COMPARATIVE CLINICO-PATHOLOGY AND HAEMATO-BIOCHEMISTRY RESPONSES IN SHEEP FED WITH FRESH AND ENSILED *Brachiaria decumbens*

By

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

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

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

Over 80% of Malaysia's native grasslands include *Brachiaria decumbens*, which serves as primary feed for ruminants. Despite possessing good forage potential, there were cases reported regarding its toxicity to animals, especially sheep. The objective of this study is to assess the clinical signs, morphological features, hematology, and biochemistry effects of including ensiled *B. decumbens* into the diet of sheep raised under tropical climates. Eighteen Dorper cross sheep were randomly allocated to three treatments and placed in individual metabolic cages. The study applied a diet with a ratio of 70:30 roughage and concentrate, consisting of three different roughage treatments. These treatments included fresh *Pennisetum purpureum* (T1), fresh *B. decumbens* (T2), and ensiled *B. decumbens* (T3). Throughout 98 days of the experiment, the temperature, heart rate, respiration rate, rumen motility and ocular mucous membrane were gathered and observed weekly, while blood samples were taken on day 30, 60 and 90 of the experiment. All 18 sheep were slaughtered at the end of study, and the organs were collected for organ morphometric, gross morphology, and histological purposes. According to the results, there were significant differences ($p<0.05$) in the body weight and mucous membranes of the sheep across different feeding groups. T3 sheep fed with ensiled *B. decumbens* reported a significant ($p<0.05$) increase in body weight compared to T2 and control group. During the last 21 days of the trial, T2 sheep also had pale ocular mucous membranes. The hematological results indicate a significant difference ($p<0.05$) in sheep fed with ensiled *B. decumbens* compared to T2, except for red blood cells, white blood cells, and plasma protein levels. Similarly, there were also significances ($p<0.05$) in the biochemistry results of T3 sheep showing no effects towards the liver and kidney function when compared with T2 sheep. In addition, pathology reported the significance ($p<0.05$) of both the organ's width and weight, displaying lower values in T3 sheep. Histologically, sheep fed with fresh *B. decumbens* displayed significance ($p<0.05$) on the lesion scoring, generally showing mild to moderate lesions within several organs namely the lung, liver, kidney, jejunum, and ileum, while T3 showed an overall absence of lesions. Feeding ruminants ensiled *B. decumbens* is established to be significantly advantageous than feeding a diet of fresh *B. decumbens*, as evidenced by the overall health performance of the sheep.

Keywords: Blood indices; Dorper cross sheep; ensiled *Brachiaria decumbens*; histopathology; organ morphometric

SDG: GOAL 2: Zero Hunger; GOAL 3: Good Health and Well-being; GOAL 12: Responsible Consumption and Production

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

PERBANDINGAN RESPON KLINIKO-PATOLOGI DAN HEMATO-BIOKIMIA PADA BIRI-BIRI YANG DIBERI MAKAN RUMPUT *Brachiaria decumbens* SEGAR DAN SILAJ

Oleh

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Lebih 80% padang rumput asli Malaysia termasuk *Brachiaria decumbens* (Rumput signal) berfungsi sebagai makanan utama untuk ruminan. Walaupun mempunyai potensi makanan ternakan yang baik, terdapat kajian kes yang melaporkan mengenai sifat toksik rumput ini kepada haiwan, terutamanya biri-biri. Objektif kajian ini adalah untuk menilai tanda-tanda klinikal, ciri-ciri morfologi, kesan hematologi, dan kesan biokimia apabila memberi makan silaj *B. decumbens* ke dalam diet biri-biri yang diternak di bawah iklim tropika. Lapan belas biri-biri Dorper dibahagikan secara rawak kepada tiga rawatan dan diletakkan dalam sangkar metabolik secara individu. Kajian ini menggunakan diet dengan nisbah 70:30 rumput dan pelet, yang terdiri daripada tiga rawatan yang berbeza. Rawatan-rawatan berkenaan adalah *Pennisetum purpureum* (Rumput gajah) segar (T1), *B. decumbens* (T2) segar dan silaj *B. decumbens* (T3). Sepanjang 98 hari eksperimen dijalankan, data

seperti suhu, kadar denyutan jantung, kadar pernafasan, pergerakan rumen, dan membran mucus mata dikumpulkan dan dipantau setiap minggu, manakala sampel darah diambil pada hari ke 30, 60 dan 90 eksperimen. Kesemua 18 ekor biri-biri disembelih pada akhir kajian, dan organ-organ telah dikumpulkan untuk tujuan pemeriksaan morfometrik, morfologi, dan histologi. Berdasarkan keputusan eksperimen, terdapat perbezaan yang signifikan ($p<0.05$) pada berat badan dan membran mukus antara ketiga-tiga rawatan. Kumpulan biri-biri T3 yang diberi makan silaj *B. decumbens* melaporkan peningkatan berat badan yang signifikan ($p<0.05$) berbanding kumpulan T2 dan kumpulan terkawal. Pada 21 hari terakhir eksperimen, keputusan menunjukkan bahawa biri-biri T2 mempunyai membran mukus mata yang pucat. Keputusan hematologi menunjukkan perbezaan signifikan ($p<0.05$) pada biri-biri yang diberi silaj *B. decumbens* berbanding T2, kecuali beberapa parameter iaitu sel darah merah, sel darah putih, dan tahap protein plasma. Selain itu, terdapat juga perubahan signifikan ($p<0.05$) dalam keputusan biokimia di mana biri-biri T3 tidak menunjukkan implikasi terhadap fungsi hati dan buah pinggang jika dibandingkan dengan biri-biri T2. Di samping itu, patologi melaporkan keputusan signifikan ($p<0.05$) pada kedua-dua lebar dan berat organ, memaparkan nilai yang lebih rendah pada kumpulan biri-biri T3. Secara histologi, biri-biri yang diberi *B. decumbens* segar menunjukkan perubahan signifikan ($p<0.05$) pada pemarkahan luka, secara amnya menunjukkan luka ringan hingga sederhana dalam beberapa organ termasuk paru-paru, hati, buah pinggang, jejunum, dan ileum, manakala T3 menunjukkan ketiadaan keseluruhan luka. Pemberian silaj *B. decumbens* kepada ruminan terbukti lebih memberi kesan yang positif berbanding *B.*

decumbens segar, seperti yang dibuktikan melalui prestasi kesihatan keseluruhan biri-biri.

Kata kunci: histopatologi; indeks darah; kambing kacukan Dorper; morfometrik organ; silaj *Brachiaria decumbens*

SDG: MATLAMAT 2: Sifar Kebuluran; MATLAMAT 3: Kesihatan yang Baik dan Sejahtera; MATLAMAT 12: Penggunaan dan Pengeluaran Bertanggungjawab

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LIST OF ABBREVIATIONS

%	percentage
µg	micro gram
A:G	Albumin to globulin ratio
ALP	alkaline phosphatase
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
B. decumbens	*Brachiaria decumbens*
BUN	Blood urea nitrogen
BW	Body weight
BWG	Body weight gain
CBC	Complete blood count
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
DOSM	Department of Statistics Malaysia
GGT	Gamma glutamyltransferase
H&E	Hematoxylin and Eosin
Hb	Hemoglobin
IACUC	Institutional Animal Care and Use Committee
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume

ME	Metabolizable energy
N/A	Not available
PCV	Packed cell volume
PLT	Platelet
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RBC	Red blood cell
SD	Standard deviation
SEM	Standard error of mean
T1	Treatment 1
T2	Treatment 2
T3	Treatment 3
TP	Total protein
WBC	White blood cell

CHAPTER 1

INTRODUCTION

1.1 General introduction

In many developing countries, livestock production systems are of crucial importance to both household and national economies. Nevertheless, ruminants are one of the less-produced agricultural commodities in the livestock industry within Malaysia. The population of small ruminants, specifically sheep was announced at 136,800 in 2021 (DOSM, 2022), which has increased around 9.7% as of the previous year. However, sheep are regarded as the second lowest livestock population, surpassing buffalo production with a count of 66,600 animals. The growing numbers of livestock reported are crucial in ensuring the constant food supply and the need for efficient farming activities. In Malaysia, mutton exhibits the lowest per capita consumption (PCC) among other livestock, with an annual consumption of just 1 kg per person. Concurrently, mutton also demonstrates the lowest self-sufficiency ratio (SSR) compared to other livestock in Malaysia, standing at 10.7% in the year 2021, as reported by the Department of Statistics Malaysia (DOSM, 2022). It has been widely recognized that Malaysia still exhibits a high dependence on the importation of mutton from foreign countries. According to the Department of Statistics Malaysia (2022), the import dependency rate (IDR) for the year 2021 reached 89.4%, exceedingly more than half of the overall consumed produce.

Additionally, it is described that smallholder farms in developing countries are more reliant on external factors that affect the productivity of the farm, such as inefficient livestock input and output markets, poor infrastructure, and poor flow of information (McDermott et al., 1999). These factors have significant implications for animal health and production. Smallholder farmers make animal health management decisions based on limited information, which could have significant implications for animal health and production. Wanyoike (2023) further explained that the range of factors that impact the performance of the livestock sector in Somaliland include environmental degradation, inadequate infrastructure, and drought conditions that compromise water availability and the quality and quantity of grazing pastures. It was suggested that this country experiences significant economic losses as a result of insufficient pasture availability during droughts. These losses can be attributed to the failure of farmers to effectively manage their flock sizes in relation to the available stocking capacity. Apart from the financial and economic concerns, neglecting the nature of dietary and behavioral needs of the animal could result in future substantial expenses. The limitation due to nutritional factors significantly influences the production system, hence resulting in a higher risk of diseases. The recurring problem of insufficiency in seasonal pastures often results in farmers facing the loss of grazing grounds. McDermott et al. (1999) discussed that the successful application of animal health and production systems relies heavily on the incorporation of local productive pastures and the guidance of farmers on animal nutritional needs. It is essential to support local farmers in maintaining a sustainable production of mutton in Malaysia by providing them with alternative feed options derived from readily available pasture. Due to the cost of feed and land, many farmers fronted the issue by providing animals with various alternatives including local grasses. While feed makes up around 70% of the cost of raising animals (Zahari & Wong, 2009), the dependency of local farmers on imported feeds should be revised. Further research on alternative feed is needed to enhance the production of the meat industry specifically mutton.

Brachiaria decumbens, widely known as Signal grass is one of the *Brachiaria* species that has been used as ruminant feed in Malaysia. Due to its strong growth behavior and adaptability to many soil types and situations, this tropical grass is known as a highly productive pasture (Chung et al., 2018). Studies showed a great relative feed value of 74 to 84 for this grass and revealed an intermediate to high digestibility of 50-80% (Suhaimi et al., 2017). It is a promising grass species as a roughage for ruminants because of its high dry matter yield, ease of cultivation, resistance to drought, and minimal maintenance expenses (Faccin et al., 2014). Despite its abundance and its nutritive value, *B. decumbens* is commonly linked to toxicity and photosensitization effects on ruminants. This grass has been related to toxicity and photosensitization of ruminants due to its content of steroidal saponins (Brum et al., 2009; Lima et al., 2012; Muniandy et al., 2021). Steroidal saponin, an anti-nutritional component is a detrimental factor in reducing the performance of ruminants. A study mentioned that the primary steroidal saponins present in *B. decumbens* and many other species have been identified as dichotomin, protodioscin, and dioscin which it is associated with the formation of birefringent crystals that may block the bile ducts and cause an increase in liver enzyme levels (Chung et al., 2018).

Several studies have shown clinical signs developed through *B. decumbens* intoxication which includes jaundice in mucous membranes (Brum et al., 2007; Carmo et al., 2021; Muniandy et al., 2021), skin lesions as an indication of photosensitization (Melo et al., 2021; Muniandy et al., 2021), facial oedema and ocular discharges (Diamantino et al., 2020; Muniandy et al., 2021). Other studies also reveal few vital symptoms of *B. decumbens* intoxication include jaundice, weight loss, dehydration, anorexia, skin lesions in photosensitized areas, chronic neurological symptoms, and severe lesions in the liver and kidneys (Porto et al., 2013; Muniandy et al., 2020). Brum et al. (2007) also suggested that *B. decumbens* poisoning should be a crucial limiting factor within the sheep sector.

Generally, the focus of research is typically to improve the herd health of ruminants rather than the individual health; thus, the study of morphological and histopathological can provide beneficial information that may be important in helping the determination of a final diagnosis in ruminants. Similar histopathological signs have been observed between *Brachiaria* spp. and other pasture species, such as *Panicum schinzii, Panicum coloratum* (Brum et al., 2007), *P. dichotomiflorum, P. miliaceum,* and *T. terrestris*, indicating the presence of birefringent crystals (Cruz et al., 2001; Porto et al., 2013; Low, 2015). The mentioned cholestasis and crystalloid material buildup in the bile ducts are related to photosensitization in animals given *Brachiaria* spp., as suggested through a study in which the hepatogenous photosensitization induced histological alterations in buffalo liver such as biliary stasis and bile duct crystals, similar to those observed in other ruminants (de Oliveira et al., 2013). *Brachiaria* spp. intoxication in sheep can cause necrosis and enlargement of periportal hepatocytes, which is mostly due to the presence of insoluble saponin crystals, as previously reported (Porto et al., 2013). As a result, the observed histological changes are highly consistent and suggest hepatotoxic effects related with the presence of lithogenic steroidal saponins in *Brachiaria* spp. plants. The findings of these publications emphasize the importance of grass-induced hepatotoxicity in intoxication, regardless of the animals' sun exposure or absence of signs of photosensitization. Even so, all these effects can be eliminated by a comprehensive understanding of plant secondary phytocompounds and their effects through histopathological information at hand.

The anatomical and pathological features of an animal principally reveal conclusive information about a disease, while hematology and serum biochemistry are used to evaluate hepatic impairment and possible secondary lesions (Carmo et al., 2021). Additionally, the complete blood count (CBC) has become crucial as part of sheep production management through the use of sophisticated equipment for processing small ruminant blood samples, combined with a higher understanding of the benefits of clinicopathological diagnostic workup among the farmers (Polizopoulou, 2010). The hematological profile, along with morphological symptoms and biochemistry tests, can indicate a specific diagnosis or prognosis of an animal. In this regard, an increase in liver enzymes, notably GGT and AST must be evaluated as a possible cause when evaluating clinical situations since it indicates hepatic dysfunction, implying a potential sign of hepatogenous photosensitization in *Brachiaria* spp. intoxicated animal (Carmo et al., 2021). Muniandy et al. (2021) also stated that increased liver enzymes such as bilirubin levels, serum aspartate aminotransferase (AST), and serum gamma-glutamyltransferase (GGT) are the general cause of impaired liver functions in relation to feeding *B. decumbens* on ruminants. The high degree of renal susceptibility will then be caused by high bilirubin levels due to ischemia, resulting in an elevation of serum urea and creatinine, an indicator of renal impairment (Lelis et al., 2018). Several other studies also exhibit similar findings, where an increase in GGT and AST levels were shown (Gracindo et al., 2014) and in naïve sheep compared to experienced sheep (Faccin et al., 2014), as well as in lamb compared to adult sheep (Brum et al., 2007). Thus, hematological and biochemistry analysis may provide an accurate diagnostic to better understand the influence of steroidal saponin in *B. decumbens* on small ruminants.

Research on saponin's intoxicating effects on ruminants has resulted to many detoxification strategies to reduce ruminant health risks. Past research has used administrations of drugs to promote detoxification mechanisms. Phenobarbitone-type cytochrome P-450 isoenzyme induction reduces saponin toxicity in animals (Hasiah et al., 2000). In another investigation, griseofulvin boosted the animal's detoxifying enzyme but did not protect them against saponin (Haisah et al., 2003). Castro (2018) also found hereditary sheep resistance to B. decumbens pasture. lowered grazing time, ruminal transfaunation, and progressive B. decumbens pasture exposure decreased intoxication.

Overall, the increasing risk of saponin poisoning in ruminants fed on pastures containing *Brachiaria* species is a major issue, particularly among farmers who rely on this grass as their primary dietary feed. Feed conservation techniques such as silage-making and haymaking help provide a stable feed source during periods of limited feed production. According to Lima (2015), silage and hay are practical and cost-effective feeding options for both dairy and livestock farming and offer several important advantages. As a result, many farmers have begun to ensile or hay-making these tropical grasses as a roughage substitute during periods of low rainfall (Bernardes et al., 2005). However, Arroquy (2014) conducted a study of the chemical composition of preserved tropical grasses and found that the fiber content of hay and silage generally varies depending on the fresh feed. The overall results of this study have shown that preserving tropical grasses as silage improves their chemical composition and dry matter decomposition compared to hay. As a common feed preservation technique employed by farmers, ensiling is mostly reliant on the anaerobic processes of lactic acid-producing bacteria (Sun et al., 2024). Silage is usually produced as a feed reserve during drought periods and is more manageable than hay, as it significantly minimizes field losses and rain damage (González & Rodríguez, 2003). Utilizing hay as a preservation approach might be somewhat challenging (Okoye et al., 2023), therefore ensiling emerges as the most effective option for tropical nations including Malaysia. Due to its ability to mitigate nutrient loss during the harvesting and storage processes, silage has maintained its popularity among dairy farms while also facilitates more efficient and timely processing than dry forages (Okoye et al., 2023). Consequentially, the impact of ensiling towards the environment was also discussed in previous studies. The methane emissions resulting from rumen fermentation in ruminants have become a growing cause for concern. Recent studies indicate an effective method for mitigating methane emissions via nutritional intake, demonstrating succession through ensiling method (Liu et al., 2013; Xu et al., 2023). The ensiled feeds are found to be nutrient-rich, easily digestible, and effective in reducing methane emissions in ruminants (Liu et al., 2013). Overall, silage emerges as a viable option owing to its operational simplicity, cost-effectiveness, and successful outcomes towards the ruminant industry (dos Santos et al., 2022).

As described by Lima et al. (2015), using the preservation approach on *Brachiaria* spp. pasture theoretically lowers the occurrence of toxicity in ruminants when compared to those grazing directly on the pasture. The application of silage is advantageous as it effectively removes saponin content while maintaining the nutritional quality of the grass as described by Lima (2015). Despite alternative techniques that minimize photosensitization effects have been previously discussed, further elimination method to eliminate saponin is required. The potential of using silage as a way of preserving nutritional content as well as eliminating saponin toxicity may be helpful in assessing the health impact it gives towards the ruminants. Although silage provides various advantages over hay for preserving animal feed, it is crucial to highlight that these benefits can only be realized by implementing appropriate harvesting techniques and good ensiling management measures.

1.2 Problem Statement

Steroidal saponin intoxication in *B. decumbens* had been a widely known concern due to its rigorous effects towards animals. Despite the availability and sustainability of *B. decumbens* grass in Malaysia, saponin toxicity still poses a threat towards farmers to fully utilize the grass.

1. There is an absence of study to determine an effective treatment of *B. decumbens* for the consumption of sheep.

2. There has been no research on how treated *B. decumbens* may affect the clinico-pathology and haemato-biochemistry of sheep. Thus, further study is crucial to ensure good nutritional management of sheep in using *B. decumbens*.

1.3 Hypothesis

1. Ensiled *B. decumbens* have zero steroidal saponin compound within the grass.
2. Sheep fed with ensiled *B. decumbens* have better overall health performances compared to fresh *B. decumbens.*
3. Sheep fed with ensiled *B. decumbens* will have better blood hematology and biochemistry parameters compared to fresh *B. decumbens.*
4. Sheep fed with ensiled *B. decumbens* has an overall better histomorphological parameters compared to fresh *B. decumbens.*

1.4 Objectives

The main aim of this research is to measure the clinico-pathology and haemato- biochemistry responses of Dorper cross sheep fed with treated *B.*

decumbens. The specific objectives include:

1. To determine the clinical responses and health performances of Dorper cross sheep fed with ensiled *B. decumbens.*

2. To evaluate the hematology and biochemistry responses of Dorper cross sheep fed with ensiled *B. decumbens.*

3. To assess the gross pathology, organ morphometric, and histopathology of Dorper cross sheep fed with ensiled *B. decumbens.*

CHAPTER 2

LITERATURE REVIEW

2.1 *Brachiaria decumbens*

Brachiaria decumbens, known as Signal grass had been a commonly used tropical grass for pastures in Malaysia. *Brachiaria spp*. have been cultivated on over eighty percent of improved farming pastures in Malaysia, with *B. decumbens* constituting the most preferred (Low, 2015). The utilization of *Brachiaria* species as high-quality pastures for animal production has been extensively adopted (Chung et al., 2018). Many tropical regions, including Africa, Asia, Australia, and South America, are home to major *Brachiaria* species, which are used as forages (Brum et al., 2009) and thrive at altitudes from 500 to 2300 m to provide feed for ruminants (Low, 2015). Moreover, *B. decumbens* is characterized by its low-growth habit and decumbent nature. The flowering stems of this species can reach heights of up to 100 cm and originate from prostrate, multi-noded stems (Low, 2015). A study reported that the plant has the ability to spread through various means, including rhizomes, stolons, and seed production. Thus, *B. decumbens* has been extensively utilized as a forage for grazing ruminants in response to its elevated nutritive content and vigorous growth pattern, which facilitates the development of a dense ground cover capable of inhibiting weed growth (Low, 2015).

Among tropical and subtropical pasture plants, *B. decumbens* distinguishes out for its ability to provide excellent forage for grazing ruminants (Low, 2015;

Idibu et al., 2016). *Brachiaria spp.* is one of the major pastures grown because of its high dry matter output, ease of cultivation, adaptability to varied soils for year- round growth, and low maintenance cost of the farmed area (Riet-Correa et al., 2011). This tropical grass is regarded as a highly productive pasture because of its robust growth behavior and capacity to adapt to various soil types and conditions. Moreover, *B. decumbens* can survive in drought conditions, are resistant to damage by pests, and can thrive in acidic, nutrient- poor soils. In addition to their effectiveness as a ground cover in preventing soil erosion, these species can withstand extensive grazing. They are aggressive and can outcompete local flora because of their adaptability and versatility. Furthermore, the seeds have resistant characteristics, enabling proliferation and thus dominating the surrounding local grasses (Lima et al., 2012; Low, 2015). *B. decumbens* exhibits a notable capacity to effectively adapt to limited precipitation, which can be attributed to its extensive root system. The observed dry matter production of this pasture is significantly higher (5700 kg/ha) compared to other improved pastures under dry seasons or drought conditions (Chung et al., 2018). Also, a study by Low (2015) reported that this pasture displays a high degree of resilience and is cultivated across a diverse array of soil types, including infertile acid soils characterized by low pH levels of less than 3.5 and elevated concentrations of aluminum. It is also mentioned that *B. decumbens* exhibits the capacity to endure brief periods of drought through the redistribution of biomass and modulation of growth rates, thereby maintaining biomass yield without any notable variations. However, it displays an apparent vulnerability to waterlogging and can only endure for a limited duration. *Brachiaria decumbens* also exhibits favorable responses to defoliation, which can be achieved through various methods including grazing, harvesting, or complete defoliation such as burning (Riet- Correa et al., 2011; Lima et al., 2012; Low, 2015; Chung et al., 2018).

Furthermore, it is known that one of the important factors influencing the nutrients of grass includes the age of harvesting. Based on a study by de la Ribera (2008), it is concluded that *B. decumbens* and *Brachiaria hibrido* have a steady increase in yield up to 105 days of growth during rainy weather. Recent study by Hanagazaki (2022) reported that the most suitable harvesting age mentioned in the study was between 40 to 55 days depending on the season of the year with an average crude protein of 13% and dry matter digestibility of 56%, offering a substantial diet in grazing. Next, Castro (2007) also mentioned that the highest ruminal fermentability for *Brachiaria brizantha* was achieved by the ideal harvest period between 28 and 56 days. Within the study, the digestibility and maturity of the grass are the most suitable for grazing ruminants. One study was conducted to determine a good harvesting age for *Mimosa pigra* in growing goats in which the 45-day harvest interval appears to provide the most nutritional value, with a DM containing 14% crude protein (Hong et al., 2008). The morphological and physiological adaptation toward light has also allowed *B. decumbens* to tolerate moderate shading (Paciullo et al., 2014). Few studies had reported an increase in the nutritive value of *B. decumbens* grass when grown under a shaded area used as a grazing plot for animals (Paciullo et al., 2011; Paciullo et al., 2014; Faria et al., 2018). Generally, the enhanced nutritional composition of forage grass grown in the presence of tree shade, primarily characterized by increased crude protein levels and reduced lignification, plays a significant role in enhancing animal productivity. The key morphophysiological adjustments that boost the photosynthetic capability and DM production under moderate shadow are increasing leaf chlorophyll, leaf area, leaf elongation rate, and aerial/root ratio (Paciullo et al., 2014). Paciullo (2011) also found that *B. decumbens* grown in a shaded condition had higher concentrations of crude protein in comparison to full sun conditions, particularly in nitrogen-deficient soils. A study by Faria (2018) led to the determination that these positive effects can be attributed to the process of organic matter release and nitrogen cycling. The impact of shading on forage quality is significant, as it alters environmental factors that influence the morphological, anatomical, and chemical composition of the forage plant, thereby affecting dry matter digestibility and nutrient content (Faria et al., 2018). Hence, the findings of various studies have demonstrated divergent outcomes, based on the suitability within different countries and the purpose of each respective study of *B. decumbens*.

Pennisetum purpureum, commonly referred to as Napier grass, has been cultivated and utilized across Malaysia since its introduction in the 1920s (Halim et al., 2013). The study by Zailan et al. (2018) identified it as one of the most prevalent grasses in Malaysia, alongside Guinea grass. This grass has become common among farmers in tropical countries because it may serve as a food source for livestock. Given that smallholder dairy farmers and pastoralists face intermittent droughts and possess restricted access to irrigation infrastructure, the use of this grass has become a viable alternative to help farmers maintain animal performance. Zailan et al. (2018) state that this grass possesses several beneficial traits, including its perennial nature, fast growth, accelerated maturation, and superior dry matter output compared to most other tropical grasses. Furthermore, the majority of smallholder livestock producers often own small and fragmented parcels of land; hence, Napier grass is an optimal alternative to other feed options, as it is a high-yielding forage that requires few inputs and land (Atumo et al., 2022). The use of this grass by farmers, mostly through the cut-and-carry method, has been advantageous to date.

Napier was selected as the control group primarily due to its prevalent utilization among farmers in Malaysia. The treatments designated for the control group must exclude any saponin, with Napier being the most suitable choice for this purpose. The use of Guinea grass species was unsuitable for this investigation, since prior research has revealed that various species of Guinea grass have differing levels of saponin (Gracindo et al., 2014). Conversely, saponin seems to exhibit no discernible effects, as indicated by comprehensive reviews of several research on Napier. Moreover, due to its adaptability and nutritional benefits, it is the most appropriate therapy for control purposes.

2.1.1 Systematic Literature Review (SLR)

Despite the availability and nutritive value displayed in this grass, additional investigation is necessary to further determine the best utilization of *B. decumbens* in Malaysia. Therefore, the purpose of this review is to discuss the mechanism of toxicity of *B. decumbens* as well as the potential application of the treated grass and the effects on the clinico-pathology and hemato- biochemistry in ruminant species. This systematic research was finalized according to the guidelines of "Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement" (PRISMA). A systematic literature search was conducted in August 2023 via the electronic database of Scopus and Science Direct where articles were identified using those keywords related to production. Keywords included were: 'sheep' OR 'ruminant' AND '*Brachiaria spp*.' OR '*Brachiaria decumbens*' AND 'grass treatment' OR 'grass preservation' AND 'saponin' OR 'saponin toxicity'. Studies were selected based on the demonstrated inclusion and exclusion criteria accordingly, selected by one researcher through independent investigation.

Furthermore, the studies selected used a two-step approach, analyzing the title and the full text. The included criteria within the selected study must require as follows: full-text English article published within a 20-year period of 2003 until 2023; involve plants containing saponin or compound related to hepatogenous photosensitization; comprehend vital clinical or subclinical signs of saponin intoxication; include control group as part of the study. The exclusion criteria were as follows: not a full-text English article; articles prior to 2003; utilizes plants absent of saponin compound or not related to saponin toxicity; inadequate investigations of clinical or subclinical signs of saponin intoxication. A total of 93 full-text papers were reviewed for eligibility, and 82 were excluded for failing to match the appropriate criteria, resulting in 11 articles being included in this review. Among the 11 included articles, the range of year of published articles was between 2007 and 2022. All of the articles were published in Brazil with 9 articles focusing on sheep and 2 of cattle. It has been proposed that Brazil is one of the countries that actively involved in the publication of research related to saponin toxicity. This occurrence can be attributed to the widespread use of grass as a primary component of animal feed, which is particularly abundant within the country. Interestingly, all included articles did not mention any treatments or alternatives for the elimination of saponin in the *Brachiaria* species. There were no available articles mentioning the treatment of grass as part of the elimination of intoxication issues during the screening and eligibility process. Accordingly, the clinicopathology and hemato-biochemistry parameters of the study were thoroughly examined in all eleven publications.

2.1.1.1 Clinical signs related to *Brachiaria* spp. poisoning

Based on Figure 2.1, the most frequent clinical signs exhibited by the animals were dermatitis, followed by edema, jaundice, and weight loss. Studies also mentioned the death of animals, with only 2 articles mentioning the animals not affected by death due to saponin intoxication. Out of eleven included articles, 58% mentioned the significance of saponin toxicity exhibited towards animals, 33% of articles showed insignificance and 9% mentioned healthy animals throughout the experiment.

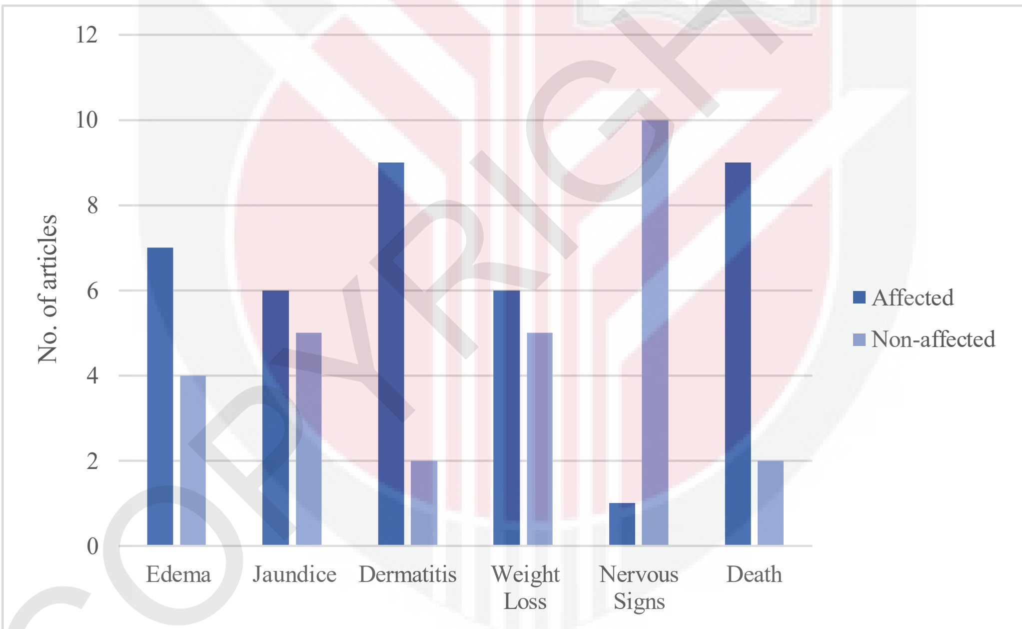

Figure 2.1 : The number of articles influencing the clinical signs exhibited by all animals in the included studies.

2.1.1.2 Blood indices related to *Brachiaria* spp. intoxication

A total of 7 articles reported blood biochemistry alterations within the result in Figure 2.2. However, there were insignificant or no reported results of hematology test within the included studies. The highest reported results of affected animals were serum GGT, followed by cholesterol. The highest number of articles mentioning the insignificance were 6 and 5 articles, which were creatinine and urea respectively. There were 4 studies with no information on the biochemistry changes in animals. Among the included articles, 32% mentioned significance of result, another 32% mentioned insignificance of result, 27% did not report any related information, and another 9% reported clinically healthy animals.

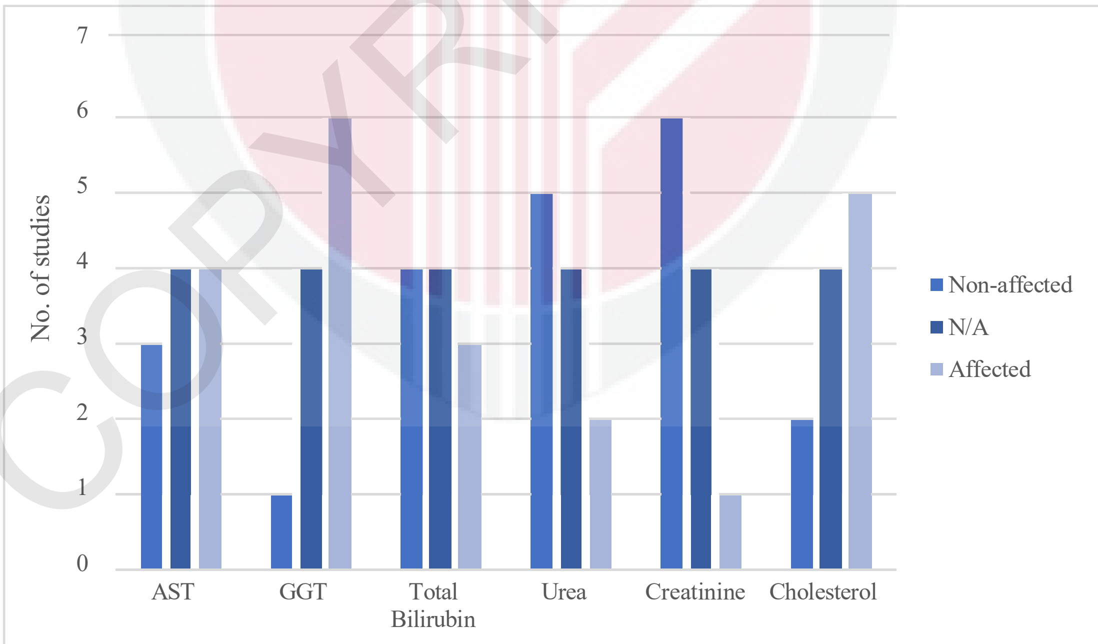

Figure 2.2 : The number of studies displaying blood biochemistry changes in all animals of included studies.

2.1.1.3 Post-mortem lesions based on *Brachiaria* spp. poisoning

Additionally, Figure 2.3 illustrates the post-mortem alterations observed in animals across each study included in the analysis. These changes include dermatitis, atrophy, hepatomegaly, liver and kidney modifications, gall bladder changes, and the presence of jaundice. The liver modifications in animals have been among one of the most frequently mentioned cases, with jaundice being the next subject of analysis. All included studies displayed post-mortem changes in animals, with 72% of studies being affected and 28.3% of studies with no significance.

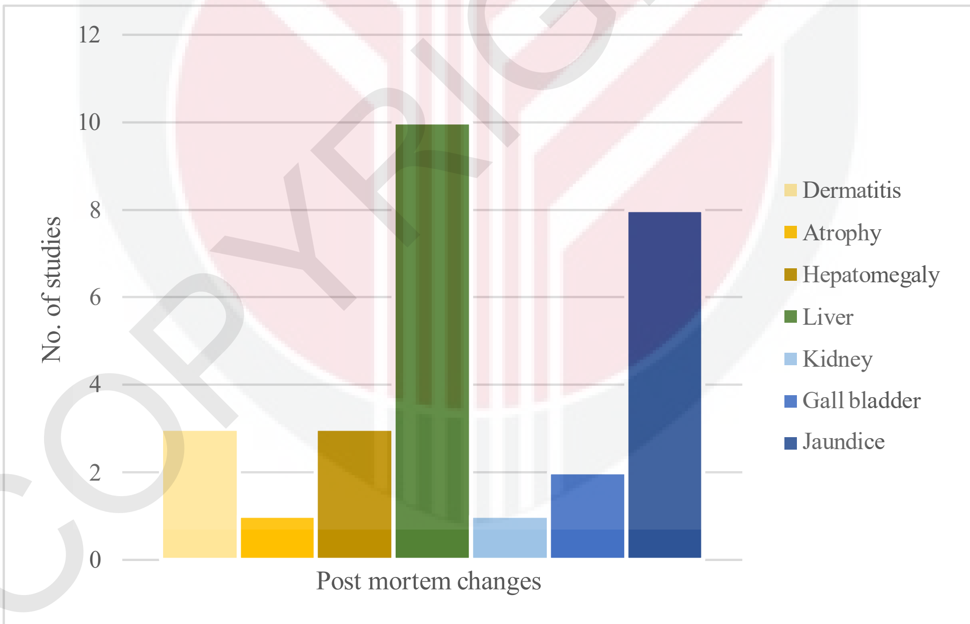

Figure 2.3 : The number of studies reporting the post-mortem changes exhibited by affected animals in all included articles.

2.1.1.4 Histopathological changes related to *Brachiaria* spp. intoxication

The histopathological changes in animals within the included eleven studies were based on Figure 2.4. The highest number of studies mentioning affected animals displayed crystal formation and hepatocellular necrosis, followed by bile duct hyperplasia and foamy macrophages within the cells. Only one study mentioned cholangitis exhibited in animals out of eleven included articles. However, 2 articles showed no information on histopathological findings, 47% reported insignificance on the result, and 35% reported significance on the result of the study.

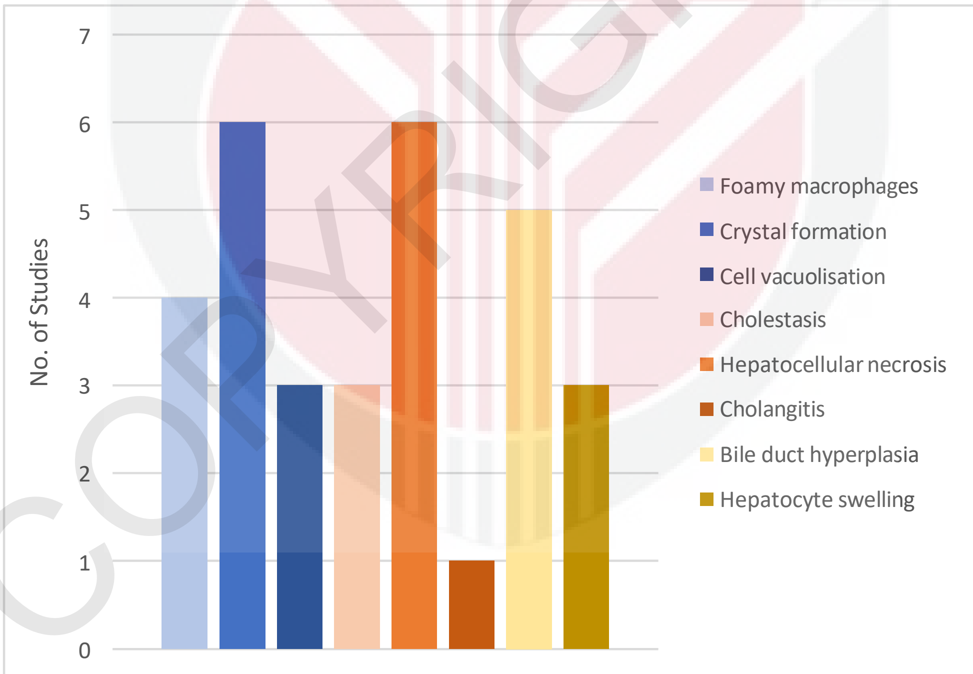

Figure 2.4 : The number of studies reporting histopathological changes of affected animals in all included studies.

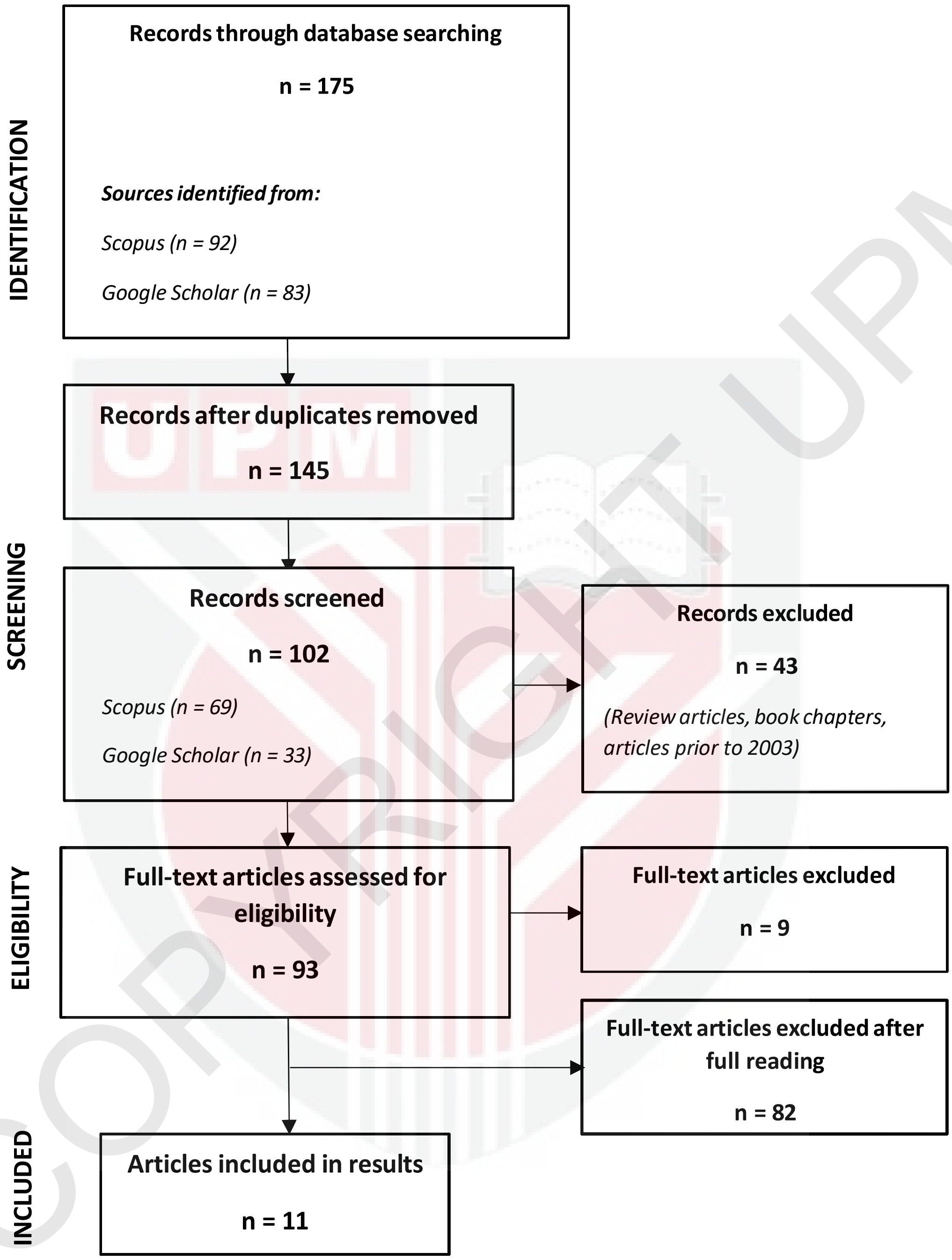

Figure 2.5 : PRISMA 2020 flow diagram of literature search for systematic review.

2.2 Toxic compounds of *Brachiaria decumbens*

The *Brachiaria* species hold significant importance as forage sources. Saponins found in *Brachiaria spp*. are widely thought to be chemical compounds that lead to liver damage (Caicedo et al., 2012). Saponins are substances found in at least 400 plant species, many of which are used as food or supplements in ruminant nutrition. Saponins have beneficial effects as growth promoters, but they sometimes are toxic to ruminants. As the derivative of secondary plant metabolism related to the plant defense system, saponins are mainly found in tissues that are most vulnerable to fungal or bacterial attack or insect predation (Lima et al., 2012).

However, a study conducted by Cruz et al. (2001) reported experimentally induced cholangiopathy in lambs through the administration of *B. decumbens* extracts in the form of crystals, which has conclusively clarified the assumption that the fungus *Pithomyces chartarum* is responsible for inducing photosensitization in *Brachiaria spp.* pastures. On the contrary, other studies conducted by Brum (2007), Castro (2007), and Caicedo (2012) have reported the presence of different concentrations of lithogenic saponins in hazardous *Brachiaria spp.* pastures, both in the absence of *P. chartarum* and in cases when the spore count is low. Nevertheless, starting from 1985, scholars have proposed that the toxicity of *Brachiaria spp.* can be attributed to the presence of lithogenic steroidal saponins inside this specific grass (Riet-Correa et al., 2011). In ruminants poisoned by *B. decumbens*, crystals in the bile ducts were in comparison to those found in *Tribulus terrestris* and *Panicum spp.*

poisoning, consisting of lithogenic saponins, suggesting that these substances are also toxic (Riet-Correa et al., 2011). The findings presented, in conjunction with prior research, provide evidence that *B. decumbens*, along with *Panicum spp., T. terrestris, Agave lechuguilla*, and *Narthecium ossifragum*, possesses the ability to induce photosensitization in animals, attributing to the presence of steroidal saponins and sapogenins (Pires et al., 2002). The primary saponin identified and found in *Brachiaria spp*. is protodioscin, as reported by Brum (2007, 2009), Riet-Correa (2011), Lima (2012), and Kono (2022). Because it has more reports of intoxication in many animal species and has the highest protodioscin concentrations, *B. decumbens* is regarded as the most toxic. According to Riet-Correa et al. (2011), *B. decumbens* is regarded as the most toxic within its species due to its potential to possess a concentration of protodioscin above 2%. Species of *B. brizantha* and *Brachiaria ruziziensis* are considered to exhibit less toxin (Kono et al., 2022). Concurrently, these studies have provided evidence to support the notion that the poisoning in question is actually caused by lithogenic saponins present in the grass.

In comparison to *B. brizantha* or *Brachiaria humidicola*, *B. decumbens* contains a greater concentration of saponins and proves to be more detrimental to livestock (Gracindo et al., 2014). The current investigation revealed that the amounts of protodioscin in *B. ruziziensis* varied between 0.001% and 0.093% in mature leaves, which are the plant tissue presumably ingested. Additionally, the levels ranged from 0.003% to 0.442% in sprouts. Hence, the observed levels of protodioscin concentrations may be deemed inadequate to induce toxicity (Diamantino et al., 2020). Protodioscin concentrations in the pastures were also comparable in those either with or without clinical manifestations when measured in *B. brizantha* and *B. ruziziensis* (Kono et al., 2022). Saponins may range within similar species grown at different regions due to environmental stress, plant age, and developmental stage (Brum et al., 2009). According to Riet-Correa (2011), the quantities of protodioscin were shown to be considerably greater in young leaves compared to mature or senescent leaves in a *B. brizantha* pasture experiment. An investigation reported a decline in saponin concentrations in both *Brachiaria spp.* plants as they progressed in their development and growth stages (Lima et al., 2012). Further research investigated the concentrations of protodioscin in several species of *Brachiaria spp.* at different stages of maturity, in which their findings indicate that younger plants in the growth phase exhibit higher levels of saponin compared to older plants. According to the results, the ingestion of protodioscin doses ranging from 0.3% to 2.56% resulted in the onset of hepatogenous photosensitization in sheep (Lima et al., 2012). During the initial stages of growth, it was observed that the concentration of saponin was greater in immature plants, where *B. decumbens* exhibited a greater saponin concentration compared to *B. brizantha.* Moreover, Brum (2009) also revealed an increase in the levels of protodioscin isomers in *B. brizantha* and *B. decumbens* as the plants matured, indicating a potential rise in toxicity during this developmental period.

