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Research Article

Mitigating Toxicity: Clinical and Pathological Effects of Ensiled *Brachiaria decumbens* in Sheep

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ABSTRACT

Brachiaria decumbens, commonly known as signal grass, is widely used as livestock feed in tropical regions due to its high nutritional value and availability. However, its high concentration of steroidal saponins poses toxicity risks, raising concerns for small ruminant farmers. Therefore, this study aimed to evaluate the effect of feeding ensiled *B. decumbens* on the health and performance of sheep under tropical conditions. A total of eighteen six-month-old male Dorper cross sheep were used in a feeding trial and assigned to three treatment groups: the control group (T1), fed *Pennisetum purpureum*; Treatment 2 (T2), fed fresh *B. decumbens*; and Treatment 3 (T3), fed ensiled *B. decumbens*. Over the 98-day experimental period, clinical data were collected weekly. At the end of the study, all 18 sheep were slaughtered for morphometric, gross morphology, and histopathological analyses of their organs. The results revealed significant differences (p<0.05) in the health performance of sheep across feeding groups. Sheep in the T3 group, fed ensiled *B. decumbens*, exhibited slightly higher body weight gain compared to both the T2 group fed fresh *B. decumbens* and the control group (T1). Additionally, only T2 sheep exhibited pale ocular mucous membranes during the final 21 days of the study. In terms of organ morphometrics, T1 and T3 sheep had lower organ width, length, and weight compared to T2 sheep. Most vital organs appeared grossly normal across all treatments; however, post-mortem examination of T2 sheep revealed lung lesions, including congestion and pus accumulation in the caudal lobes. Histopathological analysis indicated mild to moderate lesions in various organs of T2 sheep fed fresh *B. decumbens*, whereas no lesions were observed in the T1 and T3 groups. This study concludes that incorporating ensiled *B. decumbens* into the diet of small ruminants yields more favorable health and performance outcomes than feeding fresh *B. decumbens*.

Key words: Ensiled *Brachiaria decumbens*, clinical responses, gross morphology, organ morphometrics, histopathology, Dorper cross sheep

INTRODUCTION

Livestock production plays a pivotal role in food security, economic stability, and rural livelihoods, particularly in tropical and subtropical regions where small ruminants, such as sheep and goats, are primary sources of meat, milk, and income for farmers. However, the availability and quality of forage directly influence animal health and productivity. *Brachiaria decumbens* (signal grass) is widely utilized as a ruminant feed due to its adaptability, high biomass yield, and nutritional value (Chung *et al*., 2018). Despite these benefits, its use is often associated with toxicity risks, primarily due to the presence of steroidal saponins. These compounds have been linked to hepatogenous photosensitization, reduced feed intake, and organ damage in ruminants (Muniandy *et al*., 2021). Addressing these limitations is crucial for improving animal performance and ensuring the sustainability of livestock-based food systems.

Steroidal saponins, including protodioscin and dioscin, are known to induce cholestasis by forming birefringent crystals that obstruct bile flow, leading to hepatic dysfunction (Brum *et al*., 2007). Previous studies have documented clinical manifestations of *B. decumbens* intoxication, including weight loss, jaundice, dehydration, anorexia, and severe hepatic and renal damage (Porto *et al*., 2013; Melo *et al*., 2021). These adverse effects limit the widespread adoption of *B. decumbens* as a primary forage source for small ruminants, necessitating the exploration of strategies to mitigate its toxicity.

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Among the various forage preservation techniques, ensiling has emerged as a promising approach to reducing saponin concentrations while maintaining the nutritional value of grasses. Ensiling promotes anaerobic fermentation, leading to biochemical changes that degrade toxic metabolites and enhance feed digestibility (Lima *et al*., 2015). Studies have demonstrated that ensiling *B. decumbens* for more than four weeks effectively reduces its saponin content, thereby minimizing toxicity risks for ruminants (Binuomote, 2018). However, while these findings suggest potential benefits, limited research has systematically evaluated the physiological and pathological responses of sheep fed ensiled *B. decumbens* in controlled experimental settings.

B. decumbens toxicity leads to economic losses for smallholders through reduced growth rates, increased veterinary expenses, and flock mortality. Therefore, this study aims to investigate the clinical, morphological, and histopathological effects of feeding ensiled *B. decumbens* to Dorper cross sheep, compared to fresh *B. decumbens* and *Pennisetum purpureum* (control). The study hypothesizes that ensiled *B. decumbens* will enhance growth performance and minimize health complications associated with saponin toxicity. Specifically, this study will assess body weight changes, clinical parameters (e.g., mucous membrane condition, respiration rate & overall health), and post-mortem organ analysis to determine whether ensiling mitigates the deleterious effects of steroidal saponins.

By elucidating the benefits of ensiling *B. decumbens*, this research provides valuable insights for smallholder farmers and the broader livestock industry, offering a practical solution to improve forage utilization while mitigating animal health risks. Furthermore, understanding the long-term implications of saponin reduction through ensiling could inform future studies on optimizing feed preservation techniques. The findings from this study are expected to contribute to the development of sustainable feeding strategies that enhance ruminant productivity in tropical agricultural systems.

