lima bebiri dari setiap kumpulan rawatan disembelih untuk penilaian bedah siasat dan pemeriksaan histopatologi. Tidak ada perbezaan yang signifikan ($p > 0.05$) pada suhu, kadar pernafasan, dan kadar degupan jantung sepanjang kajian ini kecuali warna membran mukus. Biri-biri T3 menunjukkan membran mukus pucat bermula dari hari ke 60 hingga akhir kajian, sementara biri-biri T2 menunjukkan membran mukus pucat hanya pada hari ke-90. Di samping itu, analisis darah menunjukkan perbezaan yang signifikan ($p < 0.05$) dalam sel darah merah (RBC), rata-rata isipadu korpuskular (MCV), kepekatan hemoglobin korpuskular (MCHC), sel darah putih (WBC), neutrofil, monosit, eosinofil, basofil, platelet, dan protein plasma antara kumpulan. Kecuali PCV, terdapat perbezaan

yang signifikan dalam semua parameter hematologi pada fasa waktu yang berbeza. Semua parameter biokimia kecuali kreatinin menunjukkan perbezaan yang signifikan antara kumpulan rawatan. Terdapat perbezaan yang signifikan dalam semua parameter antara fasa. Selain itu, keputusan APP menunjukkan perbezaan yang signifikan bagi serum haptaglobin, serum SAA, dan CSF SAA antara kumpulan dan masa. Semasa bedah siasat, tidak ada kecederaan yang nyata pada semua organ dan tidak terdapat perbezaan yang signifikan pada organ morphometrik. Pada analisis histologi, organ hati and otak masing-masing daripada kumpulan T2 dan T3 menunjukkan nekrosis dan degenerasi; pendarahan, dan edema pada skor ringan dan sederhana. Kesimpulannya, kajian ini telah membuktikan kesan tahap saponin rendah dan tinggi *B. decumbens* pada fasa masa yang berbeza dalam biri-biri dan dapat dijadikan maklumat yang baru untuk penyelidikan *B. decumbens* pada masa akan datang.

ACKNOWLEDGEMENTS

I would like to offer my sincere thanks to the individuals who have directly and indirectly contributed to the completion of this work. First and foremost, my heartfelt thanks and appreciation to my supervisor, Dr. Eric Lim Teik Chung for his professional insight, skillful guidance, encouragement, suggestion, time and patience throughout my study period. Thank you for his undivided attention and constructive comments to make this study a successful one. I would also extend my sincere appreciation to my co-supervisors, Professor Dr. Faez Firdaus Jesse Abdullah and Dr. Annas Salleh for their valuable assistance and advice throughout the whole study. I sincerely acknowledge the support from Graduate Research Fellowship Scholarship awarded by Universiti Putra Malaysia. I am grateful as well to the faculty and laboratory members Mr. Jefri Norsidin, Mr. Hairulnizam Mohd Sam, Mr. Mohd Faizal Yeob Baharuddin, Mr. Hidayad Ali Arman Ali, Dr. Mohd Farhan Hanif Reduan, Farah Hanis Juhari, Mohamed M. Alghirani and many more for their assistance, supports and friendship throughout this study. A special thank you to my co-masters' mates Muhammad Hazziq Mohd Hamdan and Mimi Syazwani Jaapar for the great team works. My heartfelt thanks and love go to my family members and friends for their endless love and strong mental supports throughout the journey of my study.

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Eric Lim Teik Chung, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Faez Firdaus Jesse Abdullah, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Annas Salleh, PhD
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 14 October 2021

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No: Kalai Vaani a/p Munaindy GS54187

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Dr. Eric Lim Teik Chung

Signature: _____
Name of Member of Supervisory Committee: Professor Dr. Faez Firdaus Jesse Abdullah

Signature: _____
Name of Member of Supervisory Committee: Dr. Annas Salleh

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVATIONS

B. decumbens	*Brachiaria decumbens*
CSF	Cerebrospinal fluid
APP	Acute phase proteins
APR	Acute phase responses
Hp	Haptoglobin
SAA	Serum Amyloid A
RBC	Red blood cells
Hb	Hemoglobin
PCV	Packed cell volume
MCV	Mean corpuscular volume
MCHC	Mean corpuscular hemoglobin concentration
WBC	White blood cells
AST	Aspartate aminotransferase
GGT	Gamma-glutamyltransferase
A:G	Albumin:Globulin ratio
T1	Treatment group 1
T2	Treatment group 2
T3	Treatment group 3
H&E	Hematoxylin and Eosin
ELISA	Enzyme-linked immunosorbent assay

CHAPTER 1

INTRODUCTION

1.1 Introduction

The small ruminant sub-sector in Malaysia is very small compared to the poultry, swine and cattle industry. Most small ruminants are mainly operated by smallholder farmers. In 2019, the populations of sheep and goat consist of only 127,796 and 371,747 respectively (Department of Veterinary Services Malaysia, 2020). Although the per capita consumption (PCC) for mutton is the lowest at 1.1 kg/year compared to the other commodity, the self-sufficiency level (SSL) of mutton in Malaysia is only at 12.1% in 2019. As result, the small ruminant industry is heavily dependent on the importation of mutton and chevon from other countries. The lag in the sheep and goat industry is normally associated with several factors such as the lack of land resources; high feed price; cheaper import substitutes; poor private-sector involvement; inefficient disease prevention and control; lack of quality breeds, expertise, and workforce (National Agro-food Policy, 2011-2020). Nevertheless, feed constitutes the biggest proportion of the cost of production in any livestock industry. The small ruminant industry depends primarily on locally available feedstuffs, with only some supplementation provided by imported ingredients. The major local materials used are crop residues and other by-products such as rice bran, copra cake, palm kernel cake, oil palm frond, sago, tapioca, and broken rice as alternative feed (Zahari and Wong, 2009). Therefore, more research and development are required to identify and improve local forage that is vastly abundant in Malaysia such as the *Brachiaria decumbens*.

