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Blood indices of sheep fed with ensiled signal grass (*Brachiaria decumbens*)

Nurul Aishah Ahmad Fauzi [a], Eric Lim Teik Chung [a,b], Nurul Adilah Abu Bakar [a], Mamat Hamidi Kamalludin [a], Mohd Farhan Hanif Reduan [c], Faez Firdaus Abdullah Jesse [d] and Frank R. Dunshea [e]

[a]Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Serdang, Malaysia; [b]Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Malaysia; [c]Department of Paraclinical Studies, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Kota Bharu, Malaysia; [d]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Malaysia; [e]School of Agriculture, Food and Ecosystem, Faculty of Science, The University of Melbourne, Parkville, Australia

ABSTRACT

The *Brachiaria* species, particularly *B. decumbens*, is a key ruminant feed in the tropics but contains steroidal saponins that may cause toxicity, raising concerns for small ruminant farmers. Eighteen 6-month-old sheep were randomly assigned to three treatments over a 90-day feeding trial: Negative control (fresh *P. purpureum*), fresh *B. decumbens*, and positive control (ensiled *B. decumbens*). Blood samples were collected on days 0, 30, 60, and 90 for haematology and biochemistry analyses. Fresh *B. decumbens* group exhibited significantly elevated ($p < 0.05$) haematological parameters, including red and white blood cell counts, plasma protein, and various liver, kidney, and serum protein markers. In contrast, positive control group showed no significant differences ($p > 0.05$) compared to the negative control. Despite these variations, some values remained within normal ranges. The findings suggest that feeding ensiled *B. decumbens* mitigates the adverse effects of steroidal saponins, promoting overall healthier outcomes in sheep compared to fresh *B. decumbens*. Ensiling *B. decumbens* is a promising strategy to address saponin toxicity and improve ruminant feed safety.

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Ensiled *Brachiaria decumbens*; steroidal saponin; haematology; biochemistry; Dorper cross sheep

Introduction

Among tropical and subtropical pasture plants, *Brachiaria decumbens* distinguishes out for its ability to provide forage for grazing ruminants (Idibu et al. 2016; Low 2015). It is one of the major pastures grown due to 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). However, this type of pasture still poses a risk towards farmers due to its

CONTACT Eric Lim Teik Chung fdunshea@unimelb.edu.au

association with toxicity and photosensitization effects among ruminants from its content of steroidal saponins (Brum et al. 2009; Lima et al. 2012; Muniandy et al. 2021b). 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 is associated with the formation of birefringent crystals, causing an increase in liver enzyme levels (Chung et al. 2018; Jaapar et al. 2022).

The haematological profile, along with morphological symptoms and biochemistry tests, can indicate a specific diagnosis or prognosis of an animal (Jesse et al. 2019). The blood indices, mainly complete blood count 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 (Chung et al. 2016; Polizopoulou 2010). In certain cases, 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* in ruminants (Muniandy et al. 2021b). 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).

Consequently, there is some studies demonstrating options for the elimination of steroidal saponins in *Brachiaria* spp. grasses. For example, biological treatments using urea, activated charcoal, polyethylene glycol, and effective microorganisms proved beneficial in deactivating anti-nutritional factors and enhancing the nutritive value of the feed but require a higher cost (Chung et al. 2018). On the other hand, mechanical treatments such as ensiling and haymaking were also found effective in reducing toxins and undesirable compounds in *B. decumbens* (Chung et al. 2018; Lima et al. 2012). The novelty of ensiling *B. decumbens* lies in its ability to significantly reduce steroidal saponin content, thereby mitigating the hepatotoxic and photosensitization risks associated with fresh consumption (Liu et al. 2018). This is because 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 pH lowering during ensiling may affect various phytocompounds such as saponin through degradation, suggesting the ability to eliminate saponin by ensiling. The process of anaerobic fermentation by the depletion of sugar compounds and chlorophyll degradation consequently removes saponin compound and eliminates intoxication effects (Muniandy et al. 2021b). This preservation method enhances the grass's safety and digestibility while maintaining its nutritional value, making it a viable, cost-effective alternative for livestock feed in tropical regions.

Practically, ensiled *B. decumbens* offers farmers a sustainable strategy to improve herd health, optimise feed availability during dry seasons, and reduce dependency on expensive commercial feeds, ultimately promoting more resilient and productive livestock systems. However, no animal feeding trials were conducted before to assess the treatments' effectiveness and the safety of the improved diets. A theory from Lima et al. (2015) indicated that ruminants fed with *B. decumbens* treated as silage or hay may

experience a decreased likelihood of protodioscin poisoning. Hence, feed conservation methods including silage production and haymaking, may ensure a reliable feed supply during times of limited feed availability. Therefore, the main focus of this study was to evaluate the haematology and biochemistry responses of Dorper cross sheep fed with ensiled *B. decumbens*.