Additionally, the primary meteorological factors that showed an influence on the concentration of saponin were the time span of maximum daylight and the cumulative precipitation (Lima et al., 2012). Outbreaks are common during pasture growth at the onset of the rainfall season (Brum et al., 2009). As reported in Brazil from May to July, when rain occurrences were higher, protodioscin concentrations were much lower than in August to October, when rain rates were lower and clinical cases occurred (Pupin et al., 2016). The study displays higher protodioscin content during the dry season, contrary to what was reported earlier by Faccin (2014), which mentioned the negative correlation between the concentration of protodioscin and cumulative precipitation. The findings presented in the study align with previous research conducted by Melo (2019), reporting a positive correlation between lower rainfall levels and increased protodioscin concentration in plants. This relationship suggests that sheep consuming larger quantities of these toxic compounds may experience heightened toxicity. In situations when the water supply is limited, the forage plant reveals a longer leaf lifespan, resulting in older leaves containing a greater concentration of toxic substances (da Costa et al., 2021). Further investigation by da Costa (2021) displayed that the dry matter intake (DMI) of lambs was affected by the protodioscin content present in *Brachiaria spp*. This influence can be attributed to the grazing activity of the lambs, which stimulates regrowth on various leaf offers. Consequently, this regrowth leads to an increased concentration of protodioscin in the forage.

Moreover, the previously reported findings, although limited in scale, indicate a potential link between sheep poisoning and elevated levels of saponins in the pasture, typically above 1% (Riet-Correa et al., 2011). However, it is important to note that a definitive toxicity threshold has not yet been determined. In addition to the limited availability of data, other factors also contribute to the prevailing uncertainty. These considerations encompass the heightened vulnerability of young animals and the increased resilience observed in animals reared on *Brachiaria spp*. pastures. Similar study also reported that it is likely that the saponin concentrations observed in the pasture samples collected during the clinical observation did not accurately reflect the quantities present at the time of poisoning. There is a lack of available evidence to determine a toxic threshold for cattle and goats, which exhibit greater resistance compared to sheep (Riet-Correa et al., 2011).

In short, the majority of prior research demonstrates varying degrees of hepatogenous photosensitization in animals. The lack of intoxication effects was attributed to insufficient levels of protodioscin concentration within *Brachiaria spp*. Based on Table 2.1, the lowest protodioscin concentration (% DM) reported was 0.002%, and 0.001% within *Brachiaria spp.* and *Andropogon gayanus* respectively (Gracindo et al., 2014). Consequently, *Brachiaria spp.* exhibited the highest known protodioscin concentration of up to 2.58%, which was identified in the young leaves of the plant (Faccin et al., 2014). Only one study revealed the absence of intoxication effects when animals were fed with *B. brizantha* hay (Lima et al., 2012). Therefore, this observation highlights the importance of additional studies in determining the most effective method to prevent saponin toxicity in animals, especially through the method of preservation.

Table 2.1 : The concentration of protodioscin identified in plants

No.	Plants	Animals	Protodioscin levels	References	Intoxication occurrence
1	*B. brizantha*	Sheep	The protodioscin concentration was 1.64% in young leaves, 1.67% in mature leaves, and 1.52% in old leaves.	Faccin et al., 2014	Yes
2	*B. brizantha* hay	Lamb	The *B. brizantha* hay contained 0.86% protodioscin, with a daily protodioscin administration of 200 mg/kg BW.	Lima et al., 2012	No
3	*B. decumbens*	Sheep, cattle	The protodioscin levels were 2.36% and 1.63% for the sheep and cattle pasture, respectively.	Brum et al., 2007	Yes
4	*B. decumbens*	Sheep	The protodioscin levels were 0.98% and 1.37% for the pasture under silvopastoral and under full sunlight respectively.	Lelis et al., 2018	Yes
5	*B. decumbens*, *B. brizantha*	N/A	The protodioscin level in both pastures ranged from 0.53% to 2.09%, having the highest level at the end of their developmental stages.	Brum et al., 2009	N/A

Table 2.1 : Continued

6	*B. decumbens, B. brizantha, Panicum maximum, Andropogon gayanus*	Sheep	Protodioscin concentration ranged from 0.003 to 0.01% in *B. decumbens*. The concentrations varied from 0.002 to 0.009% in *B. brizantha*. Protodioscin concentrations ranged from 0.001 to 0.0015% in *A. gayanus*.	Gracindo et al., 2014	Yes
7	*B. ruziziensis*	Sheep	Protodioscin levels are 0.020% in mature leaves and 0.065% in sprouts, with high *P. chartarum* spore counts of 479,844 spores/g plant.	Diamantino et al., 2020	Yes
8	*B. ruziziensis, B. brizantha*	Cattle	*B. ruziziensis* pasture had 0.21% concentration of protodioscin. *B. brizantha* pasture had protodioscin concentration range of 0.25% to 0.40%.	Kono et al., 2022	Yes
9	*Brachiaria spp.*	Sheep	Protodioscin content ranges from 0.74% to 1.81%.	Pupin et al., 2016	Yes
10	*Brachiaria spp.*	Sheep	Protodioscin levels in *B. decumbens* ranges from 0.62% to 1.18%. The protodioscin levels in *B. brizantha* and *B. humidicola* pastures were 0.74% and 0.11% respectively.	Castro et al., 2018	Yes
11	*Brachiaria spp.*	Sheep	The protodioscin levels ranged from 0.45% to 0.70%, higher in *B. decumbens* compared to *B. brizantha.*	Gaspar et al., 2021	Yes

2.3 Mechanism of Toxicity

The active toxic compound found in *Brachiaria spp*. is steroidal saponins, which have been observed to induce liver damage. Diosgenin and yamogenin sapogenins were chemicals found in affected animals' rumens or pastures in earlier studies (Cruz et al., 2000). In numerous instances, this liver damage progresses to liver failure and a condition known as hepatogenous photosensitization (Montoya-Ménez et al., 2019). Principally, hepatogenous photosensitization develops when hepatotoxic substances, such as drugs, infectious agents, mycotoxins, or poisonous plants, cause enough liver damage to prevent the excretion of phylloerythrin, a metabolite produced in the digestive tract as a result of chlorophyll metabolism. The liver gets rid of the photodynamic agent phylloerythrin, as sunlight can induce skin lesions when the liver is damaged because phylloerythrin builds up in the bloodstream (De Oliveira et al., 2013). The histopathological lesions of cholangiohepatopathy are evident in ruminants that have hepatogenous photosensitization as a result of ingesting *Brachiaria spp*., and are recognized by birefringent crystals in the bile ducts and hepatocytes (Rosa et al., 2016).

It has been observed that adult cattle exhibit a higher level of resistance to *Brachiaria spp.* intoxication compared to sheep (Kono et al., 2022). Riet-Correa (2011) also explained the susceptibility of sheep to poisoning compared to cattle and also mentioned the coexistence of goats and sheep without the occurrence of photosensitization implies that goats possess a greater resistance to this condition than sheep. Younger individuals exhibit a higher vulnerability to adverse conditions compared to their adult counterparts. In addition, it is worth noting that ruminant animals, particularly those that have not been exposed to forage, also referred to as naïve animals, including adult animals, exhibit a higher susceptibility to intoxication as compared to experienced animals, which are animals that have developed an adaptation to forage (Pupin et al., 2016; Kono et al., 2022). There is also evidence to suggest that buffalo and potentially certain sheep species possess resilience, as they are capable of being poisoned without exhibiting any observable signs of intoxication (Riet-Correa et al., 2011).

Furthermore, sheep that were fed *Brachiaria spp.* containing varying concentrations of protodioscin, ranging from 0.3% to 2.56%, were found to experience outbreaks of hepatogenous photosensitization as mentioned by Lima (2012). Similarly, hepatogenous photosensitization outbreaks were observed in sheep given *Brachiaria spp.* with a protodioscin concentration of 2.36% (Brum et al., 2007), and 1.28% (Pupin et al., 2016). Nevertheless, previous studies conducted by Brum et al. (2007) has shown that calves who were fed with *Brachiaria spp.* containing 1.63% protodioscin did not exhibit any noticeable clinical indicators of intoxication. Due to the greater susceptibility of sheep compared to cattle towards lithogenic saponins, the potential poisoning caused by *Brachiaria spp.* assumes significant importance as a constraining factor for the sheep business. The presence of elevated concentrations of protodioscin saponin in *B. decumbens*, which has been consumed by sheep exhibiting crystal-associated cholangiopathy, suggests that this steroidal saponin is primarily accountable for the photosensitization induced by this particular grass species (Brum et al., 2007). The toxicity of *Brachiaria spp*. in Brazil could be substantially reduced by using the saponin concentration as a measure in selecting for *Brachiaria spp.* variations and/or species to be established in the livestock industry (Riet-Correa et al., 2011). There is also a possibility of animals reared in *Brachiaria spp.* pastures to exhibit better metabolic and degradative capabilities towards steroidal saponin, the toxic component of this grass. This may be attributed to the development of a specialized ruminal flora that enables them to limit the adverse effects of this toxin (Pupin et al., 2016).

In order to gain a better comprehension of the mechanism underlying the intoxication, Stegelmeier (2002) conducted a study on horse photosensitization, identifying three distinct characteristics that are necessary for the manifestation of clinical photosensitivity. First, phylloerythrin excretion must be sufficiently inhibited by liver injury and impaired biliary secretion of the animal. Second, the animal requires the consumption of enough feed that is high in chlorophyll for the production of phylloerythrin. Lastly, for the dermal phylloerythrin to be photoactivated, the animal must subsequently be exposed to adequate sunlight. As discussed earlier, the toxic components in *Brachiaria spp*. that led to hepatogenous photosensitization are steroidal saponins (Cruz et al., 2000), which became lithogenic when they mix with glucuronic acid in the liver, consequently after being exposed to calcium in the bile (Brum et al., 2007; Riet-Correa et al., 2011; Caicedo et al., 2012). Thus, in a study of cattle fed with *B. decumbens*, the main severe injury reported was hepatic damage (Caicedo et al., 2012). It was also explained that saponins undergo hydrolysis into sapogenins within the rumen and subsequently, upon entering the intestine, can be transported to the liver via the portal vein. This mechanism affects mainly the liver's periportal region, accounting for the majority of severe injuries as observed in the study. These saponins have the ability to induce the development of crystals in the biliary system, which were documented as being insoluble salts that form as a result of the metabolic breakdown of saponins within the gastrointestinal system of ruminant animals (Brum et al., 2009; Rosa et al., 2016). The occurrence of crystals in the liver of sheep (Lima et al., 2012; Pupin et al., 2016; Diamantino et al., 2020), goats (Rosa et al., 2016), and cattle (Kono et al., 2022) affected by *Brachiaria spp*. poisoning has been documented by various authors.

The poisonous components of *B. decumbens* have been considered two steroidal sapogenins called diosgenin and yamogenin (Brum et al., 2007). Diosgenin and yamogenin are converted into epismilagenin and episarsasapogenin in the rumen, where these compounds are subsequently conjugated with glucuronic acid and combined with salts of calcium to form crystals in the biliary ducts (Cruz et al., 2000; Riet-Correa et al., 2011). An animal's ability to excrete phylloerythrin, a photoactive metabolite of chlorophyll, is then compromised by the deposition of these crystals, which affects the biliary system and contributes to the development of photosensitization in animals (Caicedo et al., 2012). The development of crystals in the biliary canaliculi, which led to hepatogenic photosensitization, has also resulted in cholangiopathy in ruminants grazing on *B. decumbens* (Caicedo et al., 2012; Montoya-Ménez et al., 2019). Correspondingly, the formation of crystals in the cytoplasm of macrophages is thought to be the cause of the foamy characteristic (Rosa et al., 2016). Montoya-Ménez (2019) further described the mechanism of photosensitization within the study. The poisonous compound found in *Brachiaria spp*., known as protodioscin, is synthesized by the deglycosylation of saponins by the microorganism present in the rumen. Protodioscin selectively targets hepatocytes and biliary cells, resulting in the development of crystal-associated cholangiohepatopathy mentioned earlier. The sheep exhibit impaired hepatic elimination of phytoporphyrins due to hepatocyte and biliary cell damage, resulting in the rapid accumulation of these substances in the dermal tissue. Upon penetration into the skin, phytoporphyrins undergo activation upon exposure to ultraviolet (UV) light from the sun. This activation process triggers epidermal necrosis by inducing the local production of free radicals, which subsequently leads to mast cell degranulation and the release of inflammatory mediators. The pattern of cutaneous lesions observed in sheep was consistent with photosensitization commonly observed in ruminants in other studies (Driemeier et al., 2002; Gracindo et al., 2014). This condition mostly affects areas of the skin that are weakly pigmented or have minimal wool coverage when exposed to sunlight (Montoya-Ménez et al., 2019).

Extensive research on the intoxicating effects of saponin on ruminants has led to the development of several detoxification methods aimed at mitigating health risks of ruminants. This was done particularly through the hepatic detoxification pathways, which are regarded as the primary mechanism for resistance to plant poisoning. The administration of drug to induce these detoxification pathways have been introduced in the past research. It has been demonstrated that phenobarbitone-type cytochrome P-450 isoenzyme induction enhances resistance to saponin toxicity, resulting in reduced intoxication effects in animals (Hasiah et al., 2000). Another study found that administering griseofulvin increased the enzyme responsible for the animal's detoxifying mechanism, but did not protect the animals against saponin effects (Haisah et al., 2003). Furthermore, Castro (2018) reported the involvement of inherited resistance of sheep grazing under *B. decumbens* pasture. Multiple strategies were identified to mitigate the incidence of intoxication, including reduced grazing duration, implementation of ruminal transfaunation, and genetic resistance via gradual exposure to *B. decumbens* pasture. Among these strategies, the suggested approach involved the selective breeding of resistant sheep (Castro et al., 2018).

In general, the majority of studies observed evidence of toxicity effects including liver lesions or occurrences of photosensitization, throughout the course of the experimental period. In order to prevent further intoxication of animals, it is essential to have a thorough understanding of the mechanism behind saponin intoxication. Despite the background knowledge of the mechanism of toxicity, the elimination of saponin remains a crucial issue that requires improvement. The use of preservation techniques such as ensiling and hay-making, can be considered as a potential strategy to minimize the adverse effects of saponins on animals, as concluded by Lima (2012). Additional research is required to mitigate the effects of saponin on ruminants, particularly related to preservation techniques in *B. decumbens*.

2.4 Preservation technique of grasses

Several species of *Brachiaria spp.* have been documented as being responsible for inducing hepatogenous photosensitization in ruminants. At first, the intoxication was ascribed to the *P. chartarum* fungus; however, recent research indicates that the toxic components responsible for photosensitization may be traced to the steroidal saponins found in the grasses. The limitations associated with this productive grass species caused farmers to adopt preservation techniques, utilizing them as a substitute rather than a primary source of nutrition for ruminants. Forage preservation is widely recognized as an important factor in maintaining the productivity and efficiency of livestock across different regions worldwide. Forage preservation techniques, including silage making and haymaking, aid the distribution of a consistent feed source during periods of limited forage production. According to Lima (2015), silage and hay serve as practical and cost-effective feed options for both the dairy and beef sectors, displaying several important benefits. Firstly, the process of harvesting may be primarily mechanized, allowing for increased efficiency and reduced labor requirements. Next, hay also has the capacity to be stored effectively when provided with sufficient protection, ensuring its long-term preservation. Furthermore, both types of feed possess the ability to fulfill the nutritional needs of various categories of livestock (Lima et al., 2015). Hay is the most common method employed for forage conservation in feeding practices. However, there are various aspects that provide significant constraints when it comes to producing high-quality hays (Arroquy et al., 2014). However, the issue of saponin decrease during haymaking poses a challenge due to the absence of defined methods for drying grass samples prior to analysis (Chung et al., 2018). More importantly, various grasses species offer the potential to produce silage, serving as a valuable source of roughage throughout the late winter and early spring seasons during the low nutritional value of pasture (van Niekerk et al., 2009). As a result, many farmers have begun to apply the ensiling or hay-making of tropical grasses as a substitute for roughage supplementation in periods of low rainfall (Bernardes et al., 2005).

As explained by Binuomote (2018), the fundamental concept underlying the practice of silage is to preserve excess forage in a manner that maintains its stability and nutritional composition until it is needed for animal use. The process occurs in anaerobic conditions, wherein the growth of spoilage microorganisms, which have a lower tolerance to acidic conditions, is inhibited by the lactic acid generated by lactic acid bacteria (LAB). Therefore, it can be observed that when the pH values decrease, there is a corresponding decrease in silage losses. This may be attributed to the increased conversion of plant- soluble carbohydrates, which serve as the primary substrate for lactic acid bacteria (LAB), into lactic acid. This conversion process results in a remarkable energy recovery rate of 96.9% (Binuomote et al., 2018). Furthermore, a way of improving the fermentation process was by a range of silage additives, employed to enhance the retrieval of nutrients and energy in silage which frequently resulted in enhanced animal performance (Binuomote et al., 2018). The study above also revealed an olive-green color that closely resembled the original color of the grass, suggesting favorable silage qualities and effective preservation.

In assessing silage quality, pH serves as an effective and efficient method, as explained by Binuomote (2021). Fermented silage that has undergone proper fermentation typically has a reduced pH level, which has been associated with improved fermentation quality and increased stability throughout the feed-out process. The presence of soluble carbohydrates plays a crucial role in ensuring the formation of high-quality silage, as it serves as the primary nutrient supply for the development of lactic acid-producing microorganisms (Binuomote et al., 2021). Tropical forages are recognized for their tendency to contain a lower quantity of water-soluble carbohydrates, hence limiting the rapid reduction of pH during ensiling. The reduced dry matter content of silage promotes the growth of *Clostridium* bacteria in the ensiled material, particularly when it is collected at an early stage. In contrast to other tropical grass species, the *Brachiaria spp.* grasses exhibit a greater leaf proportion and higher dry matter content, thus having a greater advantage in the ensiling process (Cezário et al., 2015). Moreover, the production of high-quality fodder needs an effective microbial fermentation process. In the case of tropical grass species, the occurrence of favorable anaerobic fermentation may not be guaranteed, which is essential for achieving an adequate generation of lactic acid characterized by a low pH, as well as minimal amounts of acetic acid and ammonia nitrogen. Tropical grass species typically exhibit a greater buffering capacity (BC) and/or reduced levels of water-soluble carbohydrates (WSC) compared to optimal silage crops which enables the promotion of growth and proliferation of lactic acid fermenting bacteria (van Niekerk et al., 2010). Also, in a study of tannin elimination mentioned in Chung (2018), the most efficient method for reducing phenolic chemicals in acacia leaves was determined to be a combination of leaving the leaves unchopped, soaking them in water, and storing them under anaerobic conditions for a duration of 14 days. It was concluded that physical treatments, including chopping, soaking in water, and storing under anaerobic conditions, were the most economically efficient approaches for processing acacia leaves. The use of urea, activated charcoal, and polyethylene glycol also has been shown to effectively deactivate tannins. However, the primary constraint hindering their practical application is their cost of production, which causes drawbacks for farmers in utilizing the significant findings (Chung et al., 2018).

Nonetheless, haymaking, as well as ensiling, are forage conservation techniques that enable a more reliable supply of high-quality feed (Chung et al., 2018). In a prior investigation, Lima (2012) investigated the protodioscin levels of *B. brizantha* hay fed to the lambs, given a daily dose of 200 mg/kg BW of protodioscin, which was present in the *B. brizantha* hay at a concentration of 0.86% (Lima et al., 2012). It was observed that the administration of a 200mg/kg BW dose of protodioscin did not result in any adverse effects for the lambs. This observation also suggests that *B. brizantha* hay provided did not exhibit any evident symptoms of poisoning. The findings related to hay demonstrate a resemblance to another profound study that measures the effect of silage and hay in *B. decumbens* and *B. brizantha* on the concentrations of protodioscin (Lima et al., 2015). It was reported that after a period of 24 days of ensiling, *Brachiaria spp*. grass exhibited low amounts of saponin protodioscin. It was observed that the concentration of protodioscin in *B. decumbens* exhibited a decline of 48% within the initial three days subsequent to the process of haymaking, after which it stabilized at a consistent level. The findings of this study indicate that ruminants fed under *Brachiaria spp*. grasses treated as silage or hay may experience a decreased likelihood of protodioscin poisoning. In a separate investigation, it was found that sheep of the same breed experienced toxicity when exposed to feed levels of protodioscin above 0.3% (Castro et al., 2011).

Nevertheless, a study of the chemical composition in the preservation of tropical grasses was made by Arroquy (2014), mentioning that typically, the fiber content of hay or silage varies with fresh feed. Based on this study, the levels of hemicellulose and cellulose were found to be significantly lower in silage compared to hay and fresh grass, and the dry matter disappearance of silage was found to be significantly higher than that of hay. The dry matter content of silage and fresh grass was found to be comparable, however lower than that of hay. Both hay and silage exhibited comparable crude protein (CP) levels, while fresh grass had a lower concentration of CP compared to hay but was similar to silage. It was implied that the concentration of CP in stored fodder indirectly increased by 14% in comparison to fresh forage due to the metabolism of carbohydrate fractions, such as respiration in hay or fermentation in silage, during the process (Arroquy et al., 2014). In addition, the concentration of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in the hay was also found to be higher compared to that of fresh grass and silage (Arroquy et al., 2014). The overall findings of this study demonstrated that the conservation of tropical grasses as silage resulted in improvements in the chemical composition and dry matter decomposition as compared to hay (Arroquy et al., 2014). According to Chung (2018), one mentioned study discussed the inclusion of bamboo charcoal in the diet of goats that were fed wilted acacia leaves resulted in a notable augmentation of both dry matter intake and weight gain in comparison to the remaining experimental groups. The application of efficient microorganisms to rice straw treated with fungi resulted in a notable reduction in dry matter, organic matter, natural detergent fiber, acid detergent fiber, and cellulose content. In contrast, there was a notable increase in both crude protein and dry matter degradability. It was also proposed that the fungus possesses the potential to degrade and utilize cell walls as carbon sources, resulting in a shift in the ratio of insoluble to soluble carbohydrates in the roughage. This altered composition was subsequently consumed by the effective bacteria (Chung et al., 2018). Next, the chemical composition of cactus-alfalfa silages includes a dry matter content of 31.4%, crude protein content of 12.7%, and neutral detergent fiber content of 48.6% (Ravari et al., 2022). Ravari (2022) carried out a study on the utilization of cactus-alfalfa silage (CABS) as an alternative feed for dairy goats. The findings of this study suggest that the administration of CABS did not have a significant impact on dry matter intake, milk yield, and feeding behavior. Nevertheless, the observed rise in milk protein content and the decrease in somatic cell count (SCC) indicate a favorable impact of CABS on enhancing the efficacy of the mammary gland. Consequently, it can be concluded that CABS has the potential to function as a viable substitute for up to 30% of dry matter in the diet of dairy goats, particularly in semi-arid areas, hence replacing ensiled cactus as a feedstuff option (Ravari et al., 2022). This improvement in silage dry matter intake can have positive effects on the overall quality of the silage. Other than that, one report from Binuomote (2018) uses silage as a treatment for both *B. decumbens* and *Manihot esculenta* (Cassava leaves), along with millet additives as feed for ruminants. The findings derived from the study indicated that the combination of ensiled *B. decumbens*, cassava leaves, and millet additive exhibited elevated levels of crude protein, metabolizable energy, and short-chain fatty acids. As a result, these findings suggest that the formulated feed may potentially supply the nutritional needs of small ruminants in tropical regions, particularly during periods of dry weather. Additionally, there were no significances in terms of organic matter intake regardless of whether ensiling takes place during the boot or full bloom stage in a study of ensiled *Panicum maximum* and *Digitaria eriantha*. The flow of nitrogen (N) and non-ammonia nitrogen (NAN) contribute to the optimal stage of ensiled *D. eriantha*, leading to a greater nutritional value for lambs in comparison to *P. maximum* silage (van Niekerk et al., 2009). In the most recent study, no alterations in the chemical composition of diets were detected (Ravari et al., 2022). Consequently, the observed effects may be attributed to various secondary metabolites found in opuntia, such as tannins, saponins, and flavonoids. These compounds may have contributed to the increased molar proportion of propionic acid in the rumen. Hence, the incorporation of cactus cladodes and alfalfa hay into animal diets during periods of drought or feed scarcity may serve as a viable strategy for mitigating feed expenses (Ravari et al., 2022).

Melilotus alba and *Melilotus officinalis*, a variation of sweet clover plants frequently serve as leguminous plants within agricultural practice in Argentina as reported by Riet-Correa (2023). When these plants are utilized for the purpose of producing hay and silage in conditions of elevated humidity, certain types of fungi establish colonies within the material, leading to the conversion of coumarins present in sweet clover into dicumarol. Prolonged ingestion of hay or hazardous silage leads to alterations in the proenzymes essential for the synthesis of prothrombin, hence causing a hemorrhagic state. In addition, instances of dicumarol poisoning in cattle that have consumed sweet clover hay in Argentina have been intermittently documented (Riet-Correa et al., 2023). In any case, doing direct comparisons with other trials proves to be challenging due to variations in the stage of maturation and growth conditions, as well as the utilization of fresh forage instead of hay or silage in that research.

Furthermore, the utilization of efficient microorganisms has been discovered to effectively inhibit the spread of deleterious toxin-producing fungi that contaminate rice straws, as reported in Chung (2018). A study was also reported to investigate the impact of phenobarbitone treatment on the toxicity of *B. decumbens* grass in sheep (Chung et al., 2018). Sheep that received phenobarbitone treatment exhibited delayed and comparatively less severe manifestations of poisoning. The researchers reached the conclusion that phenobarbital offered a certain level of protection from the harmful impact of signal grass. Furthermore, the researchers also made reference to the utilization of zinc sulfate and other chemoprotective drugs, such as oltipraz, as potential preventive measures against *B. decumbens* toxicity. However, no additional clarification was provided on this matter. The acquisition and safe and effective utilization of these products by local farmers may prove to be impractical. In a further study by Cezário (2015), the varying ages of forage regrowth in *B. brizantha* were found to not have any discernible impact on the quality of silage. It was found that harvesting *B. brizantha* grass after 35 days of regrowth did not result in any significant improvements in the nutritional value of silage, nutrient digestibility, microbial or nitrogen utilization efficiency, or beef cattle performance compared to a later harvest at 70 days. The inclusion of microbial inoculant also did not result in any significant improvement in dry matter recovery, fermentation process, or have any apparent effect on nutritional intake and performance of beef cattle, as well as exhibited an inability to effectively minimize fermentation losses. The duration of this development period was probably inadequate to induce substantial modifications in the lignification of the cell wall. These scientific findings provide further support to conduct a harvest at 70 days of regrowth, as it corresponds to a notable increase in the yield of fresh biomass per unit area. Emphasizing the significance lies in the fact that tropical grasses, when collected at more advanced stages of growth, exhibit better characteristics of silage fermentation (Cezário et al., 2015).

The use of various treatments for plants may have contributed to the mitigation of toxic compounds. However, it is important to note that poor storage methods and the harvesting of hay or silage could potentially present additional risks, such as the presence of mycotoxins. To get the best yield, DM content, and nutritional value, harvest should be efficiently planned (Ogunade et al., 2018). As a result of the buffer capacity, elevated humidity levels above 70%, and limited soluble carbohydrate content, tropical grasses exhibited exposure to losses during the ensilage process. The aforementioned traits have a predisposition to negatively impact the generation of lactic acid and therefore lead to a decrease in pH levels within silage which in turn, affects the efficiency of the fermentative process. The incorporation of absorbent substances, such as citric pulp, facilitates the increase in dry matter concentration of the silage and provides better fermentation circumstances (Bernardes et al., 2005). According to one study, molds have the ability to thrive in moist feed, such as silage, as long as there is an adequate supply of oxygen (Ogunade et al., 2018). The occurrence of mold growth and mycotoxin production can be facilitated by various factors such as delayed harvesting, the sluggish or delayed filing of silos, insufficient packing and sealing of silos, poor feed-out rates, bridging in silage bags, and damage to plastic wrap, bags, or silo coverings. Additional factors that could potentially increase the likelihood of mold growth and mycotoxin formation in feeds involve the occurrence of damage inflicted upon plants and silo coverings by rodents, rainfall, hail, and drought (Ogunade et al., 2018).

Hence, a reduction of issues related to mycotoxins in silage can be achieved by the prevention of fungal development both prior to and subsequent to the ensiling process. Effective management of silage is crucial in minimizing the presence of mycotoxins in dairy cow feed. The use of specific chemical additives or microbial inoculants can further aid in lowering the levels of contamination (Ogunade et al., 2018). Additionally, it is necessary to harvest forages at the recommended dry matter concentration or maturity stage to reduce or inhibit the proliferation of mycotoxins or undesirable microorganisms in silage. Silos should also be efficiently filled with densely packed forage immediately after harvest, following suggested densities. Furthermore, it is crucial to ensure the complete sealing of the silos to maintain anaerobic conditions, thereby preserving the quality of the silage (Ogunade et al., 2018). Further study by van Niekerk (2010) mentioned that the economic value of silage is based upon factors such as the type, extent, and level of fermentation, as well as the resulting fermentation end products. The influence of the forage crop type and quality, as well as the techniques employed in harvesting and ensiling, play a significant role in determining the preservation process, nutritional value, and voluntary feed intake by the animal. Silage quality is frequently evaluated by monitoring the final products of fermentation (van Niekerk et al., 2010). This was supported by van Niekerk (2010) mentioning that repeated harvesting at the boot growth stage yielded a favorable fermentation process characterized by good fermentation end products, including a low pH level and a higher concentration of lactic acid, over a duration of 21 days. The observed impact of the silage type on the bacterial population in ruminants may be attributed to the varying types of roughage applied. The influence of the chemical composition of roughage on the microbial community has been established in previous research, which revealed variations in ether extracts and fiber contents among different types of roughages (Remling et al., 2017). The recurring occurrence of saponin intoxication in ruminants fed on pastures under *Brachiaria* species poses a significant concern. Regardless, the utilization of ensiling and haymaking methods for the preservation of *Brachiaria spp.* pasture has the potential to reduce the occurrence of toxicity in ruminant animals as compared to those grazing directly on *Brachiaria spp*. pastures (Lima et al., 2015). Although silage has numerous benefits compared to hay for preserving animal feed, it is important to note that these benefits can only be achieved by the implementation of appropriate harvesting techniques and effective ensiling management practices. To conclude, there is yet limited available data about the impact of ensiling or haymaking techniques on saponin levels in *Brachiaria spp*. No additional discourse is provided concerning the correlation between the health implications of sheep when administered ensiled *B. decumbens*. Based on Table 2.2, no study was found mentioning the use of ensiling of *B. decumbens* towards mitigating the saponin toxicity towards ruminants. It is further suggested to necessitate this issue to further assist the farmers in improving the quality of the ruminant industry.

Table 2.2 : The preservation technique of grasses

No.	Plant	Preservation type	Toxicity effects/ Summary of effects/ Ruminant feeding	References
1	*B. brizantha*	Silage	Citric pulp addition during ensiling was recommended, considering the economic advantages.	Bernardes et al., 2005
2	*B. brizantha*	Hay	*B. brizantha* hay containing 0.86% of protodioscin showed no decrease in performance and did not show clinical signs of poisoning.	Lima et al., 2012
3	*B. brizantha* cv. Marandu grass	Silage	Silage is most effective at 70 days of regrowth. Microbial inoculant did not improve DM recovery, beef cattle nutritional intake, performance and reduce fermentation losses.	Cezário et al., 2015
4	*B. brizantha, P. maximum, Chloris Gayana, Cenchrus ciliaris*	Silage, Hay	The grasses conserved as silage showed desirable significance in chemical composition and dry matter degradation compared with hay.	Arroquy et al., 2014
5	*B. decumbens*	Silage	Ensiled *B. decumbens* added with cassava leaves with millet additive had high CP, ME, and short chain fatty acid values, thus meeting the nutrient requirements of small ruminants in the tropical regions during dry season.	Binuomote et al., 2018

Table 2.2 : Continued

6	*B. decumbens*	Silage	During the period of high yield, ensiling of grass with brewer's dried grain and cassava peels provide high protein supplement for ruminant during the dry season.	Binuomote et al., 2021
7	*B. decumbens*, *B. brizantha*	Silage, Hay	Ensiling and hay-making reduced the protodioscin levels in *B. brizantha* silage and in *B. decumbens* silage and hay.	Lima et al., 2015
8	Cactus-alfalfa blend	Silage	Cactus-alfalfa silage can serve as an alternative feedstuff up to 30 % on DM basis, especially in semi-arid regions in the diet of dairy goats.	Ravari et al., 2022
9	*P. maximum*	Silage	Harvesting at the boot growth stage consistently resulted in a good fermentation process with desirable fermentation end products up to a period of 21 days.	van Niekerk et al., 2010
10	*P. maximum, Digitaria eriantha*	Silage	The nitrogen and non-ammonia nitrogen flow favored the full bloom ensiled stage of *D. eriantha* silage, resulting in a higher nutritive value for lambs compared to *P. maximum* silage.	van Niekerk et al., 2009

2.5 Clinical signs related to saponin

Clinical signs are one of the important indicators of detecting a disease or symptoms which is observed physically during an examination. The poisoning of *B. decumbens* has been a widely known concern due to its detrimental clinical manifestations and performances. Generally, the main clinical manifestations exhibited towards intoxicated animals are hepatogenous photosensitization, which involves shade seeking, facial edema, jaundice, dermatitis, and weight loss. This condition is caused by a photodynamic agent named phylloerythrin, which sensitizes the skin to ultraviolet radiation. Phylloerythrin, a product from microbial degradation of chlorophyll causes the liver unable to digest it fully and causes an accumulation of the compound. Accumulation of phylloerythrin and constant exposure to the sun would cause dermatonecrosis and inflammation of the skin (Hooser & Wilson, 2010).

Moreover, sheep exhibit a higher susceptibility to clinical manifestation of saponin toxicity when compared to other ruminants (Riet-Correa et al, 2011; Gracindo et al., 2014). The clinical manifestations and deaths caused by saponin toxicity depend on the plant production factors and animals' susceptibility (Gracindo et al., 2014). The susceptibility of sheep to photosensitization, when compared with cattle, had been associated with lithogenic saponin content in *B. decumbens* (Brum et al., 2007). Apart from *B. decumbens*, there were also no other studies found that reported an outbreak of *B. ruziziensis* intoxication in cattle (Guizelini et al., 2021). As explained by Lozano (2017), both protodioscin and dioscin are digested to create diosgenin within the rumen. Epismilagenin is created when diosgenin is saturated and epimerized in the rumen and then conjugated with glucuronic acid in the liver. The conjugated epismilagenin is very lithogenic and may deposit in canaliculi, which can harm the liver and block the bile flow. The result of the blockage of biliary flow may induce the skin lesion observed in animals.

Additionally, animals have been associated with neurological symptoms when exposed to grass containing saponin. Nervous clinical symptoms include movement incoordination, head shaking, lethargy, ataxia, circling movement, forelimb stamping, and reverse locomotion (Cruz et al., 2001; Albernaz et al., 2010; Lelis et al., 2018). Animals observed showing these neurological signs had been followed with rapid death after approximately two days of showing manifestations (Lelis et al., 2018). It was explained that the toxic substances from the grass were unable to discard normally due to liver dysfunction. Hence, toxic substances such as ammonia, short-chain fatty acid, and mercaptans reach the brain, leading to these neurological manifestations by releasing false neurotransmitters (Albernaz et al., 2010). Thus, the presence of neurological symptoms may vary depending on several factors such as the severity of animals during the secondary photosensitization, the genetic makeup of animals as well as the levels of steroidal saponin fed to animals (Badiei et al., 2009).

Further studies show that a lithogenic steroidal saponin contained in *Brachiaria spp.* was responsible for the intoxication effects exhibited by ruminants (Riet- Correa et al., 2009). The clinical signs were typically seen

on animals after approximately 7-10 days of grazing (Muniandy et al., 2020). Brum (2007) conducted an experiment to investigate the source of hepatogenous photosensitization outbreak that occurred within a herd located in Brazil. It was found that 7 animals had displayed clinical signs such as anorexia, depression, dry muzzle, ears, and face dermatitis, and ocular and nasal yellowish secretion when fed with *B. decumbens*. Approximately 5 days after the exhibition of clinical signs, six of these animals died due to severe conditions (Brum et al., 2007). Based on this study, it is conclusive that photosensitization and crystal cholangiopathy outbreaks were caused by steroidal saponins present in *B. decumbens,* and there is an absence of *P. chartarum* spores within the pasture areas (Brum et al., 2007). In support of the significant findings, two studies mentioned the absence of *P. chartarum* fungus and found a significant presence of saponin in the *Brachiaria spp.* grass fed to the sheep (Albernaz et al., 2010; Porto et al., 2013). The high amount of protodioscin within the plant indicated the source of the outbreaks as well as the formation of crystals within the biliary ducts. Other reports mentioned similar clinical signs when fed with
B. decumbens which include diarrhea, lethargy (Cruz et al., 2001), hyperemia, alopecia, and apathy (Lelis et al., 2018).

Additionally, several other plants exhibiting saponin intoxication include *Brachiaria spp., N. ossifragum, Panicum miliaceum* and *Stryphnodendron fissuratum* (Riet-Correa et al., 2009; Lozano et al., 2017; Riet-Correa et al., 2011). Few studies had reported several clinical manifestations similar to *Brachiaria spp.* which mainly include hepatogenous photosensitization (Riet-

Correa et al., 2009; Pollock et al., 2015; Ubiali et al., 2022). A study by Kono (2022) was conducted using Brazilian experienced cattle which were placed in different pastures of *B. brizantha* and *B. ruziziensis*. The results reported significance in clinical signs for both pastures, with protodioscin levels ranging from 0.21% to 1.65%. The affected animals reported showed clinical signs including jaundice, hyperemia, alopecia, and swelling of ears and dewlaps, and further progressed into skin ulcers at the abdominal area, axillae, groin, thoracic area, and pelvic limbs (Kono et al., 2022).

On the contrary, a study from Lima (2012) had shown no clinical signs in lambs at the end of the study, with a protodioscin dose of 200 mg/kg BW, indicating that the dose level was inadequate to display any clinical signs. Furthermore, Muniandy (2021) reported the health effects of sheep when fed with different levels of *B. decumbens.* The clinical investigation showed that only mucous membrane color was significantly affected by different levels of *B. decumbens* diets at different time phases in this study. The mentioned insignificance in other clinical signs was presumably due to the shaded areas provided, thus delaying the poisoning effects. Badiei (2009) reported a study using *P. miliaceum,* a plant cultivated as an annual cereal crop in Iran. It was found that *P. miliaceum* had exhibited photosensitization signs similar when using other *Panicum spp.* plants as grazing pastures for small ruminants. Findings showed that there was no significance in the clinical signs when fed with *P. miliaceum* due to the time of the experiment, as well as the susceptibility of the sheep (Badiei et al., 2009).

Consumption of non-toxic doses daily has the potential to cause some degree of adaptation in animals (Guizelini et al., 2021). Only one patient showed clinical signs of photodermatitis, which appeared very late in the course of the poisoning they had received. It is possible that this was due to the experiment being carried out during the autumn season when the days are getting shorter and the sun was less bright (Guizelini et al., 2021). There is a possibility that these conditions did not provide sufficient irradiation to cause severe photodermatitis. In addition, the severity of clinical signs varied among animals that were affected, which may point to the fact that different animals have varying susceptibilities to the plant (Badiei et al., 2009). There is also a possibility that the incidence of the disease and its severity were affected by factors such as the individual susceptibility of the animals, the varying levels of circulating photodynamic agents and bile pigments, and the varying degrees of exposure to sunlight (Badiei et al., 2009). It has also been hypothesized that the animals may have been able to adapt in some way, either by increasing their rate of consumption over time or by the transfer of rumen microbiota (Castro et al., 2018).

In brief, most of the articles mentioned above had significance in the clinical investigations observed, while few of them showed insignificance. Based on Table 2.3, only three studies showed neurological signs, whereas the majority of it has reported photosensitization issues. Despite the diverse clinical investigation made in order to understand the saponin intoxication effects, many of the articles do not include ensiling as part of the feeding regime. Only one study mentioned clinical investigations when using *B. brizantha* hay containing steroidal saponins, which had reported clinically healthy animals throughout the study (Lima et al., 2012). There is still an unidentified amount of knowledge concerning silage as part of treatment to reduce the saponin amount and its effects on ruminants. In addition, there is still an absence of resources regarding the association between silage as feed and its effects on the health of sheep. While most of the article focuses on the production performances, it is important to determine if the ensiling process could minimize saponin effects, thus helping farmers to fully utilize *B. decumbens* as a highly nutritive grass for the animals.

Table 2.3 : The effects of saponin toxicity on the clinical signs of animal

No.	Plant	Toxic compound	Animal	Clinical signs	References	Significance
1	*Brachiaria spp.*	Contains 0.003% to 0.01% of protodioscin	Sheep	Two sheep showed lethargy, depression, and weight loss.	Gracindo et al., 2014	No
2	*Brachiaria spp*	Contains 0.21% to 1.65% of protodioscin	Brazilia n cattle	Jaundice, hyperemia, alopecia, and swelling of ears and dewlaps, and further progressed into skin ulcers at the abdominal area, axillae, groin, thoracic area, and pelvic limbs	Kono et al., 2022	Yes
3	*B. decumbens*	Presence of saponin	Sheep	Head shaking, seeking shade, reddening and swelling of ears and eyelids, diarrhea, and lethargy.	Cruz et al., 2001	Yes
4	*B. decumbens*	Contains 0.94% of protodioscin	Sheep	Apathy, weight loss, photophobia, ocular mucosal hyperemia, eye secretion, and jaundice.	Porto et al., 2013	No
5	*B. decumbens*	Presence of protodioscin	Sheep	Weight loss or low weight gain, apathy, hyperemia and secretion in the conjunctive mucosa, nasal secretion, photophobia, facial, ear and tail edema, dermatitis, jaundice, alopecia, ataxia, death.	Lelis et al., 2018	Yes

Table 2.3 : Continued

6	*B. decumbens*	Contains 0.3% to 1.3% of protodioscin	Lactating lambs	Hepatogenous photosensitization, with lesions restricted to specific sites of unpigmented skin, mainly on the face, and consisted of alopecia, erythema, crusting with sloughing of the crusts, apathy, anorexia, icterus and loss of weight.	de Melo et al., 2018	Yes
7	*B. decumbens*	Presence of protodioscin	Sheep	No signs of edema, jaundice, photosensitization, and neurological signs observed in all treatment groups.	Muniandy et al., 2021	No
8	*B. ruziziensis*	Contains 0.02% of protodioscin	Sheep	Signs involving hepatogenous photosensitization.	Diamantino et al., 2020	Yes
9	*B. brizantha* hay	Contain 0.86% of protodioscin in hay	Sheep	Clinically healthy throughout the experiment.	Lima et al., 2012	Yes
10	*B. brizantha*	Contains 1.13% to 1.62% of methylprotodioscin and protodioscin	Sheep	Pruritus, hepatogenous photosensitization, motor incoordination, head tremor, lethargy, ataxia, depression and coma before death.	Albernaz et al., 2010	Yes
11	*Stryphnodendron fissuratum*	Contains 13.3-345 µg/g of carboxy-parquin, and parquin	Cattles, buffaloes, goats	Apathy, anorexia, and ruminal arrest.	Ubiali et al., 2022	Yes

Table 2.3 : Continued

12	*Narthecium ossifragum*	Presence of protodioscin	Ruminants	Skin sloughing, oedema, serum exudation, ulceration, and necrosis.	Pollock et al., 2015	Yes
13	*Panicum dichotomiflorum*	Presence of methylprotodioscin and protodioscin	Sheep	Edema of the head, followed by dermatitis, mainly in the face, ears, and croup, ocular discharge, corneal opacity with blindness, and redness of the coronary band and hoof.	Riet-Correa et al., 2009	Yes
14	*Panicum miliaceum*	Presence of steroidal saponins	Sheep	Signs of photosensitization including hyperemia, swelling of eyelids, face, and submandibular edema were seen in only one animal.	Badiei et al., 2009	No

2.6 Hemato-biochemistry alterations related to *B. decumbens*

The definitive diagnosis of *Brachiaria spp.* toxicity was made based on several factors including biochemistry analysis. Serum biochemistry is an effective method for assessing hepatic impairment and potential secondary lesions, whereas anatomopathological characteristics provide definitive diagnostic information regarding the disease (Carmo et al., 2021). In sheep poisoned with sporidesmin, gamma-glutamyl transferase (GGT) serum activity measurement was applied as a phenotypic indicator of liver injury, which is also regarded as significant in the detection of animals affected by *Brachiaria spp*. intoxication (Faccin et al., 2014; Gracindo et al., 2014; Castro et al., 2018). The ingestion of safe quantities of protodioscin allows animals to improve the hepatic toxin detoxification metabolism. Consequently, this led to a reduction in the adverse effects of grass during the designated period of examination (Castro et al., 2018). GGT serves as a reliable biomarker for assessing liver dysfunction, biliary hyperplasia, and the proliferation of bile ducts (Castro et al., 2011). Elevated levels of GGT and aspartate aminotransferase (AST) in the serum, along with increased concentrations of total, direct, and indirect bilirubin, have been observed in both natural and experimental studies of *Brachiaria spp.* poisoning in cattle and sheep. The serum biochemistry results thus demonstrated evidence of hepatic injury (Montoya-Ménez et al., 2019). Elevations in serum bilirubin levels are commonly observed in cases of hepatic dysfunction, hemolysis, and cholestasis. These elevations serve as valuable and effective indicators for diagnosing hepatogenic intoxication and identifying the primary causes of jaundice (Lelis et al., 2018). A report conducted in 2015 by Low explained that animals with secondary photosensitization commonly demonstrate increased levels of both AST and GGT. The occurrence of common clinical indicators of toxicity may not always be present, and the confirmation of diagnosis often relies on histopathological analysis of the liver in conjunction with alterations in AST and GGT levels (Porto et al., 2013). GGT is associated with the cellular membrane and is primarily found in canaliculi, bile ducts, as well as hepatocytes. In cases of hepatic diseases characterized by cholestasis, the elevation of GGT levels can be attributed to the solubilization of cell membranes induced by the accumulation of bile salts within the liver and ducts (de Oliveira et al., 2013).

It has been proposed that buffalo and sheep potentially exhibit resilience, as evidenced by the presence of histologic lesions and elevated GGT serum concentrations following poisoning, without displaying visible clinical manifestations (Riet-Correa et al., 2011). The serum chemistry analysis conducted on the affected animals demonstrated a notable increase in GGT, AST, and bilirubin levels. These findings provided additional evidence to support the preliminary diagnosis of hepatoxicity, which was further clinically suggestive of hepatogenous photosensitization (Montoya-Ménez et al., 2019). In a study using buffaloes grazing on *B. decumbens* pastures (de Oliveira et al., 2013), the serum levels of the GGT enzyme displayed significance between intoxicated and healthy buffaloes, suggesting the presence of liver damage. The occurrences of the outbreaks during the start of the rainy season and a significant association were observed between saponin levels and both the quantity of rainfall and green vegetation in the pasture. The results of this study suggest that during this specific time period, the toxicity of the grass was higher. This study presents the first evidence of photosensitization caused by *B. decumbens* in buffalo (de Oliveira et al., 2013). The results of the complete blood count of steer reared under *B. decumbens* pasture displayed the presence of anemia, hyperfibrinogenemia, leukocytosis with neutrophilia, and elevated levels of GGT, AST, urea, total bilirubin, direct bilirubin, and indirect bilirubin. The animal has shown reports of bilirubin crystals, urobilinogen, and bilirubin in the urinalysis (Carmo et al., 2021). Although the intoxication report of adult cattle is more uncommon, it may still be affected by photosensitization caused by *Brachiaria spp*. The elevation of liver enzymes, specifically GGT and AST, is indicative of hepatic dysfunction, thereby suggesting a potential indication of hepatogenous photosensitization. While hepatogenous photosensitization is a relatively uncommon occurrence in adult cattle, it is necessary to include it as a potential cause when evaluating clinical conditions (Carmo et al., 2021).