MATERIALS AND METHODS

Forage planting and harvesting

The study was conducted at Farm 15, Research Farm, Department of Animal Science, Universiti Putra Malaysia. *B. decumbens* and *P. purpureum* were cultivated under tropical environmental conditions, with average temperatures ranging from 26°C to 33°C and annual rainfall between 2000 and 2500 mm. Fertilization was performed using NPK (15:15:15) to support consistent regrowth. Fresh samples were collected weekly from week 1 to week 10 of regrowth for saponin quantification (Figure 1). The lowest saponin concentrations were recorded from week 7 onwards, aligning with optimal forage harvesting (Castro *et al*., 2007; de la Ribera *et al*., 2008).

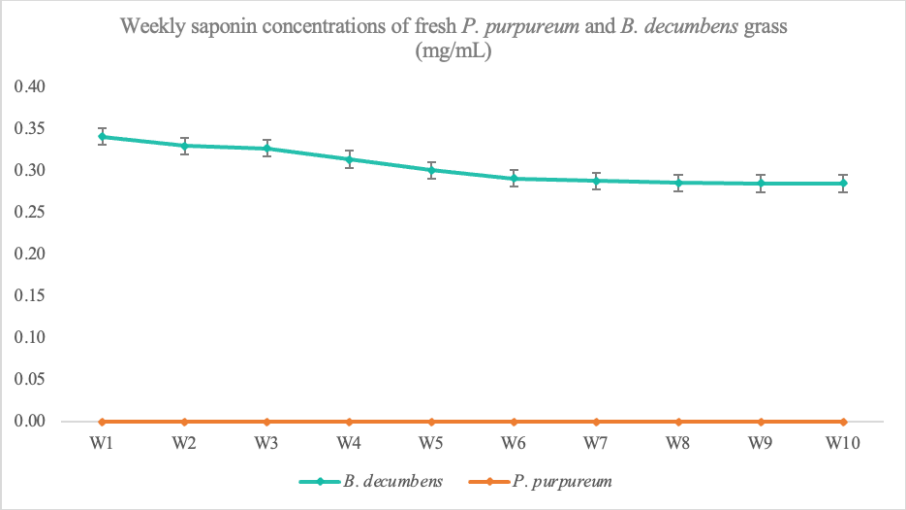

Fig. 1. Saponin concentration of fresh *P. purpureum* and *B. decumbens* at different ages.

Ensiling process

B. decumbens was harvested at seven weeks of regrowth, chopped into 7–10 cm pieces, and mixed with molasses at a concentration of 1.00% of the grass's weight to facilitate fermentation and improve microbial activity. The mixture was packed into high-density polyethylene (HDPE) bags, vacuum-sealed to ensure anaerobic conditions, and stored at 25–30°C. Saponin concentrations were monitored weekly for ten weeks using a spectrophotometric method adapted from Le Bot *et al*. (2022) at a wavelength of 540 nm. Saponins were extracted from *B. decumbens* leaves using a maceration method involving initial defatting with hexane, followed by extraction with 50% aqueous methanol and separation using n-butanol. The butanol layer containing saponins was concentrated, dried at 45°C, and the total steroidal saponin content was determined. The results showed undetectable level of saponins by spectrophotometry from week 4 onwards, confirming the selection of silage aged more than four weeks for the feeding trial (Figure 2).

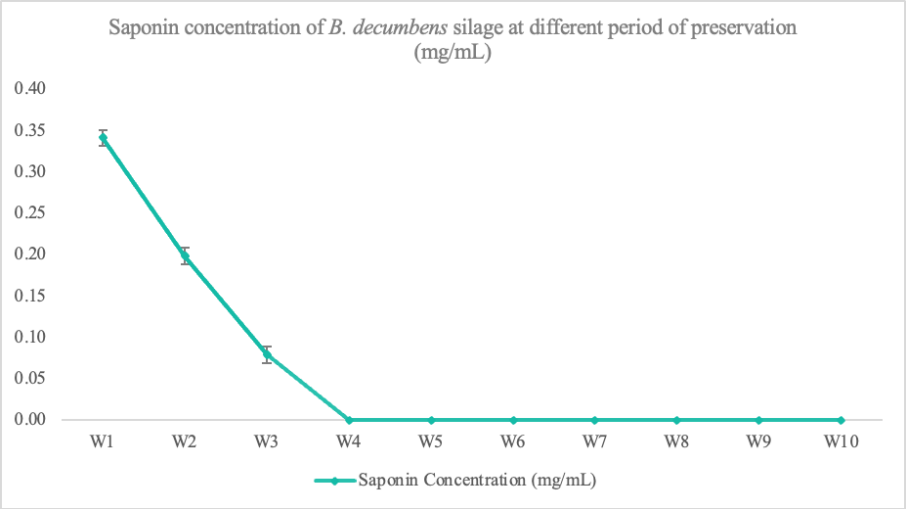

Fig. 2. Saponin concentration of ensiled *B. decumbens* at different ages of ensiling, measured using a spectrophotometric method at 540 nm.

Animal model

Eighteen (*n*=18) healthy male Dorper cross sheep, aged six months and weighing approximately 20 kg, were selected for the study. The sample size was estimated using G*Power version 3.1, based on a one-way ANOVA with α = 0.05, power = 0.80, and effect size f = 0.40, which determined that six animals per group (18 total) were sufficient, with no extras added due to housing and ethical limits. The sheep were randomly assigned to three treatment groups (*n*=6 per group) to ensure balanced distribution. The sheep were ranked by initial body weight and then randomly assigned to the three treatments using a stratified randomization method to ensure comparable average weights across groups at the start of the experiment. A two-week acclimatization period preceded the experiment, during which the sheep were dewormed with Ivermectin (1 mL per 50 kg body weight). Animals were housed individually in metabolic crates to monitor feed intake and prevent cross-contamination.