Materials method

Forage planting and harvesting

Two grass species were included in this study: *Pennisetum purpureum* (Common Napier) and *Brachiaria decumbens* (Signal grass), which were used from already existing pasture at Farm 15, Research Farm, Department of Animal Science, Universiti Putra Malaysia from January 2023 until June 2023. The estimated area allocated for pasture regrowth in this study was 12.5 hectares per plant type, utilising a cut-and-carry approach for feeding. NPK green fertiliser (15:15:15) was also periodically used monthly to ensure constant grass regrowth during the feeding trial. The amount of fertiliser required throughout the trial was based on the formula shown below:

$$Amount\ of\ fertilizer\ kg/ha = [(kg/ha\ nutrient) \div (\%\ nutrient\ in\ fertilizer)]x100$$

Saponin concentrations were further determined by collecting fresh samples of *P. purpureum* and *B. decumbens* every week from week one to week ten of regrowth. Saponin was quantified using a spectrophotometric technique derived from Le Bot et al. (2022) in which the lowest saponin concentrations were observed starting from week 7 (Figure 1). Based on earlier research on fresh *B. decumbens* and *P. purpureum*, the grasses were harvested during week 7 to establish a balance of production and nutritional content (Castro et al. 2007; de la Ribera et al. 2008).

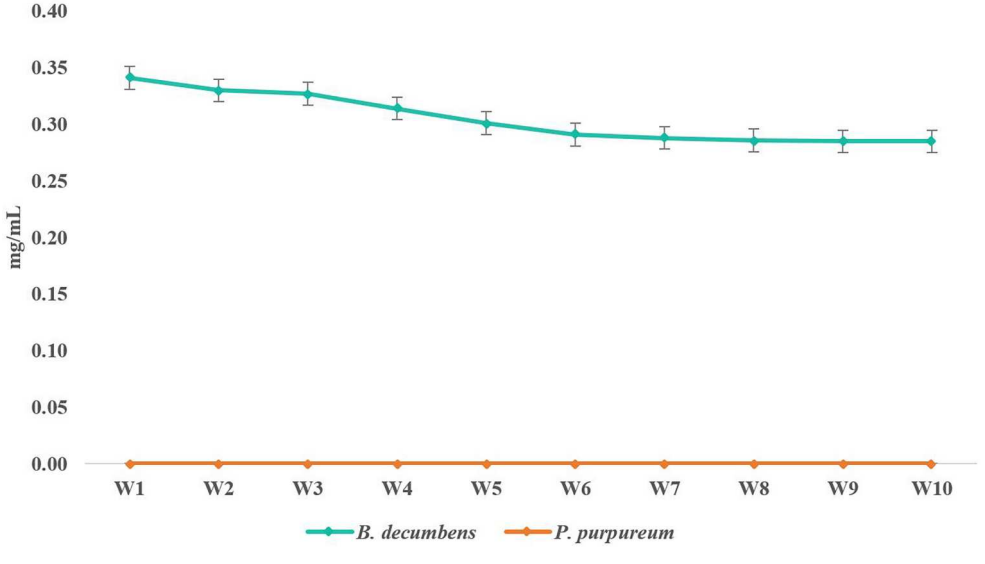

Figure 1. Weekly saponin concentration of fresh *P. purpureum* and *B. decumbens* at different ages.

Ensiling process

B. decumbens pasture were subjected to harvesting after a period of 7 weeks of regrowth after the initial cut at the start of study. These grasses were subsequently chopped into pieces approximately 7–10 cm in length, at a height of 5 cm above the soil level. *B. decumbens* silages were prepared in high-density polyethylene (HDPE) plastic packaging, incorporated with molasses at a concentration of 1.00% relative to the forage weight per bag. The silage mixture (approx. 10 kg) was compressed, vacuumed, and sealed to establish an oxygen-free environment. One bag is opened weekly throughout a ten-week period to acquire silage samples, which are shortly oven dried at 60°C for subsequent analysis. Sample was collected from different areas within the bag to achieve uniformity, with six replicates each. No saponin was detected after 4 weeks of ensiling *B. decumbens* grass (Figure 2). Hence, the silage was prepared in a standard manner and was utilised as feed after a period of more than 4 weeks of ensiling.