B. decumbens or known as signal grass is the most favoured species and covers more than 80% of pasture land in Malaysia and other tropical regions due to its tolerance to low soil fertility, high dry matter production, and drought resistance (Faccin et al., 2014; Low, 2015; Chung et al., 2018). Even though *B. decumbens* is an important source of forages for ruminant, there have been many reports of toxicity in grazing livestock including sheep due to the naturally occurring toxic compounds found in *B. decumbens* (Graydon et al., 1991; Cruz et., 2001; Brum et al., 2007; Castro et al., 2011; Assumaidee and Mustapha, 2012). Protodioscin is the main steroidal saponins found in *B. decumbens* which is associated with secondary hepatogenous photosensitization in ruminants (Low, 2015). Sheep are more susceptible than other ruminants and the young are more predisposed than adults (Riet-Correa et al., 2011; Faccin et al., 2014). Additionally, young leaves of *Brachiaria spp.* contain higher saponins concentration than mature plants (Castro et al., 2011; Riet-Correa et al., 2011).

The clinical signs of *B. decumbens* intoxication are jaundice, hepatogenous photosensitization, anorexia, facial or submandibular oedema and neurological signs at the later stage as the disease progresses (Assumaidaee et al., 2010: Lelis et al., 2018). Secondary or hepatogenous photosensitization in ruminants occurs as a result of liver

damage that is caused by the toxicity of *B. decumbens* (Knight and Walter, 2003). The damaged liver is incapable of removing chlorophyll (phylloerythrin), therefore leads to the development of skin lesions when exposed to sunlight (De Oliveira et al., 2013).

Previous studies on *B. decumbens* reported that increased liver enzymes such as serum aspartate aminotransferase (AST), serum gamma-glutamyltransferase (GGT), and bilirubin levels are the cause of impaired liver functions (Castro et al., 2011; Riet-Correa et al., 2011; De Oliveira et al., 2013). High bilirubin will also cause a high degree of renal susceptibility due to ischaemia. As a result, an increase in the concentrations of serum urea and creatinine are related to the decrease in glomerular filtration rate or indicators of renal impairment (Gracindo et al., 2014; Lelis et al., 2018). In the meantime, acute phase proteins (APPs) are blood proteins produced by the liver during acute phase responses (Jesse et al., 2019). This is an early defense system that is activated by inflammation, infection, stress, or trauma (Cray et al., 2009). In ruminants, haptoglobin (Hp) and serum amyloid A (SAA) are the major APPs identified as a marker during infection and inflammation, being more sensitive and specific than blood profile analysis (Eckersall et al., 2007; Ceciliani et al., 2012).

At post mortem, the most remarkable lesions associated with *B. decumbens* toxicosis could be observed in the liver and kidney (Castro et al., 2011; Riet-Correa et al., 2011). Gross lesions such as enlarged, mottled, brown discolouration of liver with distended gall bladder (De Oliveira et al., 2013; Faccin et al., 2014) and swollen, grey yellowish kidney may be observed in *B. decumbens* intoxicated animals (Assumaidee and Mustapha, 2012).

Histopathologically, the lesions are also more appreciable in the liver and kidneys as these organs are the major sites of cellular damage. Dominantly, the liver histopathology is characterised by hydropic degeneration of hepatocytes, hyperplasia of bile duct epithelium, and mononuclear inflammatory cells infiltration (Graydon et al., 1991; Faccin et al., 2014). Furthermore, there will be the presence of birefringent crystals within the bile ducts and accumulations of foamy macrophages during the chronic stage (Driemeier et al., 2002; Riet-Correa et al., 2011). Besides, degenerative and necrotic changes of the renal epithelial cells will also be observed in the kidneys in the later stage of intoxication (Graydon et al., 1991).

1.2 Problem statements

1. *B. decumbens* is a highly productive tropical grass that present abundantly in Malaysia, but there were many reports of sporadic outbreaks of general ill-thrift and deaths in ruminants attributable to the presence of steroidal saponins.
2. Farmers are not be able to utilise this grass effectively as a feed source for ruminants because information on the low and high levels of *B. decumbens* toxicity is still unknown.

3. In addition, information on the clinical responses, haematology changes, and APPs responses are not available, which could be used as potential biomarkers for early diagnosis for *B. decumbens* toxicity.

1.3 Hypotheses

It is hypothesized that there will be different responses in sheep fed with various level of *B. decumbens* and at different time phases as compared to the control group.

1. Sheep fed with high and low level of *B. decumbens* diets will show changes in clinico-pathological, haemato-biochemical changes, and APPs responses as compared to the control group.

2. Sheep in chronic stage will show more severe clinico-pathological, haemato-biochemical changes and APPs responses as compared to sheep in the acute stage.