Diet and feeding management

Sheep were fed a roughage-to-concentrate ratio of 70:30, ensuring a daily dry matter intake of at least 3% of body weight. The daily feeding allocation per sheep was as follows: Treatment 1 (T1): fresh *P. purpureum*; Treatment 2 (T2): fresh *B. decumbens*; and Treatment 3 (T3): ensiled *B. decumbens*. Fresh forages were chopped before feeding, and silage quality was monitored for pH, ammonia-nitrogen content, and dry matter composition. Feed and water were provided *ad libitum*, and refusals were recorded daily. Table 1 presents the nutritional composition of the three 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 fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and gross energy were analyzed in accordance with methods from Official Methods of Analysis (Jaapar *et al*., 2022).

Table 1. Nutrient content of fresh *P. purpureum*, fresh *B. decumbens*, and 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. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens*.

Experimental design

A completely randomized design (CRD) was employed for the 98-day feeding trial. However, genetic susceptibility to steroidal saponin toxicity inherent to *Brachiaria* spp. was not specifically assessed due to the lack of available genetic markers or prior phenotypic evaluations for sensitivity within the study population. Thus, any genetic predisposition influencing individual animal responses remains an unmeasured variable, which may contribute to observed inter-individual variation. On day 98, all sheep were slaughtered for organ morphometric, gross morphology, and histopathology analyses. The slaughtering process was performed according to the ethical guidelines outlined by the International Animal Care and Use Committee (UPM/IACUC/AUP-R003/2023), using the Halal slaughter method.

Health performance monitoring

Clinical parameters, including rectal temperature, heart rate, respiration rate, mucous membrane condition, and rumen motility, were monitored weekly (Jesse *et al*., 2019). Clinical signs of toxicity were assessed based on the presence of jaundice, photosensitization, facial edema, anorexia, or weight loss.

Gross morphology and morphometric analysis

Post-mortem examinations were performed at the Department of Animal Science research abattoir. The brain, heart, lungs, liver, kidneys, spleen, and gastrointestinal tract were inspected for abnormalities. Morphometric measurements included organ length, width, and weight (Chung *et al*., 2016).

Histopathology analysis and lesion scoring

Tissue samples from the brain, heart, lungs, liver, kidneys, spleen, duodenum, jejunum, and ileum were collected and fixed in 10% neutrally buffered formalin. Standard histological procedures, including paraffin embedding, sectioning, and hematoxylin-eosin staining, were conducted. Lesion scoring followed Chung *et al*. (2016) using a four-point scale: 0 = normal (no lesion), 1 = mild (up to 25% of tissue affected), 2 = moderate (26–50% affected), and 3 = severe (more than 50% affected). To ensure scoring consistency, two independent pathologists assessed the slides, and inter-observer agreement was evaluated.

Statistical analysis

Data were analyzed using R software (version 4.1.2). Normality was confirmed using the Shapiro-Wilk test. One-way ANOVA was used for treatment comparisons, followed by Tukey's post hoc test for mean separation ($p<0.05$). Non-parametric data, such as histopathology scores, were analyzed using the Kruskal-Wallis test. Results were expressed as mean ± SEM.

RESULTS

Health performance

The results showed significant differences ($p<0.01$) in weekly body weight measurements among treatment groups throughout the 98-day trial (Table 2). Sheep in the T3 group, fed ensiled *B. decumbens*, showed slightly higher weight gain over time compared to the control group (T1), while T2 sheep fed fresh *B. decumbens* had the lowest gains. Respiratory rates also differed significantly ($p<0.01$) among treatments, with the highest rate observed in T2 sheep, particularly towards the latter stages of the study (Figure 3). No significant differences were observed in rectal temperature, heart rate, or rumen motility among groups. Pale mucous membranes were observed in 100% (6/6) of the T2 sheep during the final 21 days of the study, whereas none of the animals in the T1 (0/6) or T3 (0/6) groups exhibited this sign (Figure 4). This suggests that feeding fresh *B. decumbens* may contribute to subclinical health issues.

Table 2. Body weight of sheep fed with fresh *P. purpureum*, fresh *B. decumbens*, and 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*.

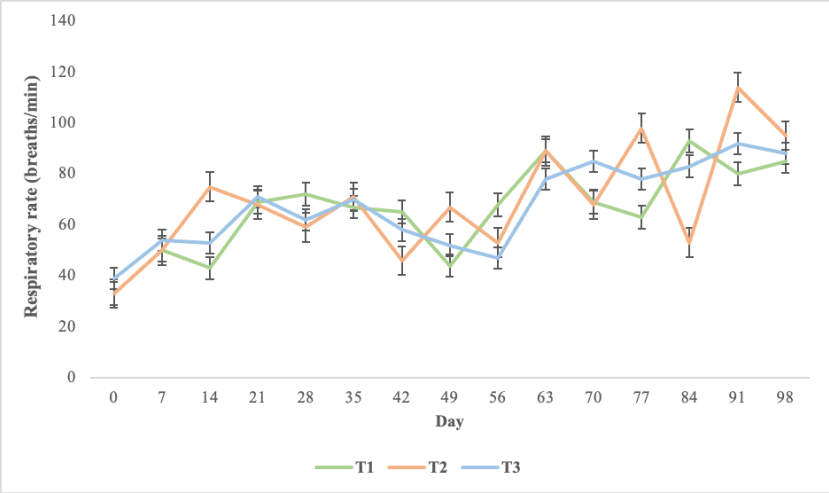

Fig. 3. The respiratory rate of sheep fed with different grass meals at various time intervals was expressed as mean ± SEM. T1: Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). Asterisk (*) indicates statistical significance among groups on the presented day.