Animal model

Eighteen six-month-old, non-castrated male Dorper sheep crossed with local sheep (15.0 ± 0.23 kg BW) were purchased from the same farm for this study. The initial weight was notably lower than reported values for purebred Dorper sheep at this age (typically 30 + kg) but was comparable to the local Malin sheep in Malaysia, which generally weigh 15–20 kg under similar extensive grazing conditions (Mahdzar et al. 2023). Each sheep was individually and randomly placed in a metabolic crate following a completely randomised design with replicates. The metabolic crates, measuring 1.3 × 0.7 × 2.2 metres, provided adequate space for the animals to turn around, while all sheep were housed in a shaded, well-ventilated area under regular surveillance. All sheep underwent a two-week acclimatisation period and were administered orally with antiparasitic drug

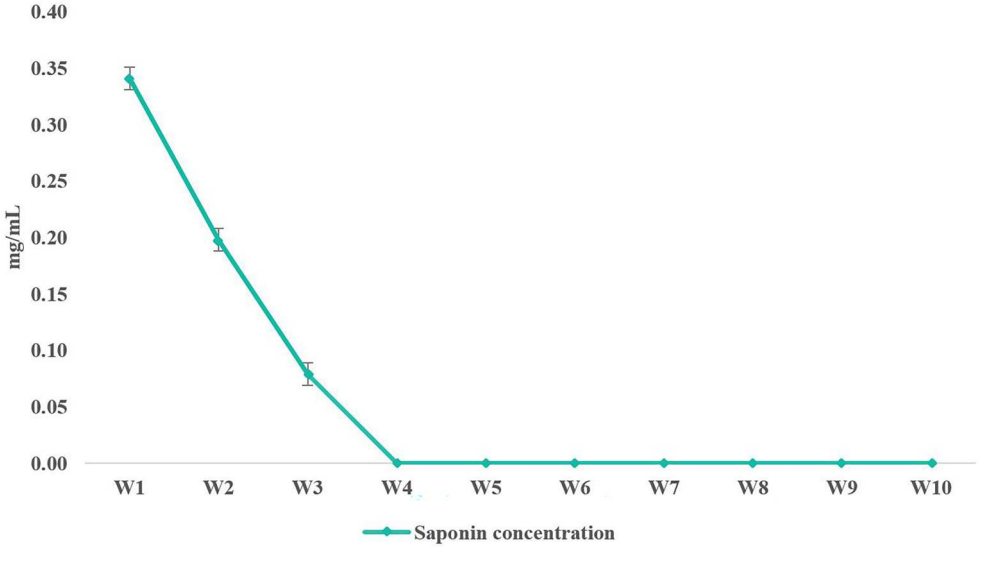

Figure 2. Weekly saponin concentration of ensiled *B. decumbens* at different ages of ensiling.

(Ivertin 1%; Range Pharma Pvt. Ltd., Malaysia). Their body weight was measured during the acclimatisation phase and statistically analysed using ANOVA in R Software to confirm the absence of significant differences among the sheep, ensuring standardisation before the start of the trial. Feed was introduced during the start of experiment, following a diet of 70:30 ratio of roughage to concentrate with at least 3.00% dry matter intake per kg of body weight of the sheep. Feeding routine was done twice daily by providing the allocated feed for each treatment along with concentrates, at 0900 and 1700hours, with water given *ad libitum* (Jaapar et al. 2023; Muniandy et al. 2021b).

Experimental design

A 90-day study was conducted where all 18 Dorper cross sheep were allocated into three treatment groups, consisting of 6 animals per treatment groups. The three treatments include sheep fed with fresh *P. purpureum* grass (negative control), sheep fed with fresh *B. decumbens* grass, and sheep fed with ensiled *B. decumbens* (positive control). Table 1 presents the nutritional composition of the three various treatments: fresh *P. purpureum*, fresh *B. decumbens*, and ensiled *B. decumbens*. The dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and gross energy were analysed in accordance with methods from Official Methods of Analysis (Jaapar et al. 2022). Daily harvesting and chopping of fresh forages were conducted for the consumption of the animals. Sheep fed with ensiled *B. decumbens* showed the highest body weight gain (↑38.5%), followed by the control group (↑37.5%) and those fed with fresh *B. decumbens* (↑34.3%), with a significant difference ($p < 0.05$) observed throughout the experiment. This indicates that feeding ensiled *B. decumbens* positively influences body weight gain in sheep. Blood collection was done at a monthly interval on day 0, 30, 60 and 90 during the study period for further analyses. The experimental protocols received approval from the International Animal Care and Use Committee at Universiti Putra Malaysia (UPM/IACUC/AUP-R003/2023).

Blood parameters

Blood samples were collected through jugular venipuncture using a vacuum collection system to conduct a comprehensive analysis of serum biochemistry and blood count.

Table 1. Nutrient content of fresh P. purpureum, fresh B. decumbens, & ensiled B. decumbens.

Composition (%)	Negative control	Fresh *B. decumbens*	Positive control	*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 fibre (CF)	30.65 ± 0.16	28.22 ± 0.26	27.15 ± 0.11	0.132
Neutral detergent fibre (NDF)	62.78 ± 0.88	60.91 ± 0.27	60.10 ± 0.41	0.257
Acid detergent fibre (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. There were no significant differences among the different feed at $p > 0.05$. Negative control: Fresh *P. purpureum*, Positive control: Ensiled *B. decumbens*.