1.4 Objectives

The general aim of this study was to determine the clinico-pathology, haemato-biochemistry, and acute phase proteins (APPs) responses of sheep fed with low and high levels of *B. decumbens* diets at different time phases. The specific objectives were:

1. To determine the clinical signs of sheep fed with low and high levels of *B. decumbens* diets and at different time phases.

2. To determine the haemato-biochemistry changes in sheep fed with low and high levels of *B. decumbens* diets and at different time phases.

3. To measure the APPs responses of both serum and cerebrospinal fluid (CSF) in sheep fed with low and high levels of *B. decumbens* diets and at different time phases.

4. To evaluate the gross pathology, organ morphometric, and histopathology in sheep fed with low and high levels of *B. decumbens* diets and at different time phases.

REFERENCES

Abdullah, A.S., Rajion, M.A. 1997. Dietary factors affecting entero-hepatic function of ruminants in the tropics. *Animal Feed Science and Technology*. 69, 79– 90.

Agha, I., Mahoney, R., Beardslee, M., Liapis, H., Cowart, R.G., and Juknevicius, I. 2002. Systemic amyloidosis associated with pleomorphic sarcoma of the spleen and remission of nephrotic syndrome after removal of the tumor. *American Journal of Kidney Diseases*. 40(2), 411-415.

Akhtar, T., Sheikh, N., and Abbasi, M.H. 2014. Clinical and pathological features of Nerium oleander extract toxicosis in wistar rats. *BMC Research Notes*. 7(1), 947.

Albernaz, T.T., Silveira, J.A.S.D., Silva, N.D.S., Oliveira, C.H.S., Belo Reis, A.D.S., Oliveira, C.M.C., Reis, A.D.S.B., Oliveira, C.M.C., Duarte, M.D., Barbosa, J.D. 2010. Photosensitization of sheep kept on *Brachiaria brizantha* pasture in the state of Pará. *Pesquisa Veterinária Brasileira*. 30(9), 741-748.

Aregheore, E.M. 2001. Nutritive value and utilization of three grass species by crossbred Anglo-Nubian goats in Samoa. *Asian-Australasian Journal of Animal Sciences.* 14 (10), 1389–1393.

Aslani, M.R., Movassaghi, A.R., Mohri, M., Ebrahim-Pour, V., Mohebi, A.N. 2004. Experimental *Tribulus terrestris* poisoning in goats. *Small Ruminant Research*. 51(3), 261-267.

Aslani, M.R., Movassaghi, A.R., Mohri, M., Pedram, M., Abavisani, A. 2003. Experimental *Tribulus terrestris* poisoning in sheep: clinical, laboratory and pathological findings. *Veterinary Research Communications*. 27(1), 53-62.

Assumaidaee, A.A., Zamri-Saad, M., Jasni, S., and Noordin, M.M. 2010. The role of oxidative stress in *Brachiaria decumbens* toxicity in sheep. *Pertanika Journal of Tropical Agricultural Science*. 33(1), 151-157.

Assumaidee, A.J.M. and Mustapha, N.M. 2012. Toxicity of signal grass (*Brachiaria decumbens*): a review article. *Journal of Advanced Medical Research*. 2(2012), 18–39.

Baber, R. 1989. Photosensitisation: a note of caution in the use of *Brachiaria* pastures—a review. *Tropical Animal Health and Production*. 21, 277–280.

Badenes-Perez, F.R., Gershenzon, J., Heckel, D.G. 2014. Insect attraction versus plant defense: young leaves high in glucosinolates stimulate oviposition by a specialist herbivore despite poor larval survival due to high saponin content. *PLoS One 9* (4), e95766.

Badiei, K., Mostaghni, K., Nazifi, S., Tafti, A.K., Ghane, M., Momeni, S.A. 2009. Experimental *Panicum miliaceum* poisoning in sheep. *Small Ruminant Research.* 82, 99–104.

Bastos, B.L., Meyer, R., Guimaraes, J.E., Ayres, M.C., Guedes, M.T., Moura-Costa, L.F., de Burghgrave, U.S., Sena, L., Azevedo, V., Portela, R.W. 2011. Haptoglobin and fibrinogen concentrations and leukocyte count in the clinical investigation of caseous lymphadenitis in sheep. *Veterinary Clinical Pathology*. 40, 496–503.

Beesley, N.J., Caminade, C., Charlier, J., Flynn, R.J., Hodgkinson, J.E., Martinez-Moreno, A., Martinez-Valladares, M., Perez, J., Rinaldi, L., Williams,

D.J.L. 2018. *Fasciola* and fasciolosis in ruminants in Europe: identifying research needs. *Transboundary and Emerging Diseases*. 65, 199–216.

Biswas, U., Sarkar, S., Bhowmik, M.K., Samanta, A.K., and Biswas, S. 2000. Chronic toxicity of arsenic in goats: clinicobiochemical changes, pathomorphology and tissue residues. *Small Ruminant Research*, 38(3), 229-235.

Braun, J.P., Benard, P., Burgat, V., and Rico, A.G. 1983. Gamma glutamyl transferase in domestic animals. *Veterinary Research Communications*, 6(1), 77-90.

Brum, K.B., Haraguchi, M., Garutti, M.B., Nobrega, F.N., Rosa, B., Fioravanti,

M.C.S. 2009. Steroidal saponin concentrations in *Brachiaria decumbens* and *B. brizantha* at different developmental stages. *Ciencia Rural*. 39, 279– 281.