Also, a study by Muniandy (2021) revealed similar biochemistry results of sheep fed with graded levels of *B. decumbens*. Significant differences were observed among treatment groups for all biochemistry and hematology parameters, with the exception of creatinine and packed cell volume across different time phases. While the PCV did not exhibit any notable alteration, the plasma protein levels experienced a significant decrease in all sheep subjected to treatment. This decline can potentially be attributed to a reduction in synthesis caused by hepatic disease (Muniandy et al., 2021). It was also mentioned that sheep that did not exhibit any clinical symptoms upon being introduced to *B. decumbens* pastures displayed elevated levels of AST and GGT, comparable to those sheep that did show clinical signs, suggesting a demonstrated resilience to *Brachiaria spp*. poisoning (Castro et al., 2011). Based on a study by Lelis (2018), only three animals exhibited no clinical signs. Nevertheless, even in the absence of clinical manifestations, these animals also demonstrated hepatic changes and an elevated serum concentration of hepatic enzymes. Moreover, a study by Gracindo (2014) reported that sheep grazing on *Brachiaria spp*. exhibit significant increases in GGT levels, despite the absence of clinical symptoms of intoxication. This suggests that these animals possess a remarkable ability to withstand the toxic effects of *Brachiaria spp*., as they can be exposed to the poison without displaying any observable clinical signs, which also shows that this resilience does not result in a decline in productivity. The average levels of serum GGT and AST were also found to be significantly elevated in the naïve flock, indicating a greater vulnerability to the toxic substance (Faccin et al., 2014). In one of the experiments by Castro (2018), it was observed that the groups subjected to continuous grazing in *B. humidicola* and in *B. brizantha* exhibited significantly elevated serum activities of GGT and AST compared to the groups that received an adaptation period of grazing on alternate days and two hours daily in *B. decumbens* paddocks. The previous observation indicates that the management of sheep grazing in *B. decumbens* pastures plays a role in facilitating sheep adaptation, thereby preventing grass-induced intoxication in sheep. On the other hand, the continuous supply of lambs in *B. decumbens, B. humidicola*, and *B. brizantha* is likely to induce toxicity and does not seem to be an effective method for adapting lambs to grazing in highly toxic pastures (Castro et al., 2018). It was observed that the impact of *Bracharia spp.* consumption varies among ruminants within a herd. The occurrence of genetic adaptation, coupled with prolonged exposure to this particular grass, provides better resistance to toxicity in animals. Notably, younger sheep exhibit greater susceptibility compared to their older counterparts (Faccin et al., 2014; Pupin et al., 2016; Castro et al., 2018). These various factors may account for the inconsistent impact observed among the sheep in the two Mexican flocks that were fed *Brachairia spp*. reported earlier (Montoya-Ménez et al., 2019).

In a study to evaluate the sun exposure towards saponin intoxication in sheep, Porto (2013) reported a greater mean serum AST and GGT activities in the sun- exposed group fed with *B. decumbens*, whereas the sheltered group had higher GGT activities than the control group. The findings of this study indicate that sun exposure does not serve as a determining factor for intoxication, but rather intensifies the toxicity of the grass. According to Lelis (2018), animals within the silvopastoral system and the full sun treatment engaged in grazing activities on *B. decumbens* exhibited clinical symptoms, liver abnormalities, and elevated levels of GGT, AST, and bilirubin throughout the entire duration of the experiment. Despite the lower protodioscin content observed in *B. decumbens* within the silvopastoral system compared to the full sun treatment, it still proved to be adequate in inducing secondary photosensitization in the animals. The presence of natural shading within the silvopastoral system does not effectively prevent or alleviate symptoms of photosensitization in young, non- adapted sheep that are grazing on *B. decumbens* (Lelis et al., 2018).

According to Low (2015), animals experiencing secondary photosensitization typically exhibit elevated levels of AST and GGT. GGT exhibits significant enzymatic activity within the liver and kidneys. Elevated levels of GGT in the bloodstream can potentially be attributed to cholestasis and damage to the biliary epithelium. The enzyme AST is found in both the cytoplasm and mitochondria of hepatocytes, as well as in muscle cells. Consequently, liver damage leads to an increase in the concentration of AST in the bloodstream. Additionally, the presence of jaundice, as observed during clinical examination and necropsy, is indicative of hepatoxic injury resulting from the consumption of *Brachiaria spp*. This is further supported by the elevated levels of serum AST and GGT activities. The primary mechanism associated with these alterations is the release of biliary pigments resulting from the obstruction of the bile ducts by crystalline structures formed through the conjugation of saponin with calcium. Badiei (2009) discovered that there were observed increases in total protein levels in the serum of the affected animals throughout the duration of the experiment. This phenomenon can be associated with the dehydration of animals under the influence of intoxication, resulting in a reduction in the volume of distribution. Dehydration could potentially be a contributing factor to the observed elevation in serum protein levels and serum urea levels. This is because dehydration leads to hemoconcentration, which subsequently results in an increase in protein and urea levels. There was also no observed correlation between elevated levels of AST and GGT and the degree of poisoning as mentioned in a report by Riet-Correa (2011). Nevertheless, the study observed minimal fluctuations in the creatinine concentration, potentially attributable to the quantity of *B. decumbens* toxin administered and/or the insufficient duration of intoxication to induce renal damage (Driemeier et al., 2002). The observed decline in urea levels in sheep affected by *B. decumbens* toxicity can likely be attributed to hepatic insufficiency and a decrease in total protein, resulting in reduced urea synthesis. Conversely, the levels of total protein, albumin, and globulin exhibited an increase during the acute phase of the *B. decumbens* intoxicated groups, potentially attributed to host cell responses (Muniandy et al., 2021). Recent clinical cases revealed elevated levels of the AST and GGT enzymes, consistent with prior research indicating hepatobiliary system damage associated with the disease (Carmo et al., 2021). This finding demonstrates the importance of serum biochemistry in diagnosing hepatogenous photosensitization. The observed elevation in total bilirubin levels, along with its individual fractions, indicates the presence of pre-hepatic and hepatic jaundice. This can be attributed to the impact of saponins on the bilirubin conjugation process and the obstruction of the bile duct. The observed elevation in urea levels can likely be attributed to a state of malnutrition, which leads to the depletion of bodily protein reserves. The identification of urobilinogen, free bilirubin, and crystals during urinalysis is indicative of hepatobiliary diseases characterized by cholestasis (Carmo et al., 2021). The primary preventive measures involve the careful selection of animals that are resistant or resilient, as well as the development of *Brachiaria* species or varieties with a reduced concentration of saponins

(Castro et al., 2011).

The results of the investigations in feeding *B. brizantha* hay in lambs by Lima (2015) did not indicate any apparent differences or significant changes observed in the levels of red blood cells (RBC), hemoglobin, hematocrit, white blood cells (WBC), or fibrinogen among the animals that received treatment. The elevated levels of serum GGT activity observed in both groups indicated the presence of damage to the biliary epithelium, a finding that was also confirmed through histological analysis. In contrast, the saponins derived from fresh *B. decumbens* possess the capability to cause damage to red blood cells, which is associated with their hemolytic activity (Muniandy et al., 2021). The adverse effects were observed to be more pronounced when exposed to higher levels of toxicity and over a prolonged period of time (Muniandy et al., 2021). The findings of this study lead us to the conclusion that the administration of *B. brizantha* hay does not result in any intoxication effects on lambs. It is worth noting that Cruz et al. (2001) conducted an experiment where they administered fractionated extracts of *B. decumbens* which surprisingly did not observe any alterations in serum GGT activity or clinical manifestations. Nevertheless, their animal subjects did exhibit cholangiohepatopathy that was induced experimentally. In the current study, the duration between the introduction of the naïve sheep to the *B. brizantha* pasture and the onset of clinical symptoms of poisoning varied from 35 to 137 days (Faccin et al., 2014). The appearance of clinical manifestations of saponin toxicity in animals exhibited a range spanning from 7 to 38 days. The data presented in this study suggest that the time needed for continuous protodioscin intake to affect the liver explains why pasture protodioscin levels did not immediately elevate GGT mean serum activity (Porto et al., 2013; Faccin et al., 2014). In addition, the application of rumen transfaunation has been utilized for the treatment of indigestion and to facilitate the restoration of normal rumen function after a left- displaced abomasum surgery. This method has been employed for the purpose of introducing distinct rumen microorganisms into animals that have been subjected to toxic compounds found in plants (DePeters & George, 2014). According to a study by Castro (2018), the process of ruminal transfaunation appears to have a significant role in the transfer of resistance or adaptation, potentially leading to a reduction in liver damage. The report demonstrated that the administration of multiple transfaunation may potentially minimize the toxicity of *B. decumbens* in transfaunated groups. This was achieved by comparing the GGT and AST serum activities between these groups. Despite the fact that the group yielded comparable outcomes, it was observed that the occurrence of sheep exhibiting elevated serum GGT levels in the single transfaunation group was lower compared to the control group. This study suggests that the ability to resist grass poisoning could potentially be transmitted to vulnerable animals through the transfer of ruminal content from adapted animals (Castro et al., 2018).

Generally, multiple studies mentioned the significance of biochemistry analysis in detecting cases of hepatogenous photosensitization. Based on Table 2.4, the significance displayed within the studies shows an elevated serum level of GGT enzymes as well as AST enzymes. In contrast, the insignificance of the results was only mentioned in one investigation using *B. brizantha* hay as the feed (Lima et al., 2012). Despite the abundant studies focusing on the toxicity of this grass, there is still an absence of research on the treatments of *Brachiaria spp.* grass used as feed to reduce the intoxication effects on the animals. Additional research is required to investigate the preservation methods of *Brachiaria spp*. grass in order to effectively mitigate the presence of its poisonous saponin component.

Table 2.4 : The effects of saponin toxicity on haemato-biochemistry alterations of animals

No.	Plant	Animal	Haemato-biochemistry findings	References	Significances
1	*B. brizantha*	Sheep	The mean activities of serum GGT and AST were significantly higher in the naïve flock.	Faccin et al., 2014	Yes
2	*B. brizantha* hay	Lamb	There were no significances in both blood and biochemistry parameters.	Lima et al., 2012	No
3	*B. decumbens*	Sheep	The hematology results revealed significance in the red blood cells, mean corpuscular volume, mean corpuscular hemoglobin concentration, white blood cells, neutrophils, monocytes, eosinophils, basophils, platelets, and plasma proteins. All biochemistry parameters except creatinine showed significance.	Muniandy et al., 2021	Yes
4	*B. decumbens*	Steer	The CBC revealed anemia, hyperfibrinogenemia; leukocytosis with neutrophilia; increased GGT, AST, urea, and total, direct, and indirect bilirubin.	Carmo et al., 2021	Yes
5	*B. decumbens*	Sheep	There were significances in serum urea, creatinine, cholesterol, triglycerides, AST, GGT, total bilirubin, direct bilirubin, and total protein as the time exposed to steroidal saponins increased.	Lelis et al., 2018	Yes

Table 2.4 : Continued

6	*B. decumbens*	Buffaloes	Only the GGT enzyme presented significantly different serum levels between intoxicated animals and healthy animals.	de Oliveira et al., 2013	Yes
7	*B. decumbens*	Sheep	Mean serum AST and GGT activities were significantly higher in the group exposed to sun than in the other two groups, and the GGT activities were significantly higher in the group sheltered than in the control group.	Porto et al., 2013	Yes
8	*B. decumbens*	Sheep	Two surviving lambs and one without symptoms showed elevated GGT, bilirubin, and cholesterol levels.	Brum et al., 2007	Yes
9	*B. decumbens*	Sheep	GGT and bilirubin levels remained unchanged throughout the experimental period.	Cruz et al., 2001	No
10	*B. decumbens, B. brizantha, P. maximum,* & *A. gayanus*	Sheep	The mean serum activities of GGT in the sheep grazing on *B. decumbens* were higher than other groups.	Gracindo et al., 2014	Yes
11	*Brachiaria spp.*	Sheep	There was a significant increase in GGT, AST, and glutamate dehydrogenase, as well as hyperbilirubinemia and azotemia.	Montoya- Ménez et al., 2019	Yes

Table 2.4 : Continued

12	*Brachiaria spp.*	Sheep	The control group, the group grazing continuously in *B. humidicola*, and group grazing continuously in *B. brizantha* showed significantly higher serum activities of GGT and AST than other treatment groups.	Castro et al., 2018	Yes

2.7 Post-mortem findings related to *B. decumbens*

In order to perform a thorough analysis of gross and macroscopic pathology, it is necessary to have a full understanding of the physical and chemical changes that occur within the body of the animal. The species of *Brachiaria* is widely recognized for its ability to induce intoxication in ruminant animals, primarily due to the presence of saponin, which serves as the principal toxic compound affecting animals at both microscopic and macroscopic levels. In particular, the primary necropsy findings in recent investigations include dermatitis, widespread jaundice, and liver atrophy or hepatomegaly with distinct lobular patterns in intoxicated animals (Faccin et al., 2014; Pupin et al., 2016; Rosa et al., 2016; Montoya-Ménez et al., 2019; Diamantino et al., 2020; Carmo et al., 2021; Kono et al., 2022). The presence of birefringent crystals in the bile ducts and crystals in enormous cells and foamy macrophages supported the conclusion that this condition is caused by poisoning by *Brachiaria spp*., as explained in previous studies (Porto et al., 2013; Faccin et al., 2014; Rosa et al., 2016).

Accordingly, multiple studies have reported the post-mortem associated with intoxication in ruminants, a finding that provides major significance to further understand subclinical saponin intoxication. One of the reported studies is the identification of sheep susceptibility in *Brachiaria spp*. toxicity by Pupin (2016), reporting that among the susceptible group, 11 lambs exhibited varying degrees of *B. decumbens* poisoning including yellowish liver, whereas none of the lambs in the resistant group were affected. This variation between the two groups was statistically significant, indicating an inherited susceptibility for resistance to *Brachiaria spp.* poisoning in sheep. Porto (2013) also reported that the most significant findings observed in necropsied sheep were generalized jaundice, hepatomegaly with an enlarged gallbladder, and a yellow liver. The presence of jaundice observed during clinical examination and necropsy from these studies are indicative of hepatoxic injury resulting from the consumption of *B. decumbens*, which is further supported through the elevated levels of serum AST and GGT activities. The primary mechanism implicated in the manifestation of these alterations is the release of biliary pigments resulting from the obstruction of the bile ducts by crystallized compounds formed through the conjugation of saponin with calcium (Brum et al., 2009; Porto et al., 2013). A necropsy of sheep when fed with *B. ruziziensis* revealed solid, greenish- colored kidneys and liver, generalized jaundice as well as crusts on the face and ears (Diamantino et al., 2020). The clinical manifestations and postmortem observations in this study are comparable to those stated in sheep that graze on *B. ruziziensis* pastures (Brum et al., 2007; Faccin et al., 2014).

Additionally, a study of induced cholangiohepatopathy was reported, mentioning that except for one, all lambs exhibited the presence of pale foci distributed multifocally throughout the hepatic parenchyma during necropsy, suggesting saponin intoxication in sheep fed with *B. decumbens.* While the recurrence of photosensitization was absent, the clinical findings and hepatic lesions observed exhibit consistent hepatic disease and align with the characteristics described for plants linked to photosensitization. The absence of photosensitization symptoms and increased enzyme activity can likely be attributed to the multifocal distribution of lesions and the lack of significant hepatocellular damage (Cruz et al., 2001). The findings of this survey, along with those of other research, provide further evidence that *B. decumbens* is capable of inducing hepatic lesions through ingestion (Cruz et al., 2001). Moreover, icterus was also reported in previous goat outbreaks (Rosa et al., 2016). During a study of spontaneous poisoning in *B. decumbens*, the most common lesion discovered during a necropsy is liver discoloration, which can be yellow or orange (Rosa et al., 2016). These findings were found in five of the seven goats studied. The liver was reduced in size and firmness at the cut surface in two goats, with several punctuate to starry regions in the capsular surface. The etiology of icterus in this saponin intoxication is supported by Cruz (2001), which attributed the obstruction of the bile ducts was caused by the aggregation of saponin crystals, thereby indicating the presence of post-hepatic icterus. The presence of elevated concentrations of protodioscin saponin in *B. decumbens*, which has been consumed by sheep affected by crystal-associated cholangiopathy, suggests that this furostanol steroid saponin is primarily accountable for the photosensitization induced by this particular grass species (Brum et al., 2007).

Further study also revealed the occurrence of green or brownish liver and kidney, which was consequently linked to saponin toxicity. Following Montoya-Ménez (2019), both natural and experimental groups fed with *Brachiaria spp*. showed the presence of green discoloration in the kidneys upon necropsy as well as the observation of nephrosis and crystals in the tubules and interstitium under microscopic examination correspond with the renal alterations that were previously shown in sheep. Another recent study in steer also revealed that the necropsied liver exhibited an increased volume of protruding edges and an appearance of greenish in nature. The kidneys exhibited a pale brown hue, displaying an irregular and mottled subcapsular texture, characterized by areas of blackened and depressed regions (Carmo et al., 2021). It was reported that the renal lesions caused by this tropical grass can be attributed to the nephrotoxic effect of the saponins, as well as the cholemic nephrosis resulting from liver disease, which leads to hyperbilirubinemia (Montoya-Ménez et al., 2019). The relative clinical significance of the renal lesions observed in sheep affected by *Brachiaria spp.* intoxication is thus likely to be less significant when compared to the dermal and hepatic lesions.

Moreover, other livestock linked to the intoxication of *Brachiaria spp.* includes cattle, buffaloes, and goats. Adult cattle are prone to developing photosensitization by *Brachiaria spp.* despite being less prevalent. Sensitive indicators that show an active inflammatory or infectious process in cattle are leukocytosis with neutrophilia and hyperfibrinogenemia (Carmo et al., 2021). The necropsy examination within this study displayed evidence of widespread bruises, erythema, and severe jaundice affecting the mucous membranes, eyeballs, and corneas, which exhibited opacity. These characteristics, together with macroscopic lesions described in the kidneys and liver brought on by the action of saponins, can be explained through subsequent bacterial infection and purulent discharge in skin lesions. Thus, serum biochemistry is an effective method for assessing hepatic impairment and potential secondary lesions, whereas microscopic and macroscopic characteristics provide definitive evidence for diagnosing the disease (Carmo et al., 2021). In another study by Rosa (2016), respiratory signs were observed in three goats with a case of bronchopneumonia, unilateral on the right side. This condition was characterized by the blackening and consolidation of the parenchyma, primarily in the right cranial lobe, with adhesion to the costal pleura. Additional observations during the necropsy examination revealed the presence of hydropericardium and ascites. The reason behind this condition is attributed to the highly irritating nature of the ruminal contents, which subsequently results in the development of fibrinous, necrotizing pneumonia. A diagnosis of severe malnutrition in these goats may have contributed a role in the occurrence of aspiration pneumonia (Rosa et al., 2016). The right cranial lung lobe is typically more significantly impacted due to the anatomical positioning of the right cranial bronchus, being the most anterior branch entering the ventrolateral region of the trachea (López, 2017).

In contrast, there were also reports showing no necropsy findings, indicating insignificance when *Brachiaria spp*. was fed to the animals. The present study showcased young weaned sheep reared in *Brachiaria spp*. pastures exhibiting comparable performance in both toxic and non-toxic pastures. These findings imply that the majority of sheep belonging to flocks acclimated to grazing in *Brachiaria spp*. pastures can be safely maintained in these grasses without encountering issues related to toxicity or compromised weight gain (Gracindo et al., 2014). There were also no observable macroscopic alterations in the liver of any animal that was fed with *B. brizantha* hay as reported by Lima (2012). Moreover, animals afflicted with pneumonia exhibited pulmonary manifestations characterized by regions of consolidation, hyperemia, emphysema, and occasionally, the formation of abscesses within the lungs. However, the findings indicate that lambs that were provided with *B. brizantha* hay containing 0.86 percent protodioscin did not exhibit any decline in performance and did not manifest any observable symptoms of poisoning (Lima et al., 2012). There is a potential for animals raised on *Brachiaria spp*. pastures to reduce the effects of the toxin due to a greater ability to metabolize and degrade the steroidal saponin, the toxic principle of this grass (Castro et al. 2007; Albernaz et al. 2010). Additionally, it is plausible that the animals may develop a distinct ruminal flora (Albernaz et al., 2010) that allows them to mitigate the adverse effects of this toxin. Furthermore, the lithogenic steroidal saponins were also found in *Panicum spp.*, which are primarily dichotomin and upon hydrolysis, produce the sapogenins diosgenin and yamogenin. The initial reports of infection manifested approximately 15 to 20 days subsequent to the introduction of the livestock into the grazing area. However, the presence of donkeys, goats, and cattle fed in the same pasture did not yield any apparent effect (Riet-Correa et al., 2009).

Several approaches have been suggested with the aim of mitigating or minimizing the deleterious effects of *Brachiaria* species-induced poisoning in livestock. Included in these options are the utilization of less toxic species of *Brachiaria spp.*, such as *B. ruziziensis* and *B. humidicola*, which exhibit lower concentrations of protodioscin (Pupin et al., 2016). Moreover, it is important to note that *B. brizantha* may collect saponins high enough to intoxicate sheep, given that there is evidence found of animal intoxication (Faccin et al., 2014; Diamantino et al., 2020). Since *Brachiaria spp*. poisoning can vary within a herd, identifying resistant animals is an alternate management method. An additional approach to controlling *Brachiaria spp*. is through the identification of animals that exhibit resistance to intoxication. Since *Brachiaria spp*. poisoning can vary within a herd, identifying resistant animals is an alternate management method to prevent toxicity (Riet-Correa et al., 2011; Pupin et al., 2016). These findings show that there exists an inherited resistance to *Brachiaria spp.* poisoning in sheep, as evidenced by the absence of poisoning symptoms in the offspring of resistant sheep. The implementation of resistant flocks within *Brachiaria spp.* pastures is proposed as a valuable strategy for mitigating the risk of *Brachiaria spp.* poisoning in sheep (Pupin et al., 2016). The utilization of animals that possess resistance or resilience to *Brachiaria spp*. poisoning may also potentially support the treatment. However, it is important to note that additional strategies, such as the cultivation of *Brachiaria* species or varieties that have low levels of saponins, are also imperative for the prevention of intoxication (Gracindo et al., 2014). Nevertheless, it is likely that reports of subclinical poisoning are present, along with cases where the clinical manifestations go unnoticed. There is a prevailing belief that the incidence of poisoning is subject to underreporting, resulting in less acknowledgment of an accurate diagnosis within the study.

Briefly, post-mortem findings are relatively less mentioned within studies, irrespective of the significance. According to the findings presented in Table

2.5, a significant number of research have indicated the association between the consumption of *Brachiaria spp.* by animals and the occurrence of necropsy findings. One study has demonstrated the manifestation of a yellowish liver in animals fed with *Panicum dichotomiflorum* (Riet-Correa et al., 2009). The occurrence of macroscopic or post-mortem findings is limited, most likely attributed to the higher accuracy and significance of microscopic and biochemistry findings reported in the studies. The majority of animals reported with saponin intoxication involved sheep since it was revealed as the most susceptible among other ruminants fed with *Brachiaria spp.* pasture (Pupin et al., 2016). Fewer reports are observed in these investigations since post-mortem findings are essentially confirmation of microscopic and blood data. Despite the implementation of various preventive measures to reduce the detrimental effects of toxicity, the current efforts remain insufficient in guaranteeing optimal animal health. More importantly, there has been an insufficient number of measures taken to address the issue of saponin presence in the pasture by including the use of preservation techniques. Thus, additional research is required to investigate the potential of ensiled *Brachiaria spp*. as a means of alleviating the adverse effects of intoxication in ruminant animals, thereby providing significant support to the livestock industry.

Table 2.5 : The effects of saponin toxicity on the post-mortem findings of animal

No.	Plant	Animal	Post mortem findings	References	Significances
1	*B. brizantha*	Sheep	Two naïve sheep had moderate to severe jaundice, a reddish brown and highly firm liver, a dilated gall bladder at necropsy, and dark-brown kidneys. One sheep was edematous.	Faccin et al., 2014	Yes
2	*B. brizantha, B. ruziziensis*	Cattle	All outbreaks included moderate jaundice, necrotizing dermatitis, and ventral tongue glossitis. An enlarged, lobular, brown-yellowish liver and viscous lumpy gallbladder were observed.	Kono et al., 2022	Yes
3	*B. decumbens*	Goats	Six goats had orange, yellow, or gray liver discoloration, indicating hepatomegaly or liver atrophy. Two exhibited lobular accentuation and punctate, linear, or stellar fibrous depressions on the capsular surface. Macroscopic liver failure alterations were reported.	Rosa et al., 2016	Yes
4	*B. decumbens*	Steer	Necropsy revealed extensive areas of bedsores, erythema, severe jaundice in the mucous membranes, eyeballs, and opaque corneas. An enlarged volume, bulging edges, greenish color liver, and pale brownish, irregular, mottled subcapsular surface, with blackened and depressed spots in the kidney	Carmo et al., 2021	Yes

Table 2.5 : Continued

5	*B. decumbens*	Sheep	One adult sheep presented unilateral a distended gallbladder.	Brum et al., 2007	Yes
6	*B. decumbens*	Sheep	At necropsy, all lambs except lamb 1 had pale foci multifocally distributed throughout the hepatic parenchyma.	Cruz et al., 2001	Yes
7	*B. ruziziensis*	Sheep	At necropsy, the main gross findings include a yellowish discoloration of the liver, icteric mucosa, marked lobular pattern, and biliary vesicles.	Diamantino et al., 2020	Yes
8	*Brachiaria spp.*	Sheep	Necropsy findings in six lambs included pale mucous membranes, emaciation, dermatitis, scar deformation of the ears, and large yellow livers with marked lobular pattern.	Pupin et al., 2016	Yes
9	*Brachiaria spp.*	Sheep	The post-mortem examination revealed jaundice, craniofacial edema, and an enlarged, orange-brown discolored liver; the hepatic parenchyma had a subtle zonal pattern.	Montoya-Ménez et al., 2019	Yes
10	*P. dichotomiflorum*	Sheep	Only one affected lamb reported yellowish liver.	Riet-Correa et al., 2009	No

2.8 Histopathology changes related to *B. decumbens*

Histopathological analysis is one of the methods used to observe alterations or cell changes consequential to diseases or infections. Generally, *Brachiaria spp.* intoxication leads to photosensitization issues sourcing from cellular changes in the organ of ruminants, mainly affecting the liver and skin. It was already established that the findings of a previous survey provide evidence that *B. decumbens* has the potential to induce hepatic lesions irrespective of sporidesmin derived from *P. chartarum* spores (Cruz et al., 2001). Previous reports also concluded that protodioscin, a steroidal saponin has been found in high concentrations in a *B. decumbens* pasture, where it is presumed to be responsible for photosensitization (Brum et al., 2007). Steroidal saponins also have been observed to inhibit the proliferation of ciliated protozoa. Consequently, the reduction in protozoa populations results in elevated volatile fatty acid synthesis, potentially exacerbating oxidative stress and precipitating hepatic damage to the animal (Montoya-Ménez et al., 2019), which is definitively reported in the histopathological analysis mentioned.

The presence of saponins causing hepatogenic photosensitization by *Brachiaria spp.* has been ascribed to the accumulation of crystals within the biliary canaliculi, resulting in the development of cholangiopathy (Cruz et al., 2001; Brum et al., 2007; Lima et al., 2012; de Oliveira et al., 2013). Other pasture species including *Panicum schinzii, Panicum coloratum* (Brum et al., 2007), *P. dichotomiflorum, P. miliaceum*, and *T. terrestris*, have been found to display comparable histopathological signs with *Brachiaria spp.* indicating the presence of birefringent crystals (Cruz et al., 2001; Porto et al., 2013; Low, 2015). A report mentioned that the histological changes in buffalo liver with hepatogenous photosensitization were comparable to those seen in other ruminants, including biliary stasis and crystals in the bile ducts. This finding supports the suggestion that photosensitization in animals fed *Brachiaria spp.* is linked to crystalloid material accumulation in the bile ducts and cholestasis (de Oliveira et al., 2013). In order to facilitate the process of crystal formation, it is necessary for the animal's diet to include a source of steroid substance, which coincides with the presence of steroidal saponins found in *Brachiaria spp*. (de Oliveira et al., 2013). Montoya-Ménez (2019) explained that the process of lithogenic crystal formation begins during ruminal digestion, at which the saponins found in *Brachiaria spp*. undergo conversion into β-D-glucuronides and steroidal sapogenins compounds. These compounds subsequently precipitate into crystals when calcium is present. The observed ultrastructure alterations, specifically the identification of cleft-shaped crystalline structures in the liver and kidney, were in accordance with findings within the study. Moreover, Faccin (2014) reported that sheep with birefringent crystals in the bile ducts and negative pictures of crystals in large cells and foamy macrophages had *Brachiaria spp*. induced liver cirrhosis. *Brachiaria*-fed sheep have not experienced this problem despite having cirrhosis and photosensitivity at three months old. This sheep simply showed exercise intolerance without weight loss during the experiment. Due to the chronic nature of the lesions, it was not possible to determine whether cirrhosis was caused by continuous ingestion of protodioscin in the pasture during the experiment or by the progression of lesions that started eight months before the experiment when the animal showed photosensitization (Faccin et al., 2014). However, few studies have reported an absence of negative images of crystals inside the bile ducts during histological analysis (Riet-Correa et al., 2009; Lima et al., 2012; Araújo et al., 2017; Lelis et al., 2018). It has been observed that the impact of *Bracharia spp*. on ruminants within a herd is not uniform (Araújo et al., 2017; Montoya-Ménez et al, 2019; Carmo et al., 2021). The phenomenon of genetic adaptation, coupled with prolonged exposure to this particular type of grass, provides a stronger resistance to toxic effects in animals. Additionally, younger sheep exhibit a greater susceptibility to these toxic effects compared when compared with older sheep (Montoya-Ménez et al, 2019). These various factors may account for the varying impact observed among the sheep fed with *Brachiaria spp.*

In addition to crystal formation, multiple investigations revealed foamy macrophages in the liver, notably in the central zones of the lobule, hepatic and mesenteric lymph nodes, spleen, and small intestine mucosa of ruminants poisoned by *Brachiaria spp*. (Riet-Correa et al., 2002; Rosa et al., 2016). The foamy appearance of macrophages is suggested to be a result of cytoplasmic crystal accumulation (Rosa et al., 2016). Araújo (2017) suggested that the presence of foamy macrophage aggregates in the livers of these animals has been observed solely based on their morphological features. This may pose challenges in cell identification and lesion determination. The low number of foam cell aggregates observed in the livers of cattle that were grazed on *Andropogon spp*. pasture provides further evidence that the occurrence of these aggregates is specifically linked to the animals' exposure to *Brachiaria spp.* pastures (Araújo et al., 2017). Furthermore, foamy macrophages take time to develop; therefore, they may not yet be seen in the livers of animals who died suddenly from severe hepatic failure in the absence of photosensitization. Foamy macrophages are absent in sheep or are so few and inconspicuous that they are easily noticed, possibly due to the larger species' sensitivity. As a result of this increased vulnerability, the clinical course is significantly shorter in this species, and the production of foamy macrophages requires more time (Rosa et al., 2016). As explained by Caicedo (2012), the presence of foamy macrophages in the liver and lymph nodes is frequently observed in ruminants grazing on *Brachiaria spp*., potentially indicating the manifestation of clinical symptoms associated with the disease. Additionally, there have been reports indicating the existence of a negative association between weight gain and the abundance of foamy macrophages in the livers of cattle that engage in grazing activities on *B. decumbens*. This observation implies the presence of a subclinical condition that carries significant economic implications (Caicedo et al., 2012).

Therefore, these histological alterations observed are highly consistent and indicative of the hepatotoxic effects associated with the presence of lithogenic steroidal saponins in plants within *Brachiaria spp*. Less frequent microscopic findings observed in intoxicated sheep also include hepatocellular swelling and vacuolization, individual necrosis of hepatocytes, and bile duct hyperplasia (Porto et al., 2013). Sheep intoxication caused by *Brachiaria spp.* can lead to necrosis and swelling of periportal hepatocytes, which is primarily attributed to the presence of insoluble saponin crystals as mentioned in the previous study. Ductal hyperplasia is widely recognized as a prevalent reaction to various hepatopathies, yet its underlying mechanism remains unclear. Further understanding of histological changes of intoxicated animals is necessary to ensure a better understanding of hepatogenous photosensitization of *Brachiaria spp.*

In contrast, several studies mentioned the potential of animals displaying healthy clinical conditions despite the presence of histopathological reports. One study described that the foamy macrophages can be observed in both healthy animals residing in pastures of *Brachiaria spp*. that are subsequently slaughtered, as well as in intoxicated animals within different states in Brazil. The observed contrast may be attributed to the elevated levels of saponins found in the grasses within that particular region. These variations in toxicity and climate, as well as the presence of more toxic cultivars or other unidentified regional factors, could potentially account for this discrepancy (Araújo et al., 2017). Moreover, buffaloes bred on *Brachiaria spp*. grasslands in Brazil revealed foamy macrophages and hepatocellular enlargement but showed no clinical evidence of photosensitization (Riet-Correa et al., 2010). In another investigation by Faccin (2014), the weight loss of two black-coated sheep that did not have photodermatitis lesions could also be linked to liver impairment caused by swallowing *B. brizantha*, based on histological abnormalities seen in the hepatic biopsies. It is crucial to note that although some remaining healthy animals have not exhibited clinical symptoms, they may still possess notable liver alterations that could lead to the condemnation of the livers during future slaughter. There is a possibility that cattle, upon experiencing liver damage, could stop the ingestion of toxic substances

(Carmo et al., 2021). The heritability transmission of toxin resistance is a significant mechanism that accounts for the extensive range of animal susceptibility to poisoning. In an investigation by Pupin (2016), three lambs had poor development but no gross or histological diseases that could explain it, such as widespread hepatic fibrosis or foamy macrophages in the intestine. This study found that the lambs' offspring were resistant to *Brachiaria spp.* poisoning, suggesting that investing in genetically resistant flocks may avoid sheep poisoning by *Brachiaria spp*. (Pupin et al., 2016). The specific mechanisms underlying resistance to the toxin remain unclear; however, it is hypothesized that this resistance may be attributed to enhanced hepatic metabolism of the toxin, potentially facilitated by the differential expression of specific cytochrome P450 (CYP) system enzymes (Castro et al., 2018). The findings from these reports highlight the significance of grass-induced hepatotoxicity in intoxication, regardless of the animals' sun exposure and the absence of photosensitization.

Overall, the majority of the studies showed a considerable significance of histopathological changes in ruminants when fed with *Brachiaria spp.* Few studies also reported insignificances due to insufficient values of saponin within the pastures as well as successful adaptation of ruminants towards pastures. Although the occurrence of photosensitization has been frequently observed in animals under various research, the histological examination of liver samples in one study suggested that the amount of saponins contained in the *Brachiaria spp.* ingested by the tested animals was insufficient to induce the formation of crystals (Caicedo et al., 2012). Based on Table 2.6, only half of the study mentioned necropsy findings within the animals, with another half mentioning both histological and pathological findings. All of these studies have provided *Brachiaria spp.* as their main feeding or grazing pastures of choice. However, no studies revealed the use of *Brachiaria spp.* as silage, as well as reporting its intoxication effects on histopathological analysis. To what extent silage can be used as a treatment to lessen saponin levels and mitigate the effects on ruminants is still a mystery. There are still insufficient resources concerning the relationship between silage as a feed and its consequences on sheep's health. The higher susceptibility of sheep compared to cattle towards lithogenic saponins implies poisoning by *Brachiaria spp.* is a significant limitation for the sheep industry (Brum et al., 2007). Thus, an additional study demonstrating the histopathological changes of sheep fed with ensiled *B. decumbens* will further give insight into the improvement of the ruminant diet.

Table 2.6 : The effects of saponin toxicity on the histological changes of animal

No.	Plant	Animal	Histopathological findings	References	Significances
1	*B. brizantha*	Sheep	In sheep livers, the microscopic lesions revealed structural disorganization and major sinusoidal infiltrations of foamy macrophages. Hepatocytes, macrophages, and bile ducts were seen to have crystals and negative pictures of crystals. The sheep displayed mild periportal fibrosis with Bowman's gap and renal cortical tubules showed amorphous eosinophilic material. There was significant coagulation necrosis, cell debris, basophilic material suggesting bacterial colonies, neutrophilic infiltration, hyperkeratosis, and parakeratosis on the epidermal lesions.	Faccin et al., 2014	Yes
2	*B. brizantha* hay	Lamb	The liver analyzed showed preserved hepatocytes and light multifocal infiltration of mononuclear inflammatory cells, which indicated mild cholangitis in lambs.	Lima et al., 2012	No
3	*B. brizantha*, *B. ruziziensis*	Cattle	The hepatic periportal regions had moderate lymphocyte and macrophage infiltration, bile duct hyperplasia, cholestasis, and connective tissue fibrosis in histological observations. There were occasional birefringent crystals with a pale yellow color. There were negative intracytoplasmic crystal images on hepatocytes and macrophages.	Kono et al., 2022	Yes

Table 2.6 : Continued

4	*B. decumbens*	Cattle	The main lesions observed were mononuclear cell cholangiohepatitis, foamy macrophages, moderate bile pigment accumulation, hepatocyte death, binucleated hepatocytes, moderate bile duct hyperplasia, and multiple foci of mild fibrosis in portal areas.	Caicedo et al., 2012	Yes
5	*B. decumbens*	Goats	Histopathological findings were the accumulation of refringent crystals in the bile ducts and infiltration of foamy macrophages in the liver, with foamy macrophages in the hepatic and mesenteric lymph nodes.	Rosa et al., 2016	Yes
6	*B. decumbens*	Steer	Histologically, the cytoplasm of the hepatocytes was occasionally refringent with an abundant presence of bile pigment. Multiple foci of accumulation of macrophages filled with vacuoles of different sizes containing saponins and crystals of saponins inside bile ducts. Furthermore, it was possible to observe hypertrophy and hyperplasia of Kupffer cells, disarrangement of hepatocytes with individual necrosis of hepatocytes.	Carmo et al., 2021	Yes
7	*B. decumbens*	Sheep	The main histological changes observed included hepatocellular degeneration, hepatocyte necrosis, inflammatory infiltrates, and biliary ductal hyperplasia. Alteration in liver coloration and the evident lobular pattern observed in animals in this study may be due to hepatocyte swelling,	Lelis et al., 2018	No

Table 2.6 : Continued

			inflammatory infiltrates, and hepatocyte degeneration.		
8	*B. decumbens*	Buffalo	Microscopy of the liver showed foamy macrophages and lesions of liver disease associated with the presence of crystals in the bile ducts.	de Oliveira et al., 2013	Yes
9	*B. decumbens*	Sheep	Histopathological evaluation of liver samples from sheep with clinical signs evidenced swelling and vacuolization of hepatocytes, individual hepatocytes necrosis, presence of foamy macrophages, crystal negative images within bile ducts and foamy macrophages, biliary duct hyperplasia and periportal mononuclear infiltration.	Porto et al., 2013	Yes
10	*B. decumbens*	Sheep	Histological lesions were epithelial degeneration, necrosis, and hyperplasia of small bile ducts. Mild amounts of foamy macrophages were observed, mainly in the centroacinar zone. Diffuse swelling and vacuolation were observed in hepatocytes. Crystal-negative images were found within bile ducts, foamy macrophages, and the lumen of some renal tubules. The heart showed multifocal areas of degeneration and necrosis of the muscle fibers.	Brum et al., 2007	Yes

Table 2.6 : Continued

11	*B. decumbens*	Sheep	Histologic examination revealed marked, multifocal cholangitis in portal triads with bile duct proliferation and infiltration by macrophages and lymphocytes. Crystals were observed within the bile ducts or surrounded by macrophages.	Cruz et al., 2001	Yes
12	*Brachiaria spp.*	Cattle	The main alterations found were random aggregates of foamy macrophages, fibrosis, proliferation of bile ducts, periportal mononuclear infiltrate, and negative image of material crystalloid in the cytoplasm of macrophages.	Araujo et al., 2017	Yes
13	*Brachiaria spp.*	Sheep	Histopathological changes include disruption of hepatocellular trabecula, clusters of foamy macrophages forming multinucleated giant cells, swollen and vacuolated hepatocytes, crystals or negative images of crystals in the biliary system, bilestasis, bile duct proliferation and lymphoplasmacytic infiltrate in portal triads.	Pupin et al., 2016	Yes
14	*Brachiaria spp.*	Sheep	Histopathological findings were severe necrotizing dermatitis, lymphoplasmacytic cholangiohepatitis, and renal tubular necrosis. Acicular crystals were evident in hepatocytes, Kupffer cells, biliary ducts, renal tubules, and interstitium.	Montoya-Ménez et al., 2019	Yes
15	*Panicum dichotomiflorum*	Sheep	Upon histologic examination, scattered necrotic hepatocytes were observed in the liver, focal areas of necrosis of myocytes appeared in the heart.	Riet-Correa et al., 2009	No

CHAPTER 3

MATERIALS AND METHODS

3.1 Ethical Approval

The experiment ethics and protocols were approved by the International Animal Care and Use Committee, Universiti Putra Malaysia (Approval number: UPM/IACUC/AUP-R003/2023).

3.2 Forage Preparation and Analysis

3.2.1 Forage Planting & Harvesting

This study utilized two grass species, including *Pennisetum purpureum* and *Brachiaria decumbens*. Both pastures were planted in Farm 15, Research Farm, Department of Animal Science, Universiti Putra Malaysia, at 2°59'15.1"N 101°44'08.3"E and 52 m altitude, prior to the animal trial. *B. decumbens* pasture was planted in a 70m x 30m area, and NPK green fertilizer 15:15:15 was applied periodically to maintain consistent grass regrowth throughout the trial. The ideal harvesting age was 7 weeks as derived based on previous studies for fresh *B. decumbens* and *P. purpureum* grass (Manyawu et al., 2003; Castro et al., 2007; de la Ribera et al., 2008). *B. decumbens* were then harvested at 7 weeks of age using a harvester machine before feeding the animals. The *P. purpureum* pasture was also cultivated in a 70m × 30m plot, and NPK green fertilizer 15:15:15 was periodically used to ensure constant grass regrowth during the experiment. In fresh *P. purpureum* pasture, manual harvesting was carried out at 7 weeks of age and further processed using a grass chopper machine before being given to the animals. *B. decumbens* silage was collected for the use of animals after a period of 4 weeks of ensiling. Samples from all three treatment groups were collected and dried in a 60°C oven for 72 hours before proceeding to grind into powder (1mm). The ground samples were subsequently used for analysis. The dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and gross energy were analyzed in accordance with methods from AOAC (2006). Table 3.1 presents the nutritional composition of the three various treatments: fresh *P. purpureum*, fresh *B. decumbens*, and ensiled *B. decumbens*.

Table 3.1: Nutrient content of fresh *P. purpureum*, fresh *B. decumbens*, & ensiled *B. decumbens*.

Composition (%)	Treatment 1	Treatment 2	Treatment 3	p-value
Dry matter (DM)	25.11 ± 0.53	24.61± 0.23	24.87 ± 0.30	0.411
Organic matter (OM)	90.22 ± 1.89	89.78 ± 1.51	90.71 ± 1.19	0.567
Crude protein (CP)	16.23 ± 0.33	15.71 ± 0.38	16.36 ± 0.81	0.078
Ether extract (EE)	2.31 ± 0.11	1.95 ± 0.33	2.11 ± 0.45	0.892
Crude fiber (CF)	30.65 ± 0.16	28.22 ± 0.26	27.15 ± 0.11	0.132
Neutral detergent fiber (NDF)	62.78 ± 0.88	60.91 ± 0.27	60.10 ± 0.41	0.257
Acid detergent fiber (ADF)	41.11 ± 0.25	38.91 ± 0.34	37.83 ± 0.76	0.121

Acid detergent lignin (ADL)	5.67 ± 0.22	4.87 ± 0.12	4.88 ± 0.31	0.069
Gross energy (kJ/kg DM)	17.87 ± 0.31	17.21 ± 0.20	17.88 ± 0.27	0.089

Note: All values were expressed as mean ± SEm; a,b values with superscript within row are significantly different at $p < 0.05$.

3.2.2 Ensiling Process

B. decumbens was harvested after 7 weeks of regrowth and chopped into pieces roughly 3-4 inches in length, at a height of 5 cm above the ground using a forage harvester. *B. decumbens* silages were prepared in high-density polyethylene (HDPE) plastic bags. Prior to ensiling, the molasses was manually added at a concentration of 1% of the forage amount per bag. The silage mixture was compressed, vacuumed, and sealed to establish an oxygen-free environment. Silages were prepared on a monthly basis for animal consumption. After a period of 4 weeks, the silage bags were unsealed and a feeding trial was carried out. The standard procedure was designed adhering to the established methodology in prior studies (Binuomote et al., 2018; Lima et al., 2015; Luo et al., 2021).

3.2.3 Saponin Extraction and Quantification

Fresh *B. decumbens* were collected and analyzed for saponin quantification every week until week ten of regrowth. Weekly samples were also collected for the ensiled *B. decumbens* from week 1 to week 10 of ensiling. Both samples were subjected to a complete randomized design, with three replicates for each sample. Approximately 500 grams of sample material were dried in a forced-air oven at a temperature of 60 degrees Celsius for a duration of 72 hours. The dried samples were then pulverized in a Laboratory Rotor Mill Pulverisette 14 with a 1-mm mesh and stored in tightly sealed plastic containers for further chemical analysis. The saponin quantification method used 60 degrees Celsius in a water bath for 20 minutes. Subsequently, the reaction was halted by placing the capped test tube into an ice-water bath for 10 minutes. The solution was transferred to the spectrophotometer. An absorbance measurement was taken at 230 nm by comparing it to a blank sample. The extract's total steroidal saponin content was determined by utilizing a standard calibration curve with protodioscin as the standard. Based on the concentration and wavelength values, the standard curve linear regression equation was calculated and concentration of saponin was determined.

3.3 Animal Model

The experimental procedures were conducted at Farm 15, located in the Faculty of Agriculture, Universiti Putra Malaysia. In this investigation, a total of eighteen (n=18) male Dorper cross sheep, aged six months, were used within 98 days of rearing. Throughout the acclimatization period, all 18 animals were raised collectively in an enclosure. The sheep underwent a 2-week period of acclimatization and were provided with *P. purpureum* grass along with ad libitum water. Ivermectin (1ml/50kg) was administered to all sheep as a deworming medication prior to the start of the experiment. Regular monitoring was conducted to ensure the well-being of the animals. The sheep's body weight was also measured during the acclimatization phase and was statistically analyzed using ANOVA statistical analysis by R Software to confirm the absence of significant differences among the sheep within the 2 weeks, ensuring standardization before the start of trial. All sheep had an average weight of approximately 15.0kg before the start of the experiment and were placed in a metabolic crate, randomly assigned for each treatment. The sheep were randomly divided into three treatment groups in a completely randomized design (CRD) and fed with three different types of grass consisting of fresh *P. purpureum*, fresh *B. decumbens*, and ensiled *B. decumbens*. Commercial concentrates were also provided, along with ad libitum water. The animals were provided with a diet equivalent to 3% of their body weight, with a feeding ratio of 70% roughage and 30% concentrate (Muniandy et al., 2021).

3.4 Experimental Design

The 18 sheep were divided into three treatments and a feeding trial was carried out within a 98-day period. The 18 sheep were divided into three treatment groups, each consisting of 6 Dorper sheep: Control group (T1) fed with *P. purpureum*; Treatment 2 group (T2) fed with fresh *B. decumbens*; and Treatment 3 group (T3) fed with ensiled *B. decumbens* (T3) grass. Fresh forages were harvested and chopped daily for the consumption of animals. Throughout the course of the study, clinical responses were monitored on a daily basis. Weekly data for the health performance of the sheep were also collected throughout the experiment. These data include the body weight, rectal temperature, respiration rate, heart rate, and clinical responses. Blood was also collected at a monthly interval during the animal trial. At day 98 of the experiment, all sheep were slaughtered and further processed to collect samples for morphological and histological analysis.