Blood samples collected on days 0, 30, 60, and 90 were submitted to the Haematology and Biochemistry Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia for haematology and biochemistry analysis. The manufacturers' guidelines were followed in the execution of these measurements and processing through a semi-automatic biochemical analyser. Haematocrit, total solid, and white blood cell counts were analysed through haematology analyser (Cell-Dyn 3700 Automatic Analyzer, United States). The haematological parameters included red blood cells (RBC), haemoglobin, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), white blood cells, band neutrophils, segmented neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelet count, and plasma protein. The PCV, MCV and MCHC were calculated according to the formula shown below:

$$PCV(L/L) = [(RBCcountxMCV) \div 10] \div 100$$

$$MCV(fL) = (PCV \times 1000)/RBC$$

$$MCHC(g/L) = Hb/PCV$$

Blood serum was processed by an automated chemistry analyser (HITACHI 902 Automatic Analyzer®, Japan) to obtain the biochemistry parameters including the aspartate aminotransferase (AST), total bilirubin, gamma-glutamyl transferase (GGT), creatinine, phosphate, urea, total protein, albumin, globulin, and albumin to globulin ratio (Muniandy et al. 2021a).

Statistical analysis

Data analysis was conducted using a one-way analysis of variance (ANOVA) based on a completely randomised design model, with the dataset uploaded into RStudio version 4.1.3. All datasets were analysed repeatedly for each parameter on a monthly basis, with different treatments included as the independent variable, while the month of the experiment served as the dependent variable. The dataset was measured independently within each month, and no treatment × month interaction was included for any parameter. The Shapiro–Wilk test was applied to assess the normality of the body weight gain dataset, and significant differences among treatments were identified using Tukey's HSD post-hoc test. Statistical significance was set at $p < 0.05$.

Results

Haematology

Several parameters showed statistical significance ($p < 0.05$) among treatments as shown in Table 2. Both positive and negative control group exhibited no notable differences for most parameters, indicating that ensiled *B. decumbens* is as safe as the control treatment, while fresh *B. decumbens* group showed more pronounced variations.

The red blood cell analysis revealed a decline in Hb levels (0.9%–10.7%) following the feeding of fresh *B. decumbens* relative to the control group, suggesting a slight decrease in oxygen-carrying capacity. Feeding fresh *B. decumbens* also showed a significantly higher PCV values (3.2%–13.3%) than those in control group during days 60 and 90 ($p < 0.01$),

Table 2. Hematology profile of sheep fed with fresh *P*. purpureum, fresh B. decumbens, and ensiled B. decumbens.

	Negative control	Fresh *B. decumbens*	Positive control	*p*-value
RBC ($x10^{12}$/L) (normal range: 9–15)				
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 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 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 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 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
WBC ($x10^{10}$/L) (40–120)				
Day 30	60.50 ± 0.00	58.43 ± 2.80	60.00 ± 1.40	0.84
Day 60	55.40 ± 0.00^{b}	72.61 ± 8.40^{a}	55.60 ± 2.90^{b}	<0.01
Day 90	41.20 ± 0.00^{b}	76.47 ± 4.20^{a}	45.10 ± 3.10^{b}	<0.01
Band Neutrophils ($x10^{10}$/L) (normal range: 0)				
Day 30	0.00 ± 0.00^{b}	0.90 ± 0.10^{a}	0.00 ± 0.00^{b}	<0.01
Day 60	0.00 ± 0.00^{b}	0.70 ± 0.20^{a}	0.00 ± 0.00^{b}	<0.01
Day 90	0.00 ± 0.00^{b}	0.40 ± 0.20^{a}	0.00 ± 0.00^{b}	<0.01
Segmented Neutrophils ($x10^{10}$/L) (normal range: 7.0–60.0)				
Day 30	36.90 ± 1.00^{a}	24.07 ± 1.40^{b}	30.90 ± 1.30^{a}	<0.01
Day 60	29.90 ± 7.00^{b}	36.00 ± 4.60^{a}	23.80 ± 7.50^{b}	<0.01
Day 90	26.20 ± 1.07^{b}	42.10 ± 2.36^{a}	15.78 ± 1.18^{b}	<0.01
Lymphocytes ($x10^{10}$/L) (normal range: 20.00–90.00)				
Day 30	14.20 ± 3.30^{b}	29.16 ± 1.31^{a}	30.07 ± 9.30^{a}	0.01
Day 60	22.20 ± 4.40	29.73 ± 3.10	28.97 ± 4.60	0.25
Day 90	13.90 ± 20.40^{c}	37.27 ± 11.60^{a}	29.57 ± 11.90^{b}	<0.01
Monocytes ($x10^{10}$/L) (normal range: < 7.50)				
Day 30	2.40 ± 0.60^{a}	1.37 ± 0.30^{b}	2.80 ± 0.10^{a}	<0.01
Day 60	0.60 ± 1.20^{b}	3.10 ± 0.20^{a}	2.83 ± 0.20^{a}	<0.01
Day 90	1.30 ± 2.00^{b}	4.33 ± 1.30^{a}	2.77 ± 0.90^{b}	<0.01
Eosinophils ($x10^{10}$/L) (normal range: < 7.50)				
Day 30	0.40 ± 0.40^{c}	1.24 ± 0.10^{a}	0.97 ± 0.20^{b}	<0.01
Day 60	0.80 ± 1.00^{b}	1.91 ± 0.90^{a}	0.67 ± 1.20^{b}	<0.01
Day 90	0.00 ± 0.10^{b}	1.30 ± 1.10^{a}	0.40 ± 0.20^{b}	<0.01
Basophils ($x10^{10}$/L) (normal range: < 3.0)				
Day 30	0.00 ± 0.00^{b}	1.03 ± 0.20^{a}	0.20 ± 0.10^{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}	0.73 ± 0.10^{a}	0.00 ± 0.00^{b}	<0.01
PLT ($x10^{9}$/L) (normal range: 260–1000)				
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 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