Brum, K.B., Haraguchi, M., Lemos, R.A., Riet-Correa, F., and Fioravanti, M.C.S. 2007. Crystal-associated cholangiopathy in sheep grazing *Brachiaria decumbens* containing the saponin protodioscin. *Pesquisa Veterinária Brasileira*. 27(1), 39-42.

Button, C., Paynter, D.I., Shiel, M.J., Colson, A.R., Paterson, P.J., Lyford, R.L. 1987. Crystal-associated cholangiohepatopathy and photosensitisation in lambs. *Australian Veterinary Journal*. 64, 176–180.

Caicedo, J.A., Ospina, J.C., Chaves, C.A., Pena, J., Lozano, M.C., Doncel, B. 2012. Hepatic lesions in cattle grazing on *Brachiaria decumbens* in Mesetas, Meta (Colombia). *Revista de la Facultad de Medicina Veterinaria y de Zootecnia*. 59, 102–108.

Cardona-Álvarez, J., Vargas-Vilória, M., and Paredes-Herbach, E. 2016. Clinical and histopathological study of the phototoxic dermatitis in Zebu calves in grazing of *Brachiaria decumbens*. *Revista Medicina Veterinaria Zootecnia Córdob.* 21(2), 5366-5380.

Castro, M.B., Gracindo, C.V., Landi, M.F.A., Filho, S.L.S.C., Filho, N.J.R., Lima, E.M.M., Riet-Correa, F. 2018. Sheep adaptation management, and investigation of inherited resistance to prevent *Brachiaria spp.* poisoning. *Small Ruminant Research*. 158, 42–47.

Castro, M.B., Santos Jr, H.L., Mustafa, V.S., Gracindo, C.V., Moscardini, A.C.R., Louvandini, H., Paludo, G.R, Borges, J.R.J, Haraguchi, M., Ferreira, M.B., Riet-Correa, F. 2011. *Brachiaria spp.* poisoning in sheep in Brazil: experimental and epidemiological findings. *Poisoning by Plants, Mycotoxins and Related Toxins*, 1, 110-117.

Ceciliani, F., Ceron, J.J., Eckersall, P.D., and Sauerwein, H. 2012. Acute phase proteins in ruminants. *Journal of Proteomics*. 75, 4207–4231.

Cheeke, P.R. 1995. Endogenous toxins and mycotoxins in forage grasses and their effects on livestock. *Journal of Animal Science*. 73, 909–918.

Cheeke, P.R. 1996. Biological effects of feed and forage saponins and their impacts on animal production. In: *Saponins Used in Food and Agriculture*. Springer, Boston, MA, pp. 377–385.

Chung, E.L.T., Abdullah, F.F.A., Marza, A.D., Ibrahim, H.H., Zamri-saad, M., Haron, A.W., Saharee, A.A., Lila, M.A.M., Norsidin, M.J. 2017. Clinico- pathology and hemato-biochemistry responses in buffaloes towards *Pasteurella multocida* type B:2 immunogen outer membrane protein via subcutaneous and oral routes of infection. *Microbial Pathogenesis*. 102: 89- 101.

Chung, E.L.T., Jesse, F.F.A., Marza, A.D., Ibrahim, H.H., Abba, Y., Zamri-Saad,

M., Haron, A.W., Lila, M.A.M., Saharee, A.A., and Bakar, M.Z.A. 2019. Responses of pro-inflammatory cytokines, acute phase proteins and cytological analysis in serum and cerebrospinal fluid during haemorrhagic septicaemia infection in buffaloes. *Tropical Animal Health and Production*. 51(6), 1773-1782.

Chung, E.L.T., Predith, M., Nobilly, F., Samsudin, A.A., Jesse, F.F.A., and Loh,

T.C. 2018. Can treatment of *Brachiaria decumbens* (signal grass) improve its utilisation in the diet in small ruminants? —a review. *Tropical Animal Health and Production*. 50, 1727–1732.

Cousins, R.J., Barber, A.K., and Trout, J.R. 1973. Cadmium toxicity in growing swine. *The Journal of Nutrition*. 103(7), 964-972.

Cray, C., Zaias, J., and Altman, N.H. 2009. Acute phase response in animals: a review. *Comparative Medicine*. 59(6), 517–526.

Cruz, C., Driemeier, D., Pires, V.S., and Schenkel, E.P. 2001. Experimentally induced cholangiohepatopathy by dosing sheep with fractionated extracts from *Brachiaria decumbens*. *Journal of Veterinary Diagnostic Investigation*. 13(2), 170–172.

Dargie, J.D. 1987. The impact on production and mechanisms of pathogenesis of trematode infections in cattle and sheep. *International Journal for Parasitology*. 17(2), 453-463.

De Oliveira, C.H.S., Barbosa, J.D., Oliveira, C.M.C., Bastianetto, E., Melo, M.M., Haraguchi, M., Freitas, L.G.L., Silva, M.X., Leite, R.C. 2013. Hepatic photosensitisation in buffaloes intoxicated by *Brachiaria decumbens* in Minas Gerais state, Brazil. *Toxicon* 73, 121–129.

de Zwart, L.L., Meerman, J.H., Commandeur, J.N., and Vermeulen, N.P. 1999. Biomarkers of free radical damage: applications in experimental animals and in humans. *Free Radical Biology and Medicine.* 26(1-2), 202-226.