3.5 Health Performance

The severity of clinical responses for sheep was monitored throughout the feeding trial. This was achieved through a weekly evaluation of rectal temperature, heart rate, respiration rate, mucous membrane condition, rumen motility, and regular observation for indicators of toxicity (Chung et al., 2017; Muniandy et al., 2021). Animals were manually weighed using a digital scale and an adjustable strap. Next, the rumen motility and heart rate were assessed using a stethoscope, while the respiration rate was determined through visual observation when the sheep were in a state of rest. The mucous membranes of animals were examined in both the ocular and oral regions. A digital thermometer was also used to measure the rectal temperature. All data was taken in triplicate to provide more accuracy before further analyses.

3.6 Blood Analysis

Blood samples were collected through jugular venipuncture using a vacuum collection system to conduct a comprehensive analysis of serum biochemistry and blood count. Blood was collected on days 0, 30, 60, and 90 of the animal trial. A total of 60 blood samples were submitted to the Haematology and Biochemistry Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia for hematology and biochemistry analysis. The manufacturers' guidelines were followed in the execution of these measurements and processing through a semi-automatic biochemical analyzer. Hematocrit, total solid, and white blood cell counts were conducted using the Cell-Dyn 3700 Automatic Analyzer. Serums were processed by an automated chemistry analyzer (HITACHI 902 Automatic Analyzer®, Japan) to obtain the biochemistry parameters.

3.7 Gross Morphology and Morphometric Analysis

The length and width of the organs were measured to determine organ morphometrics. The postmortem examination and organ morphometric analysis were carried out at the research abattoir of the Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia. All 18 animals were slaughtered and processed for organ collection on day 98 of the animal trial. A total of 108 organ samples were analyzed, with gross lesions observed throughout the brain, heart, lung, liver, spleen, and kidney. The GIT of the animal was additionally inspected for abnormalities. The organ morphometric was determined by measuring the length and width of the organs. Additionally, the organ weights of all sheep from the three groups were recorded.

3.8 Histology Analysis

Organ collection was carried out after the animals were slaughtered and handled at the research abattoir located in the Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia. A number of 162 samples were collected for evaluation of cellular changes including the brain, heart, lung, liver, kidney, spleen, duodenum, jejunum, and ileum. Samples were fixed in 10% neutrally buffered formalin and dehydrated using alcohol. The tissue fragments were processed through a series of steps of embedding in paraffin, sectioning, and staining with hematoxylin and eosin solution. All of the samples were examined using a light microscope. Each slide was examined under a 200x and 400x magnification, where six microscopic sections were observed. Four categories of identified lesions were studied. The categories consist of inflammatory cells, necrosis/degeneration, edematous fluid, and congestion/hemorrhage. The scoring technique was recorded in accordance with Chung et al. (2017), which used a four-point scale, with score 0 indicating normal tissue, score 1 indicating mild tissue (less than 25% affected), score 2 indicating moderate tissue (less than 50% affected), and score 3 indicating severe tissue (greater than 50% affected).

3.9 Statistical Analysis

All data was analyzed using ANOVA statistical analysis by R Software, with $p < 0.05$ as an indicator of significance. The data was tested for normality using the Shapiro-Wilk test, with all data following a normal distribution. Statistical techniques such as one-way ANOVA and multiple comparison post-hoc tests were employed to assess the differences in means across various groups. Kruskal-Wallis Test was used to compare ordinal means between the treatments in histological data. All data were depicted as mean ± standard deviation (SD).

CHAPTER 4

RESULTS AND DISCUSSION

4.0 Saponin Quantification

Upon calculation, the concentration of the saponin for both fresh and ensiled *B. decumbens* was displayed in Figure 4.1 and Figure 4.2. The weekly saponin concentration of fresh *B. decumbens* was presented, starting at 0.341 mg/mL in week 1 and decreasing to 0.285 mg/mL by week 10 (Figure 4.1). Beginning in week 7, saponin concentration exhibited a consistent level until week 10 of regrowth. Furthermore, the saponin content in *B. decumbens* silage exhibited a significant decrease from week 4 to week 10 of preservation (Figure 4.2). The saponin concentration originally measured around 0.341 mg/mL, but then exhibited a fast decline, ultimately reaching a concentration of zero within the ensiled grass.

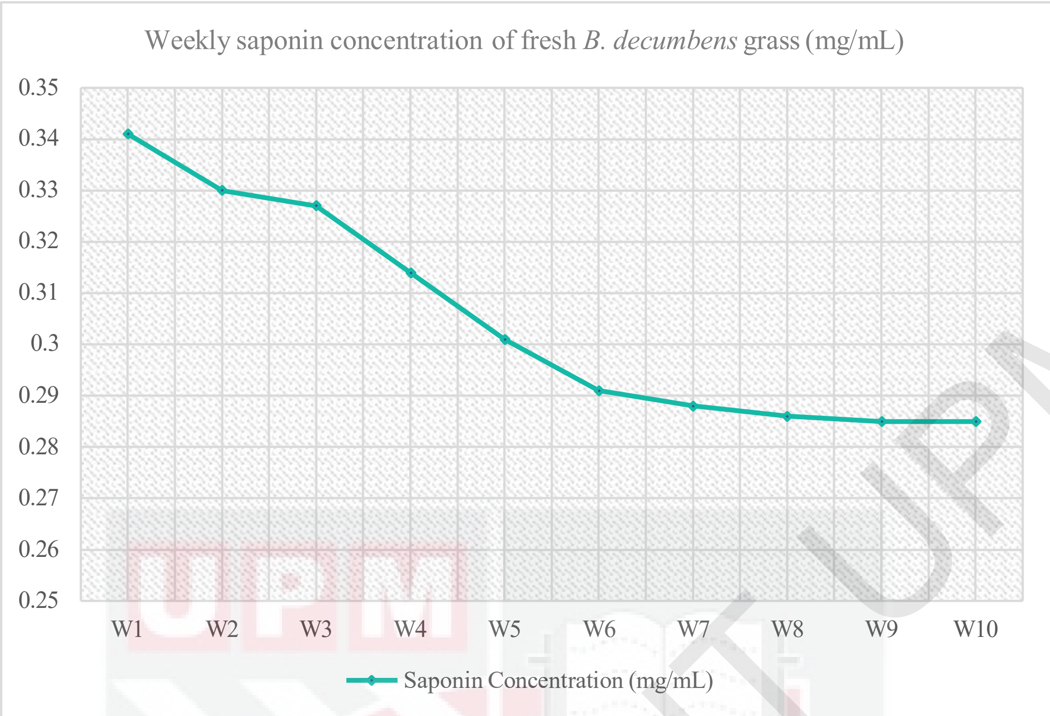

Figure 4.1 : Saponin concentration of fresh *B. decumbens* at different ages.

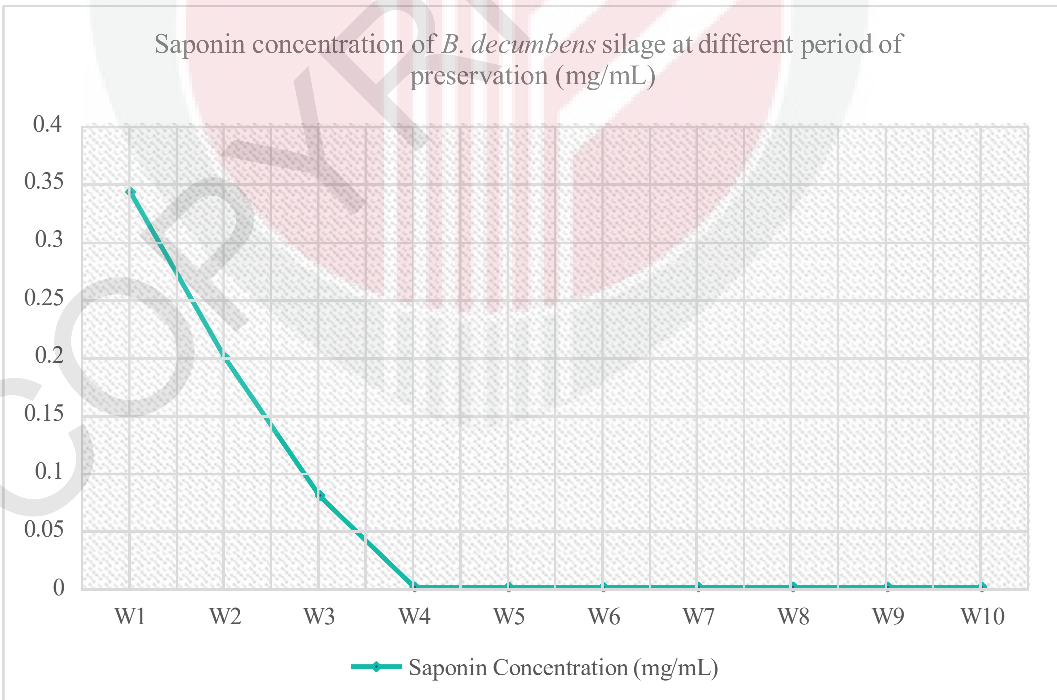

Figure 4.2 : Saponin concentration of ensiled *B. decumbens* at different ages of ensiling.

4.1 Health performances

4.1.1 Body weight

The weekly body weight of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* & ensiled *B. decumbens* is shown in Table 4.1 below. There is a significant difference ($p < 0.05$) in the body weight of sheep throughout the experiment. Generally, sheep fed with ensiled *B. decumbens* (Treatment 3) had the highest weekly body weight, followed by control group, and Treatment 2. Based on Figure 4.3, there was an increasing trend of body weight in all treatments with sheep fed with ensiled *B. decumbens* had the highest body weight gain (↑38.5%), followed by control sheep (↑37.5%), and sheep fed with fresh *B. decumbens* (↑34.3%) at the end of the study. This suggests that the body weight of sheep is positively influenced by the feeding of ensiled *B. decumbens* throughout the experiment.

Table 4.1 : Body weight of sheep fed with fresh *P. purpureum*, fresh *B. decumbens*, & ensiled *B. decumbens*.

	Treatment 1 (kg)	Treatment 2 (kg)	Treatment 3 (kg)	p-value
Day 0	23.00 ± 0.00	23.00 ± 0.00	23.00 ± 0.00	1.00
Day 7	23.15 ± 0.23^a	23.20 ± 0.23^a	22.77 ± 0.23^b	<0.01
Day 14	23.68 ± 0.05^a	23.80 ± 0.08^b	23.75 ± 0.07^b	<0.01
Day 21	24.15 ± 0.13^a	24.08 ± 0.11^c	24.45 ± 0.32^b	<0.01
Day 28	24.58 ± 0.06^a	24.33 ± 0.28^c	24.58 ± 0.06^b	<0.01
Day 35	25.78 ± 0.46^a	24.73 ± 0.56^c	25.15 ± 0.42^b	<0.01
Day 42	26.85 ± 0.46^a	25.88 ± 0.30^c	26.13 ± 0.43^b	<0.01
Day 49	27.53 ± 0.26^a	26.35 ± 0.17^c	27.30 ± 0.27^b	<0.01
Day 56	27.58 ± 0.18^a	26.50 ± 0.12^c	27.35 ± 0.09^b	0.01
Day 63	27.80 ± 0.48^b	27.43 ± 0.27^c	28.68 ± 0.32^a	<0.01
Day 70	28.40 ± 0.42^b	28.10 ± 0.41^c	28.70 ± 0.39^a	<0.01
Day 77	29.68 ± 0.62^b	28.85 ± 0.55^c	29.78 ± 0.68^a	<0.01
Day 84	30.05 ± 0.42^b	29.88 ± 0.48^c	30.33 ± 0.49^a	<0.01
Day 91	31.20 ± 0.52^b	30.60 ± 0.62^c	31.52 ± 0.65^a	<0.01
Day 98	31.63 ± 0.46^b	30.88 ± 0.33^c	31.85 ± 0.27^a	<0.01

Note: All values were expressed as mean ± SEM; $^{a, b, c}$ values with superscript within the same row are significantly different at p<0.05. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens*.

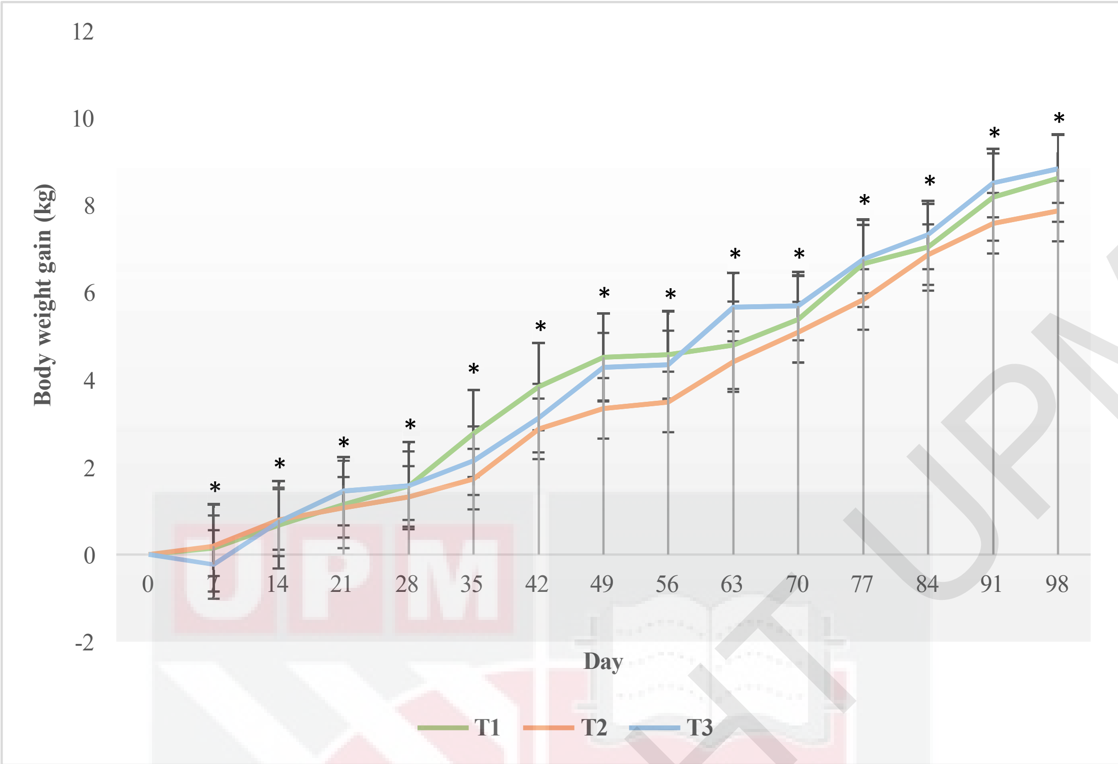

Figure 4.3 : The weekly body weight gain of sheep fed with fresh and ensiled *B. decumbens* from day 0 until day 98 was expressed as mean ± SEM. The significant differences existed between groups displayed throughout the weekly body weight of sheep. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between groups on the presented day.

Body weight gain is an essential indicator of an animal's productivity and health performance. The *Brachiaria* species are highly significant as sources of animal feed. However, only a few recent studies reported the body weight gain of animals within the experiment due to the insignificant findings shown when feeding *B. decumbens* compared to other forages. In the present study, the saponin content of fresh and ensiled *B. decumbens* was quantified at the start of the present experiment with week one of regrowth having the highest saponin concentration (0.34%), and showed zero saponin concentration by the fourth week of preservation. This shows that the treatment of grass was suggested to be a great alternative of saponin elimination, thus eliminating the hepatogenous photosensitization. This finding is similar to another extensive study that assessed how silage and hay affect protodioscin concentrations in *B. decumbens* and *B. brizantha* (Lima et al., 2015). Results from the previous study by Lima (2015) displayed *Brachiaria* spp. grass eliminating saponin protodioscin concentration after 24 days of ensiling. The saponin concentration decreased significantly by 94% from 2.28g/kg to 0.14g/kg within the initial days and reached zero after 20 days of ensiling. Similarly, the protodioscin content in *B. decumbens* decreased by 48% in the initial three days during haymaking and remained consistent. The reported saponin concentration was 8.24g/kg at the end of day 21, which was marginally lower than the concentration observed in fresh *B. decumbens* (Lima et al., 2015). Thus, it is suggested that ruminants fed *Brachiaria* spp. silage or hay will have a lower risk of protodioscin poisoning.

Throughout the experiment, sheep given ensiled *B. decumbens* (Treatment 3) had the highest weekly body weight, followed by the control group, and fresh *B. decumbens* (Treatment 2). The differing results between ensiled *B. decumbens* and fresh *B. decumbens* highlights the beneficial outcomes of ensiling as a method for eliminating saponin content in the grass. Ensiling has several advantageous effects, such as enhancing the digestibility and performance of animals, as well as improving the nutrient availability of grasses. A study using ensiled rice straw results in the physical structure of the straw to break down, causing a decrease in the levels of dry matter, neutral detergent fiber, hemicellulose, and water-soluble carbohydrates (Xu et al., 2023). The ensiling process has aided rumen microbial colonization and fiber digestion during early fermentation, increasing bacterial diversity while decreasing methane production, increasing *in vitro* nutrient degradability, and, eventually, improving the average daily gain of lambs (Xu et al., 2023). Generally, a complex of bacteria, fungi, protozoa, and archaea in the rumen fluid of ruminant animals can disrupt the three-dimensional lignocellulosic structures, allowing polysaccharides to be more accessible to microbes and enzymes and enabling high bioconversion of biomass to soluble sugars and small-molecule organic acids (Ren et al., 2021). Thus, the application of this preservation technique may enhance the degradation of cellulose and hemicellulose in the rumen, and improve the nutritional quality of the grass as reported by Xu (2023). Moreover, the ensiling process involves the consumption of water-soluble carbohydrates by lactic acid bacteria to form lactic acid. This lactic acid serves to lower the pH, which then prevents the growth of harmful microbes. Xu (2023) also reported that cellulolytic bacteria break down the cellulose components of ensiled rice straw, and cellulase stimulates the release of internal enzymes, facilitating digestion and absorption. Ruminants possess ruminal fiber-degrading microorganisms, including anaerobic fungi and bacteria, which attach and occupy the surface of fibers in forages. These microorganisms produce cellulases and hemicellulases, which efficiently break down the fibers into degraded products that serve as substrates for the synthesis of essential nutrients for ruminants. Consequentially, molasses is known as a byproduct substance produced during the sugar production process and has been discovered to increase the water-soluble carbohydrates (WSC) content available during ensiling (Bakare et al., 2023). The inclusion of molasses during ensiling is suggested to enhance the palatability of the feed, resulting in increased feed consumption by sheep. The efficacy of ensiling as a method to degrade fibers in grasses, together with the beneficial impacts of adding molasses, is supported by the reported increase in the body weight of sheep in T3. The inclusion of molasses in the ensiled *B. decumbens* has resulted in a significantly more positive outcome for T3 compared to T1 and T2. This further reinforces the importance demonstrated by the body weight of the sheep. Hence, the preservation method through ensiling within *B. decumbens* showed an improved digestion and absorption of nutrients, increased palatability as well as eliminating the saponin which may contribute to the increased intake of feed.

In line with the findings demonstrated in T2 sheep, Lelis (2018) reported low weight gain and weight loss in sheep, showing the detrimental effects of saponin on health performance when fed with fresh *B. decumbens*. It is proposed that the weight loss resulted from a decrease in overall health was caused by liver damage. In this compound, the primary adverse effects observed in animals are secondary hepatogenous photosensitization which affects the liver by inhibiting the excretion of phylloerythrin. These liver changes can also result in observable physical effects in animals, such as depression, fatigue, loss of appetite, and weight loss. This provides evidence that the reported outcomes of low weight gain have been linked to the poisonous compound present in *B. decumbens* grass. Lelis (2018) further suggested that animals experiencing a negative energy balance due to altered liver metabolism may be the cause of weight loss and low weight gain. This is supported by Albernaz (2010) explaining that liver disease prevents the normal elimination of toxic compounds from the grass, resulting in a range of intoxication symptoms. Livestock that are consistently fed with a low saponin content may still be affected and could lead to secondary hepatogenous photosensitization, even though the effect appears insignificant. Gracindo (2014) provides further backing for the suggested evidence, emphasizing that the low protodioscin content of 0.002% to 0.01% can cause weight loss in animals, potentially attributable to the harmful properties of saponins. While the saponin concentration from the present study may be comparatively lower than earlier reports, the effects of sheep fed with fresh *B. decumbens* remain evident as compared to T1 sheep fed with fresh *P. purpureum*. This demonstrates that prolonged intake of fresh *B. decumbens* grass can lead to significant health effects for the animals, potentially resulting in a decline in the growth performance.

In brief, the differing results in Treatments 2 and 3 highlight the beneficial outcomes of ensiling as a method for eliminating saponin content in the grass. The weight disparity observed between sheep that were given ensiled *B. decumbens* and those that were fed fresh *B. decumbens* provides additional evidence of the grass's nutritional value in the absence of any toxic compound.

Despite T2 sheep fed having an increased body weight throughout the study period, prolong feeding of fresh *B. decumbens* would definitely be detrimental the feed efficiency and ultimately affecting the health status of animals. The ensiled *B. decumbens* is proven to be a better choice of pasture for the ruminants as compared to fresh *B. decumbens* and fresh *P. purpureum*. Therefore, information provided on how ensiling affects *Brachiaria* spp. saponin concentrations and body weight could help farmers to utilize *B. decumbens* effectively which will eventually improve the feed efficiency of the farm.

4.1.2 Vital Signs

The rectal temperature of sheep with fresh *P. purpureum*, fresh *B. decumbens*, & ensiled *B. decumbens* is shown in Table 4.2. There were significant differences ($p < 0.05$) among the treatment sheep throughout the feeding trial at different time intervals (Day 0, 7, 14, 21, 35, 42, 49, 56, 63, and 77). Generally, T1 and T3 sheep fed with fresh *P. purpureum* and ensiled *B. decumbens* respectively had slightly higher (↑0.26 — 1.81%) rectal temperatures as compared to T2 sheep fed with fresh *B. decumbens.* Despite having significant variations, all rectal temperatures of sheep in all treatments were still within the normal range of 38.6 to 39.6°C (Figure 4.4). This signifies that all grass meals did not have any negative effects on the temperature of the sheep.

Table 4.3 displays the heart rate of sheep fed with fresh *P. purpureum*, fresh *B. decumbens*, & ensiled *B. decumbens* within the presented time intervals. A statistically significant difference ($p < 0.05$) in the heart rate of the sheep is observed among treatments at different time intervals (Day 0, 7, 14, 21, 35, 49, 56, 63, and 84). Overall, the observation revealed that sheep fed with fresh *P. purpureum* had a comparatively greater average heart rate in comparison to the other two treatments, whereas those fed with fresh *B. decumbens* exhibited the lowest average heart rate (Figure 4.5). Nevertheless, all treatments exhibit values that are similar to the established normal range, which is typically measured between 71 and 115 beats per minute. Thus, it is evident that different treatments did not affect the heart rate of sheep within the 98 days of the experiment.

Table 4.4 mentions the respiratory rate of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* and ensiled *B. decumbens* during weekly observation until Day 98 (week 14) of the experiment. Significant differences ($p < 0.05$) have been observed across the different treatments starting from day 42 of the experiment. It can be noted that the respiratory rate reveals a rising pattern within the established normal range across all treatments. Based on Figure 4.6, the increasing respiratory rate among the treatments was primarily from Treatment 2 (fresh *B. decumbens*), with the highest value observed on day 91 of the experiment. Overall, the feeding of fresh *B. decumbens* was found to affect the respiratory system as observed from the upward trend throughout the experiment, suggesting a poor health sign as compared to fresh *P. purpureum* and ensiled *B. decumbens*.

Table 4.5 presents the rumen motility of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* and ensiled *B. decumbens* over the course of 98 days of experiment. The experiment revealed no significant difference ($p > 0.05$) in rumen motility across the analyzed time intervals. In general, it can be observed that Treatment 2 exhibits the lowest value of rumen motility, as evidenced by an average frequency of 1 flush per minute, in comparison to Treatment 1 and Treatment 3 (Figure 4.5). The findings also display that the rumen motility of Treatment 3 is similar to that of Treatment 1 with 2 flushes per minute. Therefore, this indicates that fresh *B. decumbens* could decrease gut function as well as digestibility and absorptivity, while fresh *P. purpureum* and ensiled *B. decumbens* had no detrimental effects on the GIT function.

Table 4.2 : Rectal temperature of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* & ensiled *B. decumbens.*

	Treatment 1 (°C)	Treatment 2 (°C)	Treatment 3 (°C)	p-value
Day 0	39.10 ± 0.90[a]	38.40 ± 0.90[b]	38.90 ± 0.80[a]	<0.01
Day 7	39.10 ± 0.60[a]	38.60 ± 0.70[b]	39.30 ± 0.70[a]	<0.01
Day 14	39.40 ± 0.20[a]	39.10 ± 0.10[b]	39.10 ± 0.11[b]	0.01
Day 21	39.10 ± 0.40[a]	38.70 ± 0.30[b]	38.90 ± 0.32[c]	<0.01
Day 28	38.90 ± 0.10	38.70 ± 0.12	38.90 ± 0.12	0.07
Day 35	39.20 ± 0.31[a]	38.70 ± 0.30[b]	38.90 ± 0.41[c]	0.02
Day 42	38.90 ± 0.30[a]	38.60 ± 0.30[b]	38.90 ± 0.20[a]	<0.01
Day 49	38.90 ± 0.10	38.70 ± 0.20	38.90 ± 0.13	0.49
Day 56	38.80 ± 0.11[a]	38.40 ± 0.08[b]	38.60 ± 0.09[c]	0.03
Day 63	39.20 ± 0.40[a]	38.80 ± 0.30[b]	38.90 ± 0.32[b]	<0.01
Day 70	39.00 ± 0.07	38.90 ± 0.10	39.00 ± 0.12	0.69
Day 77	39.10 ± 0.02[a]	38.90 ± 0.08[c]	39.00 ± 0.10[b]	<0.01
Day 84	38.90 ± 0.07	38.60 ± 0.10	38.80 ± 0.09	0.07
Day 91	39.10 ± 0.10	39.00 ± 0.10	39.00 ± 0.11	0.40
Day 98	39.00 ± 0.08	39.10 ± 0.10	39.00 ± 0.02	0.08

Note: All values were expressed as mean ± SEM; [a, b, c] values with superscript within the same row are significantly different at $p<0.05$. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens.* Average temperature of sheep: 38.6 — 39.6°C.

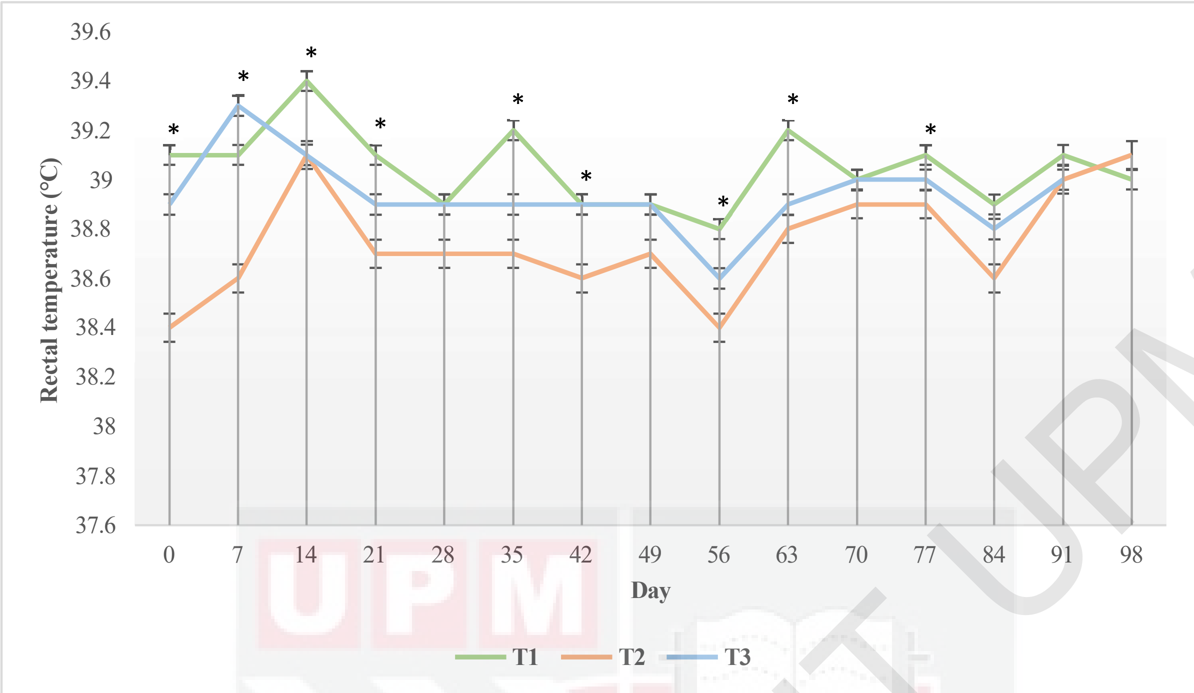

Figure 4.4 : The rectal temperature of sheep fed with fresh and ensiled *B. decumbens* at various time intervals was expressed as mean ± SEM. No significant differences existed between groups. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk indicates statistical significance between groups on the presented day.

Table 4.3 : Heart rate of sheep fed with fresh *P. purpureum*, fresh *B. decumbens*, & ensiled *B. decumbens*.

	Treatment 1 (beats/min)	Treatment 2 (beats/min)	Treatment 3 (beats/min)	p-value
Day 0	93.0 ± 4.0^{a}	88.0 ± 4.0^{b}	88.0 ± 2.0^{b}	0.01
Day 7	100.0 ± 10.0^{b}	97.0 ± 7.0^{b}	107.0 ± 4.0^{a}	<0.01
Day 14	115.0 ± 5.0^{a}	80.0 ± 9.0^{b}	88.0 ± 2.0^{b}	<0.01
Day 21	113.0 ± 4.0^{a}	101.0 ± 5.0^{b}	97.0 ± 5.0^{c}	0.01
Day 28	93.0 ± 5.0	83.0 ± 2.0	87.0 ± 5.0	0.30
Day 35	95.0 ± 2.0^{a}	84.0 ± 3.0^{b}	83.0 ± 2.0^{b}	0.01
Day 42	85.0 ± 4.0	78.0 ± 4.0	75.0 ± 2.0	0.14
Day 49	94.0 ± 5.0^{a}	76.0 ± 2.0^{b}	71.0 ± 3.0^{b}	<0.01
Day 56	90.0 ± 2.0^{a}	81.0 ± 2.0^{b}	78.0 ± 3.0^{b}	<0.01
Day 63	101.0 ± 4.0^{a}	83.0 ± 2.0^{b}	94.0 ± 3.0^{a}	<0.01
Day 70	86.0 ± 1.0	86.0 ± 1.0	84.0 ± 2.0	0.62
Day 77	101.0 ± 5.0	91.0 ± 3.0	93.0 ± 2.0	0.13
Day 84	99.0 ± 2.0^{a}	85.0 ± 2.0^{b}	85.0 ± 2.0^{b}	<0.01
Day 91	103.0 ± 1.0	100.0 ± 2.0	92.0 ± 5.0	0.07
Day 98	101.0 ± 1.0	99.00 ± 1.0	96.00 ± 2.0	0.10

Note: All values were expressed as mean ± SEM; $^{a, b, c}$ values with superscript within the same row are significantly different at $p<0.05$. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens.* Average heart rate of sheep: 70 — 80 beats per minute.

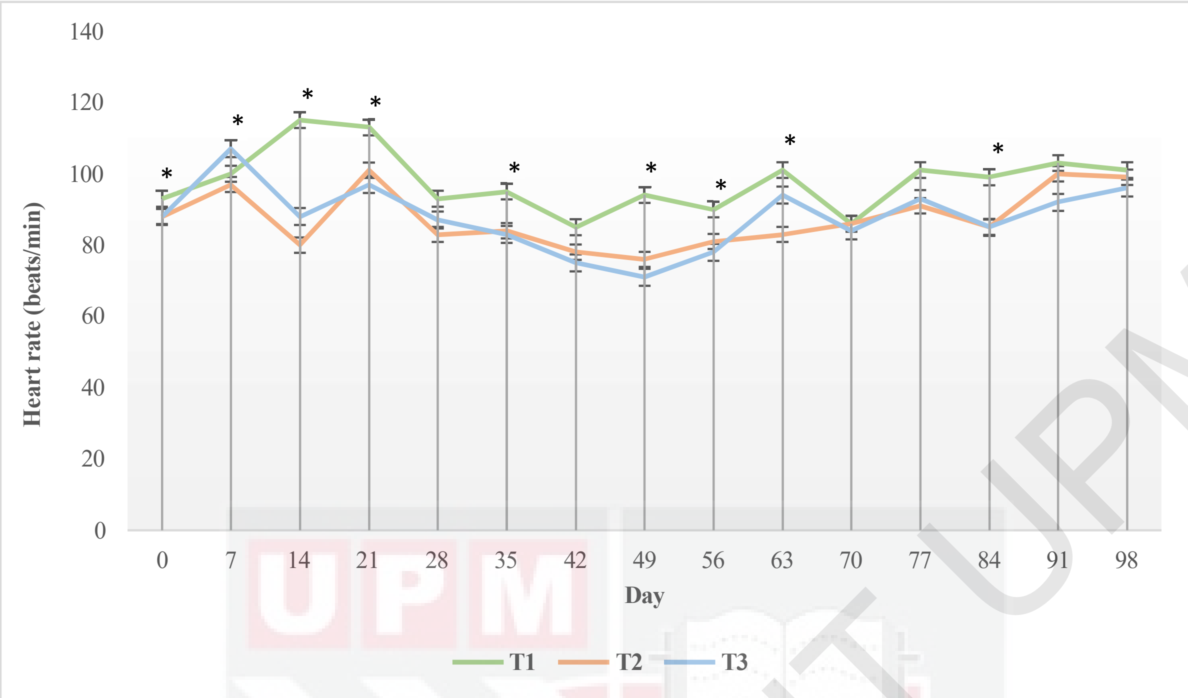

Figure 4.5 : The heart rate of sheep fed with fresh and ensiled *B. decumbens* at various time intervals was expressed as mean ± SEM. No significant differences existed between groups. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk indicates statistical significance between groups on the presented day.

Table 4.4 : Respiratory rate of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* & ensiled *B. decumbens.*

	Treatment 1 (breaths/min)	Treatment 2 (breaths/min)	Treatment 3 (breaths/min)	p-value
Day 0	33.0 ± 4.7	33.0 ± 1.7	39.0 ± 4.0	0.68
Day 7	50.0 ± 4.1^{b}	50.0 ± 2.6^{a}	54.0 ± 2.3^{b}	0.02
Day 14	43.0 ± 3.5^{b}	75.0 ± 7.0^{a}	53.0 ± 4.4^{ab}	<0.01
Day 21	69.0 ± 5.0^{a}	68.0 ± 2.0^{ab}	71.0 ± 8.0^{b}	0.03
Day 28	72.0 ± 2.2	59.0 ± 3.7	62.0 ± 6.1	0.08
Day 35	67.0 ± 4.4	71.0 ± 4.8	70.0 ± 4.9	0.84
Day 42	65.0 ± 2.2^{a}	46.0 ± 1.2^{b}	58.0 ± 2.5^{a}	<0.01
Day 49	44.0 ± 2.5^{a}	67.0 ± 2.4^{b}	52.0 ± 3.2^{ab}	<0.01
Day 56	68.0 ± 2.9^{a}	53.0 ± 2.3^{b}	47.0 ± 1.8^{b}	<0.01
Day 63	89.0 ± 2.0	89.0 ± 3.1	78.0 ± 4.4	0.07
Day 70	69.0 ± 2.1^{b}	68.0 ± 2.0^{b}	85.0 ± 5.1^{a}	<0.01
Day 77	63.0 ± 3.7^{c}	98.0 ± 6.4^{a}	78.0 ± 6.6^{b}	<0.01
Day 84	93.0 ± 3.8^{a}	53.0 ± 2.3^{b}	83.0 ± 6.2^{a}	<0.01
Day 91	80.0 ± 4.1^{b}	114.0 ± 5.4^{a}	92.0 ± 5.1^{b}	<0.01
Day 98	85.0 ± 3.4^{b}	95.0 ± 2.9^{a}	88.0 ± 4.4^{b}	<0.01

Note: All values were expressed as mean ± SEM; $^{a,\ b,\ c}$ values with superscript within the same row are significantly different at p<0.05. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens.* Average respiratory rate of sheep: 16 — 34 breaths per minute.

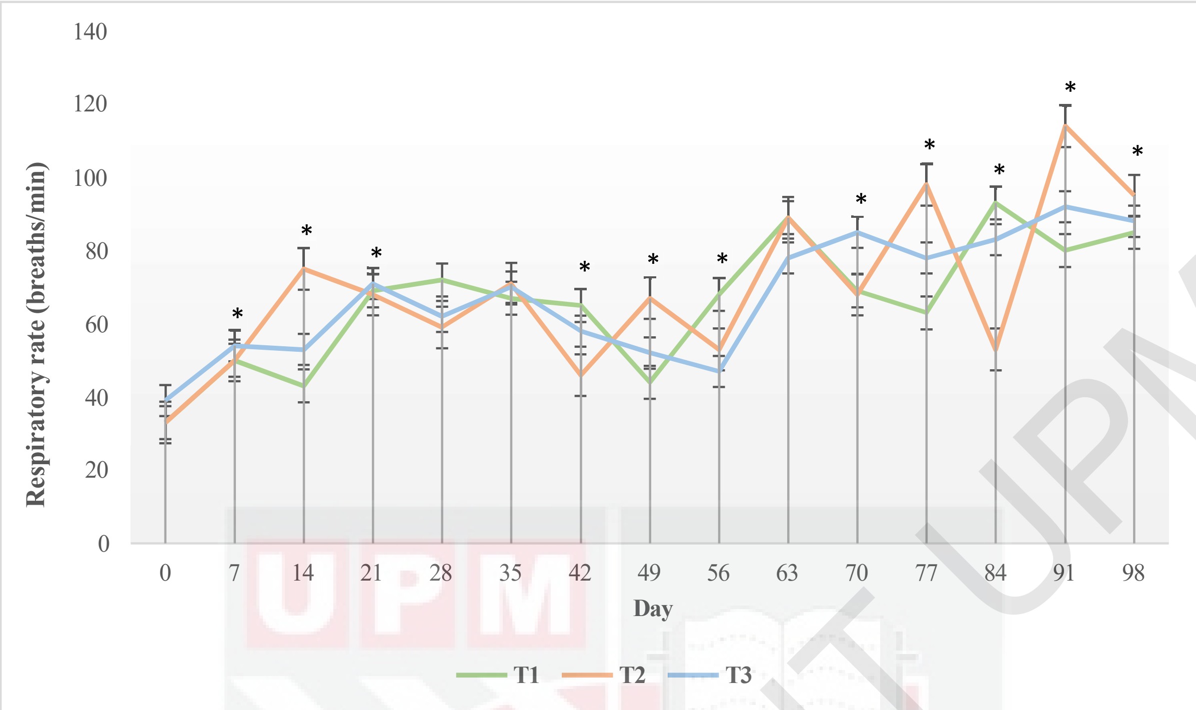

Figure 4.6 : The respiratory rate of sheep fed with fresh and ensiled *B. decumbens* at various time intervals was expressed as mean ± SEM. Significant differences were identified among the groups throughout the remaining half of the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk indicates statistical significance between groups on the presented day.

Table 4.5 : Rumen motility of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* & ensiled *B. decumbens.*

	Treatment 1 (flush/min)	Treatment 2 (flush/min)	Treatment 3 (flush/min)	p-value
Day 0	2.00 ± 0.04	2.00 ± 0.04	2.00 ± 0.04	1.00
Day 7	2.00 ± 0.05	1.00 ± 0.04	1.00 ± 0.03	0.15
Day 14	2.00 ± 0.04	1.00 ± 0.04	2.00 ± 0.04	0.13
Day 21	2.00 ± 0.03	1.00 ± 0.04	2.00 ± 0.03	0.07
Day 28	1.00 ± 0.04	1.00 ± 0.05	1.00 ± 0.04	1.00
Day 35	2.00 ± 0.04	1.00 ± 0.04	1.00 ± 0.05	0.20
Day 42	1.00 ± 0.05	1.00 ± 0.04	2.00 ± 0.06	0.09
Day 49	2.00 ± 0.07	2.00 ± 0.05	2.00 ± 0.06	0.83
Day 56	3.00 ± 0.07	1.00 ± 0.06	2.00 ± 0.07	0.05
Day 63	2.00 ± 0.06	2.00 ± 0.05	2.00 ± 0.07	0.14
Day 70	2.00 ± 0.08	1.00 ± 0.08	2.00 ± 0.08	0.17
Day 77	2.00 ± 0.07	2.00 ± 0.05	2.00 ± 0.06	0.08
Day 84	2.00 ± 0.04	1.00 ± 0.05	2.00 ± 0.05	0.12
Day 91	2.00 ± 0.04	1.00 ± 0.05	2.00 ± 0.05	0.55
Day 98	2.00 ± 0.03	1.00 ± 0.06	2.00 ± 0.04	0.06

Note: All values were expressed as mean ± SEM; $^{a, b, c}$ values with superscript within the same row are significantly different at p<0.05. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens.*

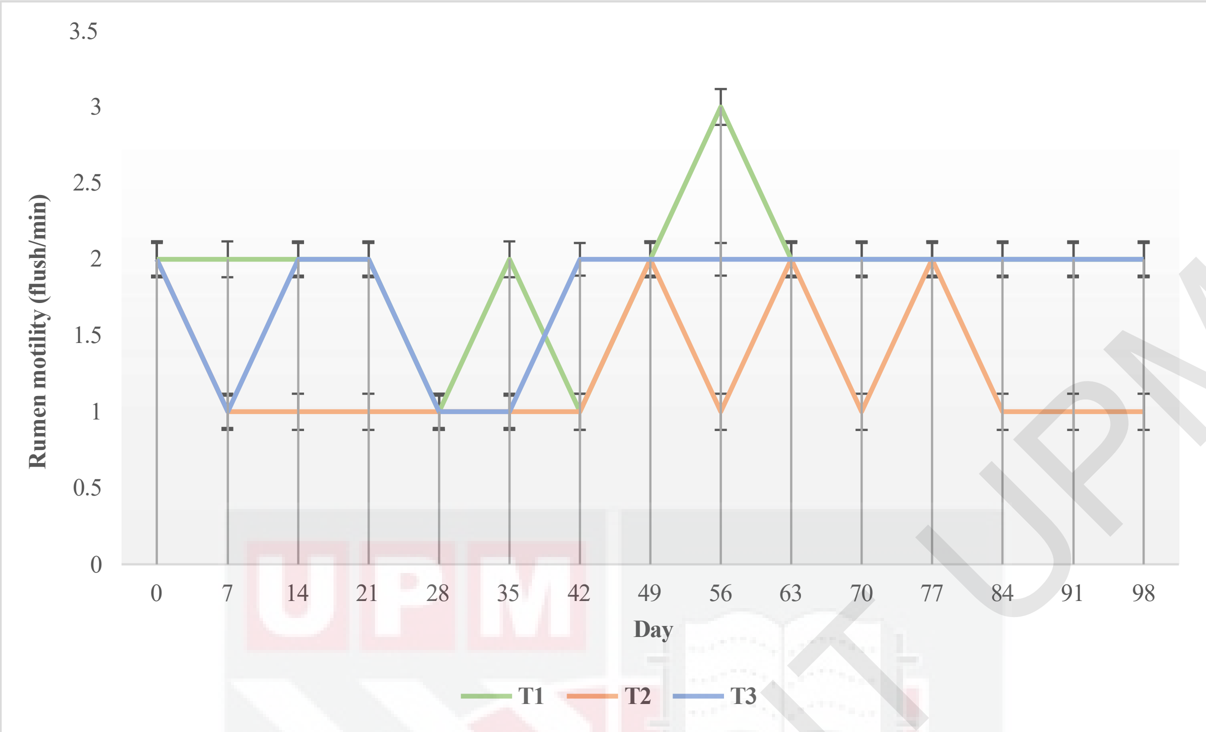

Figure 4.7 : The rumen motility of sheep fed with fresh and ensiled *B. decumbens* at various time intervals was expressed as mean ± SEM. No significant differences existed between groups. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*).

The clinical and vital signs, indicative of an animal's health performance are crucial markers for directly identifying diseases or symptoms during a physical examination, and helps identify early signs of deterioration. The current study revealed statistically significant differences ($p<0.05$) in the vital signs of Treatment 3 sheep as compared to Treatment 2 in which the vital signs were all normal. Montoya-Ménez (2019) described that rumen microorganisms undergo deglycosylation of saponins to make protodioscin, a poisonous compound found in *Brachiaria* spp. Crystal-associated cholangiohepatopathy then develops when protodioscin selectively targets hepatocytes and biliary cells causing further liver damage, leading to photosensitization. In turn, the fermentation process during ensiling was found to eliminate these saponin compounds, and ultimately remove the photosensitization by saponin which damages the liver. Generally, all saponins consist of an aglycone, a non-sugar compound connected to one or more sugar or oligosaccharide units known as glycosides. The aerobic process of ensiling depletes the sugar content in the plant through a process of breakdown of these sugar chains. These ensiling mechanisms demonstrate the absence of saponin previously intact within the sugar chains leading to the positive outcomes of T3 sheep. To simplify, the demonstrated vital signs in the current report provide additional support for the concept that ensiling, as a preservation method, may positively impact the health and welfare of animals.

Throughout the weekly clinical examination, it was observed that the fresh *B. decumbens* (Treatment 2) showed statistical significance ($p<0.05$) of respiration rate, exhibiting a higher rate compared to the remaining two feeding groups. Respiratory problems are commonly attributed to several factors, and in this particular instance, they may be induced by saponin found in *B. decumbens*. This finding provides support to studies that have reported respiratory issues in animals that consume *Brachiaria* spp. containing saponin (Lima et al., 2012; Rosa et al., 2016). Based on previous findings, steroidal saponin has the potential to induce intoxication, primarily causing liver impairment. By inhibiting the excretion of a constituent of chlorophyll called phylloerythrin, liver dysfunction induced by steroidal saponin results in hepatogenous photosensitization (Caicedo et al., 2012). This impairment will subsequently result in stress, leading to a decrease in the immune response of sheep. It is further believed that the decline in immunity is linked to the age of sheep, given that the immune system is not fully developed earlier than 12 months of age. Immunosuppression and viral infections are risk factors for respiratory diseases caused by both species, and these bacteria represent a component of the respiratory tract microbiota in sheep (Franco et al., 2019). The decreased immune response is likely to result in the development of nosocomial bacterial infection, which can manifest as pasteurellosis, leading to pneumonia. The potential factor of pneumonia could also be from environmental changes, such as transportation during the beginning phase of the experiment. Rapid changes in the environment can induce stress in animals, which in turn triggers bacterial infection, leading to the development of symptoms related to pneumonia. Pasteurellosis is a common zoonotic illness that affects both humans and animals, caused by Gram-negative bacillus classified within the genus Pasteurellae. Generally, the identified specific serotypes as the predominant bacterial agents in sheep and goats were *Mannheimia haemolytica* and *Pasteurella multocida* across the globe.

Furthermore, a study by Franco (2019) revealed a higher respiratory rate, rectal temperature, nasal discharge, cough, and dyspnea in pneumonic sheep, which is in line with the present study revealing elevated respiration rate in the sheep receiving Treatment 2. The potential inference of this phenomenon is that sheep are having pneumonic symptoms, due to the factors described above. The detected increase in respiration rate in the sheep fed with fresh *B. decumbens* may be attributed to the initial respiratory damage, further suggesting that the sheep's continuous ingestion of this forage containing saponin may contribute to the onset of pneumonia. This observation further strengthens the association between nutritional content and respiratory incidences. It is also possible that the occurrence and severity of the disease were influenced by factors such as the susceptibility of each animal, various levels of circulating photodynamic agents and bile pigments, and the varying levels of sunlight exposure (Badiei et al., 2009). The findings presented previously, although limited, suggest a possible connection between sheep poisoning and high levels of saponins in the pasture, often above 1% (Riet- Correa et al., 2011).