Note: All values were expressed as mean ± SEM; $^{a, b, c}$ values with superscript within the same row are significantly different at $p < 0.05$. Negative control: Fresh *P. purpureum*, Positive control: Ensiled *B. decumbens*. RBC: Erythrocytes, Hb: Haemoglobin, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCHC: Mean corpuscular haemoglobin concentration. WBC: Leukocytes. PLT: Platelet count.

reflecting an overall increased RBC concentration. MCV and MCHC levels of fresh *B. decumbens* group also displayed elevation ($p < 0.01$) as compared to the control treatment, particularly on days 30 and 60 in MCV levels. Despite all the differences, all treatments exhibited RBC values that were still within the stated normal range. Overall, RBC parameters were not adversely affected by any of the treatments. Feeding ensiled *B. decumbens*, in particular, was comparable to the control group, demonstrating its safety as a feed option.

All WBC parameters exhibited significant differences ($p < 0.05$), with fresh *B. decumbens* group showing notable increases starting in the first month. Fresh *B. decumbens* sheep showed a 3.5%–60.0% increase in WBC counts compared to the negative control group during days 60 and 90, indicating an immune response to fresh *B. decumbens*. Fresh *B. decumbens* sheep also exhibited a 100% increase in band neutrophils, exceeding normal range limits and suggesting a mild inflammatory response. Segmented neutrophil counts were elevated in fresh *B. decumbens* group (18.5%–46.6%) compared to the negative control group. Moreover, fresh *B. decumbens* sheep showed a 28.9%–91.4% increase in lymphocyte counts ($p < 0.01$). Monocyte and eosinophil levels were also significantly higher by 52.6%–135.1% and 81.5%–100% in fresh *B. decumbens* group respectively. Basophil counts were 100% higher in fresh *B. decumbens* group. Similar to the RBC values, most WBC parameters remained within normal ranges for all treatments, except for band neutrophils in fresh *B. decumbens* group. The results of positive control sheep mirrored those of the negative control, confirming its safety and minimal impact on immune parameters.

Platelet counts were significantly elevated in fresh *B. decumbens* group during the first 30 days, with a 52.7% increase compared to the negative control ($p < 0.01$). Plasma protein levels of sheep fed with fresh *B. decumbens* were significantly higher (4.4%–19.0%) during days 60 and 90 compared to the other treatments ($p < 0.01$). Nonetheless, all platelet counts and plasma protein levels were still within the normal range. Sheep fed with ensiled *B. decumbens*, however, displayed values similar to the negative control, highlighting its safety. On the other hand, the icterus index remained unchanged across all treatments, with a consistent value of 5 throughout the study. This indicates that none of the feeding treatments, including ensiled *B. decumbens*, affected liver function or haemolytic processes in the sheep. Nonetheless, it is worth noting that the value of the parameters above may be insignificant due to it falling below the detection limits. The detection limit in this case were 0.5 $x10^{10}$/L for white blood cell count and 20 $x10^{10}$/L for platelet count.

Biochemistry

Table 3 summarises the liver, kidney, and protein profiles of sheep fed with fresh *P. purpureum* (negative control), fresh *B. decumbens*, and ensiled *B. decumbens* (positive control). Significant differences ($p < 0.05$) were observed across several parameters. While fresh *B. decumbens* group showed elevated values in liver, kidney, and protein parameters compared to the negative control, ensiled *B. decumbens* group displayed results that closely aligned with the negative control, indicating its safety and suitability as a feed option.

Referring to the liver parameters, significant increases in aspartate aminotransferase (AST) values were observed in fresh *B. decumbens* group, with levels ranging from

Table 3. Blood biochemistry of sheep fed with fresh *P.* purpureum, fresh B. decumbens, and ensiled B. decumbens.