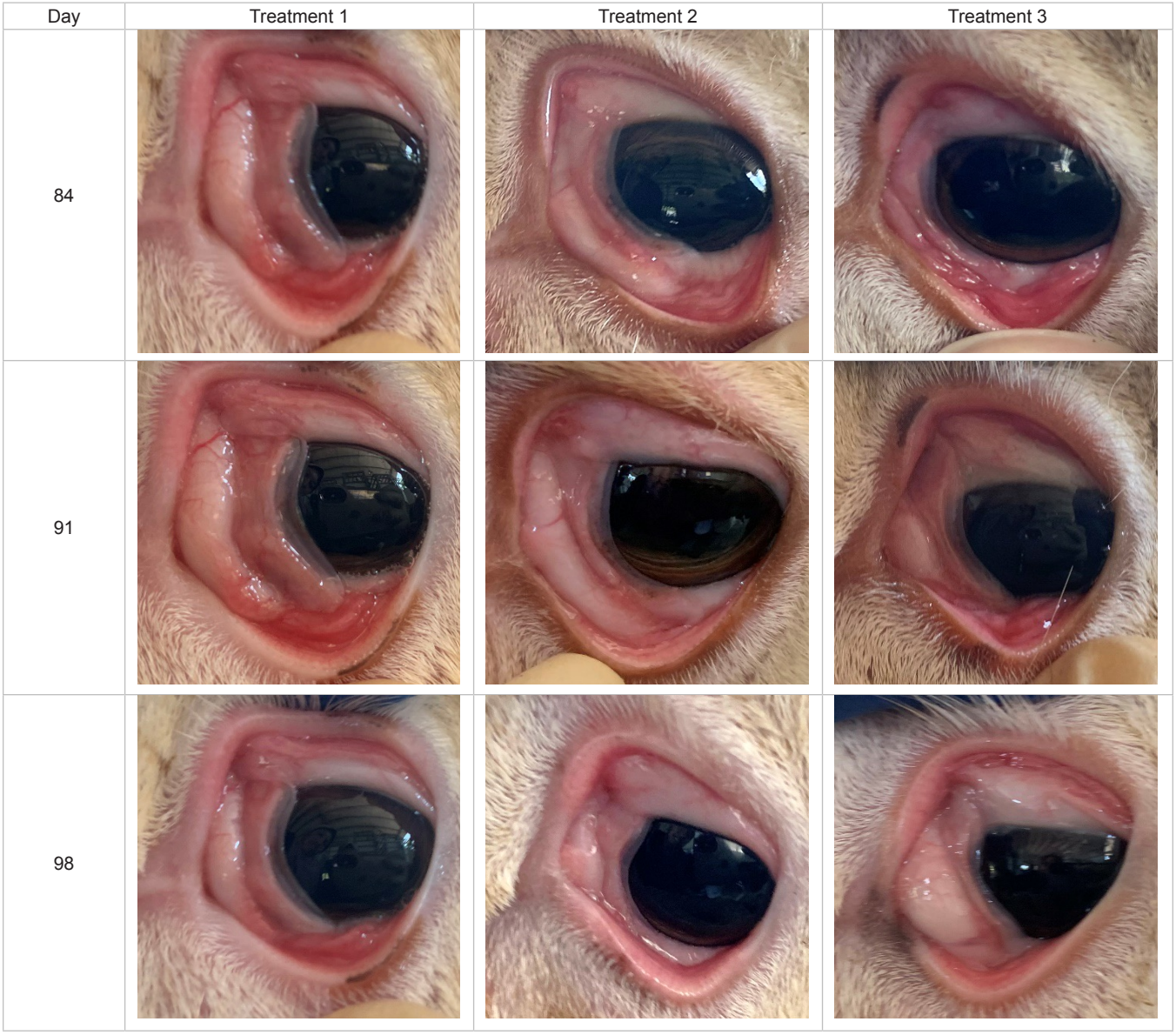

Fig. 4. The mucous membrane of sheep fed with different grass meals at various intervals. Treatment 1 (fresh *P. purpureum*) (control group), T2: Treatment 2 (fresh *B. decumbens*), T3: Treatment 3 (ensiled *B. decumbens*). The mucous membrane of T2 sheep appeared to be paler from day 84 onwards.

Gross morphology and organ morphometrics

Post-mortem examinations revealed that the brain, heart, liver, kidneys, spleen, and gastrointestinal tract were grossly normal across all treatment groups. However, five out of six T2 sheep (83.33%) showed lung lesions, characterized by congestion and pus accumulation in the caudal lobes (Fig. 5). Morphometric measurements indicated significant enlargement of the lung (p<0.01), liver (p<0.01), kidneys (p<0.01), and spleen (p<0.01) in T2 compared to T1 and T3, potentially due to saponin-induced organ inflammation (Table 3).