Department of Veterinary Services Malaysia: Perangkaan Ternakan. Available online:http://www.dvs.gov.my/dvs/resources/user_1/2020/BP/Perangkaan/Perangkaan_Ternakan_2019_2020_4_Jan_2021_final_combine.pdf(accessed on 29th July 2021).

Di Menna, M.E., Mortimer, P.H., Smith, B.L., and Tulloch, M. 1973. The incidence of the genus *Myrothecium* in New Zealand pastures and its relation to animal disease. *Microbiology.* 79(1), 81-87.

DiBartola, S.P. and Bateman, S. 2006. Introduction to fluid therapy. Fluid Therapy in Small Animal Practice, *Elsevier Health Sciences*, 2, 265-280.

Driemeier, D., Colodel, E.M., Seitz, A.L., Barros, S.S., and Cruz, C.E. 2002. Study of experimentally induced lesions in sheep by grazing *Brachiaria decumbens*. *Toxicon.* 40, 1027–1031.

Eckersall, P.D., Bell, R. 2010. Acute phase proteins: biomarkers of infection and inflammation in veterinary medicine. *The Veterinary Journal*. 185, 23–27.

Eckersall, P.D., Lawson, F.P., Bence, L., Waterston, M.M., Lang, T.L., Donachie, W., and Fontaine, M.C. 2007. Acute phase protein response in an experimental model of ovine caseous lymphadenitis. *BMC Veterinary Research.* 3(1), 35.

El-Deeb, W.M., Tharwat, M. 2015. Lipoproteins profile, acute phase proteins, proinflammatory cytokines and oxidative stress biomarkers in sheep with pneumonic pasteurellosis. *Comparative Clinical Pathology*. 24, 581–588.

Faccin, T.C., Riet-Correa, F., Rodrigues, F.S., Santos, A.C., Melo, G.K., Silva, J.A., Ferreira, R., Itavo, C.C.B.F., Lemos, R.A. 2014. Poisoning by *Brachiaria brizantha* in flocks of naïve and experienced sheep. *Toxicon* 82, 1–8.

Fernández, A., Ramos, J.J., Sanz, M.D.C., Saez, T., and Luco, D.F.D. 1996. Alterations in the performance, haematology and clinical biochemistry of growing lambs fed with aflatoxin in the diet. *Journal of Applied Toxicology*. 16(1), 85-91.

Ferreira, M., Brum, K., Fernandes, C., Martins, C., Pinto, G., Castro, V., Rezende, K.G., Riet-Correa, F., Haraguchi, M., Wysocki, H.L., Lemos, R.A.A. 2009. Variation in saponin concentration in *Brachiaria brizantha* leaves as a function of maturation: preliminary data. In: *Poisoning by Plants, Mycotoxins, and Related Toxin*, Proc. 8th. ISOPP., pp. 2–8. Joao Pessoa, Para-Iba, Brazil.

Filippo, P.A.D., Lannes, S.T., Meireles, M.A., Nogueira, A.F., Ribeiro, L.M., Graça, F., and Glória, L.S. 2018. Acute phase proteins in serum and cerebrospinal fluid in healthy cattle: possible use for assessment of neurological diseases. *Pesquisa Veterinária Brasileira*. 38(4), 779-784.

Flåøyen, A. 1996. Do steroidal saponins have of role in hepatogenous photosensitization diseases of sheep?. In: *Saponins Used in Food and Agriculture.* Springer, Boston, MA, pp. 395–403.

Flåøyen, A., Smith, B.L., Miles, C.O. 1993. An attempt to reproduce crystal- associated cholangitis in lambs by the experimental dosing of sarsasapogenin or diosgenin alone and in combination with sporidesmin. *New Zealand Veterinary Journal*. 41, 171–174.

Flåøyen, A., Wilkins, A.L. 1997. Metabolism of saponins from *Narthecium ossifragum*-a plant implicated in the aetiology of alveld, a hepatogenous photosensitization of sheep. *Veterinary Research Communications*. 21 (5), 335–345.

Francis, G., Kerem, Z., Makkar, H.P., Becker, K. 2002. The biological action of saponins in animal systems: a review. *British Journal of Nutrition*. 88, 587– 605.

Gholamzadeh, S., Zarenezhad, M., Montazeri, M., Zareikordshooli, M., Sadeghi, G., Malekpour, A., Hoseni, S., Bahrani, M., Hajatmand, R. 2017. Statistical Analysis of Organ Morphometric Parameters and Weights in South Iranian Adult Autopsies. *Medicine*. 96(21).

Giffen, P.S., Turton, J., Andrews, C.M., Barrett, P., Clarke, C.J., Fung, K.W.,

Munday, M.R., Roman, I.F., Smyth, R., Walshe, K., and York, M. J. 2003. Markers of experimental acute inflammation in the Wistar Han rat with particular reference to haptoglobin and C-reactive protein. *Archives of Toxicology*, 77(7), 392-402.

Gomar, M.S., Driemeier, D., Colodel, E.M., Gimeno, E.J. 2005. Lectin histochemistry of foam cells in tissues of cattle grazing *Brachiaria spp. Journal of Veterinary Medicine*. A 52, 18–21.