	Negative control	Fresh *B. decumbens*	Positive control	*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
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. Negative control: Fresh *P. purpureum*, Positive control: Ensiled *B. decumbens*. ALB: Albumin, AST: Aspartate aminotransferase, GGT: Gamma glutamyltransferase. A:G ratio: Albumin to globulin ratio.

4.1% to 58.9% higher than those of the negative control ($p < 0.05$). This suggests a mild liver stress response associated with fresh *B. decumbens*. Gamma glutamyltransferase (GGT) levels were significantly higher in fresh *B. decumbens* group compared to the negative control, with increases of 4.9% to 82.6% across the study period ($p < 0.05$).

Despite having significances, 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 as compared to fresh *P. purpureum* and ensiled *B. decumbens*. Bilirubin levels remained consistent across all treatments, with no significant differences observed. All values were within the normal range, suggesting no adverse effects on hepatic excretory function. sheep fed with ensiled *B. decumbens* showed no significant differences from the negative control, indicating no adverse effects on the overall liver function.

Based on the kidney parameters, the positive control group demonstrated the lowest creatinine levels (64.33 ± 7.36 μmol/L) at day 90, which was a 15.1% reduction compared to the negative control group ($p < 0.05$). sheep fed with fresh *B. decumbens* had elevated levels during earlier time points but normalised by day 90. Besides, ensiled *B. decumbens* group exhibited significantly lower phosphate levels (2.30 ± 0.14 mmol/L, ↓25.1%) compared to the negative control at day 90. Fresh *B. decumbens* group showed reductions at earlier time points but normalised by day 90. The lowest urea levels were recorded in the same group (2.30 mmol/L at day 90, $p < 0.05$). Overall, kidney function parameters exhibited significant differences across treatments. Ensiled *B. decumbens* group have consistently displayed values comparable to the negative control, indicating its non-detrimental effects on renal function.

For the protein profiles, sheep fed with ensiled *B. decumbens* displayed the highest total protein levels among all treatments, with significant increases ranging from 3.5% to 32.4% compared to the control ($p < 0.05$), while fresh *B. decumbens* sheep have the lowest total protein values ($p < 0.05$) at the end of trial. This suggests improved protein metabolism associated with ensiled *B. decumbens*. The fresh *B. decumbens* group also showed the highest albumin levels, with increases of 3.5% to 24.6% compared to the negative control group ($p < 0.05$). Globulin levels were highest in fresh *B. decumbens* group, with increases ranging from 1.3% to 30.1% in comparison. As a result, the A:G ratio was also significantly higher in fresh *B. decumbens* group, ranging from 6.3% to 49.8% compared to the negative control ($p < 0.05$). Alternately, values remained similar to those of the negative control group.

Discussion

Haematology

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). Saponins operate as a growth inhibitor in monogastric animals by reducing their feed intake due to their bitter nature and irritating the throat of animals. *B. decumbens* is also regarded as the most toxic within its species due to its potential to possess high concentration of protodioscin above 2% (Riet-Correa et al. 2011). Another related study describing the complete blood count of a steer reared in *Brachiaria* pastures revealed anaemia, hyperfibrinogenaemia, and leukocytosis with neutrophilia (Carmo et al. 2021). A study by Muniandy et al. (2021a) indicated that there is a notable decrease in plasma protein concentrations in sheep fed with *B. decumbens* while the PCV remained

unaffected, which may be ascribed to a decline in synthesis resulting from hepatic illness leading to hepatogenous photosensitization.

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. 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. In 2001, Cruz et al. experimentally induced cholangiopathy in lambs by administering *B. decumbens,* suggesting that *Pithomyces chartarum* causes photosensitization in *Brachiaria* spp. pastures. On the contrary, other studies conducted by Brum et al. (2007), Castro et al. (2007), and Caicedo et al. (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 where the spore count is low. Recent investigations indicate no instances of *P. chartarum* poisoning attributable to the low spore count, suggesting the measurement of spores is inconsequential.

Alternately, haymaking and ensiling are methods used to preserve forage, which in turn ensures a consistent feed supply as well as the safety of certain feedstuffs by removing those phytocompounds (Chung et al. 2018). Equivalently, one finding of a study on the effects of *B. brizantha* hay on lambs did not reveal any noticeable variations or significant alterations in the levels of RBC, haemoglobin, haematocrit, WBC, or fibrinogen among the treated animals (Lima et al. 2015). Another recent finding by Mako et al. (2021) also indicate that all RBC, WBC, and MCHC values in sheep fed with *Alternanthera brasiliana* silage containing phytochemicals were within the normal range, showing that the feed supported overall health, indicating the lack of anaemia in the experimental treatment groups. Similarly, the current results demonstrated no significance in the RBC values between negative and positive control groups. The application of grass preservation in eliminating steroidal saponin within feed has proven to be a successful method. Steroidal saponin is generally composed of an aglycone and glycosides. The ensiling process through anaerobic fermentation depletes sugar compounds and degrades chlorophyll by reducing the production of phylloerythrin, a phototoxic agent within chlorophyll compound, which consequently eliminates intoxication effects. As stated, an accumulation of lactic acid and the subsequent decrease in pH are factors in the degradation of chlorophyll compounds and other pigments. The successful attempt of saponin removal inhibits liver injury and biliary secretion, which in turn helps to secrete phylloerythrin through detoxification. As such, the manifestation of clinical photosensitivity and other intoxication effects are not able to take effect in ensiled *B. decumbens*. 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 anaemia, given the normal range of PCV values.