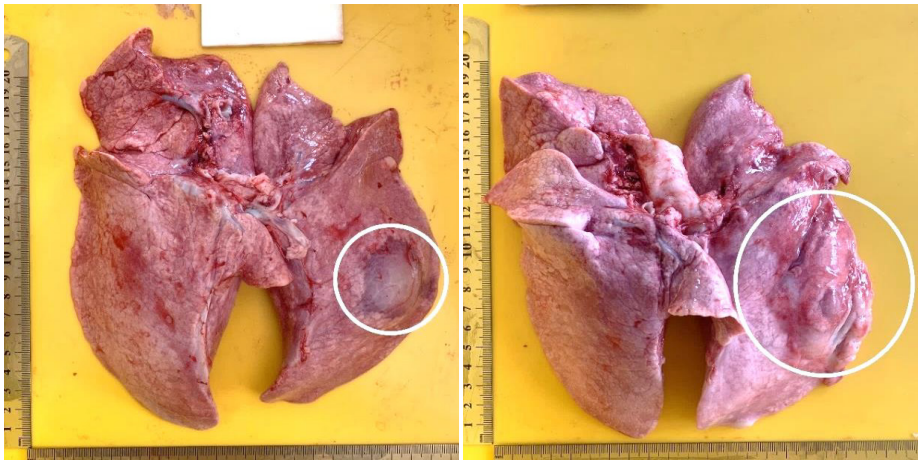

Fig. 5. General congestion and pus accumulation on the caudal lobe of the right lung of sheep in T2 in two different sheep (white circle).

Table 3. Gross morphology of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* and 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)				
Brain	990.50 ± 3.00	990.60 ± 3.01	995.60 ± 4.05	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}	81.75 ± 9.01^{a}	75.75 ± 8.01^{c}	<0.01
Spleen	43.00 ± 2.11^{b}	49.25 ± 3.37^{a}	49.00 ± 3.31^{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.*

Histopathology

Histopathological analysis revealed significant differences in inflammatory cell infiltration (p<0.01), necrosis/degeneration (p<0.01), edematous fluid (p=0.03), and congestion/hemorrhage (p<0.01) in T2 organs compared to T1 and T3 (Table 4). The lungs of T2 sheep showed moderate hemorrhage and edematous fluid accumulation, whereas T3 and T1 groups displayed no abnormalities. Liver sections from T2 sheep exhibited mild inflammatory responses and hepatocellular degeneration, while T3 and T1 groups had no significant histopathological changes (Figure 6 – 10). Overall, the results suggest that ensiling *B. decumbens* effectively reduces toxicity risks and improves the health performance of sheep compared to feeding fresh *B. decumbens*.

Table 4. Histological lesions of sheep fed with fresh *P. purpureum,* fresh *B. decumbens,* and ensiled *B. decumbens*

	Treatment 1	Treatment 2	Treatment 3	*p*-value
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
CongestionHemorrhage	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
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.*

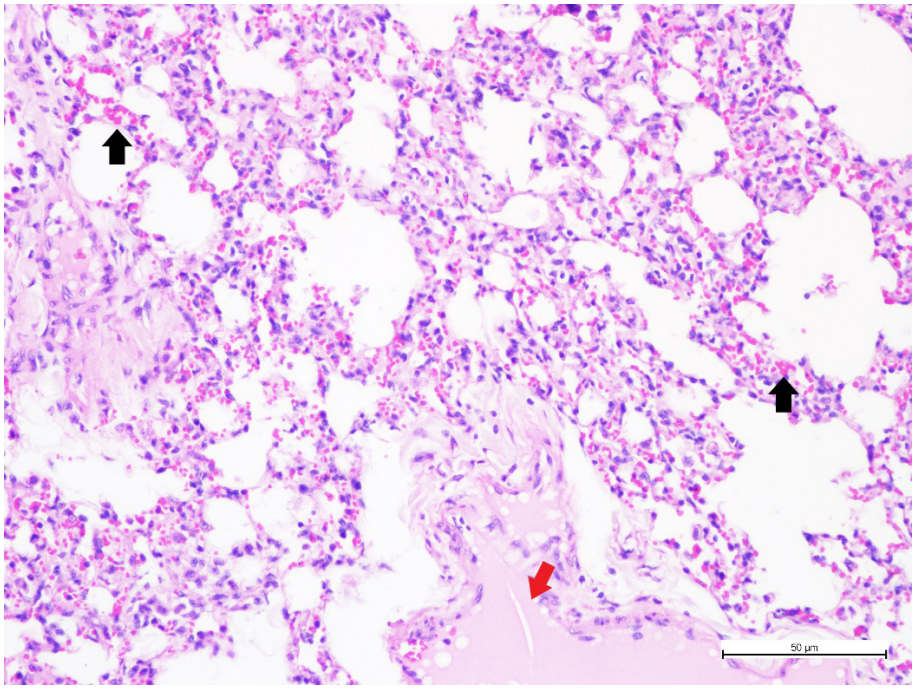

Fig. 6. A photomicrograph of the lung section of sheep within Treatment 2 displaying moderate hemorrhage (black arrow) along with edematous fluid (red arrow). H&E stain, 20x magnification, scale bar 50 µm.