Gracindo, C.V., Louvandini, H., Riet-Correa, F., Barbosa-Ferreira, M., and de Castro, M.B. 2014. Performance of sheep grazing in pastures *of Brachiaria decumbens, Brachiaria brizantha, Panicum maximum,* and *Andropogon gayanus* with different protodioscin concentrations. *Tropical Animal Health and Production*. 46(5), 733–737.

Graydon, R.J., Hamid, H., Zahari, P., and Gardiner, C. 1991. Photosensitisation and crystal- associated cholangiohepatopathy in sheep grazing *Brachiaria decumbens*. *Australian Veterinarian Journal*. 68(7), 234–236.

Haligur, M., Ozmen, O. 2011. Immunohistochemical detection of serum amyloid A, serum amyloid-P, C-reactive protein, tumour necrosis factor-and TNF-a receptor in sheep and goat pneumonias. *Revue de Médecine Vétérinaire*. 162, 475–481.

Haschek, W.M., Rousseaux, C.G., and Wallig, M.A. 2010. Hematopoietic System.

Fundamentals of Toxicologic Pathology, Academic Press, 491–512.

Horadagoda, A., Eckersall, P.D., Hodgson, J.C., Gibbs, H.A., and Moon, G.M. 1994. Immediate responses in serum TNFα and acute phase protein concentrations to infection with *Pasteurella haemolytica* A1 in calves. *Research in Veterinary Science*. 57(1), 129-132.

Iliev, P.T., Georgieva, T.M. 2017. Acute phase biomarkers of diseases in small ruminants: an overview. *Bulgarian Journal of Veterinary Medicine.*

Jackson, P.G., Cockcroft, P.D., and Elmhurst, S. 2002. Clinical examination of the nervous system. *Clinical Examination of Farm Animals*, Wiley-Blackwell.

Jesse, F.F.A., Chung, E.L.T., Abba, Y., Muniandy, K.V., Tan, A.H.A.R., Maslamany, D., Bitrus, A.A., Lila, M.A.M., and Norsidin, M.J. 2019. Establishment of lung auscultation scoring method and responses of acute phase proteins and heat shock proteins in vaccinated and non-vaccinated goats. *Tropical Animal Health and Production*. 51, 289–295.

Jones, M.L. and Allison, R.W. 2007. Evaluation of the ruminant complete blood cell count. Veterinary Clinics of North America: *Food Animal Practice*, 23(3), 377-402.

Keller-Grein, G., Maass, B.L., and Hanson, J. 1996. Natural variation in Brachiaria and existing germplasm collections. Brachiaria: biology, agronomy, and improvement, 45, 16-42.

Kempuraj, D., Thangavel, R., Selvakumar, G.P., Zaheer, S., Ahmed, M.E., Raikwar, S.P., Zahoor, H., Saeed, D., Natteru, P.A., Iyer, S., and Zaheer, A. 2017. Brain and peripheral atypical inflammatory mediators potentiate neuroinflammation and neurodegeneration. *Frontiers in Cellular Neuroscience*. 11, 216.

Kerr, M.G. 2008. Veterinary laboratory medicine: Clinical biochemistry and haematology. *John Wiley & Sons, Inc*., Hoboken, New Jersey.

Keyvanlou, M., Aslani, M.R., Mohri, M., and Seifi, H.A. 2011. Clinical, haematological and biochemical evaluation of onion (*Allium cepa*) toxicity in goats. *Revue de Médecine Vétérinaire*, 162(12), 593-598.

Knight, A.P., Walter, R.G. 2003. Plants Affecting the Skin and Liver. *A Guide to Plant Poisoning of Animals.* North America, Teton Media, Jackson WY.

Lajis, N.H., Abdullah, A.S.H., Salim, S.J.S., Bremmer, J.B., Khan, M.N. 1993. Epi-sarsasapogenin and epi-smilagenin: two sapogenins isolated from the rumen content of sheep intoxicated by *Brachiaria decumbens*. *Steroids*. 58, 387–389.

Lelis, D.L., Rennó, L.N., Chizzotti, M.L., Pereira, C.E.R., Silva, J.C.P., Moreira, L.G.T., Carvalho, F.B.P., and Chizzotti, F.H.M. 2018. Photosensitization in naïve sheep grazing signal grass (*Brachiaria decumbens*) under full sunlight or a silvopastoral system. *Small Ruminant Research*. 169, 24-28.

Lima, F.G.D., Haraguchi, M., Pifster, J.A., Guimar~aes, V.Y., Andrade, D.F., Ribeiro, C.S., Costa, G.L., Araujo, A.L.L., Fioravanti, M.C.S. 2012. Weather and plant age affect the levels of steroidal saponin and *Pithomyces chartarum* spores in *Brachiaria* grass. *The International Journal of Poisonous Plant Research.* 2, 45–53.

Lima, F.G.D., Lee, S.T., Pfister, J.A., Miyagi, E.S., Costa, G.L., Silva, R.D.D., Fioravanti, M. C.S. 2015. The effect of ensiling and haymaking on the concentrations of steroidal saponin in two *Brachiaria* grass species. *Ciencia Rural*. 45, 858–866.

Low, S. 2015. Signal grass (*Brachiaria decumbens*) toxicity in grazing ruminants. *Agriculture.* 5(4), 971–990.

Marques, F., Sousa, J.C., Coppola, G., Falcao, A.M., Rodrigues, A.J., Geschwind, D.H., Sousa, N., Correia-Neves, M., and Palha, J.A. 2009. Kinetic profile of the transcriptome changes induced in the choroid plexus by peripheral inflammation. *Journal of Cerebral Blood Flow & Metabolism*. 29(5), 921- 932.