Conversely, the present findings indicate that the MCV and MCHC values were slightly elevated in fresh *B. decumbens* group compared to the other treatments. The slight elevation in MCHC value in fresh *B. decumbens* group suggests the occurrence of haemolysis (Polizopoulou 2010), which may signal the onset of anaemia (Mangiagalli 2023) due to the concurrent increase in MCV values in fresh *B. decumbens* group. According to Javed et al. (2016), the changes in blood manifest prior to the emergence of any morphological or degenerative alterations of an animal. The changes in blood samples include fluctuations in both MCV values and MCHC values. Therefore, it is not possible for degenerative changes to occur when these conditions are not met. Additionally, a slight increase of PCV values in fresh *B. decumbens* group 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.

In addition, positive control sheep fed with ensiled *B. decumbens* displayed good overall health performance, with no significant changes when compared with control in WBC count, platelets, plasma protein, and icterus index, demonstrating advantageous results with no observed physiological changes or immunological reactions. 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 positive control sheep exhibited superior overall performance in terms of WBC indices when fed with *B. decumbens* silage.

On the other hand, sheep fed with fresh *B. decumbens* exhibited elevated values of WBC, band neutrophil, segmented neutrophil, lymphocytes, monocytes, eosinophil, and basophil when compared to the control group. However, all values were within the normal range. 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). 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. According to Mako et al. (2021), the slight increase in value may signify an effective disease resistance and indicates that the animals were not susceptible to stress or illness. In addition, sheep fed with fresh *B. decumbens* 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 hyperproteinaemia or hyperglobulinemia, which in turn can contribute to the development of chronic inflammatory conditions (Polizopoulou 2010).

Biochemistry

Serum biochemistry is a valuable method for assessing hepatic damage, renal functions, and potentially associated abnormalities (Carmo et al. 2021; Chung et al. 2016). Due to the limitations of *Brachiaria* spp. pasture, farmers have resorted to preservation techniques, using it as a secondary source of nutrition for ruminants. Tropical grass

species generally have a higher buffering capacity and lower levels of water-soluble carbohydrates which allows for the growth and proliferation of lactic acid fermenting bacteria (Van Niekerk et al. 2010), which can potentially aid in the removal of the saponin component found in *B. decumbens*, hence ensuring a safer animal feed.

The current blood biochemistry results of positive control sheep fed with ensiled *B. decumbens* mostly revealed insignificance when compared with the negative 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. Similar to the current result, Rezaei et al. (2013) 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 utilisation of dietary crude protein by the animals (Rezaei et al. 2013). The mechanism of intoxication as explained by Stegelmeier (2002), described that phylloerythrin excretion is inhibited in ruminants with diffuse liver injury, resulting in secondary photosensitization. Excess phylloerythrin is poorly eliminated by the kidneys and accumulates in tissues like the skin (Stegelmeier 2002). 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. 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, which effectively controls lipid metabolism while maintaining liver protection (Liu et al. 2018).

When an organ sustains injury, there is a noticeable rise in blood enzyme activity. Serum enzymes are thus frequently analysed for the evaluation and identification of internal organ injury. Conversely, the current blood biochemistry results revealed significance in sheep fed with fresh *B. decumbens* when compared to the control groups, indicating the possibility of a hepatic condition. The sheep exhibited slightly elevated values for both AST and GGT, but the range remained within the established reference values. In cases of hepatic diseases characterised by cholestasis, the elevation of GGT levels can be attributed to the solubilisation of cell membranes caused by the accumulation of bile salts within the liver and ducts. Additionally, a previous report found that the time between introducing naïve sheep to *B. brizantha* pasture and the onset of clinical signs of poisoning ranged from 35 to 137 days (Faccin et al. 2014). The findings of this study suggest that the delay in the effect of continuous protodioscin ingestion on the liver explains why pasture protodioscin levels did not immediately lead to an increase in mean serum GGT activity (Faccin et al. 2014). 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 early evidence of hepatic injury as documented in the previous study (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). The investigation revealed that the majority of serious liver damage is attributed to this mechanism occurring in the periportal area. Steroidal 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. The increased levels of AST enzymes may suggest a certain degree of muscle damage and haemolysis, as reported in the related hepatocyte injury in cattle (Carmo et al. 2021). Likewise, the

increase in serum GGT activity could also serve as a sign of liver damage, as demonstrated by Carmo et al. (2021) in cattle that were exposed to fresh *B. decumbens*.