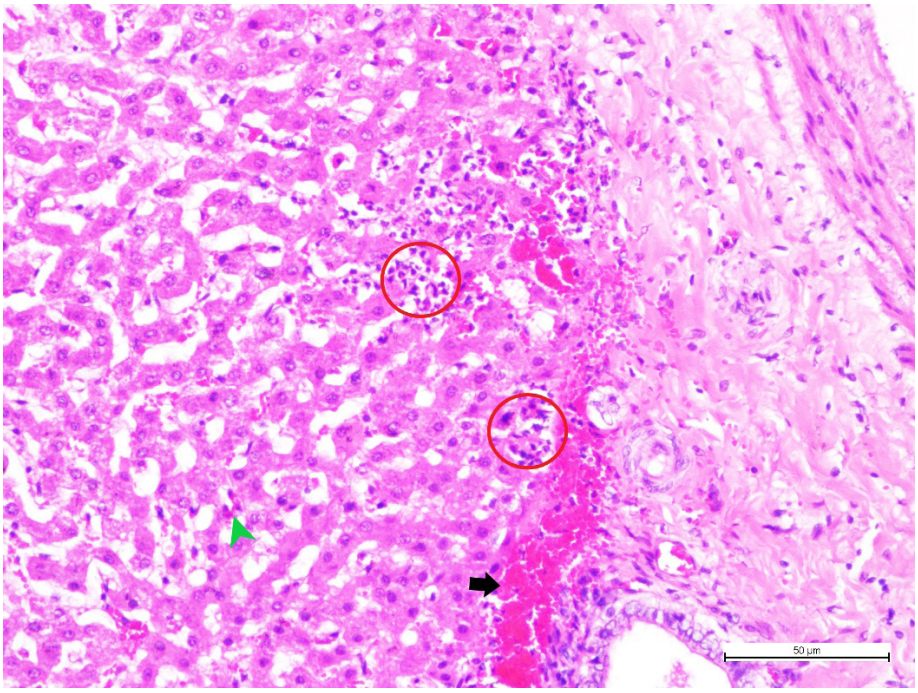

Fig. 7. A photomicrograph of the liver of sheep in Treatment 2 exhibiting mild hemorrhage (black arrow), inflammatory cell infiltration (red circle), and mild degeneration (green pointed arrow). H&E stain, 20x magnification, scale bar 50 µm.

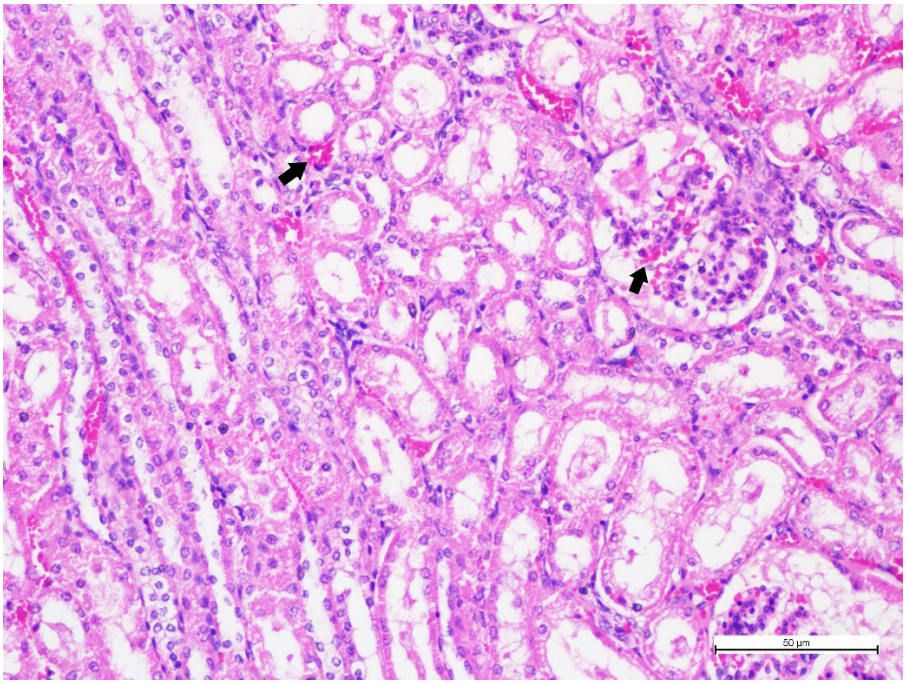

Fig. 8. The photomicrograph displays the kidney of sheep from Treatment 2 exhibiting a slight hemorrhage (black arrow) on the glomerulus and the adjacent tubules. H&E stain, 20x magnification, scale bar 50 µm.