Overall, despite the nutritional value of *B. decumbens* is significantly better than that of the control group (*P. purpureum*), the high concentration of saponins in *B. decumbens* hinders the absorption of nutrients due to its toxic effects, mostly manifested as hepatogenous photosensitization, hence negatively impacting the general health of sheep. To infer the supporting evidence, the constant intake of *B. decumbens* results in saponin intoxication that affects the liver, and may trigger respiratory problems such as pneumonia, pasteurellosis, or other respiratory infections that can adversely affect the overall health and performance of sheep. However, the nutrient content of *Brachiaria* spp. grass can be effectively utilized by reducing the availability of saponin via ensiling. This could therefore assist in maximizing the availability of forage resources in the small ruminant business, particularly concerning sheep. It is necessary to highlight that the current findings demonstrated significant changes in the health performance of sheep when fed with ensiled *B. decumbens*.

4.1.3 Ocular Mucous Membrane Colour

The findings reported the examination of the mucous membrane of sheep subjected to different treatments throughout the 98 days of the experiment. Among the treatments, sheep fed with fresh *B. decumbens* (Treatment 2) was observed to be slightly paler compared to sheep fed with fresh *P. purpureum* and ensiled *B. decumbens* starting from day 49. This finding is consistent with other health performances, the majority of which demonstrate statistical significance over the final 7 weeks of the experiment. There were also no significant changes during the clinical observations throughout the experiment. No facial dermatitis, edema, jaundice, or photosensitization issues were observed in all sheep. Generally, the clinical observation throughout the experiment showed no significant effect among treatment groups, except for pale mucous membrane color which was demonstrated in sheep fed with fresh *B. decumbens*.

Weekly clinical observation and examination are essential to maintain the well-being and welfare of animals throughout a study. In ruminants with hepatic dysfunction caused by steroidal saponin, symptoms generally include anorexia, growth retardation, weight loss, diarrhea, jaundice, and photosensitivity. The clinical manifestations often appeared in animals after around 7-10 days of feeding, as reported by Muniandy et al. (2020). Alternatively, animals reared on *Brachiaria* spp. pastures can potentially be able to decrease the effects of the toxin due to a higher ability to metabolize and breakdown the steroidal saponin, which serves as the secondary metabolite in plants (Castro et al. 2007; Albernaz et al. 2010). It is also possible that the animals acquire a distinctive ruminal flora (Albernaz et al., 2010), allowing them to overcome saponin toxicity. However, overall reports from previous studies display greater health implications as compared to the adaptability of animals. It is also emphasized that in order to produce a genetically resistant animals, further death of susceptible animals within the herd is involved (Faccin et al., 2014).

This current study demonstrated clinically healthy sheep within Treatment 3 fed with ensiled *B. decumbens* signifies the implication of steroidal saponin towards ruminants. This further supports previous studies reporting that preservation through both ensiling and hay-making can mitigate both clinical and subclinical effects of steroidal saponin. A study on sheep fed with *B. brizantha* hay found a substantial difference in improved health performance, which is believed to be attributed to the elimination of saponin from the grass (Lima et al., 2012). The preservation methods used by Lima (2015) effectively eliminated the presence of steroidal saponin, hence eliminating the hepatogenous effects and maintaining overall performance. Another study conducted by Cezário (2015) also revealed comparable results, demonstrating a significant nutrient intake and performance when fed with ensiled *B. brizantha*, with or without microbial inoculant. The sugar chain in saponin likely went through hydrolysis during anaerobic respiration, leading to a decrease in saponin concentration over time. An accumulation of lactic acid and the subsequent decrease in pH are factors in the degradation of chlorophyll compounds and other pigments (Santra et al., 2021). Due to the nature of saponin being a secondary metabolite, most saponin compound was digested and excreted through liver synthesis. Saponin also has been found to reduce nutrient utilization and conversion efficiency in ruminants and cause endogenous impairment in the liver. As a consequence, biliary lesions do not form, which would otherwise induce clinical signs such as hepatogenous photosensitization. The presented suggestions demonstrate how saponin can be removed during ensiling by hydrolyzing the sugar chain in saponin and breaking down chlorophyll in *B. decumbens* grass. Elimination efficiently removes the deleterious effects of saponin, creating a safer alternative for animal consumption. The nutritional benefits of grass remained unchanged, demonstrating that ensiling *B. decumbens* is superior to both the control group and Treatment 2 group.

Based on the observational reports, it was described that sheep given fresh *B. decumbens* (Treatment 2) appeared to have paler mucous membranes than sheep that were given fresh *P. purpureum* and ensiled *B. decumbens*, starting from day 49. In consistence, multiple studies reported various clinical signs related to ocular issues, including eye discharge, redness, and jaundice in the ocular region, during either the experimental period or in post-mortem reports (Badiei et al., 2009; Porto et al., 2013; Pupin et al., 2016; Lelis et al., 2018; Diamantino et al., 2020; Carmo et al., 2021; Kono et al., 2021), with only one study showing similar result of pale mucous membrane in sheep (Muniandy et al., 2021). The observed pale mucous membrane in the results provided can be attributed to the regulated blood flow of sheep in Treatment 2. In general, blood distribution typically exhibits variability across the circulatory system, showing that the internal mechanism adjusts the diameter of arterioles based on the metabolic needs of the assessed tissue. An acute inflammation, for instance, can induce the rise of blood volume (hyperemia) across the body (Mosier et al., 2017). Lungs, gastrointestinal tract, and liver receive substantially higher blood flow than required to meet metabolic demands, which in this instance would be the hepatic injury. The present study revealed saponin intoxication in Treatment 2 sheep, as indicated by the respiratory issues observed through the lung lesions, as well as various histological regions displaying inflammatory cell lesions. The observed pale mucous membrane in the presented data can be attributed to the regulated blood flow in the sheep that were fed with fresh *B. decumbens*. These injuries result in localized vasodilation, leading to reduced blood flow in other areas. It has been proposed that the occurrence of vasoconstriction such as in the ocular region, leads to the appearance of pale membranes.

Regardless, other clinical observations conducted during the trial revealed insignificances, indicating that there was no evidence of face dermatitis, edema, jaundice, or photosensitization problems in all eighteen sheep. The absence of statistical significance in other clinical indicators can be attributed to the shading area, which might delay the manifestation of saponin toxicity. Similarly, in a study conducted by Muniandy (2021), the impact of *B. decumbens* levels on sheep health was examined, showing the significance of only mucosal membrane color towards the intoxicated animals. On the contrary, Lelis (2018) reported that the natural shade within the silvopastoral system is unable to prevent or mitigate photosensitization symptoms in young, non-adapted sheep grazing *B. decumbens*, which contradicts the previous claim. Given that it was a single occurrence, it is inferred that the animal's susceptibility and preferences are likely a cause (Carmo et al., 2021). Besides, the absence of reported clinical signs in the present study may be attributed to the short duration of the rearing period, insufficient to cause severe intoxication effects towards the animals. For instance, the rearing period ranged from 35 days (Badiei et al., 2009) to 63 days total, with the protodioscin level of 0.94% from *B. decumbens* (Porto et al., 2013) have been insufficient to cause significant clinical manifestations. However, the investigation conducted by Faccin (2014) mentioned the time span between the introduction of the naïve sheep to the *B. brizantha* pasture and the first display of clinical indications of poisoning ranged from 35 to 137 days. In brief, higher saponin levels and prolonged exposure periods were found to result in significant adverse effects (Muniandy et al., 2021); however, it is sufficient to conclude that the lack of significant clinical symptoms is caused by multiple factors, as evidenced by several research.

The difference in clinical outcomes between Treatment 2 and Treatment 3 demonstrated the efficacy of ensiling as a preservation technique to eliminate saponin content in *B. decumbens*. While sheep fed with fresh *B. decumbens* did not exhibit any clinical signs other than pale mucous membrane, there is a possibility of severe saponin poisoning occurring if the consumption of forage is prolonged. As a result of chlorophyll degradation and sugar loss during the fermentation process, protodioscin levels decreased significantly through preservation, demonstrating the effectiveness of ensiling as a method to combat saponin intoxication. It is worth noting that the presence of saponin, regardless of the concentration will induce hepatogenous issues, thus causing ocular problems as well as other clinical signs, which can be eradicated through the ensiling of grass. This alternative leads to a more effective way for farmers to reduce the harmful effects of toxicity in *B. decumbens*, making it safer for ruminants to consume. In emphasis, the hypothesized explanation provides additional confirmation that sheep fed with ensiled *B. decumbens* in Treatment 3, showed no clinical indications due to the lack of saponin compound. Therefore, the method of ensiling *B. decumbens* has been found to be a better substitute for fresh *B. decumbens* in order to eliminate saponin toxicity, thereby mitigating the harmful health effects observed in ruminants.

4.2 Blood profile

4.2.1 Hematology (Red Blood Cells)

Table 4.6 displays the erythrocytes (RBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular value (MCV), and mean corpuscular hemoglobin concentration (MCHC) of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* & ensiled *B. decumbens.* Several parameters showed significance ($p < 0.05$) including Hb, PCV, MCV, and MCHC. Within these parameters, Treatment 2 showed a lower value of Hb (0.9 - 10.7%) but higher values of PCV (3.2 - 13.3%), MCV (0.7 - 20%), and MCHC (0.3 - 3.6%) when compared to Treatment 1 (Figure 4.8 — 4.12). Despite the differences, all treatments exhibited values that were still within the stated normal range as mentioned in Table 4.6. Overall, all red blood cell of sheep were not affected by the different feeding treatments.

Table 4.6 : Red blood cells of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* & ensiled *B. decumbens.*

	Treatment 1	Treatment 2	Treatment 3	p-value
RBC ($x10^{12}$/L) (normal range: 9-15)				
Day 0	11.20 ± 0.19	11.20 ± 0.19	11.20 ± 0.19	1.00
Day 30	10.57 ± 0.20	10.10 ± 0.24	10.88 ± 0.22	0.08
Day 60	11.75 ± 0.40	11.10 ± 0.40	10.98 ± 0.39	0.32
Day 90	10.40 ± 0.60	10.20 ± 0.47	9.29 ± 0.61	0.29
Hb (g/L) (normal range: 90-150)				
Day 0	109.50 ± 1.13	109.50 ± 1.13	109.25 ± 1.13	1.00
Day 30	113.00 ± 2.24^{a}	101.56 ± 1.77^{b}	104.33 ± 2.53^{ab}	0.05
Day 60	121.00 ± 5.53	110.00 ± 3.23	109.00 ± 3.47	0.13
Day 90	112.00 ± 2.11	106.33 ± 1.10	103.67 ± 2.74	0.16
PCV (L/L) (normal range: 0.27-0.45)				
Day 0	0.32 ± 0.00	0.32 ± 0.00	0.32 ± 0.00	1.00
Day 30	0.32 ± 0.05	0.31 ± 0.02	0.31 ± 0.02	0.17
Day 60	0.28 ± 0.10^{b}	0.32 ± 0.07^{a}	0.31 ± 0.11^{a}	<0.01
Day 90	0.34 ± 0.08^{a}	0.32 ± 0.10^{a}	0.29 ± 0.12^{b}	<0.01
MCV (fL) (normal range: 28-40)				
Day 0	29.13 ± 0.63	29.13 ± 0.63	29.13 ± 0.63	1.00
Day 30	29.50 ± 2.40^{ab}	30.67 ± 1.42^{a}	28.50 ± 2.49^{b}	0.01
Day 60	24.00 ± 4.10^{b}	29.67 ± 5.28^{a}	29.33 ± 5.68^{a}	<0.01
Day 90	32.33 ± 0.81	32.56 ± 1.07	30.00 ± 0.97	0.08
MCHC (g/L) (normal range: 310-340)				
Day 0	305.50 ± 6.87	305.50 ± 6.87	305.50 ± 6.87	1.00
Day 30	342.00 ± 7.04^{ab}	344.67 ± 6.36^{a}	333.33 ± 6.50^{b}	0.02
Day 60	332.00 ± 6.21^{a}	344.00 ± 6.66^{b}	343.00 ± 9.81^{b}	<0.01
Day 90	329.00 ± 5.70	323.67 ± 5.87	333.00 ± 6.74	0.41

Note: All values were expressed as mean ± SEM; $^{a, b, c}$ values with superscript within the same row are significantly different at p<0.05. All parameters showed significance except RBC and Hb. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens,* RBC: Erythrocytes, Hb: Haemoglobin, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCHC: Mean corpuscular haemoglobin concentration.

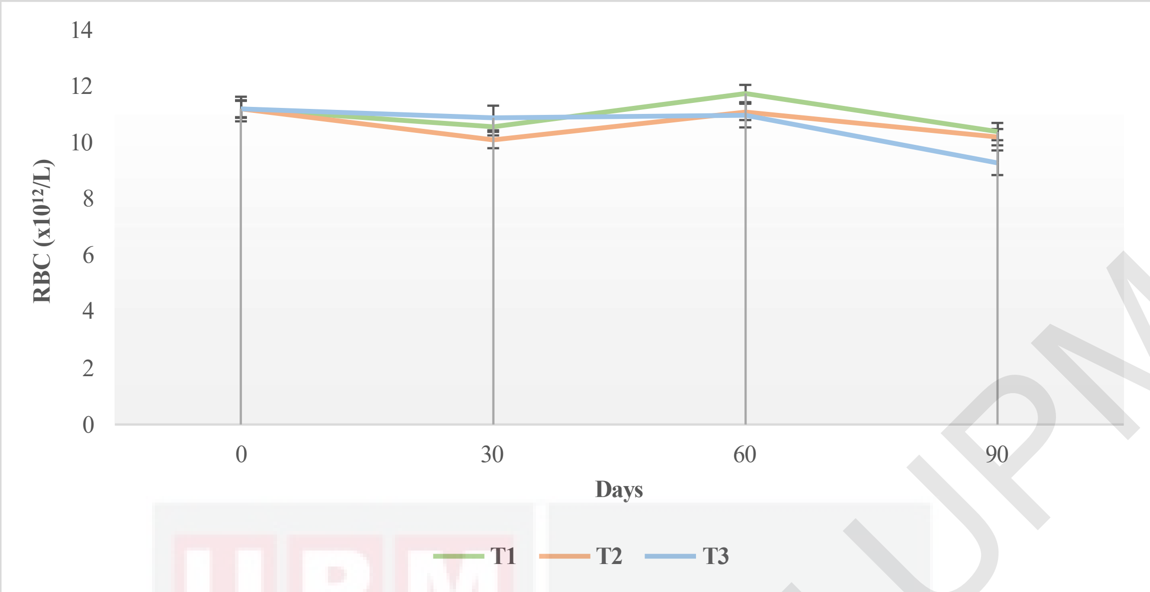

Figure 4.8 : The RBC of sheep fed with fresh and ensiled *B. decumbens* at various time intervals was expressed as mean ± SEM. No significances were identified among the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*).

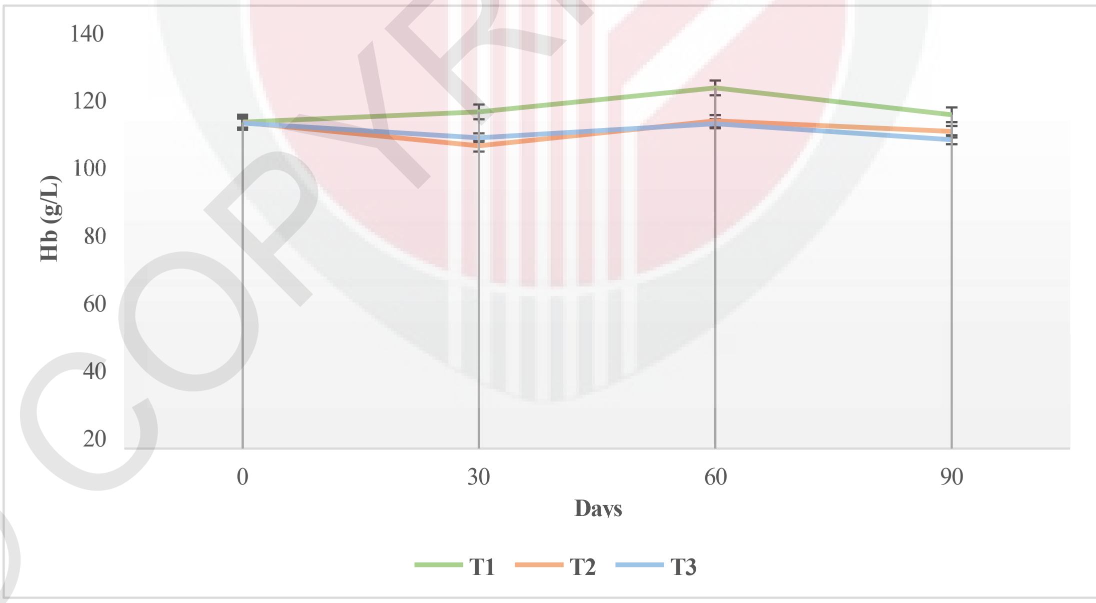

Figure 4.9 : The hemoglobin of sheep fed with fresh and ensiled *B. decumbens* at various time intervals was expressed as mean ± SEM. No significances were identified among the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*).

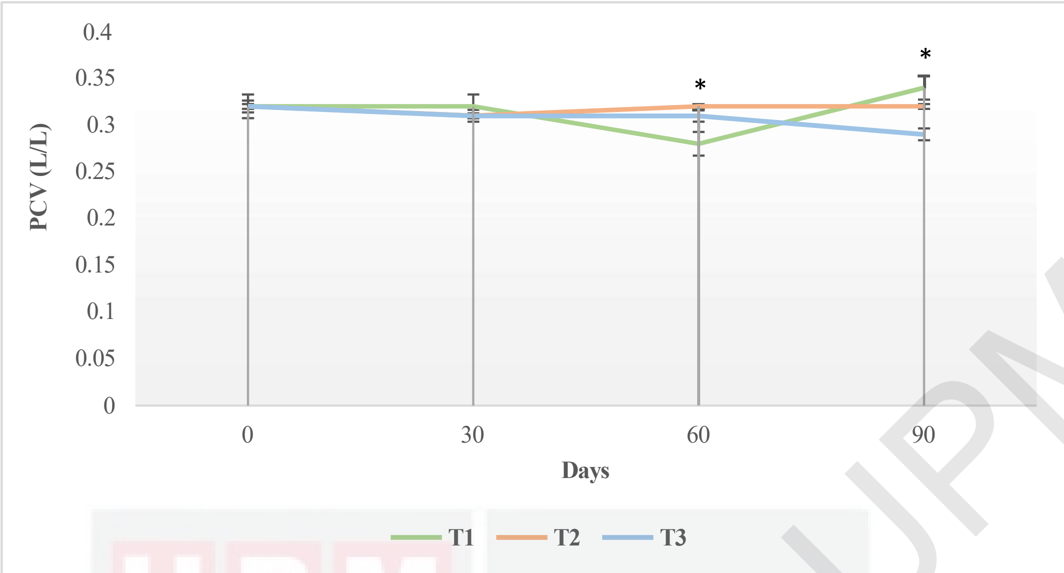

Figure 4.10 : The packed cell volume (PCV) of sheep fed with fresh and ensiled *B. decumbens* at various time intervals was expressed as mean ± SEM. The significances were identified among the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

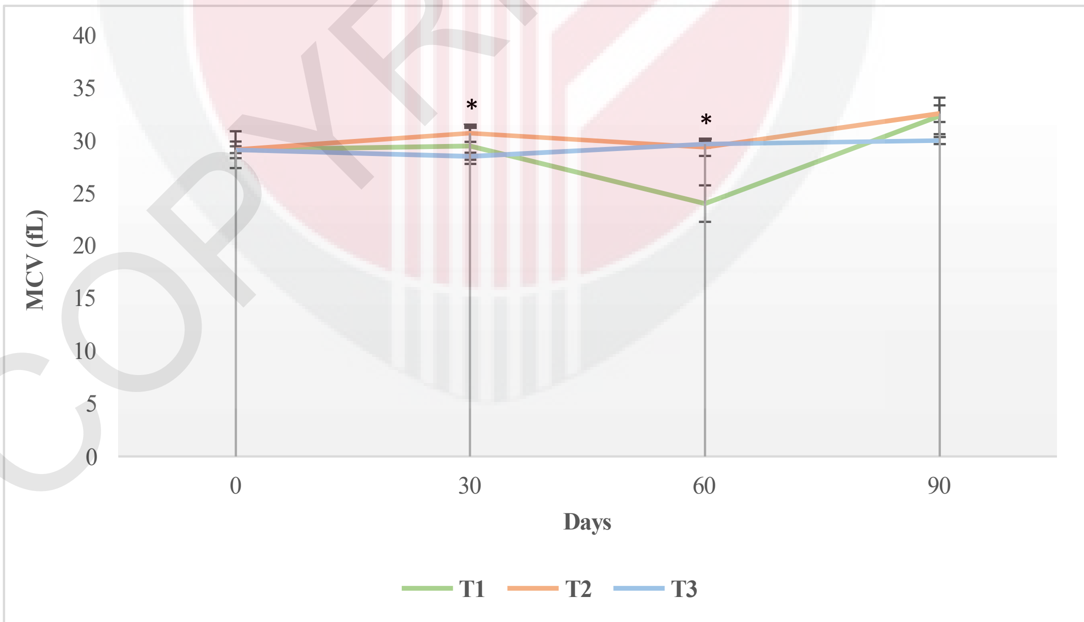

Figure 4.11 : The mean corpuscular value (MCV) of sheep fed with fresh and ensiled *B. decumbens* at various time intervals was expressed as mean ± SEM. The significances were identified among the groups. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

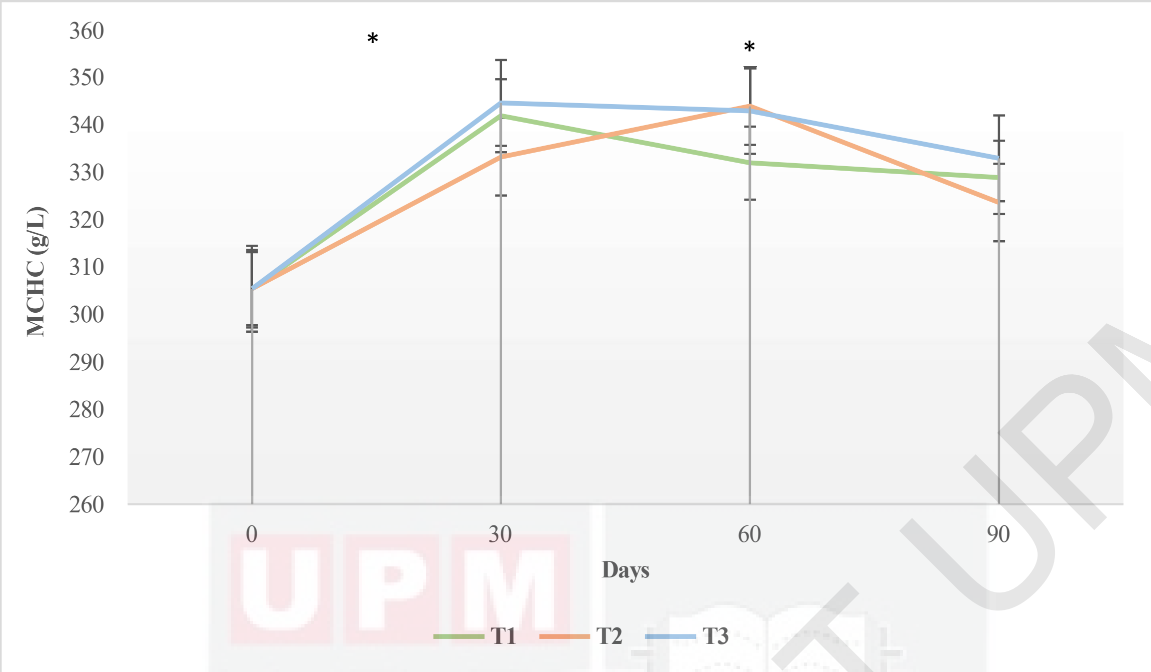

Figure 4.12: The mean corpuscular hemoglobin concentration (MCHC) of sheep fed with fresh and ensiled *B. decumbens* at various time intervals was expressed as mean ± SEM. The significances were identified among the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

Understanding the mechanism of toxicity through hematology is crucial in ensuring the accuracy of diagnosis. However, there is limited research discussing the hematological outcomes of ensiled grass, due to its reported insignificance as mentioned earlier. The current results demonstrated no significance in the RBC values between Treatment 3 and Treatment 1. The application of grass preservation in eliminating steroidal saponin within feed has proven to be a successful method. Stegelmeier (2002) mentioned the characteristics that are necessary for the manifestation of clinical photosensitivity, which garnered the attention towards the mechanism of saponin elimination by ensiling. The toxic component, namely steroidal saponin consists of an aglycone, a non-sugar compound that also connects to one or more sugar or oligosaccharide units known as glycosides. Non-volatile organic acids, including lactic acid, protein degradation compounds, and other microbial metabolites are formed during the aerobic fermentation and feed-out phases of ensiling and silage use. The ensiling process through anaerobic fermentation depletes sugar compounds and degrades chlorophyll, consequently removes saponin compound and eliminates intoxication effects. *B. decumbens* silage also reduces the production of phylloerythrin, a phototoxic agent within chlorophyll. Based on earlier reports, steroidal saponins in *Brachiaria spp*. led to hepatogenous photosensitization (Cruz et al., 2000), which became lithogenic when they mix with glucuronic acid in the liver, consequently after being exposed to calcium in the bile (Brum et al., 2007; Riet-Correa et al., 2011; Caicedo et al., 2012). The successful attempt of saponin removal inhibits liver injury and biliary secretion, which in turn helps to secrete phylloerythrin through detoxification. Photosensitization cannot be induced as the animals are exposed to the sunlight due to the absence of phylloerythrin, the main component responsible for the intoxication. As such, the manifestation of clinical photosensitivity and other intoxication effects are not able to take effect in ensiled *B. decumbens* (Treatment 3). Supporting this, Mako et al. (2021) also states that the gradual rise in Hb and PCV values of the ensiled *A. brasiliana* group reveals minimal levels of anti-nutritional factors, specifically phenols and condensed tannins that suggests that the treatment promoted the sheep's blood formation. Roba et al. (2022) also reported similar results, revealing that sheep fed with biologically treated rice husks did not exhibit anemia, given the normal range of PCV values.

Conversely, the present findings indicate that the MCV and MCHC values were slightly elevated in T2 compared to T1 and T3, demonstrating the impact of saponin levels on the blood indices of sheep. A higher MCV, known as macrocytosis, is a sign of a regenerative response. Among erythrocyte indicators, the MCHC has been shown to be most reliable (Polizopoulou, 2010). The slight elevation in MCHC value in T2 suggests the occurrence of haemolysis (Polizopoulou, 2010), which may signal the onset of anemia (Mangiagalli, 2023) due to the concurrent increase in MCV values in T2. According to Harvey (2012), degenerative changes in blood samples occur when there is an increase in MCV values and a drop in MCHC values. Therefore, it is not possible for degenerative changes to occur in blood samples when these conditions are not met. Moreover, the PCV is a metric used to assess the level of toxicity in the bloodstream. A low PCV value is frequently associated with the presence of anemia, in which decreases the ability to carry oxygen, leading to an elevated heart rate and, as a result, heart failure (Mako et al., 2021). Additionally, a slight increase of PCV values in T2 could indicate the beginning of jaundice; although, additional investigations are necessary to validate this possibility. It is plausible for *B. decumbens* to influence blood parameters through stress if saponin concentration is sufficiently increased to induce liver injury and photosensitization.

Definitively, the presented findings described similar outcomes across all treatments. Despite the similarities, there were still significances within the study period, which could suggest influence from saponin compound within *B. decumbens*. It is possible that the effectiveness of ensiling in removing saponin compound was a contributing element in the removal of this component that was altering the blood indices, particularly the PCV, MCV, and MCHC.

4.2.2 Hematology (White Blood Cells)

Table 4.7 displays the leukocytes (WBC), band neutrophil, segmented neutrophil, lymphocytes, monocytes, eosinophil, basophil, platelet count (PLT), plasma protein, and icterus index of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* & ensiled *B. decumbens.* All parameters exhibited statistically significant results, typically beginning in the first month of the experiment. Sheep within Treatment 2 fed with fresh *B. decumbens* exhibited elevated values including the WBC (3.5 – 60.0%), band neutrophil (↑100%), segmented neutrophil (18.5 — 46.6%), lymphocytes (28.9 — 91.4%), monocytes (52.6 — 135.1%), eosinophil (81.5 – 100.0%), and basophil (↑100%) when compared to the control group (Figure 4.13 - 4.19). However, these parameters showed values that remained within the normal range, except the band neutrophils in sheep from Treatment 2. Therefore, it can be concluded that the feeding of fresh *B. decumbens* will influence the white blood cells parameters specifically the band neutrophils, while both fresh *P. purpureum* and ensiled *B. decumbens* had no influence on the white blood cells parameters.

Moreover, there were significant differences seen in both platelet count (PLT) and plasma protein levels among the treatment groups (Table 4.7). The results indicate that the platelet counts were highest (↑52.7%) during the initial 30 days of the experiment. During day 60 and 90 of the experiment, Treatment 2 exhibited higher levels of plasma protein compared to the other groups, with a difference ranging from 4.4% to 19.0%. Nevertheless, both platelet count and plasma protein levels were still within the normal range throughout the study. The icterus index for sheep across all treatments remained consistent at a value of 5 throughout the study. Generally, the PLT, plasma protein, and icterus index of sheep were not affected by the different feeding treatments (Figure 4.20 - 4.22).

Table 4.7 : White blood cells, platelets, plasma protein & icterus index of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* & ensiled *B. decumbens.*

	Treatment 1	Treatment 2	Treatment 3	p-value
WBC (x10^9/L) (4-12)				
Day 0	7.45 ± 0.25	7.45 ± 0.25	7.45 ± 0.25	1.00
Day 30	6.05 ± 0.00	5.84 ± 0.28	6.00 ± 0.14	0.84
Day 60	5.54 ± 0.00^b	7.26 ± 0.84^a	5.56 ± 0.29^b	<0.01
Day 90	4.12 ± 0.00^b	7.65 ± 0.42^a	4.51 ± 0.31^b	<0.01
Band Neutrophils (x10^9/L) (normal range: 0)				
Day 0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Day 30	0.00 ± 0.00^b	0.09 ± 0.01^a	0.00 ± 0.00^b	<0.01
Day 60	0.00 ± 0.00^b	0.07 ± 0.02^a	0.00 ± 0.00^b	<0.01
Day 90	0.00 ± 0.00^b	0.04 ± 0.02^a	0.00 ± 0.00^b	<0.01
Segmented Neutrophils (x10^9/L) (normal range: 0.7-6.0)				
Day 0	4.18 ± 0.28	4.18 ± 0.28	4.18 ± 0.28	1.00
Day 30	3.69 ± 0.10^a	2.41 ± 0.14^b	3.09 ± 0.13^a	<0.01
Day 60	2.99 ± 0.70^b	3.60 ± 0.46^a	2.38 ± 0.75^b	<0.01
Day 90	2.62 ± 1.07^b	4.21 ± 2.36^a	1.58 ± 1.18^b	<0.01
Lymphocytes (x10^9/L) (normal range: 2.0-9.0)				
Day 0	3.26 ± 0.13	3.26 ± 0.13	3.26 ± 0.13	1.00
Day 30	1.42 ± 0.33^b	2.92 ± 1.31^a	3.01 ± 0.93^a	0.01
Day 60	2.22 ± 0.44	2.97 ± 0.31	2.90 ± 0.46	0.25
Day 90	1.39 ± 2.04^c	3.73 ± 1.16^a	2.96 ± 1.19^b	<0.01
Monocytes (x10^9/L) (normal range: <0.75)				
Day 0	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	1.00
Day 30	0.24 ± 0.06^a	0.14 ± 0.03^b	0.28 ± 0.01^a	<0.01
Day 60	0.06 ± 0.12^b	0.31 ± 0.02^a	0.28 ± 0.02^a	<0.01
Day 90	0.13 ± 0.20^b	0.43 ± 0.13^a	0.28 ± 0.09^b	<0.01
Eosinophils (x10^9/L) (normal range: <0.75)				
Day 0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Day 30	0.04 ± 0.04^c	0.12 ± 0.01^a	0.10 ± 0.02^b	<0.01
Day 60	0.08 ± 0.10^b	0.19 ± 0.09^a	0.07 ± 0.12^b	<0.01

Day 90	0.00 ± 0.01[b]	0.13 ± 0.11[a]	0.04 ± 0.02[b]	<0.01
Basophils (x10^9/L) (normal range: <0.3)				
Day 0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Day 30	0.00 ± 0.00[b]	0.10 ± 0.02[a]	0.02 ± 0.01[b]	<0.01
Day 60	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Day 90	0.00 ± 0.00[b]	0.07 ± 0.01[a]	0.00 ± 0.00[b]	<0.01
PLT (x10^9/L) (normal range: 260-1000)				
Day 0	447.25 ± 36.08	447.25 ± 36.08	447.25 ± 36.08	1.00
Day 30	394.00 ± 102.03[b]	683.00 ± 94.19[a]	581.33 ± 83.61[c]	<0.01
Day 60	637.00 ± 30.02	669.00 ± 40.71	626.00 ± 20.58	0.64
Day 90	478.00 ± 70.21	558.67 ± 67.19	562.67 ± 72.15	0.75
Plasma Protein (g/L) (normal range: 60-75)				
Day 0	73.75 ± 0.83	73.75 ± 0.83	73.75 ± 0.83	1.00
Day 30	72.00 ± 2.33	68.67 ± 2.20	72.00 ± 0.94	0.35
Day 60	70.00 ± 0.80[c]	80.67 ± 0.83[a]	77.22 ± 0.88[b]	<0.01
Day 90	62.00 ± 1.93[b]	75.00 ± 1.78[a]	70.00 ± 1.09[a]	<0.01
Icterus Index (Units) (normal range: <15)				
Day 0	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	1.00
Day 30	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	1.00
Day 60	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	1.00
Day 90	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	1.00

Note: All values were expressed as mean ± SEM; [a, b, c] values with superscript within the same row are significantly different at p<0.05. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens,* WBC: Leukocytes, PLT: Platelet count.

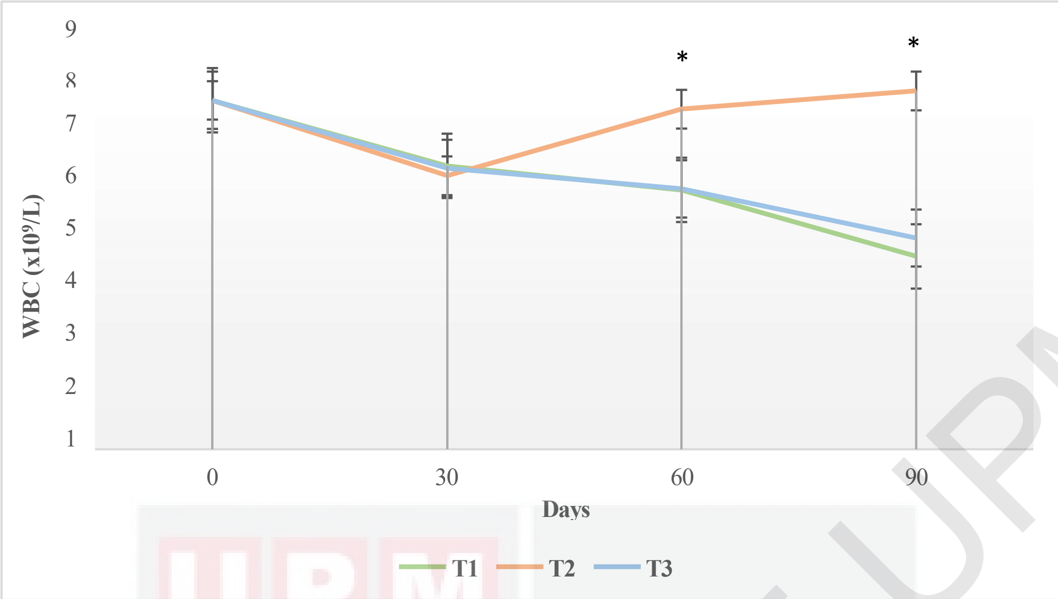

Figure 4.13: The white blood cells (WBC) of sheep fed with fresh and ensiled *B. decumbens* at various time intervals was expressed as mean ± SEM. The significance was identified among the groups at the end of the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

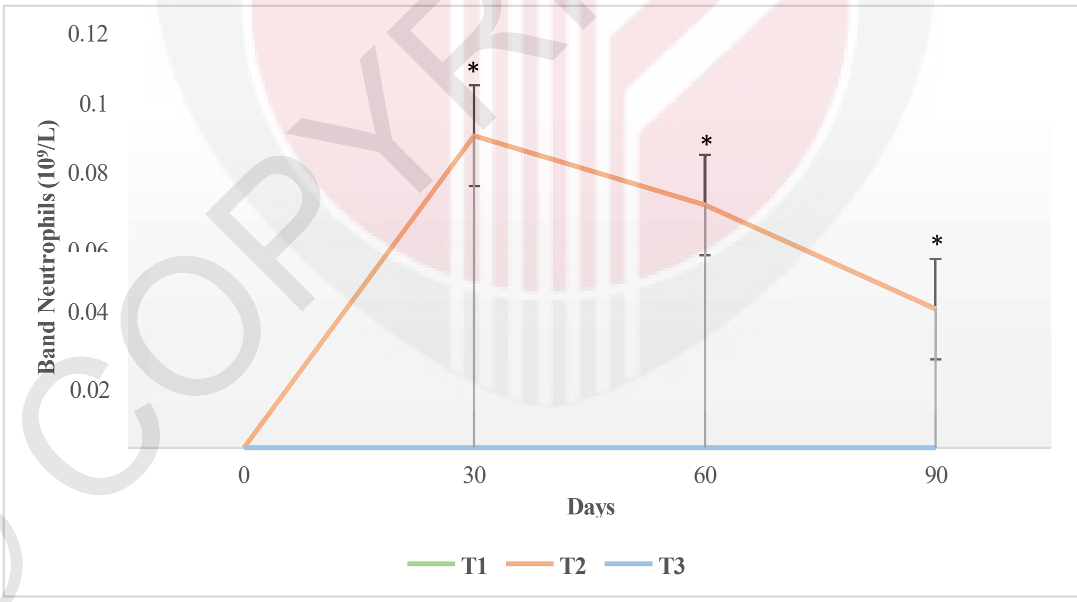

Figure 4.14 : The band neutrophils of sheep fed with fresh and ensiled *B. decumbens* at various time intervals was expressed as mean ± SEM. The significance was identified among the groups. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

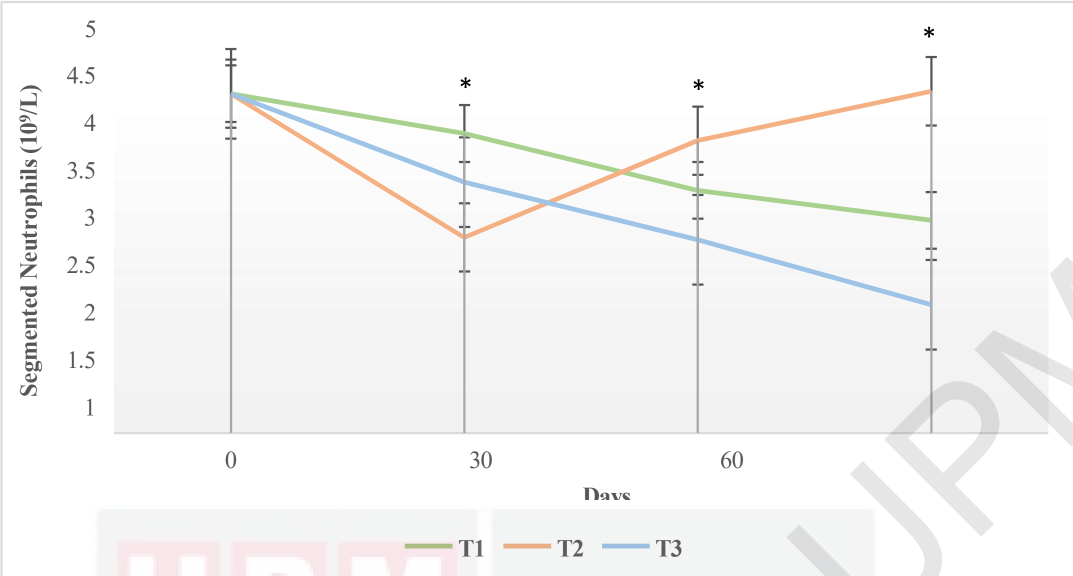

Figure 4.15 : The segmented neutrophils of sheep fed with fresh and ensiled *B. decumbens* at various time intervals was expressed as mean ± SEM. The significances were identified among the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

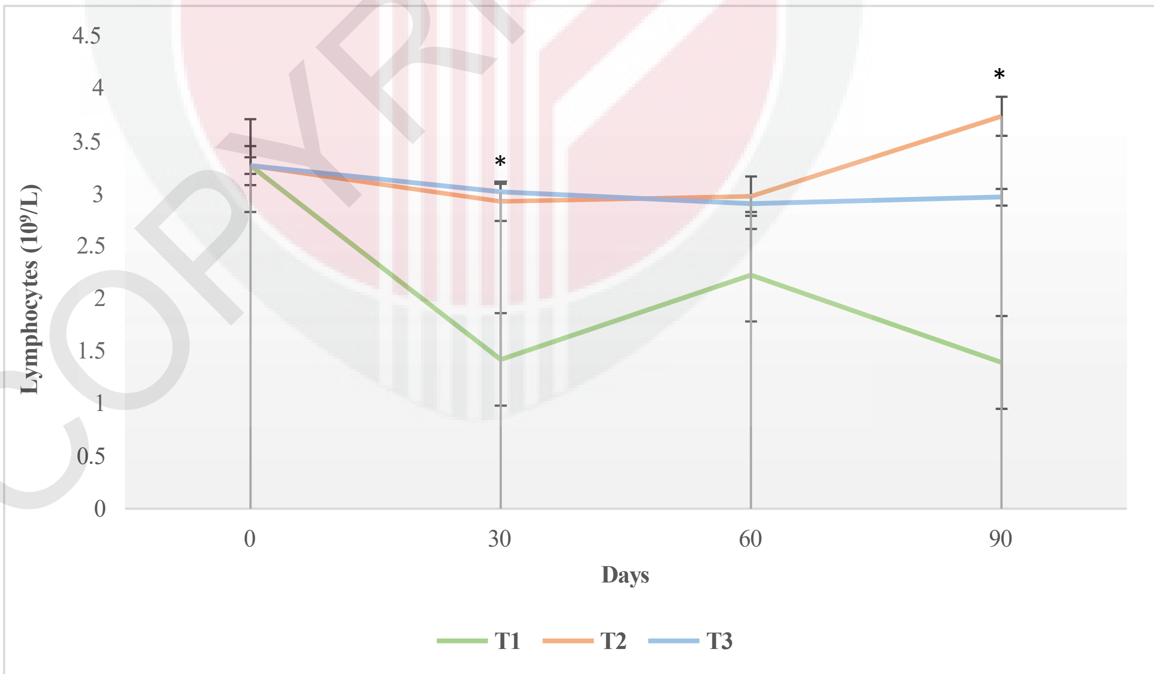

Figure 4.16 : The lymphocytes of sheep fed with fresh and ensiled *B. decumbens* at various time intervals was expressed as mean ± SEM. The significances were identified among the groups throughout the experiment, with the lowest value from T1. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3:

Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

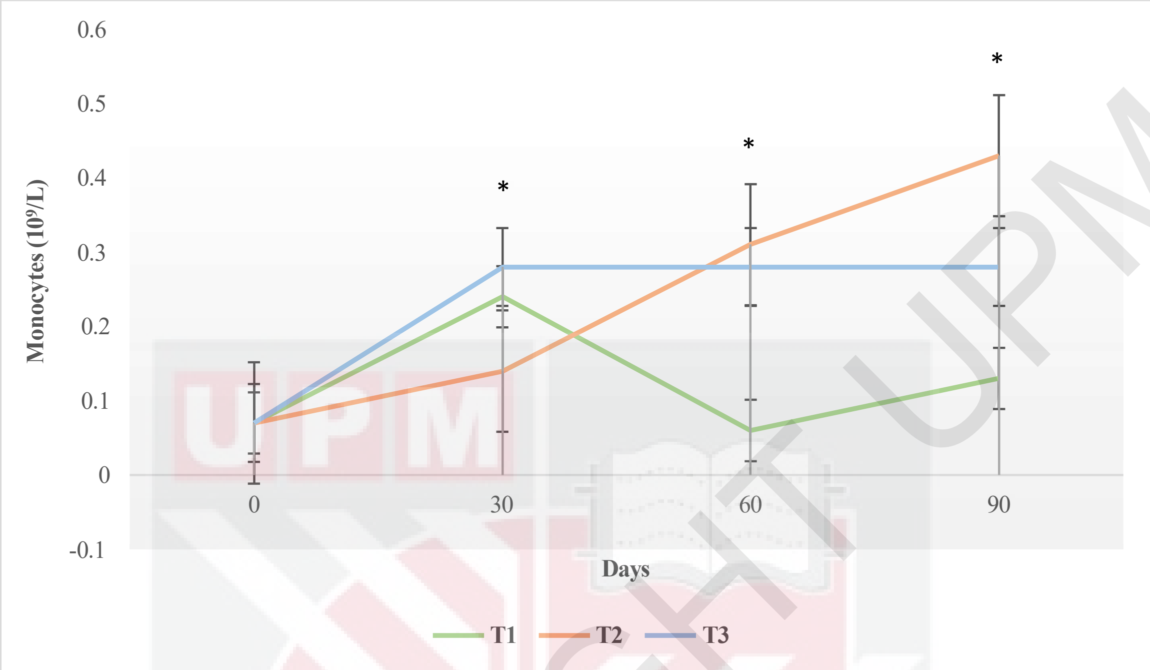

Figure 4.17 : The monocytes of sheep fed with fresh and ensiled *B. decumbens* at various time intervals was expressed as mean ± SEM. The significances were identified among the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

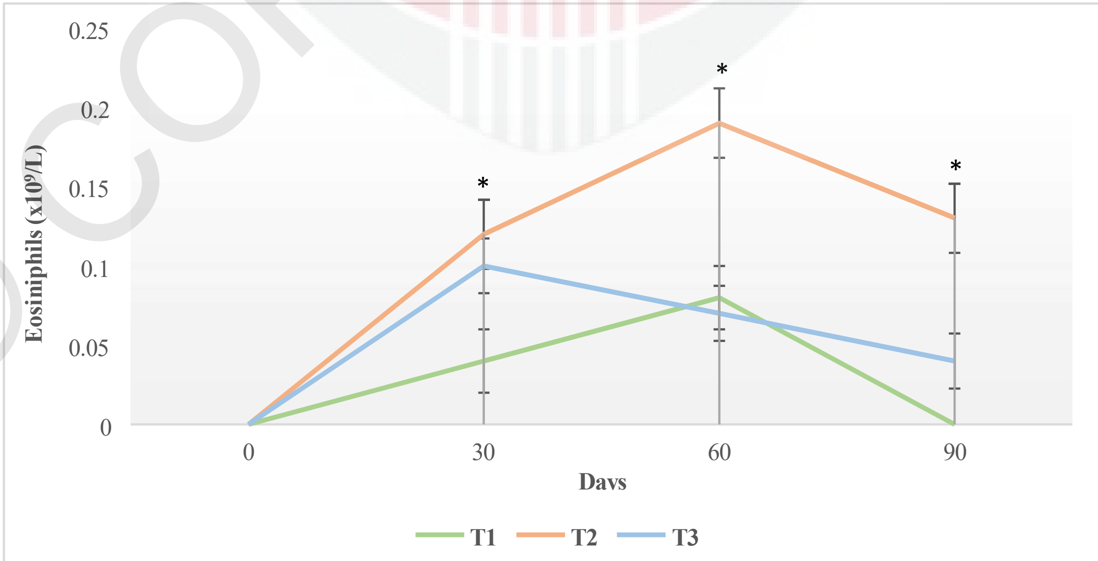

Figure 4.18 : The eosinophils of sheep fed with fresh and ensiled *B.*

decumbens **at various time intervals was expressed as mean ± SEM. The significances were identified among the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.**