Based on the present study, the biomarkers for kidney function exhibited statistical significance in both creatinine and phosphate, indicating lower levels in treatment sheep as compared to the negative control. The present study described comparable findings to a study conducted by Muniandy et al. (2021a), 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. 2021a). Moreover, the process by which ensiling mitigates the harmful consequences of intoxication is achieved by the elimination of steroidal saponin from the grass. The anaerobic processes during ensiling may eliminate the primary component responsible for intoxication, through the loss of sugar normally associated with sapogenin (Lima et al. 2015). Certain metabolites were produced and others were broken down as a result of the anaerobic ensiling conditions stimulating the activity of fermentative bacteria. This process results in steroidal saponin becoming less toxic and more soluble in water. In comparison, the levels of serum urea and creatinine of sheep fed with fresh *B. decumbens* 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). Moreover, the significance changes of creatinine, phosphate, and urea in sheep fed with fresh *B. decumbens* were consistent with previous research findings. 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.

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). Positive control sheep exhibited higher levels of total protein and globulin, but lower levels of albumin and A:G ratio. A similar outcome was observed in a prior investigation, suggesting that the diet did not increase the animals' disease susceptibility (Mako et al. 2021). A low concentration of serum albumin is indicative of poor health, namely liver dysfunction. Although the present study observed reduced levels of albumin within the positive control sheep, 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, sheep fed with fresh *B. decumbens* demonstrated lower levels of total protein and globulin, but higher levels of albumin and A:G ratio. Saponin can form complexes with enzymes such as proteases, influencing intestinal digestion by interfering with enzymatic function. 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). Additionally, saponin compounds could inhibit microbe development by compromising membrane integrity, resulting in the disruption of the intestinal mucosal cell lining. The lower total protein and globulin could be caused by the disrupted digestion and absorption along the gastrointestinal tract. Besides, the current study showing elevations of these albumin and A:G ratio in those sheep may signify dehydration, hyperthermia, renal or hepatic dysfunction, or heightened consumption of grains. A study conducted by Omidi et al. (2015) also described that animal fed with *Setaria italica* exhibited a significant change in albumin levels. This further provides evidence, as displayed by the rise in mean corpuscular volume (MCV) of sheep fed with fresh *B. decumbens* in the present study.

Generally, feeding fresh *B. decumbens* may influence liver and kidney function, as evidenced by elevated AST, GGT, and creatinine levels, as well as altered protein metabolism. However, these values remained within normal ranges, suggesting a mild, non-pathological response. In contrast, ensiled *B. decumbens* demonstrated values similar to the negative control group across all parameters, confirming its safety and suitability as a feed option. Both negative and positive control groups maintained blood biochemistry parameters within normal ranges, highlighting their non-detrimental impact on the overall health of the sheep.

Conclusion

This work emphasises the significance of preserving *B. decumbens* in tropical regions, in particular to the removal of steroidal saponin compounds. The blood indices indicate that although the animals exhibit no visible indications of intoxication, their internal systems can still be affected due to the ingestion of steroidal saponin toxicity. This study demonstrates that both haematological and biochemistry suggest an overall healthier sheep while consuming ensiled *B. decumbens* compared to fresh *B. decumbens*. Ensiled *B. decumbens* is a more advantageous grazing option for sheep than fresh *B. decumbens*. In summary, ensiling *B. decumbens* successfully eradicates detrimental saponin chemicals, consequently improving the general health and performance of sheep. The use of *B. decumbens* silage may enhance the consumption of grass by farmers in tropical areas, reducing potential risks to health and thus promoting the small ruminant sector.

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N. N. A. Fauzi: Investigation, Writing – original draft. E. L. T. Chung: Funding acquisition, Resources, Supervision, Conceptualization, Methodology, Investigation, Writing – review & editing. N. A. A. Bakar: Investigation. M. H. Kamalludin: Writing – review & editing. M. F. H. Reduan: Writing – review & editing. F. F. A. Jesse: Writing – review & editing. F. R. Dunshea: Writing – review & editing.

ORCID

Nurul Aishah Ahmad Fauzi http://orcid.org/0009-0004-8974-3456
Eric Lim Teik Chung http://orcid.org/0000-0003-1038-1057
Nurul Adilah Abu Bakar http://orcid.org/0009-0000-6692-6285
Mamat Hamidi Kamalludin http://orcid.org/0000-0001-9728-8212
Mohd Farhan Hanif Reduan http://orcid.org/0000-0003-0815-8761
Faez Firdaus Abdullah Jesse http://orcid.org/0000-0001-8119-3331
Frank R. Dunshea http://orcid.org/0000-0003-3998-1240

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