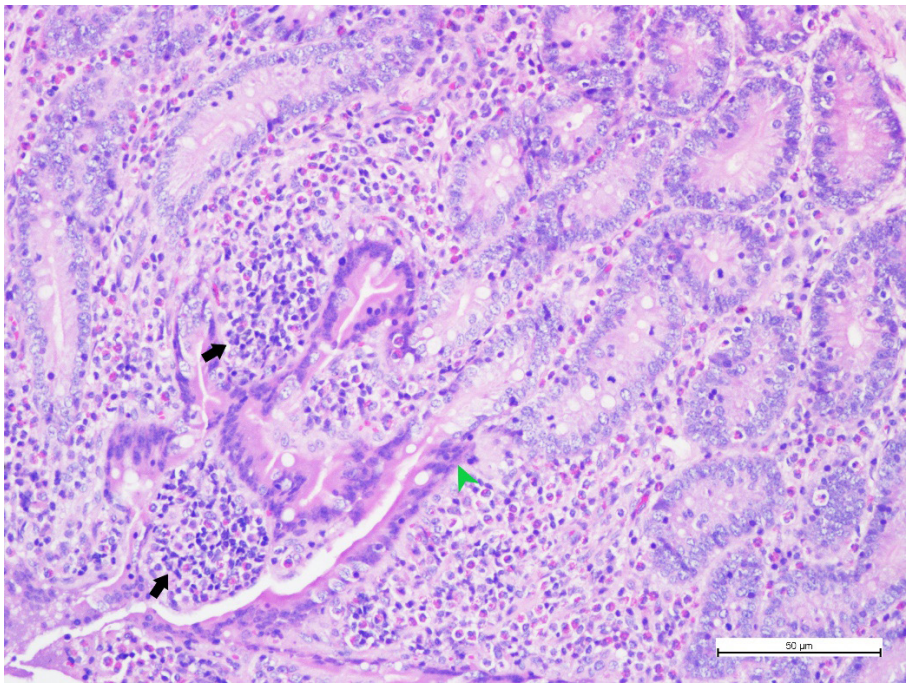

Fig. 9. A photomicrograph of the jejunum section of sheep within Treatment 2 displaying inflammatory cell infiltration (black arrow) and mild degeneration (green pointed arrow). H&E stain, 20x magnification, scale bar 50 µm.

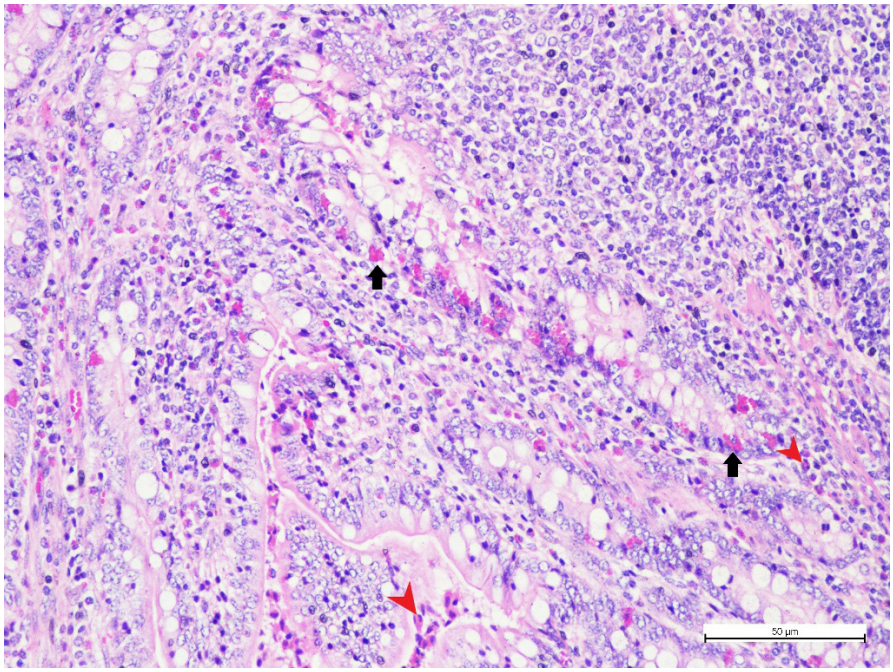

Fig. 10. The photomicrograph illustrates the ileum of sheep in Treatment 2 with evidence of inflammatory cell infiltration (red pointed arrow) and slight hemorrhage (black arrow). H&E stain, 20x magnification, scale bar 50 µm.

DISCUSSION

Health performance

Individual animals vary in their sensitivity and adaptation to steroidal saponins in *Brachiaria* spp., influenced by genetics, prior exposure, physiological status, and metabolic capacity (Lima *et al*., 2015). Repeated exposure can promote adaptation, allowing animals to develop tolerance through improved metabolic detoxification or microbial changes in the rumen. In contrast, naive animals often show greater susceptibility and more severe clinical signs when first exposed (Muniandy *et al*., 2020; 2021). These differences in response can affect health and growth, making body weight gain a useful indicator of overall productivity. Few studies have evaluated weight gain in animals fed *B. decumbens*, as its effects are often minimal compared to other forages. However, steroidal saponins in *Brachiaria* species have been linked to hepatotoxicity, which can impair liver function and, in turn, negatively impact growth performance and overall productivity (Caicedo *et al*., 2012).

Sheep fed ensiled *B. decumbens* (T3) showed the highest weight gain, followed by the control group, while those fed fresh *B. decumbens* (T2) had the lowest gains. This indicates that ensiling effectively reduces harmful saponins, improving growth and lowering the risk of saponin-related issues such as hepatogenous photosensitization (Ran *et al*., 2021). In addition to reducing toxicity, ensiling enhances digestibility and rumen health through fermentation, which increases lactic acid and beneficial bacteria. These changes help break down fiber and complex carbohydrates into simpler, more digestible nutrients, making the feed more efficient (Ran *et al*., 2021; Xu *et al*., 2020). Improved nutrient availability and rumen stability likely explain the better health and performance observed in T3 sheep fed ensiled *B. decumbens*.

Thus, the application of this preservation technique may not only enhance the degradation of cellulose and hemicellulose in the rumen but also improve the nutritional quality of the grass while effectively eliminating steroidal saponins (Chung *et al*., 2018). Although ensiling significantly reduces saponin content and enhances digestibility, haymaking has also been investigated as an alternative preservation method. However, studies indicate that haymaking is less effective at reducing saponin toxicity, as the drying process lacks the microbial fermentation necessary to degrade toxic metabolites (Lima *et al*., 2015). Ensiling is particularly advantageous in humid tropical regions, where rapid spoilage limits the feasibility of haymaking. Nevertheless, further research is needed to compare the economic viability and labor requirements of these preservation techniques across different farming systems.