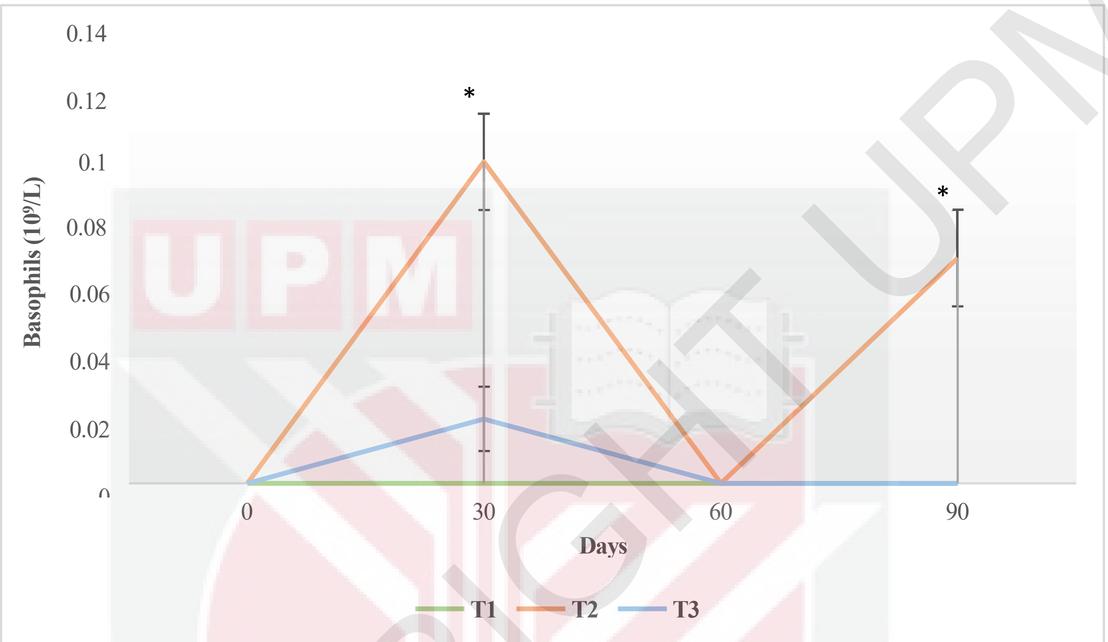

Figure 4.19 : The basophils of sheep fed with fresh and ensiled *B. decumbens* at various time intervals were expressed as mean ± SEM. The significances were identified among the groups throughout the experiment, with the highest value from T2. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

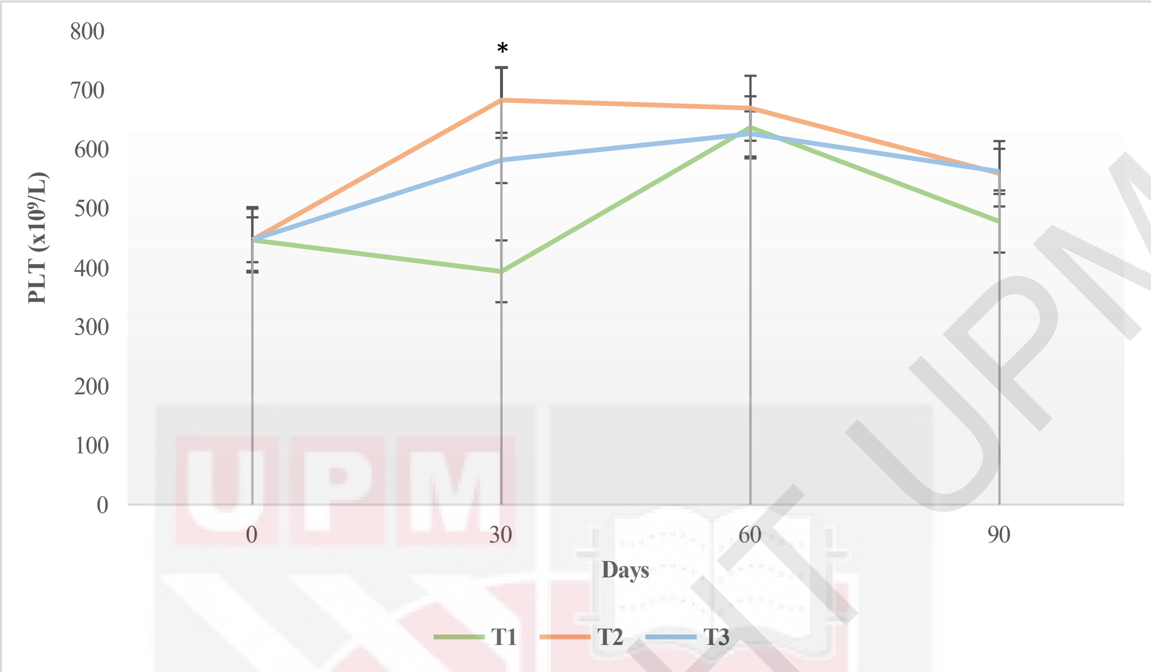

Figure 4.20 : The PLT of sheep fed with fresh and ensiled *B. decumbens* at various time intervals were expressed as mean ± SEM. The significances were identified among the groups during day 30 of the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

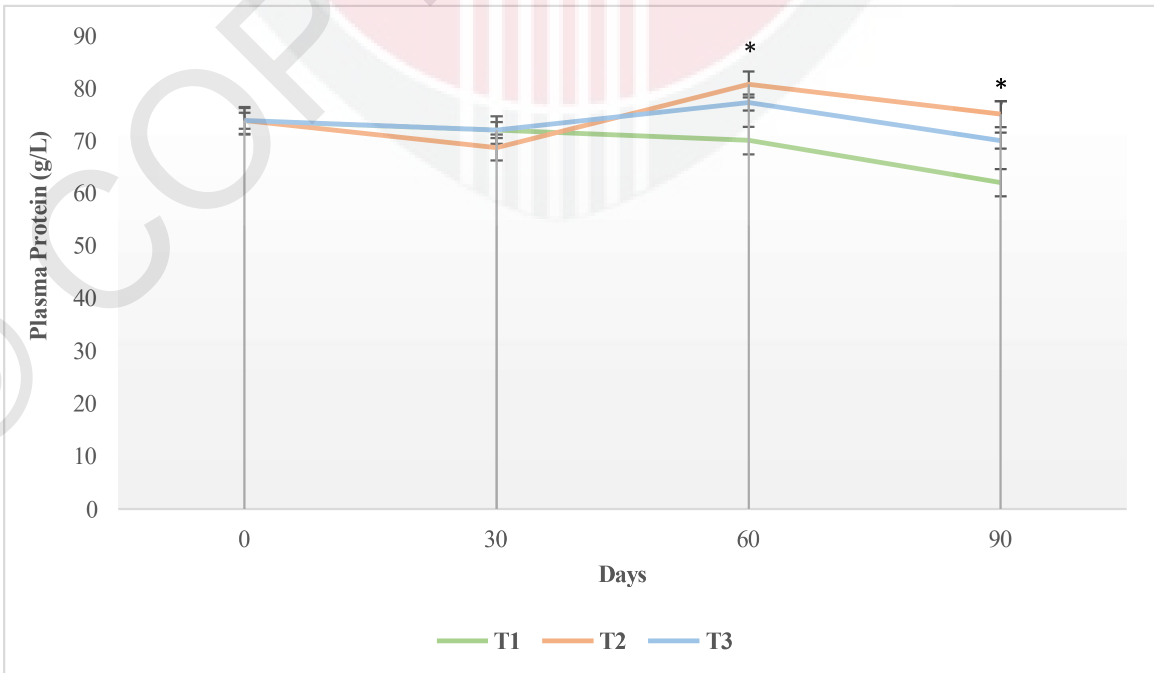

Figure 4.21 : The plasma protein of sheep fed with fresh and ensiled *B. decumbens* at various time intervals were expressed as mean ± SEM. Significant differences were identified among the groups during day 60 and 90 over the course of the experiment, with the highest observed value recorded at T2. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

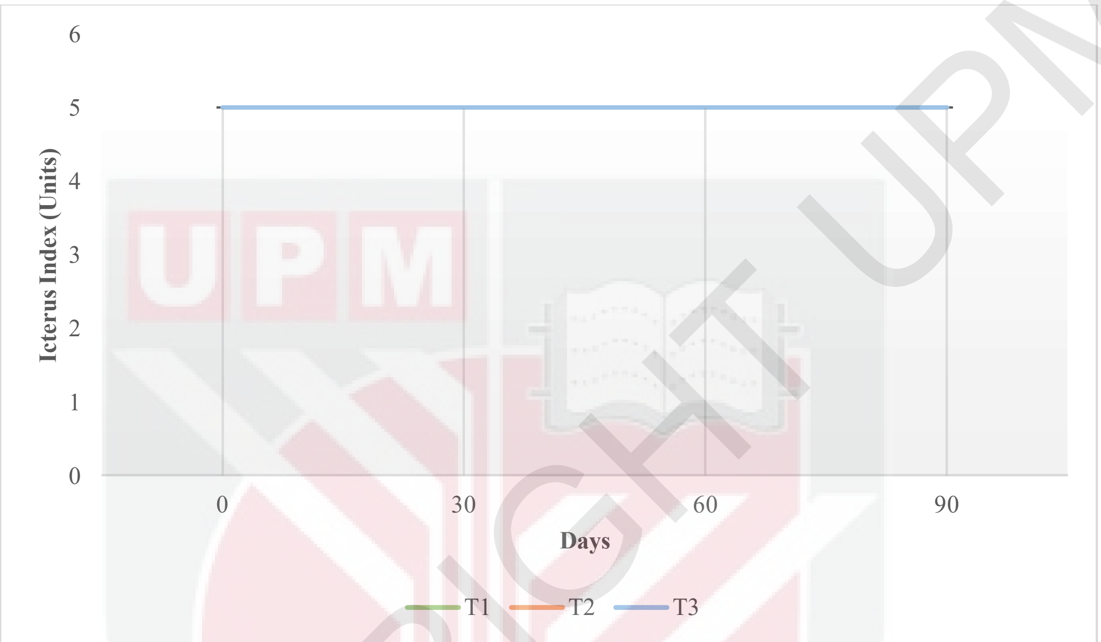

Figure 4.22 : The icterus index of sheep fed with fresh and ensiled *B. decumbens* at various time intervals were expressed as mean ± SEM. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*).

It is widely acknowledged that an excessive presence of phytochemicals specifically saponins can lead to bitterness, decreased nutrient absorption, reduced palatability, and the potential for toxicity in animals if consumed above certain levels (Mako et al., 2021). A hematological analysis enables a review of saponin's impact on the blood components and immune system of ruminants. In certain instances, sheep fed with *B. decumbens* reported a significant drop in plasma protein concentrations in all treated sheep, while the PCV remained unaffected. The decrease in production may be ascribed to a decline in synthesis resulting from hepatic illness (Muniandy et al., 2021). This study reported T3 sheep displaying good overall health performance, with no significant changes when compared with T1 in WBC count, platelets, plasma protein, and icterus index. This demonstrates that the preservation method of *B. decumbens* grass through ensiling can lead to advantageous results, with no observed physiological changes or immunological reactions. The nutritional content of *B. decumbens* can be thoroughly utilized by animals without concern for the adverse effects as a result of the saponin elimination within T3. Similarly, these findings were consistent with a report from Mako et al. (2021) using ensiled *A. brasiliana* and Guinea grass, which described elevated WBC counts within the normal range. In brief, the T3 sheep exhibited superior overall performance in terms of WBC indices when fed with *B. decumbens* silage. The grass preservation method did not impact the immune response of the sheep as it effectively eliminated saponins, thereby preventing any further intoxication in the sheep. Therefore, preserved *B. decumbens* can be utilized as a substitute feed for the small ruminant sector, replacing the need for fresh *B. decumbens* grass.

Instead, sheep within T2 fed with fresh *B. decumbens* exhibited elevated values including the WBC, band neutrophil, segmented neutrophil, lymphocytes, monocytes, eosinophil, and basophil when compared to the control group. Elevated levels of WBC in sheep are indicative of infection and inflammation (Roba et al., 2022). As evidenced by Rosa et al. (2016), the study revealed that goats fed with *B. decumbens* exhibited bronchopneumonia, which was caused by bacterial infection resulting from saponin poisoning. Affected goats also developed inflammatory lesions affecting liver, including neutrophilic and lymphoplasmacytic cholangitis and pericholangitis (Rosa et al., 2016). Previous investigations have also indicated that the consumption of steroidal saponin resulted in cholangitis, as evidenced by the infiltration of macrophages and lymphocytes detected in histological examinations (Cruz et al., 2001; Lima et al., 2012), as well as renal lesions (Montoya-Ménez et al., 2019) displaying eosinophilic materials (Faccin et al., 2014).

In addition, skin lesions found within one affected steer exhibited a secondary bacterial infection with the presence of purulent discharge (Carmo et al., 2021). It was explained that the ingestion of *B. decumbens* leads to the production of phylloerythrin by chlorophyll, which in turn generates free radicals with the ability to cause cellular damage and inflammation when excretion is impaired (Carmo et al., 2021). The pathological characteristics, along with the histological damage in the kidneys and liver caused by saponins, can be attributed to bacterial infection. These findings pose as additional evidence that the toxic effects of steroidal saponins can result in inflammatory responses, as indicated by changes in WBC in the present investigation. Given that lymphocytes are the most numerous types of leukocytes found in the blood of ruminants (Harvey, 2012), it is crucial to assess them to gain a more comprehensive understanding of the health of sheep. Nevertheless, the slight increase in value may signify an effective disease resistance and indicates that the animals were not susceptible to stress or illness (Mako et al., 2021). Also, it is uncommon to observe lymphocytosis, which may be present in chronic viral infections and autoimmune disorders (Polizopoulou, 2010). In addition, T2 exhibited an increasing trend in the plasma protein value, suggesting a potential onset of an inflammatory response. Elevated levels of plasma protein can result in hyperproteinemia or hyperglobulinemia, which in turn can contribute to the development of chronic inflammatory conditions (Polizopoulou, 2010).

Hence, it is reasonable to state that although T2 and T3 produced almost similar outcomes in the blood indices; ensiled *B. decumbens* remains an advantageous option due to its reliable removal of steroidal saponins, hence eliminating concerns of feed intoxication that could impact animal productivity. To summarize, the utilization of *B. decumbens* silage is a safer option for removing saponins due to its ability in maintaining the nutritional contents within the grass and showing no effects on the blood indicators. Regarding the blood indices, sheep fed with ensiled *B. decumbens* have the best results among the treatments, highlighting the effectiveness of this grass in the absence of saponin. With this, sheep were able to efficiently utilize the nutritional value of their feed, while also offering farmers a consistent supply of quality feed throughout the year.

4.2.3 Blood Biochemistry (Liver function)

Table 4.8 below mentions the aspartate aminotransferase (AST), total bilirubin, and gamma glutamyltransferase (GGT) of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* & ensiled *B. decumbens.* Throughout the experiment, the results observed within the treatment showed significance in the AST and GGT ($p < 0.05$) at various time intervals. Treatment 2 sheep displayed the highest values in AST (4.1 — 58.9%) and GGT (4.9 — 82.6%) in contrast to the control sheep. Despite having significance, AST and GGT values are still within the normal range of values within the 90 days of study. Nonetheless, the feeding of fresh *B. decumbens* could eventually affect the liver functions as the values were showing an increased trend line as compared to fresh *P. purpureum* and ensiled *B. decumbens* (Figure 4.23 — 4.25).

Table 4.8 : Liver function of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* & ensiled *B. decumbens.*

	Treatment 1	Treatment 2	Treatment 3	p-value
AST (U/L) (normal range: 50-100)				
Day 0	105.60 ± 6.56^{b}	148.45 ± 6.56^{a}	126.65 ± 6.56^{c}	0.02
Day 30	73.40 ± 0.76^{c}	76.50 ± 1.23^{a}	70.40 ± 1.16^{b}	<0.01
Day 60	48.00 ± 10.10^{b}	70.27 ± 30.36^{a}	63.17 ± 30.35^{a}	<0.01
Day 90	42.60 ± 52.15^{a}	78.20 ± 74.64^{b}	60.93 ± 52.48^{b}	<0.01
Total Bilirubin (umol/L) (normal range: 1.7- 6.8)				
Day 0	1.26 ± 0.23	0.96 ± 0.23	1.11 ± 0.23	1.00
Day 30	0.22 ± 0.05	0.17 ± 0.03	0.21 ± 0.03	0.66
Day 60	5.17 ± 0.45	3.52 ± 0.36	4.14 ± 0.35	0.09
Day 90	4.70 ± 0.67	5.40 ± 1.37	4.56 ± 0.51	0.27
GGT (U/L) (normal range: 30-50)				
Day 0	10.5 ± 0.32	10.5 ± 0.32	10.5 ± 0.32	1.00
Day 30	20.00 ± 0.79^{b}	32.67 ± 1.93^{a}	31.33 ± 1.10^{a}	<0.01
Day 60	33.00 ± 2.90	34.67 ± 3.06	29.67 ± 5.47	0.73
Day 90	19.25 ± 15.80^{b}	46.33 ± 29.26^{a}	25.83 ± 23.57^{ab}	0.04

Note: All values were expressed as mean ± SEM; $^{a, b, c}$ values with superscript within the same row are significantly different at p<0.05. All parameters showed significance except Total bilirubin. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens,* ALB: Albumin, AST: Aspartate aminotransferase, GGT: Gamma glutamyltransferase.

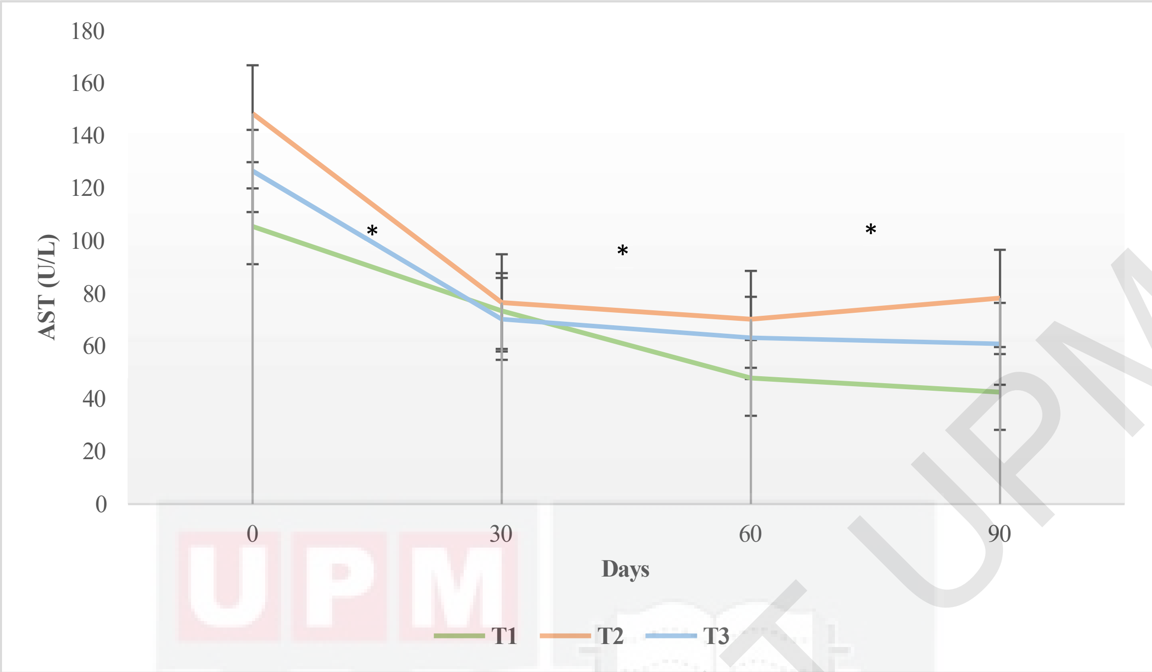

Figure 4.23 : The AST of sheep fed with fresh and ensiled *B. decumbens* at various time intervals were expressed as mean ± SEM. Significant differences were identified among the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

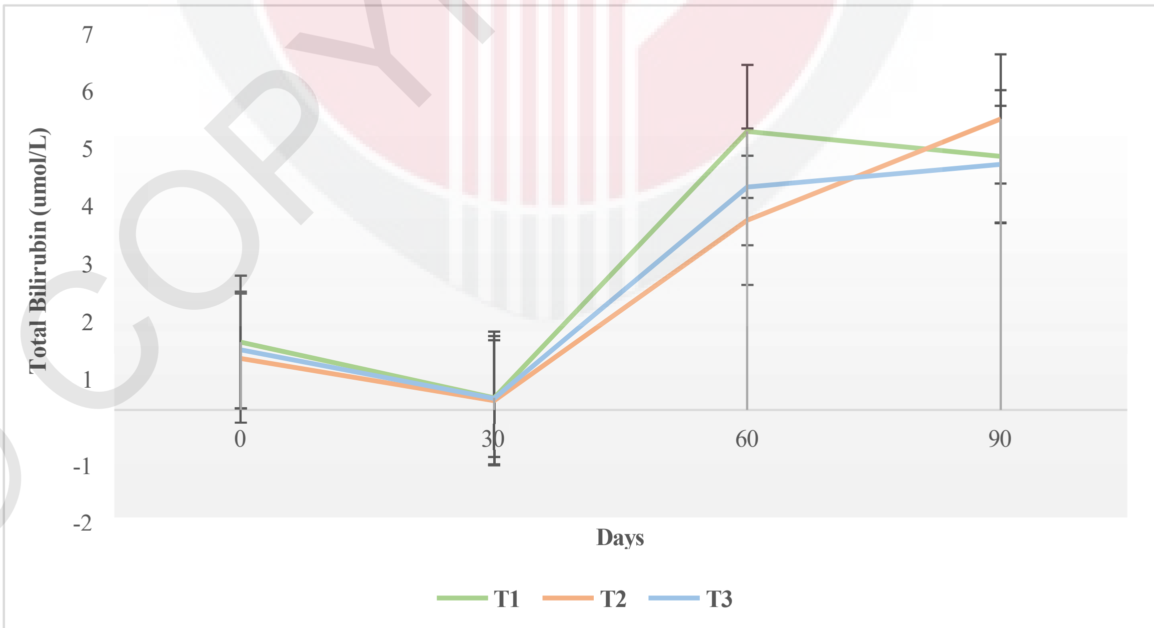

Figure 4.24 : The total bilirubin of sheep fed with fresh and ensiled *B. decumbens* at various time intervals were expressed as mean ± SEM. No significant differences were presented among the groups

throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*).

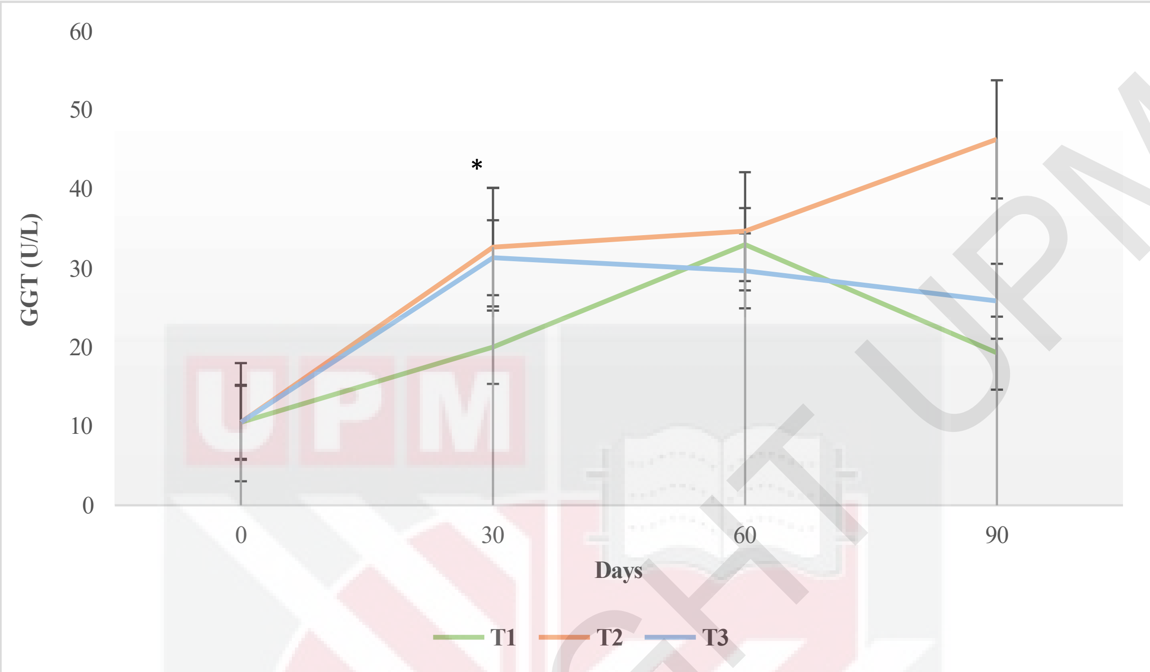

Figure 4.25 : The GGT of sheep fed with fresh and ensiled *B. decumbens* at various time intervals were expressed as mean ± SEM. Significant differences were identified among the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

Serum biochemistry is a valuable method for assessing hepatic damage, renal functions, and potentially associated abnormalities (Carmo et al., 2021). The intoxication effects on sheep grazing on *Brachiaria* spp. pasture highlighted the severity and concerns about the steroidal saponin compound described in biochemistry parameters. Due to the limitations of *Brachiaria* spp. pasture, farmers have resorted to preservation techniques, using it as a secondary source of nutrition for ruminants. The current blood biochemistry results of T3 sheep fed with ensiled *B. decumbens* mostly revealed insignificance ($p > 0.05$) when compared with the control group. Similar to a study by Ran et al. (2021), the analysis of serum enzyme activity indicates that substituting a part of corn silage with sweet sorghum silage did not affect the internal organs. Moreover, a reported study measuring the variations in blood metabolites (glucose, BUN, total protein) did not show any significant differences across the experimental groups fed with a combination of cactus-alfalfa silage, which suggests a steady metabolic condition and a moderate level of homeostatic status of the animals (Ravari et al., 2022). Similar to the current result, Rezaei et al. (2015) mentioned that the blood albumin concentration in the experimental cows remained consistent, indicating that the dietary treatment did not impact the health of the animals. In addition, the consistent blood albumin levels observed across the different treatments may be attributed to the comparable utilization of dietary crude protein by the animals (Rezaei et al., 2013).

The mechanism of intoxication as explained by Kreutzer et al. (2008), described that phylloerythrin excretion is inhibited in ruminants with diffuse liver injury, resulting in secondary photosensitization. This photodynamic agent is produced from ingested chlorophyll compound, delivered by the portal system to the liver, where it is conjugated and excreted in bile. Excess phylloerythrin is poorly eliminated by the kidneys and accumulates in tissues like the skin (Kreutzer et al., 2008). During the process of ensiling the grass, saponin is removed through an anaerobic process in which the glucose component within saponin breaks down, resulting in a higher quality feed for the sheep. The process of ensiling facilitates the cellular breakdown of saponin, resulting in the formation of easily digestible components that can be safely excreted by animals. It is presumed that enzymatic or microbiological breakdown results in the production of the aglycone without any sugar chains (Jiang et al., 2018). Through the consumption of ensiled *B. decumbens* in this study, the elimination of saponin compound revealed a better result of biochemical indices, indicating a healthier physiology of animals as well as effectively controls lipid metabolism while maintaining liver protection (Liu et al., 2018). Thus, it is necessary to state that ensiling *B. decumbens* is a practical and cost-effective feed choice for the ruminant sectors, offering significant advantages.

Conversely, the current blood biochemistry results revealed significance ($p < 0.05$) in the T2 sheep when compared to the control group, indicating the possibility of a hepatic condition. T2 exhibited slightly elevated values for both AST and GGT, but the range remained within the established reference values. Generally, the liver, heart, and muscle express the presence of enzymes such as AST, ALT, ALP, and creatine kinase. These enzymes maintain stable relationships in the bloodstream at low levels during normal activities. Besides, hepatocytes, erythrocytes, cardiac myocytes, and skeletal myocytes typically contain AST enzymes. Due to its elevated cellular activity in hepatic and muscular cells, it is more effective in measuring damage (Kreutzer et al., 2008). When an organ sustains injury, there is a noticeable rise in blood enzyme activity. Serum enzymes are thus frequently analyzed for the evaluation and identification of internal organ injury. The result of sheep fed with fresh *B. decumbens* showing a sudden increase in AST and GGT levels on the final day of the experiment could represent evidence of hepatic injury (Montoya-Ménez et al., 2019).

Principally, the toxicity of *Brachiaria* spp. is known to be associated with lithogenic steroidal saponins (Riet-Correa et al., 2011). Saponins are broken down by the rumen into sapogenins, which then pass into the intestinal tract and are transported to the liver by the portal vein (Rosa et al., 2016). The investigation also revealed that the majority of serious liver damage is attributed to this mechanism occurring in the periportal area. Saponins can lead to the formation of insoluble salts known as biliary system crystals in the gastrointestinal systems of ruminant animals through the metabolic breakdown of saponins. When combined with glucuronic acid in the liver and exposed to calcium in the bile, the poisonous steroidal saponins of *Brachiaria* spp. undergo lithogenesis, which induces hepatogenous photosensitization (Cruz et al., 2000). The increased levels of AST enzymes may suggest a certain degree of muscle damage and hemolysis, as reported in the related hepatocyte injury in horses and cattle (Kreutzer et al., 2008). Likewise, other studies also claimed that the increase in serum GGT activity could also serve as a sign of liver damage (Underwood et al., 2015), as demonstrated by Kreutzer et al. (2008) in cattle that were exposed to moldy hay. Overall, the study exploring the preservation of *B. decumbens* through ensiling has demonstrated its advantage over using fresh pastures. The process of ensiling, as described, has proven useful in ensuring an improved nutritional intake in ruminants. Treatment 3 demonstrated hematological and biochemical alterations indicative of a healthier sheep, successfully mitigating hepatotoxic effects associated with the consumption of fresh *B. decumbens*.

4.2.4 Blood Biochemistry (Kidney Functions, Total Protein, Albumin, Globulin, A:G Ratio)

Table 4.9 presents the measured levels of creatinine, phosphate, and urea of sheep that were provided with fresh *P. purpureum*, fresh *B. decumbens*, and ensiled *B. decumbens* during the period of the experiment. All three parameters exhibited statistical significances ($p < 0.05$) across the various treatments. These parameters demonstrated values that were either higher or lower than the established normal range. Both creatinine and phosphate exhibited comparable findings, with Treatment 3 sheep that were fed ensiled *B. decumbens* displayed the lowest values at 64.33 ± 1.36 umol/L (↓15.1%) and 2.30 ± 0.04 mmol/L (↓25.1%) respectively in comparison to the control group.

On the other hand, the result indicated that the urea levels were the lowest in Treatment 2 (fresh *B. decumbens*), with the lowest recorded value being 2.30 mmol/L at day 90. In general, the blood biochemistry associated with kidney functions exhibited significant differences, with Treatment 3 demonstrating the lowest value among the different treatments administered during the trial (Figure 4.26 — 4.28).

Based on Table 4.9, parameters including total protein, albumin, globulin, and albumin to globulin ratio showed significance ($p < 0.05$) throughout the experiment. The data indicates that Treatment 3 demonstrated the highest total protein levels across all treatments with differences in significance between control ranging from 3.5% to 32.4% (Figure 4.29). Additionally, sheep in Treatment 2 exhibited the highest levels of albumin (3.5 – 24.6%), globulin (1.3 — 30.1%) and A:G ratio (6.3 — 49.8%) in contrast to the control sheep (Figure 4.30 – Figure 4. 32). Accordingly, fresh *B. decumbens* had different effects on the total protein, globulin, and A:G ratio of sheep throughout the 98-day study.

Table 4.9 : Kidney functions of sheep fed with fresh *P. purpureum*, fresh *B. decumbens*, & ensiled *B. decumbens*.

	Treatment 1	Treatment 2	Treatment 3	p-value
Creatinine (umol/L) (normal range: 106-168)				
Day 0	84.00 ± 1.27	82.75 ± 1.27	83.25 ± 1.27	1.00
Day 30	35.00 ± 18.63[b]	51.33 ± 22.44[a]	48.00 ± 22.49[a]	0.01
Day 60	72.00 ± 2.44	73.00 ± 4.77	75.78 ± 2.36	0.83
Day 90	74.33 ± 3.90[a]	73.89 ± 3.00[a]	64.33 ± 7.36[c]	0.02
Phosphate (mmol/L) (normal range: 1.6-2.4)				
Day 0	2.85 ± 0.05	2.85 ± 0.05	2.85 ± 0.05	1.00
Day 30	1.80 ± 0.08	1.80 ± 0.11	2.03 ± 0.04	0.14
Day 60	3.10 ± 0.29[a]	2.17 ± 0.11[b]	3.07 ± 0.15[a]	<0.01
Day 90	2.50 ± 0.17[a]	2.43 ± 0.04[c]	2.30 ± 0.14[b]	0.02
Urea (mmol/L) (2.8-7.1)				
Day 0	5.80 ± 0.04	5.75 ± 0.04	5.70 ± 0.04	1.00
Day 30	2.80 ± 0.80[a]	1.97 ± 0.11[b]	2.23 ± 0.89[b]	<0.01
Day 60	4.80 ± 0.04[a]	3.43 ± 0.12[b]	4.60 ± 0.14[a]	<0.01
Day 90	4.00 ± 0.20[a]	2.30 ± 0.10[b]	3.89 ± 0.11[a]	<0.01
Total Protein (g/L) (normal range: 55-70)				
Day 0	70.95 ± 0.65	70.95 ± 0.65	70.95 ± 0.65	1.00
Day 30	32.20 ± 3.09	42.67 ± 4.91	44.67 ± 3.51	0.11
Day 60	66.30 ± 0.80[b]	70.03 ± 4.52[a]	71.63 ± 4.62[a]	<0.01
Day 90	66.70 ± 7.40[a]	60.80 ± 5.32[b]	69.10 ± 5.62[a]	<0.01
Albumin (g/L) (normal range: 25-35)				
Day 0	38.76 ± 0.35	38.84 ± 0.35	38.85 ± 0.35	1.00
Day 30	28.00 ± 0.90	29.00 ± 0.60	28.20 ± 0.99	0.75
Day 60	38.50 ± 5.02[b]	43.30 ± 2.73[a]	42.90 ± 3.93[a]	<0.01
Day 90	32.80 ± 6.50[b]	42.00 ± 7.85[a]	38.73 ± 6.84[a]	<0.01
Globulin (g/L) (normal range: 25-45)				
Day 0	30.83 ± 0.73	30.83 ± 0.73	30.83 ± 0.73	1.00
Day 30	11.00 ± 5.66[b]	15.83 ± 2.70[c]	17.43 ± 2.20[a]	0.02
Day 60	22.30 ± 0.00[b]	30.20 ± 1.01[a]	28.43 ± 0.98[a]	<0.01
Day 90	25.73 ± 1.07	26.06 ± 1.34	27.90 ± 1.16	0.52

A:G ratio (Unit) (normal range: 0.5-1.2)				
Day 0	1.25 ± 0.05	1.25 ± 0.05	1.25 ± 0.05	1.00
Day 30	2.30 ± 0.10	2.16 ± 0.16	2.02 ± 0.19	0.58
Day 60	1.10 ± 0.11[c]	1.83 ± 0.07[a]	1.43 ± 0.08[b]	<0.01
Day 90	1.45 ± 0.70[b]	1.73 ± 0.56[a]	1.28 ± 0.74[b]	<0.01

Note: All values were expressed as mean ± SEM; [a, b, c] values with superscript within the same row are significantly different at $p<0.05$. All parameters exhibit significance throughout the experiment. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens.* A:G ratio: Albumin to globulin ratio.

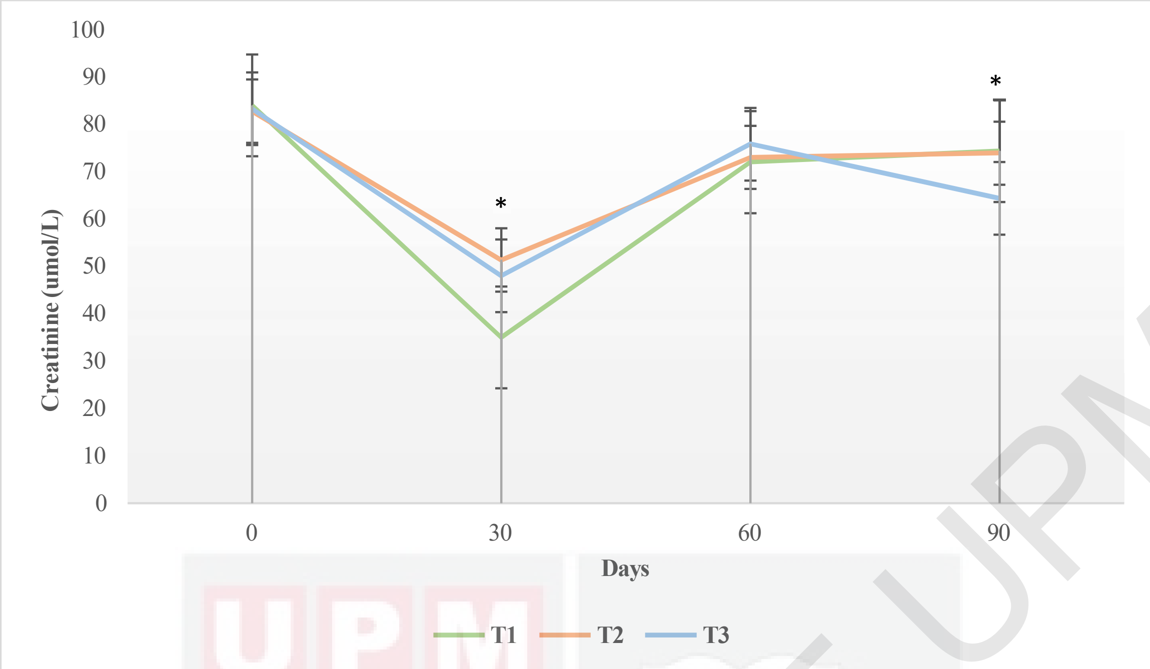

Figure 4.26 : The creatinine of sheep at various time intervals were expressed as mean ± SEM. No significances were identified among the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

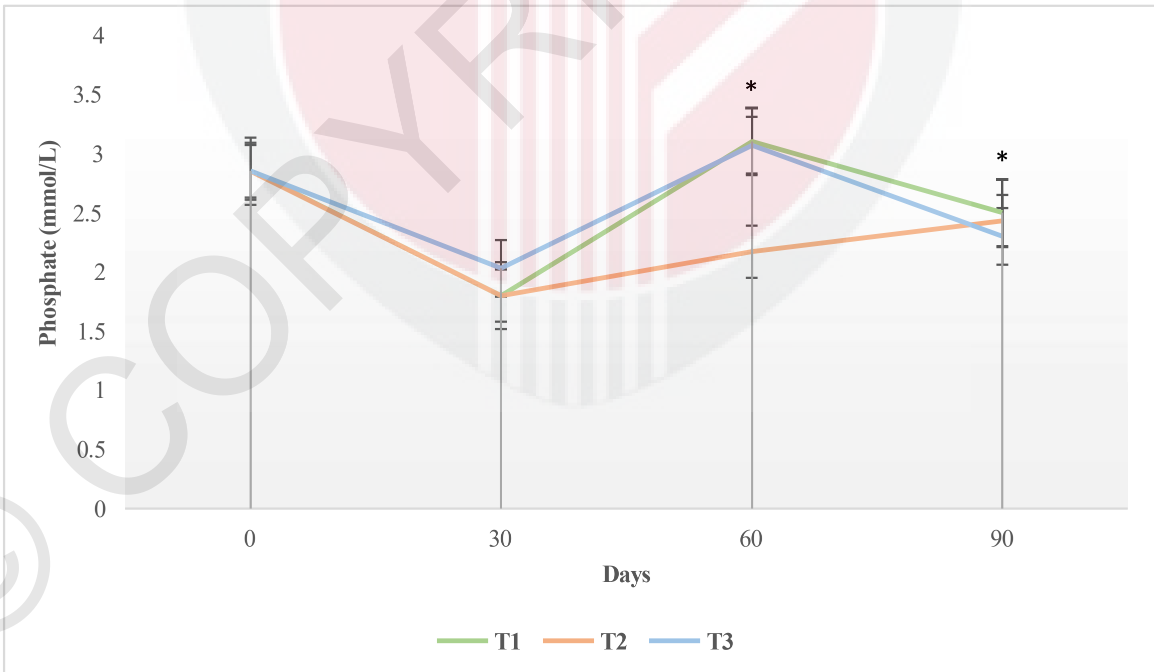

Figure 4.27 : The phosphate of sheep fed with fresh and ensiled *B. decumbens* at various time intervals were expressed as mean ± SEM. Significant differences were identified during day 60 within the groups throughout the experiment, with the highest value exhibited from T3. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2

(fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

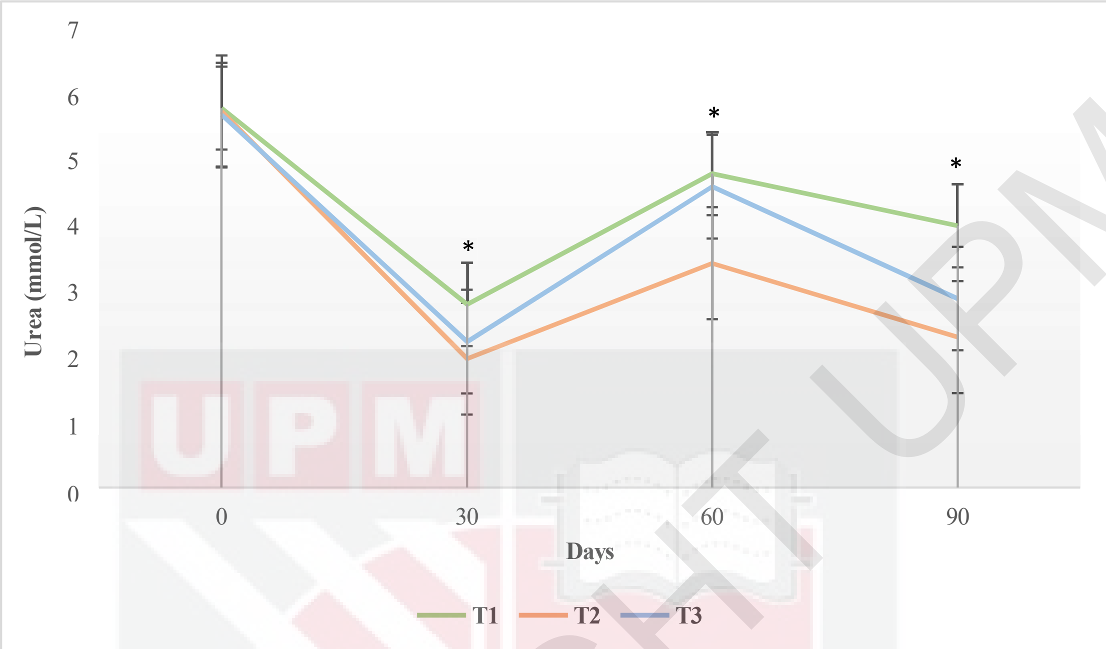

Figure 4.28: The urea of sheep fed with fresh and ensiled *B. decumbens* at various time intervals were expressed as mean ± SEM. Significant differences were identified within the groups throughout the experiment, with the lowest value exhibited from T2. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

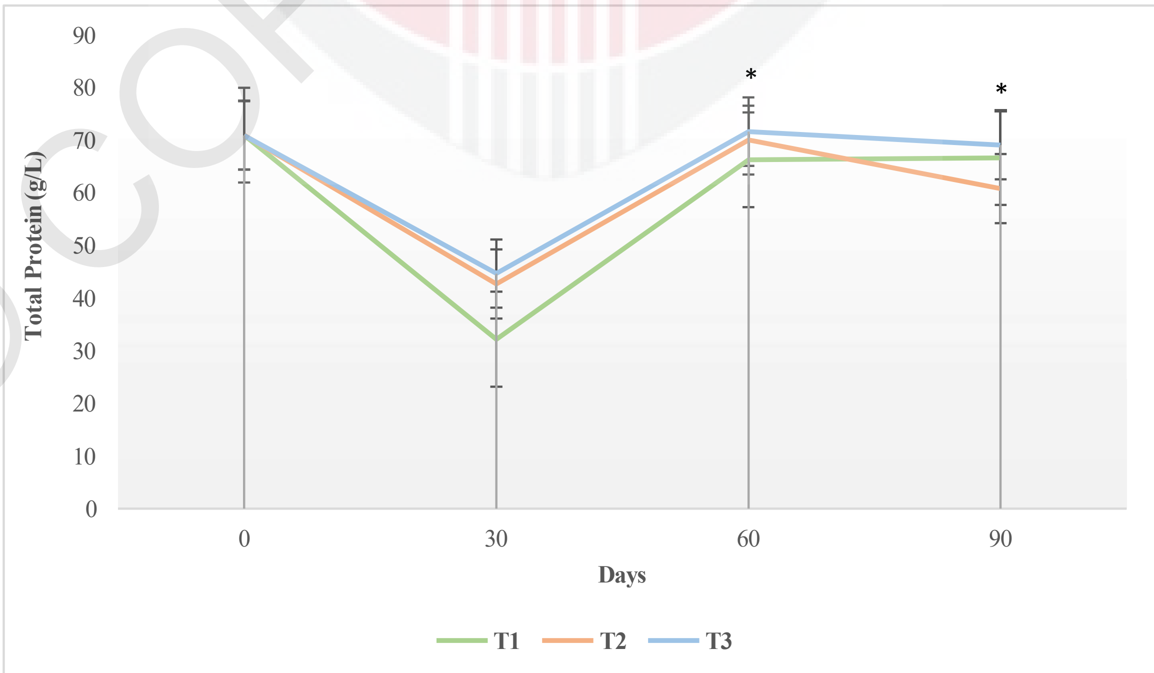

Figure 4.29 : The total protein of sheep fed with fresh and ensiled *B. decumbens* at various time intervals were expressed as mean ± SEM. Significant differences were identified within the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