Mazni, O.A., Sharif, H., Khusahry, M.M., Vance, H.N. 1985. Photosensitisation in goats grazed on *Brachiaria decumbens*. *Mardi Research*. 13, 203–206.

McEwen, B.J. 1992. Eosinophils: a review. *Veterinary Research Communications*, 16(1), 11-44.

McGavin, M.D., Zachary, J.F. 2006. Pathologic basis of veterinary disease. *Elsevier Health Sciences.*

Meagher, L.P., Smith, B.L., Wilkins, A.L. 2001. Metabolism of diosgenin-derived saponins: implications for hepatogenous photosensitisation diseases in

ruminants. *Animal Feed Science and Technology*. 91, 157–170.

Meling, S., Bårdsen, K., Ulvund, M.J. 2012. Presence of an acute phase response in sheep with clinical classical scrapie. *BMC Veterinary Research*. 8, 113.

Menkes, A., Bukchin, M., Zakharov, M., Dagan, A. 2017. Pet animal collar for health and vital signs monitoring, alert and diagnosis. *U.S. Patent No. 9,615,547*. Washington, DC: USPTO.

Miles, C.O., Wilkins, A.L., Erasmus, G.L., Kellerman, T.S., Coetzer, J.A.W. 1994. Photosensitivity in South Africa. VII. Chemical composition of biliary crystals from a sheep with experimentally induced geeldikkop. *The Onderstepoort Journal of Veterinary Research*. 61, 215–222.

Miles, C.O., Wilkins, A.L., Munday, S.C., Flaoyen, A., Holland, P.T., Smith, B.L. 1993. Identification of insoluble salts of the. beta. -D-glucuronides of episarsasapogenin and epismilagenin in the bile of lambs with alveld and examination of *Narthecium ossifragum*, *Tribulus terrestris*, and *Panicum miliaceum* for sapogenins. *Journal of Agricultural and Food Chemistry*. 41 (6), 914–917.

Miles, C.O., Wilkins, A.L., Munday, S.C., Holland, P.T., Smith, B.L., Lancaster, M.J., Embling, P.P. 1992. Identification of the calcium salt of epismilagenin. beta. -D- glucuronide in the bile crystals of sheep affected by *Panicum dichotomiflorum* and *Panicum schinzii* toxicoses. *Journal of Agricultural and Food Chemistry*. 40, 1606–1609.

Montoya-Ménez, C.B., Ruíz-Ramírez, J.A., Márquez, L.J., Méndez-Bernal, A., Morales-Salinas, E., Ramírez-Romero, R., Martinez-Burnes, J., and López-Mayagoitia, A. 2019. Hepatogenous photosensitization by Brachiaria spp. in sheep: first report in Mexico. Brazilian Journal of Veterinary Pathology, 12(3), 128-133.

Moore, M.N., Allen, J.I., and McVeigh, A. 2006. Environmental prognostics: an integrated model supporting lysosomal stress responses as predictive biomarkers of animal health status. *Marine Environmental Research*. 61(3), 278-304.

Moreira, C.N., Souza, S.N., Barini, A.C., Araújo, E.G., and Fioravanti, M.C.S. 2012. Serum γ-glutamyltransferase activity as an indicator of chronic liver injury in cattle with no clinical signs. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. 64(6), 1403-1410.

Munday, S.C., Wilkins, A.L., Miles, C.O., Holland, P.T. 1993. Isolation and structure elucidation of dichotomin, a furostanol saponin implicated in hepatogenous photosensitization of sheep grazing *Panicum dichotomiflorum*. *Journal of Agricultural and Food Chemistry*. 41 (2), 267– 271.

Murata, H., Shimada, N., and Yoshioka, M. 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. *The Veterinary Journal*. 168(1), 28–40.

Nagy, O., Tothovam C., Nagyova, V., and Kovac, G. 2015. Comparison of serum protein electrophoretic pattern in cows and small ruminants. *Acta Veterinaria Brno*. 84(2), 187-185.

National Agro-food Policy, 2011-2020. Available online: http://teebweb.org/wp-content/uploads/2020/12/10-National-Agrofood-Policy-Scope-Priorities-MAFI.pdf (accessed on 29th July 2021).

Ng, K.F., Stür, W.W., Shelton, H.M. 1997. New forage species for integration of sheep in rubber plantations. *The Journal of Agricultural Science.* 128, 347– 356.

Noordin, M.M., Abdullah, A.S., Rajion, M.A. 1989. Experimental *Brachiaria decumbens* toxicity in cattle. *Veterinary Research Communications.* 13, 491–494.

Opasina, B.A. 1985. Photosensitisation jaundice syndrome in West African Dwarf sheep and goats grazed on *Brachiaria decumbens*. *Tropical Grasslands*. 19, 120–123.

Ortolani, E.L. 2001. Sulphur deficiency in dairy calves reared on pasture of

Brachiaria decumbens. Ciencia Rural. 31, 257–261.

Othman, A.M., Haron, S. 1988. Hepatotoxicity in indigenous sheep of Malaysia stall-fed with different forms of *Brachiaria decumbens*. *Pertanika Journal of Tropical Agricultural Science.* 11, 57–61.