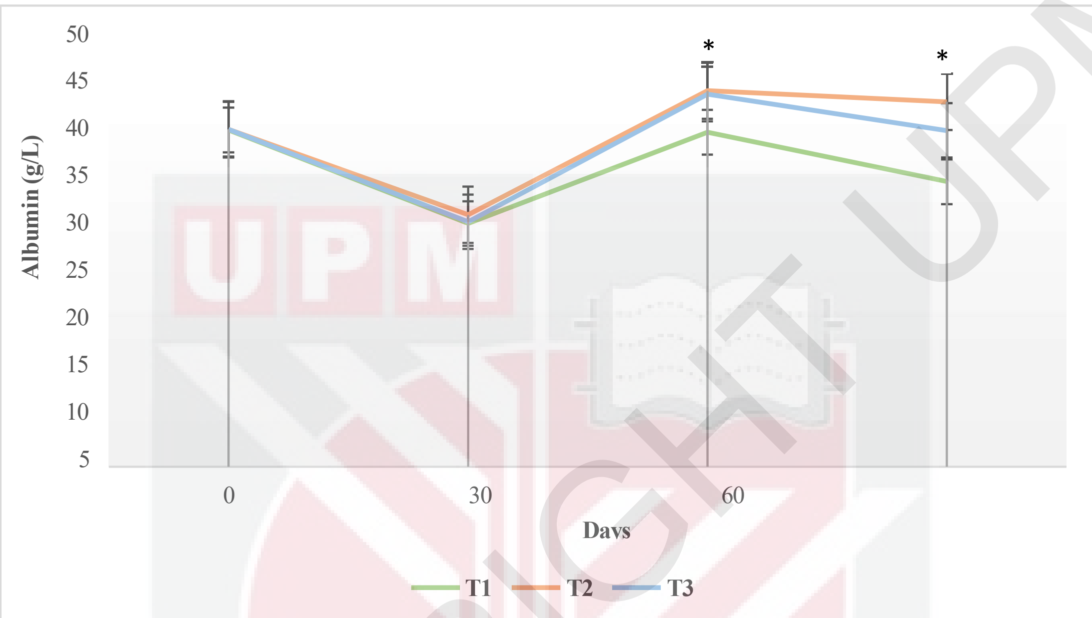

Figure 4.30 : The albumin of sheep at various time intervals were expressed as mean ± SEM. Significant differences were identified among the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

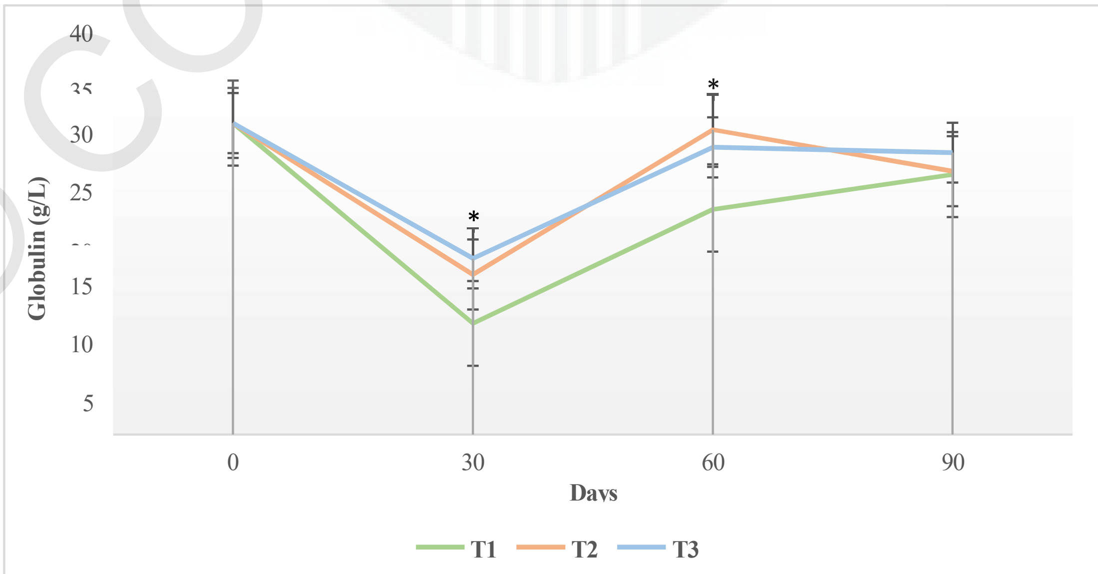

Figure 4.31 : The globulin of sheep fed with fresh and ensiled *B. decumbens* at various time intervals were expressed as mean ± SEM. Significant differences were identified during day 30 and day 60 within the groups. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

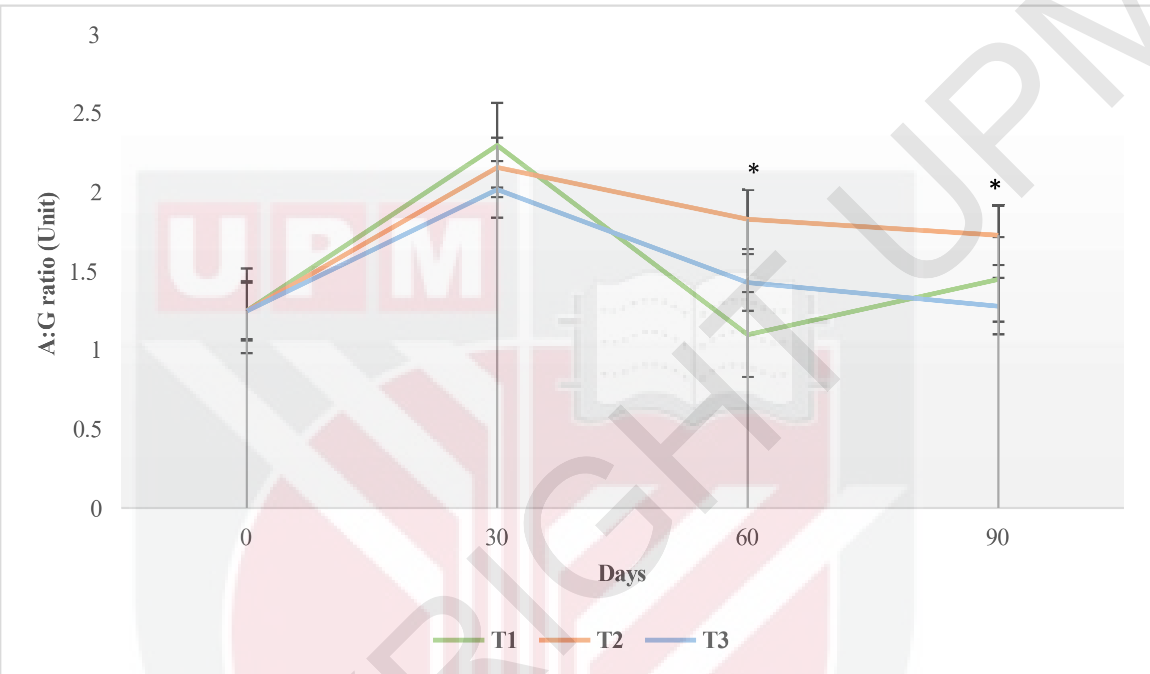

Figure 4.32 : The A:G ratio of sheep fed with fresh and ensiled *B. decumbens* at various time intervals were expressed as mean ± SEM. Significant differences were identified within the groups. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

Based on the present study, the biomarkers for kidney function exhibited statistical significance ($p < 0.05$) in both creatinine and phosphate, indicating lower levels in comparison to the T1 group. The present study described comparable findings to a study conducted by Muniandy et al. (2021), which demonstrated little variations in creatinine levels. These changes could perhaps be ascribed to the quantity of *B. decumbens* toxin or the insufficient period of intoxication to induce renal injuries (Muniandy et al., 2021). Moreover, the process by which ensiling mitigates the harmful consequences of intoxication is achieved by the elimination of steroidal saponin from the grass. Animals with liver damage may be unable to eliminate toxic substances, which could result in kidney damage. At first, conjugation processes include the addition of a polar molecule, like glucuronic acid, to carboxyl, hydroxyl, amino, or sulfhydryl groups on the metabolites (Cullen & Stalker, 2016). This process results in steroidal saponin becoming less toxic and more soluble in water. It is suggested that lipophilic molecules are converted into water-soluble molecules, which may then be transported out of the hepatocyte and eliminated from the body through the bile or the circulation (Cullen & Stalker, 2016), indicating a well-functioning kidney in T3 group. In comparison, the levels of serum urea and creatinine of sheep under T2 showed an elevation after exposure to steroidal saponin. Saponin intoxication can lead to increased levels of urea and creatinine in the bloodstream, which can adversely impact kidney function (Lelis et al., 2018). The silage treatment demonstrated no effects on kidney function, as confirmed by the histological findings in the present investigation, indicating an effective elimination of saponin.

Moreover, the significance changes of creatinine, phosphate, and urea ($p<0.05$) in T2 sheep were consistent with previous research findings. The total protein concentrations, albumin, and serum urea levels in blood serve as biomarkers of the animals' nutritional health and are directly associated with the amount of protein consumed in feed (Roba et al., 2022). According to Driemeier et al. (2002), the changes in the level of creatinine could be caused by either the amount of *B. decumbens* toxin given or the insufficient length of time for the toxin to cause kidney injury. The decrease in urea levels reported in sheep afflicted by *B. decumbens* poisoning is likely due to hepatic insufficiency and a decrease in total protein, leading to a reduction in urea production. The explanation provided thus explains the possible cause for the observed significance in the present investigation.

Additionally, serum total protein, albumin, and globulin are biomarkers that provide information about the status of animal protein, including its absorption, synthesis, and decomposition (Zhang et al., 2022). T3 sheep exhibited higher levels of total protein and globulin, but lower levels of albumin and A:G ratio. Total protein is used to quantify the intake, absorption, and use of proteins in which higher levels of total protein contribute to the growth of animals and enhance feed conversion ratio (Su et al., 2022). The total protein levels observed in the T3 group of this study are within the standard range for sheep. A similar outcome was observed in a prior investigation, suggesting that the diet did not increase the animals' disease susceptibility (Mako et al., 2021). Albumin, primarily produced by hepatocytes typically remains preserved until there is a substantial decrease in the hepatic mass (Omidi et al., 2015). The nutrient carrier albumin plays a role in regulating the osmotic pressure of plasma, promoting tissue healing, and supplying energy (Su et al., 2022). A low concentration of serum albumin is indicative of poor health, namely liver dysfunction. Although the present study observed reduced levels of albumin within T3 group, other indicators of liver function did not exhibit significant variations between the treatments, which implies that the diets did not have any detrimental effects on the liver (Mako et al., 2021). Correspondingly, Su et al. (2022) revealed a rise in the levels of total protein, albumin, as well as a decrease in the A:G ratio, which was similar to the result of using ensiled *B. decumbens* in the current study. This could be attributed to the polysaccharides present in the feed, which have the ability to facilitate protein synthesis and metabolism, increase the proliferation and differentiation of lymphocytes, and thus enhance the immunological function of the body (Su et al., 2022).

Contrariwise, T2 sheep demonstrated lower levels of total protein and globulin, but higher levels of albumin and A:G ratio. The lower TP and globulin could be caused by the disrupted digestion and absorption along the gastrointestinal tract. It is worth noting that the surfactant properties of saponins within *B. decumbens* may cause interference with cell membranes through the development of pores, vesiculation, and disrupting membrane domains (Jiang et al., 2018). Besides, the current study showing elevations of these albumin and A:G ratio in T2 sheep may signify dehydration, hyperthermia, renal or hepatic dysfunction, or heightened consumption of grains (Mako et al., 2021). A study conducted by Omidi et al. (2015) also described that animal fed with *Setaria italica* exhibited a significant change in albumin levels. The progressive elevation in albumin levels may potentially be associated with cases of diarrhea and dehydration. Increased corpuscular volume may also contribute to verifying clinical symptoms of dehydration (Omidi et al., 2016). This further provides evidence, as displayed by the rise in mean corpuscular volume (MCV) of T2 sheep in the present study. The hematological and biochemistry changes within Treatment 3 showed an overall healthier sheep, which effectively eliminated hepatoxicity effects, such as those resulting from feeding fresh *B. decumbens*. Given that the blood indices results demonstrate the numerous benefits of *B. decumbens* silage, it is crucial to acknowledge that these benefits can only be attained through the use of proper harvesting techniques, effective ensiling management practices, and optimal sheep production performance. Thus, utilizing preservation techniques like ensiling can serve as a viable strategy to mitigate the detrimental impact of saponins on animals specifically in ruminants.

4.3 Pathology

4.3.1 Organ morphometric and gross morphology

The organs length, width, and weight of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* & ensiled *B. decumbens* are shown in Table 4.12 below. For the length and width of the selected organ, there were significances observed in the heart, lung, liver, and spleen of sheep within different treatments ($p < 0.05$). The organs measured from Treatment 2 sheep was found to have the highest measurements when compared to Treatment 1 and Treatment 3. For organ length, Treatment 2 sheep had longer heart (↑4.5%) and liver (↑18.4%) as compared to the control. For organ width, Treatment 2 sheep had wider heart (↑12.9%), lung (↑17.8%), and spleen (↑16.5%) as compared to the control. Moreover, there were also significances observed in the organ's weight including lung, liver, kidney, and spleen ($p < 0.05$). Comparatively among treatments, the lung and liver of Treatment 2 sheep were the heaviest, while the kidney and spleen of Treatment 3 sheep were the heaviest. Treatment 2 lung and liver were 28.1% and 18.2% heavier, whereas Treatment 3 kidney and spleen were 16.6% and 13.6% heavier when compared to the control (Figure 4.33 - Figure 4.44). As compared to the control group, the analysis of organ morphology suggested that sheep in Treatment 3 had more favorable results compared to the sheep within Treatment 2.

For gross morphology, the brain, heart, liver, kidney, and spleen of all treatments appeared to be normal with no obvious post-mortem lesions.

However, some post-mortem lesions were observed in the lung organ of Treatment 2 sheep. The left and right lung lobes appeared to be congested with pus accumulation at the caudal lobes (Figure 4.45). Upon cutting the surface, blood was oozing out from the lung and pus from the caudal lobes.

In general, all organs excluding the brain exhibited varying degrees of morphometric effects via the feeding of fresh *B. decumbens* and ensiled *B. decumbens*. Meanwhile, only the lung was affected grossly in sheep fed with fresh *B. decumbens*. In other words, sheep that are fed with ensiled *B. decumbens* exhibit a better overall condition in terms of both organ morphometric and gross morphology, as compared to those fed with fresh *B. decumbens*.

Table 4.10 : Gross morphology of sheep fed with fresh *P. purpureum*, fresh *B. decumbens*, & ensiled *B. decumbens*.

	Treatment 1	Treatment 2	Treatment 3	p-value
Organs length (cm)				
Brain	9.8 ± 0.30	9.7 ± 0.60	10 ± 0.40	0.83
Heart	11.0 ± 1.30[a]	11.5 ± 0.90[a]	9.7 ± 0.40[b]	<0.01
Lung	19.6 ± 0.50	20.8 ± 0.30	20.2 ± 0.50	0.26
Liver	14.1 ± 0.20[b]	16.7 ± 1.30[a]	16.2 ± 1.20[a]	<0.01
Kidney	6.4 ± 0.10	6.8 ± 0.20	6.6 ± 0.10	0.08
Spleen	6.7 ± 0.30	7.6 ± 1.20	6.8 ± 0.40	0.08
Organs width (cm)				
Brain	7.0 ± 0.10	7.1 ± 0.20	7.4 ± 0.10	0.20
Heart	7.0 ± 0.10[b]	7.9 ± 0.30[a]	7.2 ± 0.30[b]	<0.01
Lung	16.3 ± 0.30[b]	19.2 ± 0.20[a]	18.0 ± 0.90[c]	<0.01
Liver	20.8 ± 0.20	21.3 ± 0.60	20.5 ± 0.40	0.11
Kidney	4.4 ± 0.10	4.5 ± 0.10	6.7 ± 1.10	0.39
Spleen	9.1 ± 0.10[b]	10.6 ± 0.30[a]	9.8 ± 0.30[c]	<0.01
Organs weight (g)				
Head (kg)	1.89 ± 0.03	1.95 ± 0.03	1.99 ± 0.04	0.15
Heart	127.25 ± 5.05	136.25 ± 8.02	133.00 ± 8.07	0.52
Lung	263.17 ± 55.01[c]	349.23 ± 25.01[a]	305.66 ± 20.01[b]	<0.01
Liver	371.75 ± 31.03[c]	446.25 ± 10.01[a]	427.00 ± 30.16[b]	<0.01
Kidney	69.25 ± 8.21[b]	75.75 ± 8.01[c]	81.75 ± 9.01[a]	<0.01
Spleen	43.00 ± 2.11[b]	49.00 ± 3.31[a]	49.25 ± 3.37[a]	<0.01

Note: All values were expressed as mean ± SEM; [a, b, c] values with superscript within the same row are significantly different at p<0.05. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens.*

Figure 4.33 : The organ length and organ width (brain) of sheep fed with fresh and ensiled *B. decumbens* were expressed as mean ± SEM. Significant differences were identified within the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*).

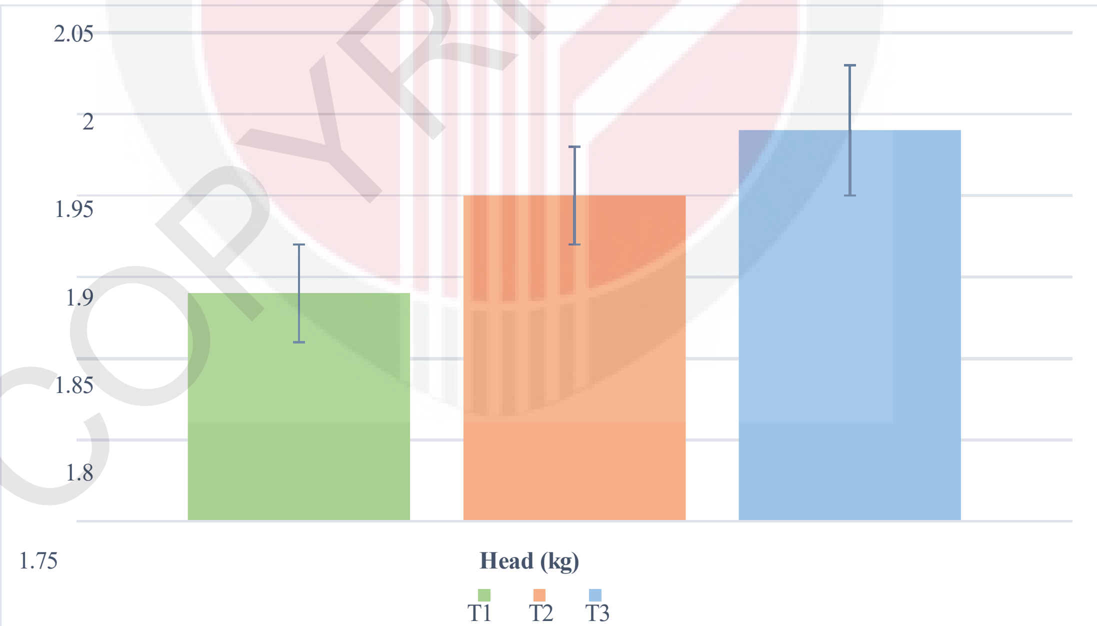

Figure 4.34 : The organ weight (head) of sheep fed with fresh and ensiled *B. decumbens* were expressed as mean. Significant differences were identified within the groups. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*).

Figure 4.35 : The organ length and organ width (heart) of sheep fed with fresh and ensiled *B. decumbens* were expressed as mean ± SEM. Significant differences were identified within the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

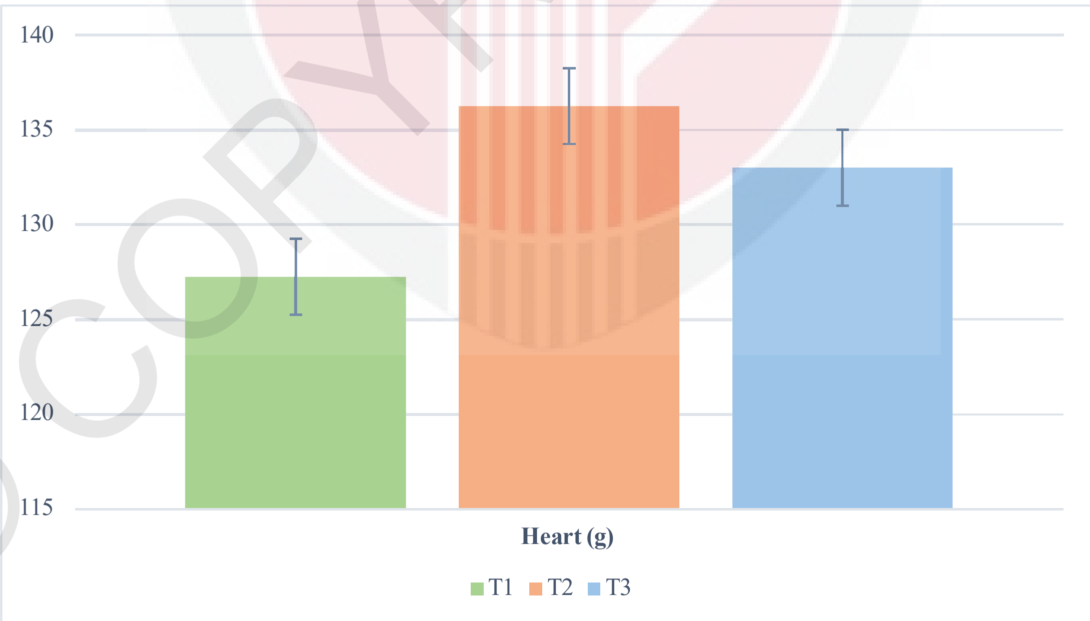

Figure 4.36 : The organ weight (heart) of sheep fed with fresh and ensiled *B. decumbens* were expressed as mean. Significant differences were identified within the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*).

Figure 4.37 : The organ length and organ width (lung) of sheep fed with fresh and ensiled *B. decumbens* were expressed as mean ± SEM. Significant differences were identified within the groups. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

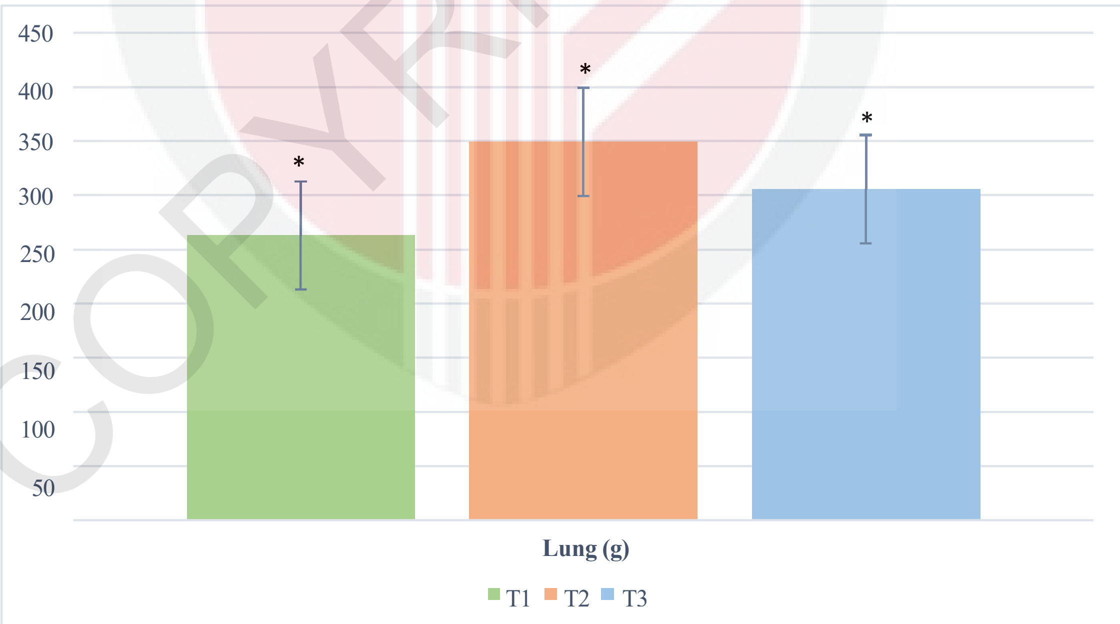

Figure 4.38 : The organ weight (lung) of sheep fed with fresh and ensiled *B. decumbens* were expressed as mean. Significant differences were identified within the groups. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

Figure 4.39 : The organ length and organ width (liver) of sheep fed with fresh and ensiled *B. decumbens* were expressed as mean ± SEM. Significant differences were identified within the groups. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

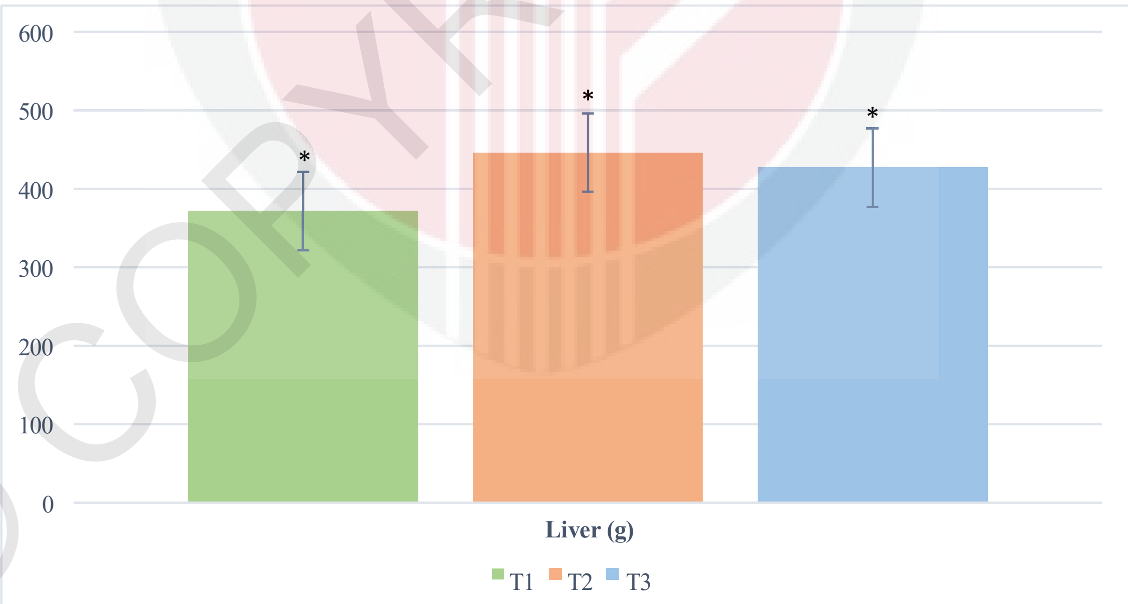

Figure 4.40 : The organ weight (liver) of sheep fed with fresh and ensiled *B. decumbens* were expressed as mean. Significant differences were identified within the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

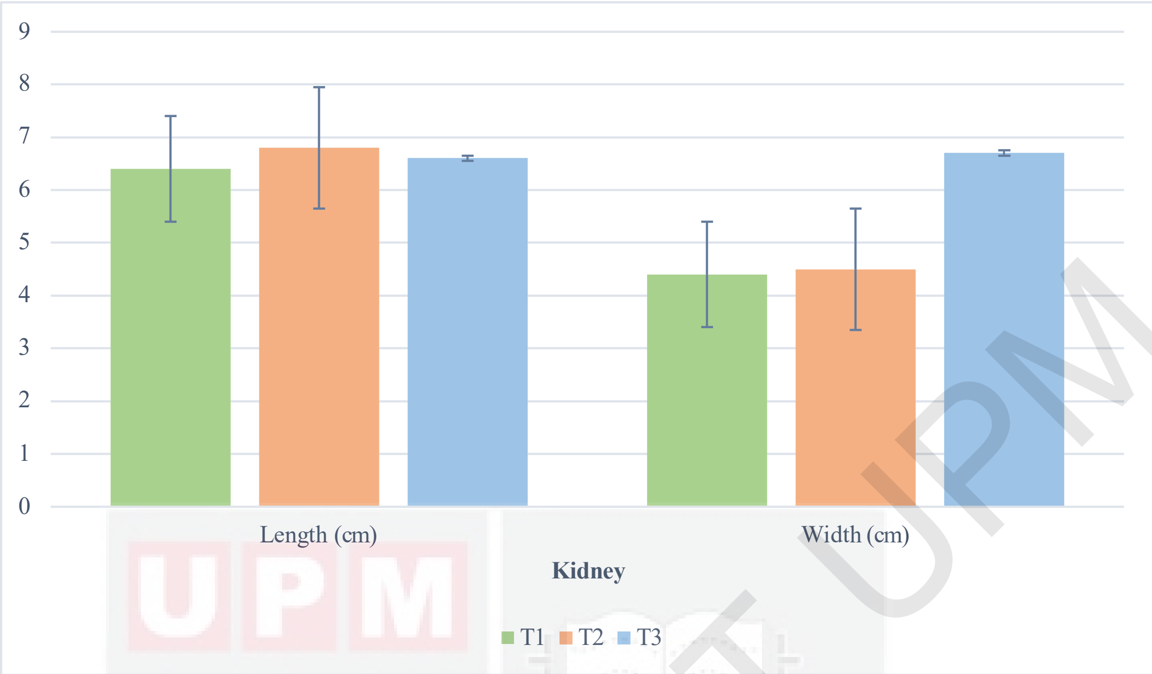

Figure 4.41 : The organ length and organ width (kidney) of sheep fed with fresh and ensiled *B. decumbens* were expressed as mean ± SEM. Significant differences were identified within the groups. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*).

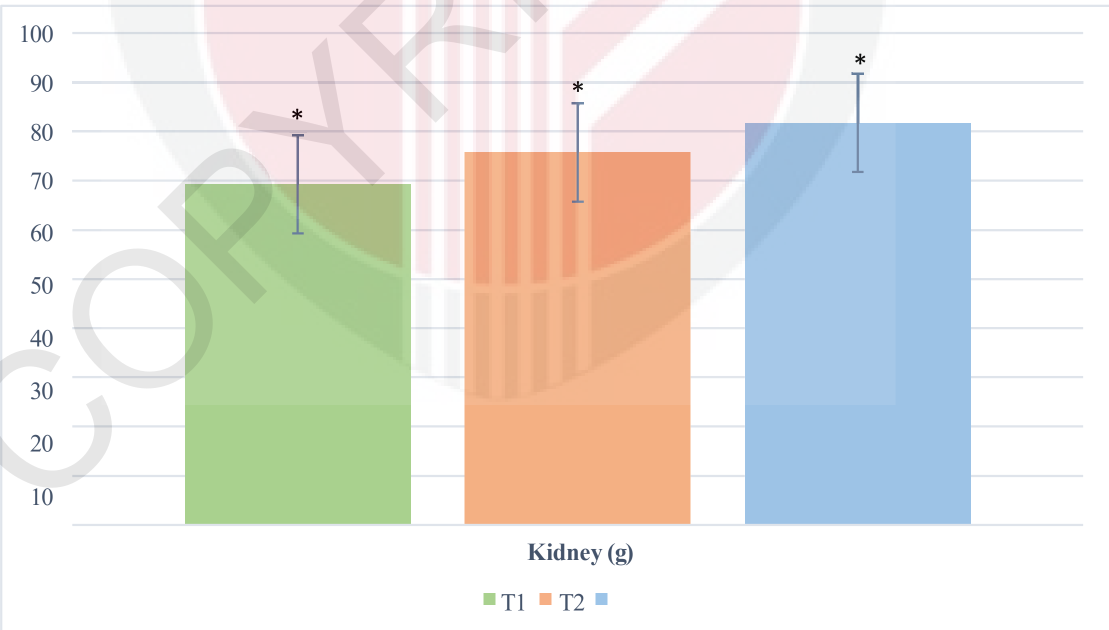

Figure 4.42 : The organ weight (kidney) of sheep fed with fresh and ensiled *B. decumbens* were expressed as mean. Significant differences were identified within the groups. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

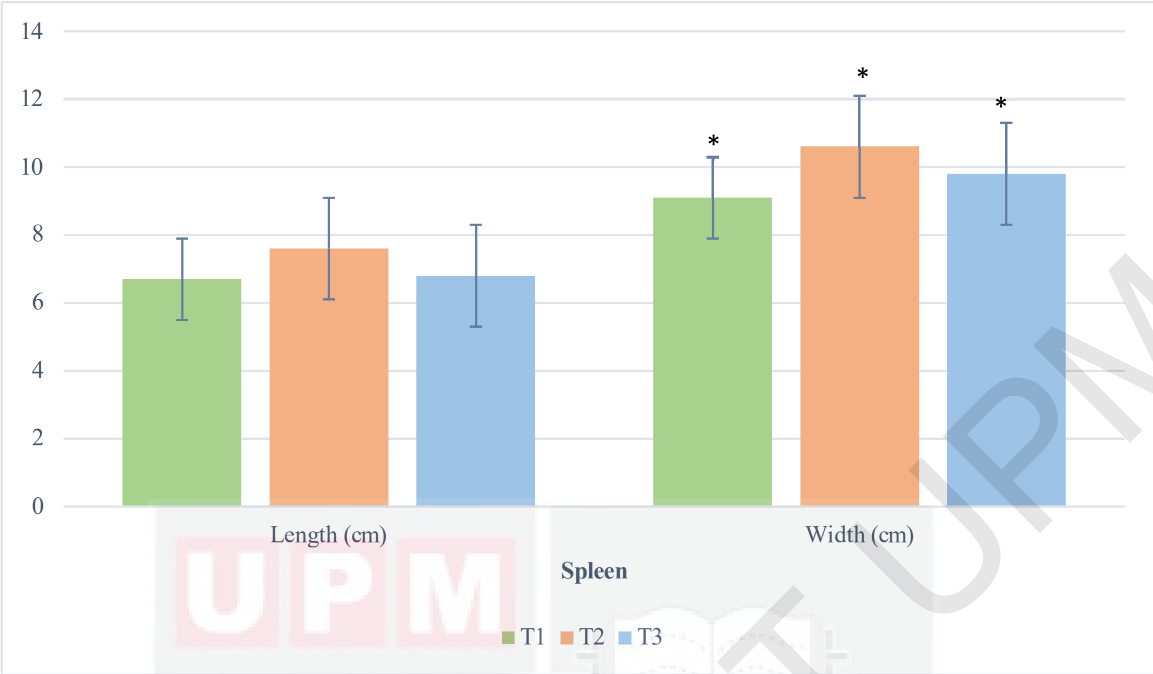

Figure 4.43 : The organ length and organ width (spleen) of sheep fed with fresh and ensiled *B. decumbens* were expressed as mean ± SEM. Significant differences were identified within the groups throughout the experiment. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

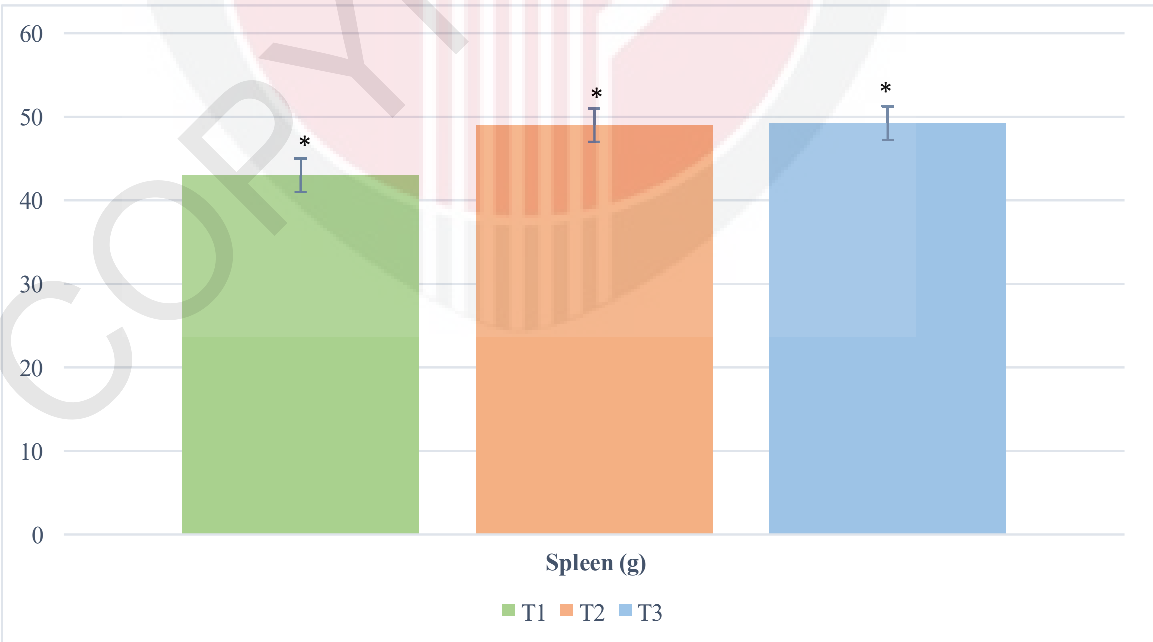

Figure 4.44 : The organ weight (spleen) of sheep fed with fresh and ensiled *B. decumbens* were expressed as mean. Significant differences were identified within the groups throughout the experiment. T1:

Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance between treatments.

Figure 4.45 : Pus accumulation on the caudal lobe of the right lung of sheep in Treatment 2 in two different sheep (white circle).

Physical and chemical changes that occur within an animal's body must be thoroughly understood in order to conduct a comprehensive analysis of gross and macroscopic pathology. In an experimental study, both organ weight and size, either absolute or relative to the body are sensitive markers of early toxicity. Overall, the examination of organ morphology in the current study indicated that sheep given ensiled *B. decumbens* exhibited an improved overall condition in terms of organ morphometric and gross morphology, compared to those given fresh *B. decumbens*. It is understood that the morphological alterations of an organ serve as indicators of animal performance. The liver plays a crucial role in the elimination of the poisonous compound known as steroidal saponin within *Brachiaria* pasture. Significant metabolic processes occur in the liver, including the conversion of exogenous substances or foreign substances into toxic metabolites. In this case, the size and relative weight of the liver within T3 group was the second highest, with an average of 1.3% of total body weight. This value acts as a general indicator of the physical performance of a young, growing animal. The average liver weight of sheep in the current study remained within the usual range of 1.3% to 1.9%, as reported by Li et al. (2021). According to Montanholi et al. (2017), there is a positive correlation between liver weight and both the nutritional plane and physiological stages. Conversely, variations in feed intake impact the metabolic rate of visceral organs, subsequently generating blood flow changes that influence the organ morphology and the metabolic activity of the tissues (Montanholi et al., 2017). Moreover, Lima (2012) similarly found no macroscopic liver changes in *B. brizantha* hay-fed animals. The elimination of steroidal saponin through the anaerobic processes during ensiling may remove the main compound responsible for causing intoxication, due to the losses of sugar typically attached to sapogenin (Lima et al., 2015). The absence of toxic compounds within the grass would then allow the nutrients to be fully absorbed and utilized, hence leading to an improvement in the health of animals. Essentially, young growing sheep often exhibit a higher liver-to-body weight proportion compared to adult sheep, indicating a healthier overall condition. Therefore, it is suggested that the overall morphological performance of sheep in T3 was from the elimination of saponin in *Brachiaria* spp. through grass preservation. The liver of T3 was substantially larger than that of the control group, indicating that *Brachiaria* grass had a higher nutritional content than *P. purpureum* grass. The observation that T2 sheep displayed the greatest overall morphological value may suggest the potential adverse effects resulting from the ingestion of saponin, as evidenced by histological findings.

Following the present findings, different treatments resulted in Treatment 2 sheep having an apparent enlargement among the heart, lungs, liver, and spleen of sheep. The organs from the sheep fed with fresh *B. decumbens* (Treatment 2) exhibited the most significant measurement in comparison to those from Treatment 1 and Treatment 3. The ingestion of foreign toxic compounds, such as steroidal saponin, has the potential to induce hepatic reactions that are not classified as intoxication effects. The liver potentially undergoes adaptation in reaction to the saponins, thereby adjusting to new environments or conditions. While several adaptive responses of hepatocytes are primarily metabolic, morphological changes can be observed as part of the observation including active enlargement of other organs (de Oliveira et al., 2013). However, it is still possible for liver damage to happen without visible clinical signs or photosensitization. Principally, the occurrence of hepatic intoxication can result in either a decrease or an increase in liver size. The decrease in size can be attributed to either acute or chronic hepatic injury, leading to the loss of cells; while an enlarged liver is a result of either hepatocyte proliferation or hepatocyte hypertrophy. In liver cell regeneration, injuries like infections or toxic damages will turn mature hepatocytes, biliary epithelium, and endothelial cells into actively proliferating cells until a normal functional mass is restored. The increase in cell proliferation, as well as the macrophages due to inflammatory responses of saponin, may contribute to the increase in organs' measurement in Treatment 2 sheep.

For instance, an outbreak was previously reported observing hepatomegaly, with lobular, brown-yellowish colored liver as well as lumpy gallbladder in the animals (Kono et al., 2022). In another study conducted by Carmo (2021), the necropsy findings involved the presence of extensive bedsores, erythema, severe jaundice, an enlarged liver with bulging edges and greenish color, as well as a pale brownish, irregular, mottled subcapsular surface which were consistent with the clinical and laboratory findings described in earlier reports identifying saponins as the source of the disease as acknowledged by histopathology. The current study suggests that organ proliferation, primarily the hepatomegaly, is likely a result of the symptoms leading to cholangiohepatitis. It is also possible that the absence of icterus observed in the present study was due to the short duration of the trial, insufficient to cause biliary duct obstruction (cholestasis) through crystal formation, thus preventing the excess of bile pigments in the liver of the animal. These findings were further substantiated through clinical and histological data, which included the identification of inflammatory cells in several organ areas and the absence of symptoms of photosensitization. In addition, the inflammation in cases of cholangiohepatitis tends to be characterized by increased cell growth rather than the presence of fluid or pus. The liver is enlarged and may exhibit either a normal shape or distortion due to uneven regions of atrophy and regenerative hyperplasia, and may be smooth on the surface. In cases of a blockage in the ducts, bile pigments can accumulate in the areas supplied by the blocked ducts; however, the occurrence of systemic icterus or photosensitization is relatively uncommon unless a significant part of the liver is damaged (Cullen & Stalker, 2016).

Additionally, the accumulated evidence also supports the current results showing pus accumulation and infection on the lungs observed in both macroscopic and microscopic reports on Treatment 2 sheep. These abnormalities included congested left and right lung lobes, buildup of pus in the caudal lobes, and hemorrhages upon cutting the surface of the lungs. Although the post-mortem report only exhibited lung abnormalities, the overall pathology correlates with the health performance and blood results of the affected sheep within other organs. Through pieces of evidence displayed in the biochemistry data of the present study, elevated levels of AST and GGT in Treatment 2 sheep indicated liver dysfunction, possibly resulting from biliary hyperplasia, bile duct proliferation, or cholestasis, as confirmed by the detected increase in organ measurement. These injuries could serve as a fundamental starting point for ongoing effects in the lungs. Hepatocytes are essential components of the innate immune system, and they play a significant part in regulating an overall inflammatory response. Despite the liver being primarily responsible for the bioactivation of metabolites of steroidal saponins through cytochrome P450 activity, other cells such as club cells in the lung may generate these metabolites (Cullen & Stalker, 2016). The bioactivation further leads to hypertrophy within the cells, potentially contributed to the inflammatory cell response in the lungs. Correspondingly, Rosa (2016) described a study where three goats displayed respiratory symptoms due to a case of bronchopneumonia, affecting the right side unilaterally when fed with *B. decumbens*. Goats suffering from pneumonia showed pulmonary symptoms defined by areas of consolidation, hyperemia, emphysema, and infrequently, the development of abscesses in the lungs. However, the findings from a prior study using lambs fed with *B. brizantha* hay indicate that animals did not experience any decrease in performance and did not display any visible signs of poisoning (Lima et al., 2012). As previously stated, the compromised immune response is likely to result in an onset of nosocomial bacterial infection, specifically pasteurellosis, which can subsequently progress to pneumonia. With clinical findings and morphological data indicating similar symptoms to pasteurellosis, it is worth mentioning that the probable explanation for the evidence was saponin poisoning from *B. decumbens* in T2 group during the study.

In summary, the preservation of *B. decumbens* grass was shown to have a positive effect on the overall morphological performance of the sheep, while the group fed with fresh *B. decumbens* identified an incidence of saponin toxicity. Ensiling was proven to have eliminated the saponin within the grass, which has also reduced the photosensitization effects on the sheep, as well as maintaining good morphological features of the animal. The elimination of saponin during the anaerobic respiration caused an improved performance within the cellular levels, which prompted animals to fully absorb the nutrients from the *B. decumbens* grass. These findings highlight the important role of grass preservations in reducing the harmful effects of steroidal saponin exposure across the morphological structure of sheep. The improved digestibility and effective absorption will further increase the overall economic viability of the small ruminant industry. As emphasized, it is a plausible replacement for the farmers to utilize the grass to avoid the intoxication effects from *B. decumbens*.

4.3.2 Histomorphology

Table 4.13 includes the histological lesion observed in the organs of sheep that were fed with fresh *P. purpureum*, fresh *B. decumbens*, and ensiled *B. decumbens*. The four histological lesions classified were the presence of inflammatory cells, necrosis or degeneration, the accumulation of edematous fluid, and congestion or hemorrhage throughout several organs involving the brain, heart, lung, liver, kidney, spleen, duodenum, jejunum, and ileum.

For the inflammatory cell lesions, significant differences ($p < 0.05$) in lesion scoring were observed in multiple organs, such as the lung, liver, jejunum, and ileum among feeding groups. Sheep fed with fresh *P. purpureum* and ensiled *B. decumbens* had no inflammatory cell lesions in all the organs mentioned. Comparatively, T2 sheep fed with fresh *B. decumbens* exhibited mild to moderate inflammatory cell infiltration in the lung organs, while the other organs demonstrated mild lesions. The presence of these inflammatory cell infiltration may be observed as indicated in the lung (inter-alveolar septum), liver (hepatocytes), jejunum (lamina propria), and ileum (lamina propria) of the sheep fed with fresh *B. decumbens* (Figure 4.49, 4.53, & 4.54).

The study also reported statistically significant changes ($p < 0.05$) in the scoring of necrosis and degeneration lesions within the liver and jejunum. It was indicated that the liver and jejunum of both T1 and T3 sheep fed with fresh *P. purpureum* and ensiled *B. decumbens* displayed no lesion scores. On the other hand, T2 sheep given with fresh *B. decumbens* presented a relatively mild lesion score, as shown in the findings. The evidence of these mild degenerations can be seen in the liver (hepatocytes) in Figure 4.49.

On the other hand, there were no significances ($p > 0.05$) on the scoring of edematous fluid among the different organs, showing zero lesion scores across all treatments except for the lung organ. Similarly, T2 sheep given with fresh *B. decumbens* exhibited mild edematous fluid accumulation in the alveolar spaces (Figure 4.48).

Furthermore, the results indicating congestion and hemorrhage lesions showed statistically significant differences ($p < 0.05$) on several organs, specifically the lung, liver, kidney, and ileum. There were no lesions reported on the organs of both T1 and T3 sheep that were fed with fresh *P. purpureum* and ensiled *B. decumbens*, showing no significant difference ($p > 0.05$) between the two groups. By contrast, T2 lambs that were given fresh *B. decumbens* exhibited mild to moderate lesion scores indicating hemorrhages on the lung, followed by none to mild lesion scores showing hemorrhages on the liver, kidney, and ileum. These hemorrhage lesions are identified in the lung (alveoli), kidney (cortex) and ileum (lamina propria) of the sheep that were given fresh *B. decumbens*, as presented in Figure 4.48, 4.50 and 4.54.

Overall, sheep fed with ensiled *B. decumbens* exhibited more favorable histological results when compared to those fed with fresh *B. decumbens*.

Table 4.11 : Histological lesions of sheep fed with fresh *P. purpureum*, fresh *B. decumbens*, & ensiled *B. decumbens*.