Ovalle, W.K., Nahirney, P.C. 2020. Netter's Essential Histology E-Book: With Correlated Histopathology. *Elsevier Health Sciences.*

Pfeffer, A., Rogers, K.M. 1989. Acute phase response of sheep: changes in the concentrations of ceruloplasmin, fibrinogen, haptoglobin and the major blood cell types associated with pulmonary damage. *Research in Veterinary Science*. 46, 118–124.

Pfeffer, A., Rogers, K.M., O'keeffe, L., Osborn, P.J. 1993. Acute phase protein response, food intake, liveweight change and lesions following intrathoracic injection of yeast in sheep. *Research in Veterinary Science*. 55, 360–366.

Pupin, R.C., Melo, G.K.A., Heckler, R.F., Faccin, T.C., Itavo, C.C.B.F., Fernandes, C.E., Gomes, D.C., Lemos, R.A.A. 2016. Identification of lamb flocks susceptible and resistant against *Brachiaria* poisoning. *Pesquisa Veterinária Brasileira*. 36, 383–388.

Riet-Correa, B., Castro, M.B., Lemos, R.A., Riet-Correa, G., Mustafa, V., and Riet-Correa, F. 2011. *Brachiaria spp*. poisoning of ruminants in Brazil. *Pesquisa Veterinária Brasileira*, 31(3), 183–192.

Rosa, F.B., Rubin, M.I., Martins, T.B., de Lemos, R.A., Gomes, D.C., Pupin, R.C., Lima, S. C., Barros, C.S. 2016. Spontaneous poisoning by *Brachiaria decumbens* in goats. *Pesquisa Veterinária Brasileira*. 36, 389–396.

Roy-Chowdhury, N., Wang, X., and Roy-Chowdhury, J. 2020. Bile pigment metabolism and its disorders. In *Emery and Rimoin's Principles and Practice of Medical Genetics and Genomics* (pp. 507-553). Academic Press United States.

Santos, J.C.A., Riet-Correa, F., Simões, S.V., Barros, C.S. 2008. Pathogenesis, clinical signs and pathology of diseases caused by hepatotoxic plants in ruminants and horses in Brazil. *Pesquisa Veterinária Brasileira*. 28(1), 1- 14.

Saxena, A. and Cronstein, B.N. 2013. Acute phase reactants and the concept of inflammation. In *Kelley's Textbook of Rheumatology* (pp. 818-829). WB Saunders, Philadelphia.

Sparg, S., Light, M.E., and Van Staden, J. 2004. Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology*. 94(2-3), 219–243.

Strimbu, K. and Tavel, J. A. 2010. What are biomarkers?. *Current Opinion in HIV and AIDS*, Vol 16, issue 4, Lippincott Williams and Wilkins Ltd, 5(6), 463.

Suriyapha, C., Ampapon, T., Viennasay, B., Matra, M., Wann, C., and Wanapat, M. 2019. Manipulating rumen fermentation, microbial protein synthesis, and mitigating methane production using bamboo grass pellet in swamp buffaloes. *Tropical animal health and production*, 1-7.

Tothova, C., Nagy, O., and Kovac, G. 2016. Serum proteins and their diagnostic utility in veterinary medicine: a review. *Veterinární Medicína*, 61(9), 475- 496.

Tothova, C., Nagy, O., Kovac, G. 2014. Acute phase proteins and their use in the diagnosis of diseases in ruminants: a review. *Veterinární Medicína*. 59, 163–180.

Ulutas, P.A., Ozpinar, A. 2006. Effect of *Mannheimia (Pasteurella) haemolytica* infection on acute-phase proteins and some mineral levels in colostrum– breast milk-fed or colostrum–breast milk-deprived sheep. *Veterinary Research Communications*. 30, 485–495.

Wells, B., Innocent, G.T., Eckersall, P.D., McCulloch, E., Nisbet, A.J., Burgess, S.T. 2013. Two major ruminant acute phase proteins, haptoglobin and serum amyloid A, as serum biomarkers during active sheep scab infestation. *Veterinary Research*. 44, 103.

Wendler, A. and Wehling, M. 2010. The translatability of animal models for clinical development: biomarkers and disease models. Current opinion in pharmacology, *Elsevier BV*. 10(5), 601-606.

Wina, E., Muetzel, S., Becker, K. 2005. The impact of saponins or saponin- containing plant materials on ruminant production – a review. *Journal of Agricultural and Food Chemistry*. 53, 8093–8105.

Xu, T., Zhang, S., Zheng, L., Yin, L., Xu, L., and Peng, J. 2012. A 90-day subchronic toxicological assessment of dioscin, a natural steroid saponin, in Sprague–Dawley rats. *Food and Chemical Toxicology*. 50(5), 1279-1287.

Yuliana, P., Laconi, E.B., Wina, E., and Jayanegara, A. 2014. Extraction of tannins and saponins from plant sources and their effects on in vitro methanogenesis and rumen fermentation. *Journal of the Indonesian Tropical Animal Agriculture*. 39(2), 91-97.

Zahari, M.W., and Wong, H.K. 2009. Research and development on animal feed in Malaysia. *Indonesian Bulletin of Animal and Veterinary Sciences*, 19(4), 172-179.

Zubair, M. and Martyniuk, C.J. 2018. A review on hemato-biochemical, accumulation and patho-morphological responses of arsenic toxicity in ruminants. *Toxin Reviews*. 38(3), 176-186.