	Treatment 1	Treatment 2	Treatment 3	p-value
Brain				
Inflammatory cells	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Necrosis/ Degeneration	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Edematous fluid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Congestion/ Hemorrhage	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Heart				
Inflammatory cells	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Necrosis/ Degeneration	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Edematous fluid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Congestion/ Hemorrhage	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Lung				
Inflammatory cells	0.00 ± 0.00^b	1.45 ± 0.10^a	0.00 ± 0.00^b	<0.01
Necrosis/ Degeneration	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Edematous fluid	0.00 ± 0.00^b	0.10 ± 0.03^a	0.00 ± 0.00^b	0.03
Congestion/ Hemorrhage	0.00 ± 0.00^b	1.60 ± 0.11^a	0.00 ± 0.00^b	<0.01
Liver				
Inflammatory cells	0.00 ± 0.00^b	0.50 ± 0.07^a	0.00 ± 0.00^b	<0.01
Necrosis/ Degeneration	0.00 ± 0.00^b	1.00 ± 0.09^a	0.00 ± 0.00^b	<0.01
Edematous fluid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Congestion/ Hemorrhage	0.00 ± 0.00^b	0.11 ± 0.04^a	0.00 ± 0.00^b	0.03
Kidney				
Inflammatory cells	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Necrosis/ Degeneration	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Edematous fluid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00

Congestion/ Hemorrhage	0.00 ± 0.00[a]	0.83 ± 0.05[b]	0.00 ± 0.00[a]	<0.01
Spleen				
Inflammatory cells	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Necrosis/ Degeneration	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Edematous fluid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Congestion/ Hemorrhage	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Duodenum				
Inflammatory cells	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Necrosis/ Degeneration	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Edematous fluid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Congestion/ Hemorrhage	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Jejunum				
Inflammatory cells	0.00 ± 0.00[b]	0.67 ± 0.09[a]	0.00 ± 0.00[b]	<0.01
Necrosis/ Degeneration	0.00 ± 0.00[b]	0.17 ± 0.05[a]	0.00 ± 0.00[b]	0.04
Edematous fluid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Congestion/ Hemorrhage	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Ileum				
Inflammatory cells	0.00 ± 0.00[b]	0.83 ± 0.11[a]	0.00 ± 0.00[b]	<0.01
Necrosis/ Degeneration	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Edematous fluid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00
Congestion/ Hemorrhage	0.00 ± 0.00[b]	0.39 ± 0.07[a]	0.00 ± 0.00[b]	<0.01

Note: All values were expressed as mean ± SEM; [a, b] values with superscript within the same row are significantly different at p<0.05. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens.*

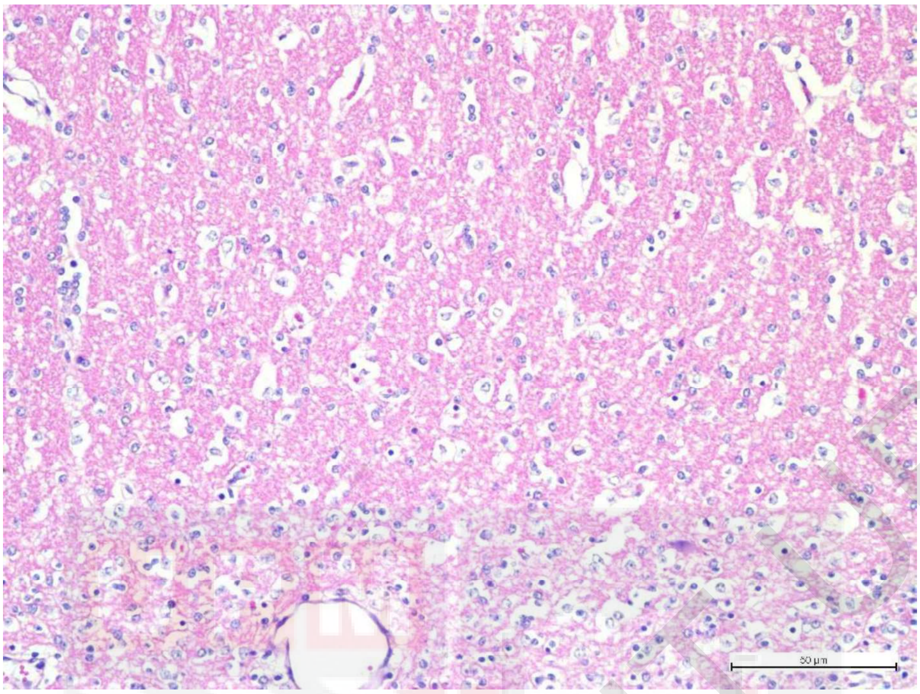

Figure 4.46 : A photomicrograph illustrating a normal cerebrum section of sheep within Treatment 3. H&E stain, 20x magnification, scale bar 50µm.

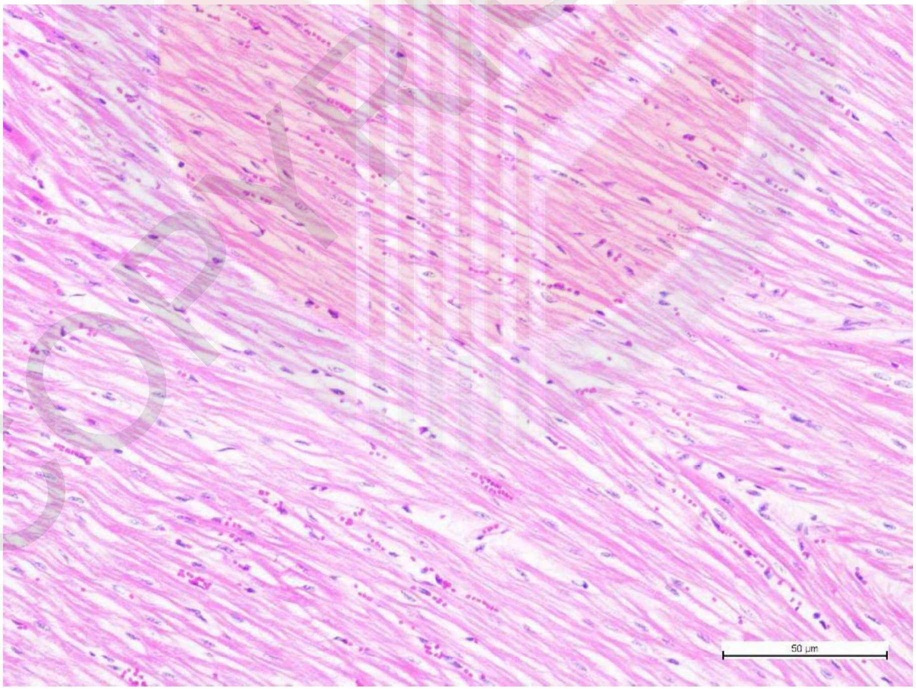

Figure 4.47 : A photomicrograph of a normal heart section of sheep within Treatment 3. H&E stain, 20x magnification, scale bar 50µm.

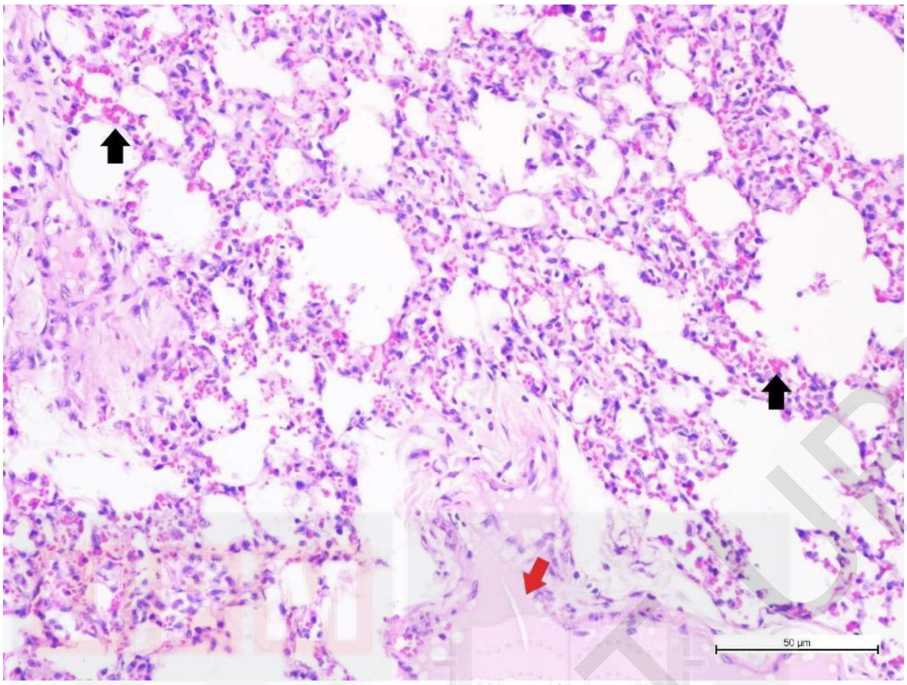

Figure 4.48 : A photomicrograph of the lung section of sheep within Treatment 2 displaying moderate hemorrhage (black arrow) along with edematous fluid (red arrow). H&E stain, 20x magnification, scale bar 50µm.

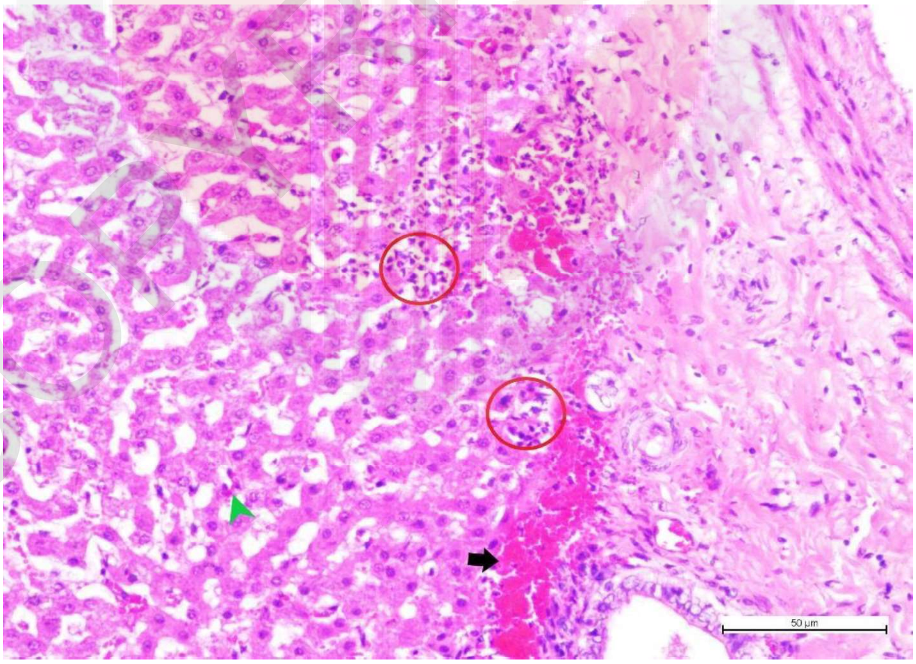

Figure 4.49 : A photomicrograph of the liver of sheep in Treatment 2 exhibiting mild hemorrhage (black arrow), inflammatory cell infiltration (red circle), and mild degeneration (green pointed arrow). H&E stain, 20x magnification, scale bar 50µm.

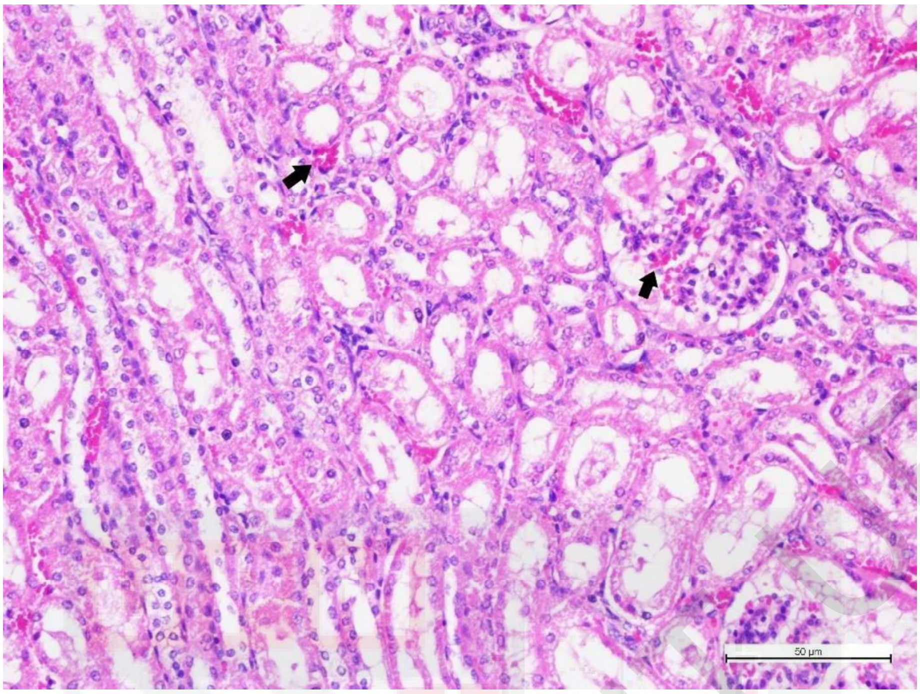

Figure 4.50 : The photomicrograph displays the kidney of sheep from Treatment 2 exhibiting a slight hemorrhage (black arrow) on the glomerulus and the adjacent tubules. H&E stain, 20x magnification, scale bar 50µm.

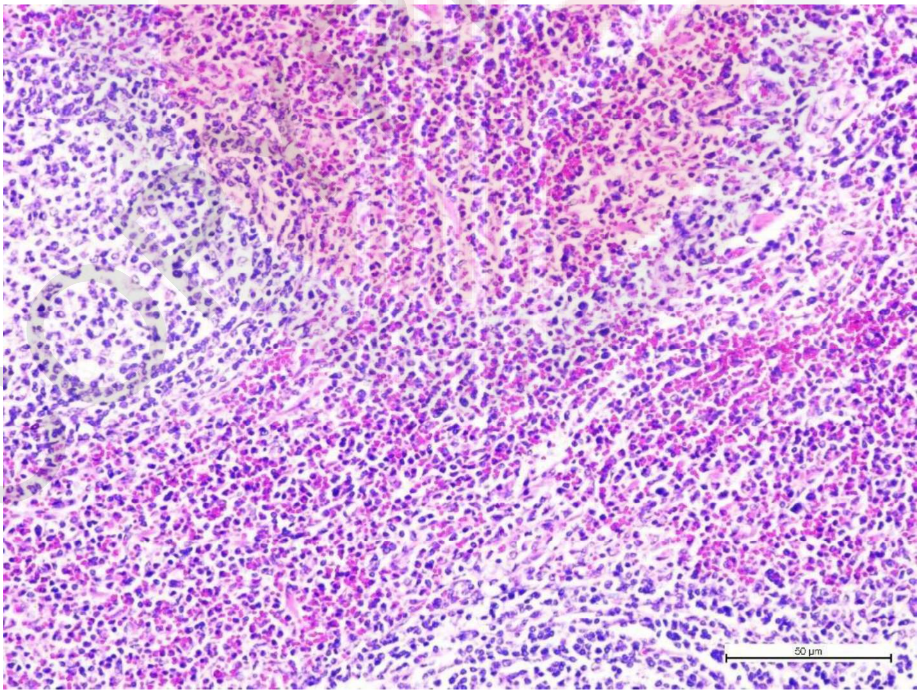

Figure 4.51 : A photomicrograph of a normal spleen of sheep from Treatment 3. H&E stain, 20x magnification, scale bar 50µm.

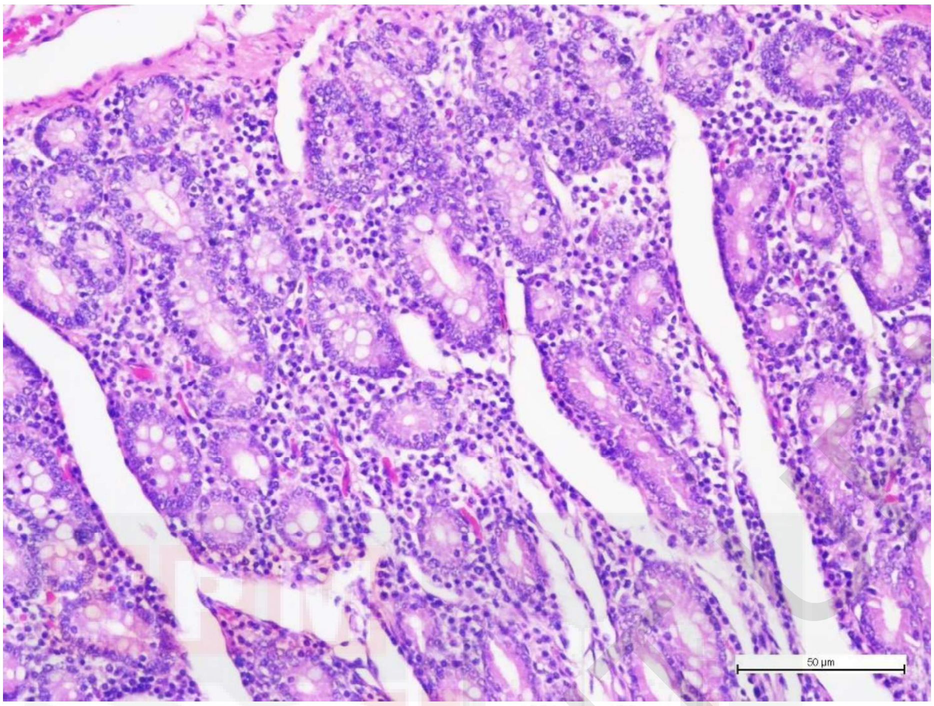

Figure 4.52 : A photomicrograph exhibiting a normal duodenum of sheep from Treatment 3. H&E stain, 20x magnification, scale bar 50µm.

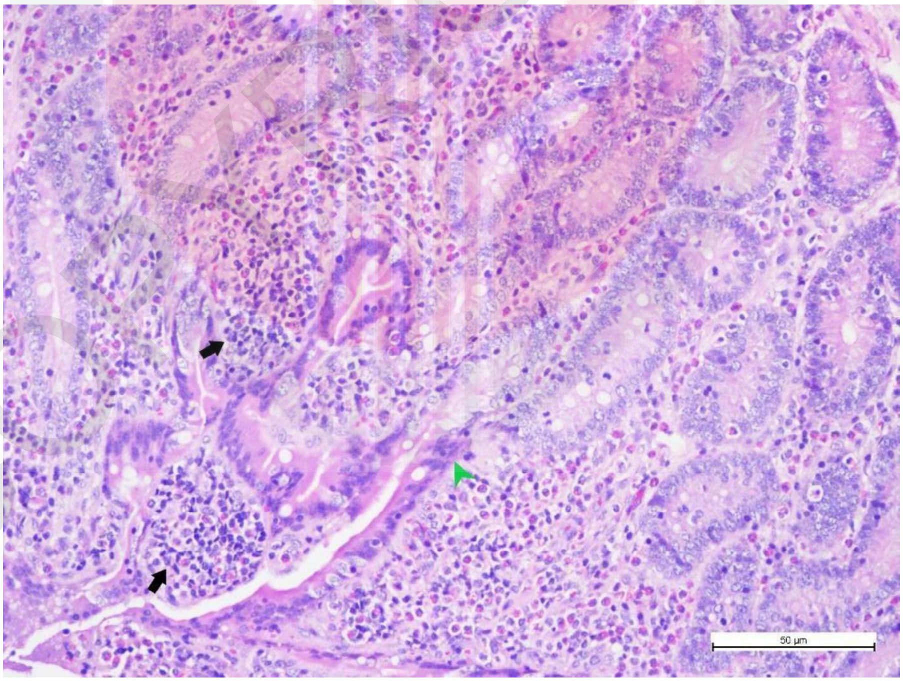

Figure 4.53 : A photomicrograph of the jejunum section of sheep within Treatment 2 displaying inflammatory cells infiltration (black arrow) and mild degeneration (green pointed arrow). H&E stain, 20x magnification, scale bar 50µm.

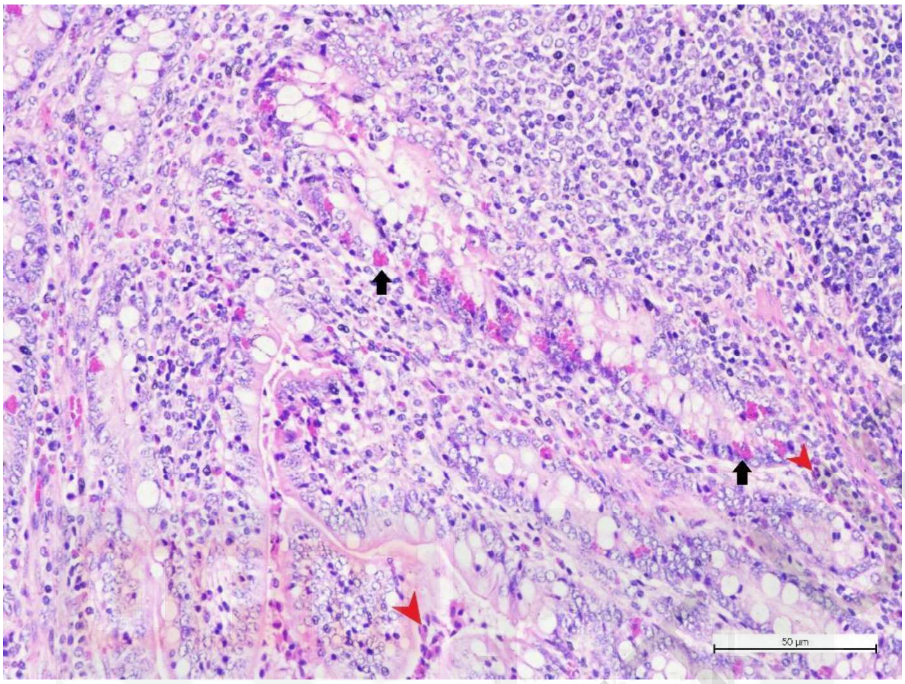

Figure 4.54 : The photomicrograph illustrates the ileum of sheep in Treatment 2 with evidence of inflammatory cell infiltration (red pointed arrow) and slight hemorrhage (black arrow). H&E stain, 20x magnification, scale bar 50µm.

The study of both macroscopic and microscopic analyses are essential to investigate the cause of a disease and provide additional proof of injury to an animal. Among all the organs, the liver maintains significant regulatory control over numerous proinflammatory damages and inflammatory mediators. The effects of saponin toxicity can result in different histological changes in the liver, which can impair the organ's function and ultimately affect the overall health of the animal. Sheep within Treatment 3 demonstrated more favorable histological outcomes in comparison to those that were administered fresh *B. decumbens*. General histological lesions caused by saponin consumption of fresh *B. decumbens* may result in cytoplasmic crystal buildup; however, this has not been seen in sheep from the T3 group. Similarly to the control sheep, no obvious histomorphological lesions were detected in all studied organs, indicating the absence of steroidal saponin. All saponins consist of an aglycone, which is a non-sugar molecule linked by glycosides. The process of breaking down sugar chains through anaerobic respiration via ensiling generally has been suggested to have gradually lowered and removed the concentration of saponin over time. The anaerobic ensiling conditions stimulated the activity of fermentative microbiota, leading to the production of certain metabolites and the breakdown of others (Ayemele et al., 2024). On the other hand, the microsomal component of the liver contains membrane-bound cytochrome P450 proteins which may facilitate bile acid synthesis and rendering toxic compounds such as steroidal saponin derived from *B. decumbens* to be less toxic and soluble for excretion. In this case, these proteins present in the interior membranes of steroidal tissues are also responsible for both the synthesis and degradation of endogenous steroid hormones, further contributing to the elimination of steroidal saponins. It is inferred that the absence of steroidal saponins through ensiling promotes a healthier development in all involved organs. The liver and other organs are protected by minimizing contact with steroidal saponins, thus maintaining its physical structure and ability to function properly. Histological examinations of the liver within T3 group revealed a reversal of the negative effects associated with hepatogenous intoxication, such as inflammatory cell lesions, necrosis, and hemorrhages across hepatocytes and other cells. Thereby, the prevention of biliary lesions through grass preservation can help reduce hepatogenous photosensitization, which has the ability to break down the sugar chain of saponins and the chlorophyll of *B. decumbens* grass. Removing saponin from the feed improves the safety of animal intake. In this regard, ensiling *B. decumbens* outperforms both the control group and Treatment 2 groups, as the grass maintains its nutritional value.

On the other hand, significant differences ($p < 0.05$) in lesion scoring for inflammatory cell lesions were reported in multiple organs, including the lung, liver, jejunum, and ileum, across different treatment groups, in which Treatment 2 sheep (fresh *B. decumbens*) displayed mild to moderate infiltration of inflammatory cells in the lungs, whereas the other organs displayed mild lesions. Kupffer cells present in the liver have a significant function in the processes of inflammation, damage, and regeneration in the liver. The reabsorption of bile components by biliary epithelium causes neutrophilic inflammation and local edema. Under microscopic examination, the current study displayed that steroidal saponin from *B.*

decumbens grass would initiate the Kupffer cell phagocytosing of the toxins, serving as the final defense of gut barrier. It is suggested that the constant intake of *B. decumbens* containing saponin leads to a disruption of the inflammatory response, which then causes moderate inflammation of the liver. The moderate severity of the microscopic injury in the liver may be due to the lower intensity of saponin concentration, which does not cause enough intoxication within the allocated time of the study. Similar responses were hypothesized for pulmonary and intestinal organs such as lung, kidney, spleen, jejunum, and ileum which showed these histological lesions. Moreover, although lesions were observed in Treatment 2 sheep, the saponin concentration was insufficient to cause additional crystallization as commonly seen in previous studies. It is also worth noting that the possibility of identifying macrophages is much more frequent compared to the crystal formation induced by saponin, which may not always be visible in bile ducts (Cullen & Stalker, 2016). This is further evidenced by a prior study reporting that the hepatic periportal areas displayed mild enlargement due to the presence of connective tissue (fibrosis), bile duct hyperplasia, and a mild infiltration of lymphocytes and macrophages (Kono et al., 2022). Extended exposure to low levels of the toxin is a more frequent issue than sudden poisoning in larger animal species. This exposure may result in decreased growth rates and mild liver enlargement, without any notable indicators of liver damage. The enlargement may be partially attributed to the hypertrophy of hepatic smooth endoplasmic reticulum and an extent of fatty change. In addition, the differences in histologic reactions between cattle and sheep may arise when they graze on plants that contain sapogenin, where it is more common to observe the presence of foamy Kupffer cells and hepatocytes as well as foamy macrophages in cattle. In contrast, crystal formation within bile ducts is more frequently observed in sheep (Cullen & Stalker, 2016).

Additionally, the present study also reported significance ($p < 0.05$) in the scoring of necrosis and degeneration lesions in the liver and jejunum where sheep fed with fresh *B. decumbens* had a relatively mild lesion score, as indicated. Saponins are recognized for their ability to inhibit the growth of ciliated protozoa, the reduction of which results in an increase in volatile fatty acid production, which in turn elevates oxidative stress and induces liver damage (Kono et al., 2022). After profound necrosis, all remaining hepatocytes are expected to replicate, but random localized necrosis will cause local proliferation. In centrilobular necrosis, which is frequent in toxic damage, complete repair is typically achieved within 24–48 hours. However, consistent intake of *B. decumbens* grass containing saponin may interfere with cell reparation, causing further necrosis and degeneration within the organs. From a histological perspective, the livers that suffer from saponin toxicity display a clear enlargement of certain hepatocytes and their nuclei (known as megalocytosis), along with localized necrosis or apoptosis. In cases of more severe intoxications, there can be coagulative necrosis of blood vessel walls in the portal triads. In situations when this necrosis is not complete, the most affected section of the vessel tends to be the one closest to the damaged bile duct. Both arteries and veins can be adversely affected, and it is plausible that necrosis is associated with both vascular insufficiency and insufficient biliary drainage. Montoya-Ménez (2019) demonstrated photosensitization in crystal-associated cholangiohepatopathy caused by protodioscin targeting hepatocytes and biliary cells. Hepatocyte and biliary cell damage induced free radicals, mast cell degranulation, and inflammatory mediator release, causing epidermal necrosis. Other ruminants also show photosensitization like sheep's cutaneous lesions (Driemeier et al., 2002; Gracindo, 2014).

Further results described that only the sheep that were given fresh *B. decumbens* showed mild edematous fluid accumulation in the alveolar spaces of the lung. Subsequently, the report also indicated statistically significant results ($p < 0.05$) in congestion and hemorrhage lesions across multiple organs, namely the lung, liver, kidney, and ileum. Lesion was absent in all sheep from Treatment 3 and control group. In cultivated plants, saponins became known to decrease nutrient utilization and conversion efficiency in ruminants. Saponins also operate as a growth inhibitor in monogastric animals by reducing their feed intake due to their bitter nature and irritating the throat of animals (Kemboi et al., 2023). It is described that saponins from several plants in the forage were discovered to induce ruminant bloat and photosensitization, and impact protein digestibility by inhibiting several digestive enzymes like trypsin and chymotrypsin. Nonetheless, pulmonary edema and other nonspecific histologic alterations can be useful in determining the pathophysiology of animals. Nevertheless, it is crucial to visually and histologically examine all tissue components before evaluating a specific characteristic such as pulmonary edema, especially in cases when lung tissue has undergone significant postmortem autolysis. If there is clinical evidence of pulmonary edema, submitting samples of the heart, liver, and lungs will aid in determining its severity.

To conclude, histological results revealed that sheep under Treatment 3 and control group did not exhibit any of the identified lesions in any of the specified organs. Additional variables, such as age, nutritional status, sex, food, previous or simultaneous exposure to environmental substances, or associated illness, all impact the body's reaction to toxic damages, thus influencing the divergences observed in the way species adapt to liver injury. The probable suggestions mentioned earlier points out the absence of saponin in ensiled *B. decumbens* after the preservation process. These reports further highlight the benefit of ensiling grasses with saponin compound, which effectively prevent the health deterioration in animals.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

In practicality, farmers are concerned with the use of *B. decumbens* due to its recognized toxicity to ruminants. Ruminant performance may be analyzed throughout the study when using ensiled *B. decumbens* as a substitute. This study describes how utilizing ensiled *B. decumbens* may aid in understanding the availability of nutrients within the silage, as evidenced by health performance, blood biochemistry, and histomorphology results.

In the health performance of the animals, the successful elimination of the toxicity effects on sheep was observed through the utilization of ensiled *B. decumbens*, where animals are clinically healthier. The steroidal saponin compound in *B. decumbens* grass is eliminated by ensiling, as evidenced by the absence of clinical responses and an overall improvement in body weight gain and vital signs. The main purpose of this study was further highlighted in hematological and biochemical indices of T3 which resulted in a healthier sheep, effectively eliminating hepatoxicity effects resembling those caused by feeding fresh *B. decumbens*. The blood indices exhibited no significant differences ($p>0.05$) across all sheep as compared to the control group. More importantly, the successful preservation of *B. decumbens* grass through ensiling may enable farmers to prevent potential impacts on ruminants, particularly sheep in the future through effective ensiling of this grass. Ensiled *B. decumbens* revealed normal blood levels, indicating an overall better outcome when compared to sheep fed with fresh *B. decumbens*. The evaluation of blood in the present study may have contributed to providing the evidence that highlights the safe consumption of ensiled *B. decumbens*. The process of ensiling *B. decumbens* completely removes the steroidal saponin compound detected in the grass, therefore addressing the concerns of farmers hesitant to use *B. decumbens* grass as the primary forage for sheep. A better understanding of how ensiling eliminates toxicity effects demonstrates the importance of this alternative over consuming fresh *B. decumbens* pasture. The present result also describes how ensiling effectively eliminated steroidal saponin compounds, thereby reducing photosensitization and maintaining good morphology. The histological lesions in the T3 group did not show any significance ($p>0.05$) as compared to the control group, indicating that incorporating ensiled grass is an effective alternative to remove steroidal saponin in *B. decumbens* grass. The removal of saponin during anaerobic respiration resulted in better performance in cellular function, allowing the sheep to fully absorb nutrients from *B. decumbens* grass. Thus, it is inferred that feeding ruminants with ensiled *B. decumbens* has been found to be more beneficial for the sheep compared to a diet consisting of fresh *B. decumbens*.

It was observed that only the sheep fed with fresh *B. decumbens* exhibited pale ocular mucous membranes throughout the final 21 days of the study. It has been suggested that the appearance of pale mucous membrane results from the presence of vasoconstriction such in the ocular area. The hepatic damage occurs throughout the lungs, gastrointestinal tract, and liver which receive more blood than needed to meet metabolic demands. These injuries cause localized vasodilation, reducing blood flow elsewhere. In addition, the gross pathology analysis revealed a statistically significant ($p<0.05$) difference between the width and weight of the organ, indicating the presence of inflammation among T2 sheep. The lung organ in T2 sheep showed a notable abnormality, particularly with the buildup of pus in the right caudal lobes. Sheep consuming fresh *B. decumbens* also had mild to moderate histological abnormalities in their lung, liver, kidney, jejunum, and ileum. The respiratory signs as observed in the group fed with fresh *B. decumbens* describes the rare occurrences of the saponin intoxication symptom present as pneumonia in small ruminants. This condition, in conjunction with liver damage caused by saponin intoxication, may contribute to the death of animals. The newfound result could assist in a more accurate diagnosis of symptoms related to saponin poisoning. This, in turn, could facilitate the correct identification and continued observation of clinical indications in saponin-affected animals. However, several parameters derived from T2 sheep exhibited subtle changes as evidenced by health performances and histomorphological data, indicating the presence of saponin within the fresh *B. decumbens* grass. The observed increase in organ size, particularly in the lungs where pus has accumulated, provides more evidence supporting the clinical symptoms observed in animals consuming fresh *B. decumbens*. Furthermore, the histological examination of the T2 group revealed mild to moderate lesions in multiple organs, suggesting that even a low concentration of steroidal saponin might cause histological damage in animals fed with *B. decumbens*. It is important to note that the presence of saponin, regardless of concentration, will cause hepatogenous complications, resulting in toxicity problems and other clinical indicators that can be eliminated through ensiling of grass.

The practical application of the results of this study has numerous advantages across different sectors of society, including academics, industry, community, and government sectors. The introduction of *B. decumbens* silage as a feed aid in understanding its value as a feed for ruminants, ensuring ideal practices for grass preservation. This study may also facilitate research opportunities, contributing through engaging with practical and applied agricultural sciences. Additionally, it may help to provide improvements in sustainable agriculture researches which maintains the resource efficiency through ensiling of grass in ruminant production. Moreover, this research findings offers the potential to improve small ruminant production through a better development of feeding strategies, resulting in increased productivity and profitability in the economic aspect. This research may assist farmers in developing innovative products using ensiled *B. decumbens*, thereby expanding feed options and promoting agricultural innovation. Utilizing local forages, such as *B. decumbens*, might decrease reliance on imported feed and enhance the economic sector. Through the use of silage, farmers may utilize the nutrient availability throughout the season, which may have helped further the animal productivity. Furthermore, this research has the potential to improve food security by addressing the nutritional requirements of small ruminants, resulting in increased meat production and higher quality of protein sources for the communities. The findings of this study might create an opportunity for training programs which focuses on grass preservation techniques, enabling farmers to improve their expertise through implementing efficient feeding strategies. The research findings may then improve the livelihood and productivity of farmers. Subsequently, improving the feeding practice through grass preservation may help to boost Malaysia's agricultural sector by supporting the smallholders or rural farmers. The improvements on the feeding practices may also diversify job opportunities through innovation of grass preservation techniques. Utilizing this information could positively impact the sheep sector in Malaysia, leading to improved sustainability and increased availability of pastures for farmers.

Nonetheless, although silage effectively eliminates saponin toxicity in *B. decumbens* grass, some limitations were found to remain linked with this preservation method. When it comes to the preparation of these grasses, the utilization of this procedure may vary depending on the availability of these grasses and the economic viability of farmers. Improper management during the ensiling process may lead to increased health risks from mycotoxins, consequently diminishing the nutritional value of the grasses. Improper storage during the ensiling process could also result in to a rapid temperature rise, which may adversely affect nutritional content and hence reducing animal performance (Adesogan & Newman, 2010). Various recommendations and new alternatives in maintaining the quality of silages have been done, but the cost of production may not be as inviting towards farmers, especially small-scale farmers.

Based on the findings of this study, several improvements could be considered to improve the availability of saponin-rich grasses. This study evaluates the effectiveness of removing saponins through preservation of *B. decumbens*. Further research can be conducted on other grasses facing the same issue, such as different *Brachiaria* spp. grasses, *Digitaria eriantha*, and other saponin-related tropical grasses. Different types of preservation method may be utlilized in the future study, such as hay-making. Further research on alternative forages could perhaps mitigate the toxicity issues that pose a threat to the industries in tropical countries. Additional studies may be conducted to explore the possible benefits of using ensiling for other toxin-related grasses to enhance the production performance of ruminants, particularly sheep.

In conclusion, the process of ensiling *B. decumbens* has been demonstrated to effectively eliminate saponin compounds present in the grass, hence enhancing the overall health performance of sheep. Introducing *B. decumbens* silage could enhance the utilization of the grass by farmers in Malaysia, mitigating any potential health risks and thus boosting the small ruminant industry.

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APPENDICES

Appendix 1 : IACUC approval letter

UPM
UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI

CERTIFIED TO ISO 9001:2015 CERT. NO.: QMS 00794

CERTIFIED TO ISO/IEC 27001:2013 CERT. NO.: ISMS 00150

PEJABAT TIMBALAN NAIB CANSELOR (PENYELIDIKAN DAN INOVASI)
OFFICE OF THE DEPUTY VICE CHANCELLOR (RESEARCH AND INNOVATION)

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Date:	28th February 2023
AUP No.:	UPM/IACUC/AUP-R003/2023
Project Title:	In Vitro Rumen Fermentation, Production and Reproductive Performance of Sheep Fed with Ensiled Brachiaria decumbens..
Principal Investigator:	Dr. Eric Lim Teik Chung
Members:	Nurul Adilah Binti Abu Bakar, Nurul Aishah Binti Ahmad Fauzi, Mohd Faizal Yeob, Hidayad Ali Arman Ali , Hairulnizam Mohd Sam.
Attending Veterinarian:	Dr. Eric Lim Teik Chung
Committee Decision:	The committee has reviewed and approved the proposed animal utilisation protocol, subject to relevant permit and/ or owner's consent.
Project Classification:	Chronic
Category of Invasiveness:	B
Source of Animals:	**Global Field Trading**, No. 12, Jalan 9/6 Taman IKS, Seksyen 9, 43650 Bandar Baru Bangi, Selangor Darul Ehsan.
Number of Animals Approved:	18 Sheep
Housing:	Metabolic crate, Ruminant Unit Ladang 15, Faculty of Agriculture. Slaughterhouse, Department of Animal Science.
Duration:	28th February 2023 – 28th February 2024

Ethical approval is required in the case of amendments to the approved animal utilisation protocol (AUP). Please apply using Form 105. Kindly submit a final/annual report (Form 106) upon study completion, or before expiry of approval.

PROF. DATO' DR. MOHD AZMI MOHD LILA
Chairman
Institutional Animal Care and Use Committee
Universiti Putra Malaysia

Pejabat Timbalan Naib Canselor (Penyelidikan dan Inovasi), Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
Pejabat Timbalan Naib Canselor (P&I) 603-9769 1002, Pejabat Pentadbiran TNCPI 603-9769 1608, Pejabat Pengarah, Pusat Pengurusan Penyelidikan (RMC) 603-9769 1610, Pejabat Pengarah, Putra Science Park (PSP) 603-9769 1291
http://www.tncpi.upm.edu.my

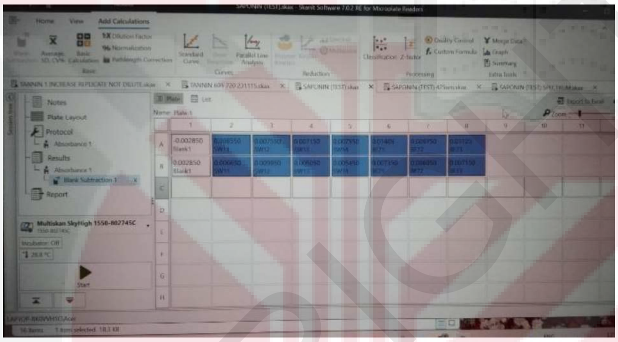
Appendix 2 : Sheep placed in a pen during 2-week acclimatization

Appendix 3 : Sheep in an individual metabolic crate

Appendix 4 : *B. decumbens* harvest in Farm 15, Universiti Putra Malaysia

Appendix 5 : *B. decumbens* ensiling process

Appendix 6 : Saponin analysis and quantification



Appendix 7 : Weekly health examination performed in all 18 sheep

Appendix 8 : Monthly blood collection via jugular venipuncture for further analyses

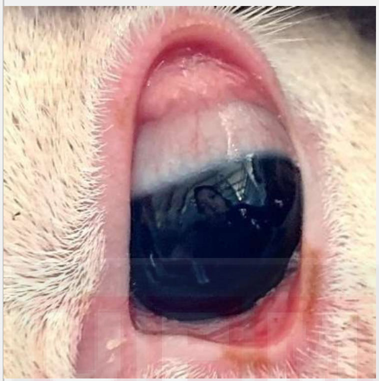
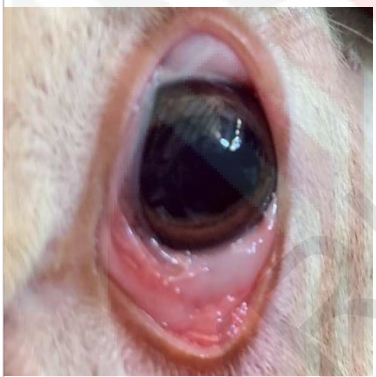
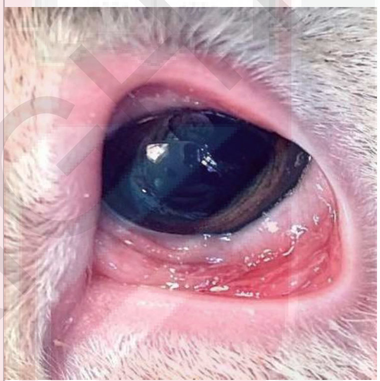
Appendix 9 : Morphological and histological sampling of all 18 sheep

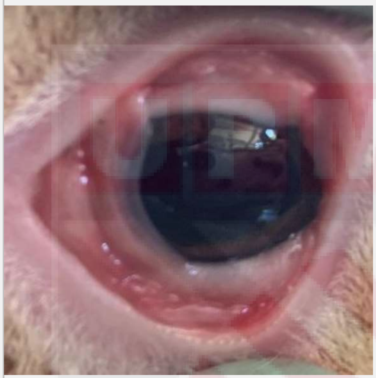
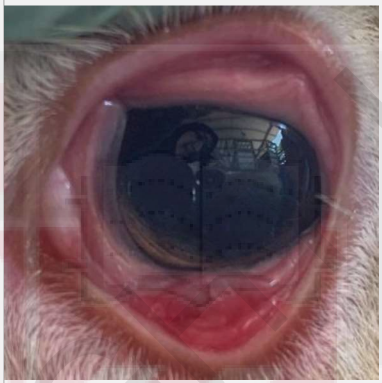
Appendix 10

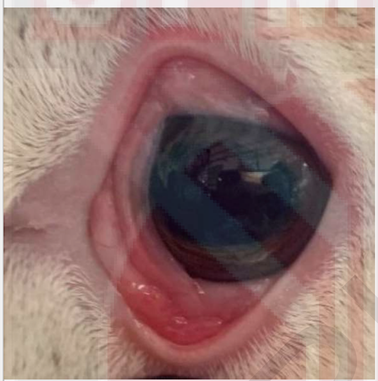
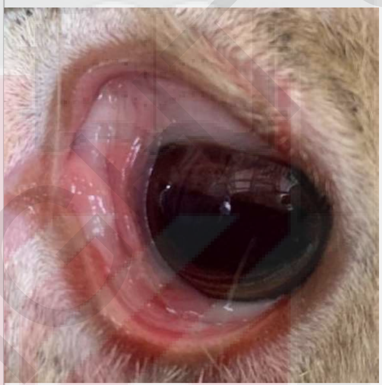
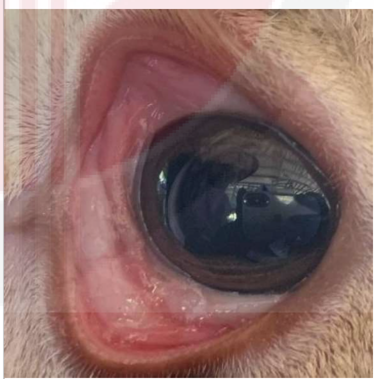
Treatment: 1 (control)

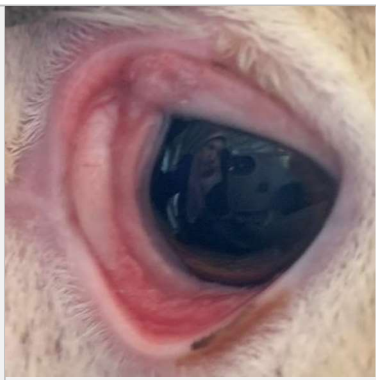
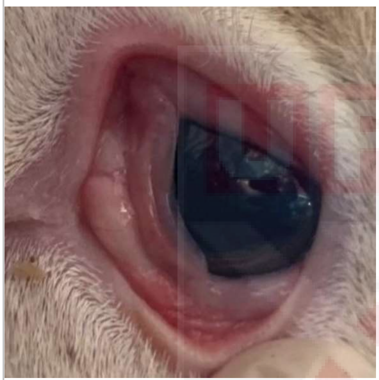
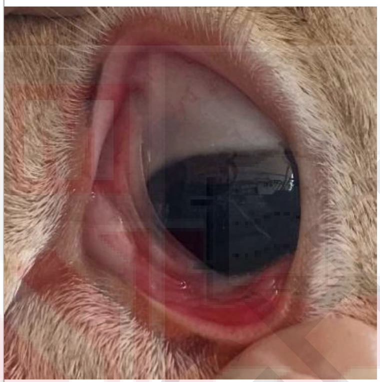
Day	ID	Cage	Rep	Temp (°C)	Pulse (bpm)	Resp. Rate (bpm)	Mucous Membrane	Rumen Motility
***baseline**				**38.6 - 39.6**	**70-90**	**20-40**	**pink**	**1**
0	T1	1	T1N1	39	88	28	pink	1
		2	T1N2	39	88	28	pink	1
		3	T1N3	39	92	28	pink	2
		12	T1N4	39.3	90	32	pink	2
		7	T1N5	38.5	95	36	pink	1
		8	T1N6	38.1	89	34	pink	2

Appendix 11 : Mucous membrane color of sheep fed with fresh *P. purpureum*, fresh *B. decumbens*, & ensiled *B. decumbens*.

Day	T1	T2	T3
0			
7			
14			

21			
28			
35			
42			
49			

			
56			
63			
70			

			
77			
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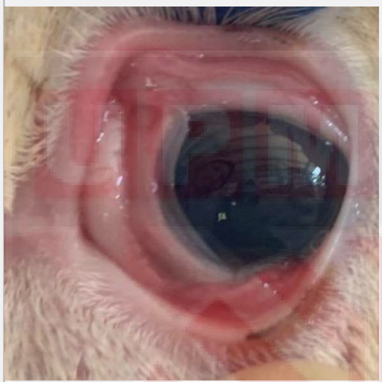
91			

			
98			

The mucous membrane of sheep fed with fresh and ensiled *B. decumbens* at various time intervals. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens*.

Appendix 12

Treatment: 1 (control) Organ: Brain

		Lesions			
Treatment	Rep.	Inflammatory cell	Necrosis/ Degeneration	Edematous fluid	Congestion/ Hemorrhage
T1-1	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
T1-2	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
T1-3	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
T1-4	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
T1-5	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
T1-6	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
Average		**0**	**0**	**0**	**0**

BIODATA OF STUDENT

The author was born in Johor, Malaysia. She completed her Bachelor's in Agriculture, majoring in Animal Science at Universiti Putra Malaysia in 2022. After graduating, she enrolled as a Master's student, majoring in Animal Nutrition at Universiti Putra Malaysia in October 2022.

LIST OF PUBLICATIONS

Fauzi, N. A. A., Chung, E. L. T., Bakar, N. A. A., Kamalludin, M. H., Reduan, M. F. H., Jesse, F. F. A., & Dunshea, F. R. (2025). Blood indices of sheep fed with ensiled signal grass (*Brachiaria decumbens*). *New Zealand Journal of Agricultural Research*, 1-18.