Consistent with the findings observed in T2 sheep, Lelis *et al*. (2018) reported low weight gain and, in some cases, weight loss in sheep, underscoring the detrimental effects of saponins on health when fresh *B. decumbens* is included in the diet. This weight loss, attributed to declining overall health, is likely a consequence of hepatocyte damage caused by secondary hepatogenous photosensitization, which impairs the liver's ability to excrete phylloerythrin (Jaapar *et al*., 2022). These findings provide further evidence that the low weight gain observed in such studies is directly linked to the toxic compounds present in *B. decumbens*. Lelis *et al*. (2018) further suggested that animals experiencing a negative energy balance, due to altered liver metabolism, may be predisposed to weight loss and reduced weight gain. The contrasting results between T2 and T3 sheep highlight the benefits of ensiling in eliminating saponins from the grass. The significant weight disparity between sheep fed ensiled versus fresh *B. decumbens* further supports the improved nutritional value of the grass when toxic compounds are removed. While T2 sheep exhibited weight gain throughout the study, prolonged feeding of fresh *B. decumbens* may ultimately impair feed efficiency and adversely affect animal health.

Clinical and vital signs serve as fundamental indicators of animal health, offering critical insights for disease diagnosis, the identification of pathological changes during examination, and the early recognition of physiological deterioration. Muniandy *et al*. (2021) reported that the vital signs of sheep fed fresh *B. decumbens*, including rectal temperature, heart rate, and respiration rate, remained within the normal range throughout the trial. However, despite these normal readings, the animals exhibited chronic brain and liver damage when consuming this grass. In the present study, statistically significant differences ($p<0.05$) were observed in the vital signs of T3 sheep compared to T2, although all measured parameters remained within the normal range. The ensiling process effectively eliminated saponin compounds, thereby preventing liver damage associated with saponin-induced photosensitization and contributing to the improved health outcomes observed in T3 sheep.

During weekly clinical examinations, T2 sheep fed fresh *B. decumbens* exhibited a statistically significant ($p<0.05$) increase in respiration rate compared to the other feeding groups. Respiratory issues can arise from multiple factors, and in this case, they may be linked to saponins in *B. decumbens*, which contribute to liver impairment. Liver damage can induce physiological stress, thereby compromising the immune response in sheep. Additionally, the reduced immunity observed may be associated with the age of sheep, as the immune system remains underdeveloped before 12 months of age. A weakened immune response increases susceptibility to nosocomial bacterial infections, such as pasteurellosis, which can lead to pneumonia (Franco *et al*., 2019). This finding is consistent with previous studies reporting respiratory complications in animals consuming *Brachiaria* species containing saponins (Rosa *et al*., 2016). The higher respiratory rate and lung lesions observed in T2 sheep may be related to liver dysfunction caused by saponins. This study hypothesizes that liver injury could contribute to increased susceptibility to respiratory issues, such as pneumonia, by weakening immune function and altering metabolic processes. However, this relationship remains speculative and requires further investigation to be confirmed.

In ruminants suffering from hepatic dysfunction induced by steroidal saponins, common symptoms include anorexia, growth retardation, weight loss, diarrhea, jaundice, and photosensitivity (Muniandy *et al*., 2020). The present study, which observed clinically healthy T3 sheep fed ensiled *B. decumbens*, further underscores the detrimental effects of steroidal saponins on ruminants. This finding aligns with previous research demonstrating that preservation methods such as ensiling and haymaking can mitigate both clinical and subclinical effects of steroidal saponins (Lima *et al*., 2015). Lima (2015) reported that these preservation techniques effectively eliminate steroidal saponins, preventing hepatotoxic effects and supporting overall health and performance.

Sheep fed fresh *B. decumbens* (T2) developed pale mucous membranes during the final 21 days of the study, unlike those fed fresh *P. purpureum* or ensiled *B. decumbens*. This sign suggests reduced oxygen-carrying capacity, which may result from anemia or chronic inflammation. Prolonged exposure to saponins can trigger hemolysis, shortening red blood cell lifespan and leading to anemia (Jaapar *et al*., 2022). In normal conditions, blood flow adjusts to meet tissue demands, but acute inflammation can cause hyperemia, increasing blood flow to organs such as the lungs, liver, and gastrointestinal tract (Vajdovich, 2008). This effect may worsen when liver and lung tissues are damaged by saponin toxicity (Kono *et al*., 2022). Although gastrointestinal parasites like *Haemonchus contortus* are a common cause of anemia in sheep, and animals were treated with ivermectin at the start of the trial, parasitism was not monitored through fecal egg counts or other tests. Therefore, it cannot be completely ruled out as a factor (Muniandy *et al*., 2020). However, T2 sheep continued to gain weight, which is unlikely in cases of severe parasitic anemia. Combined with the absence of pale mucous membranes in T1 and T3 sheep, these findings suggest that the observed signs were more consistent with low-grade saponin toxicity. Nonetheless, the absence of hematological and biochemical analyses, such as liver enzymes, bilirubin, packed cell volume, and red blood cell counts, limits the depth of interpretation regarding toxicity. Future studies should incorporate these parameters to provide a more comprehensive assessment of saponin-induced effects.

Gross morphology and organ morphometric

Both organ weight and size, whether absolute or relative to body weight, serve as sensitive markers of early toxicity. Primary necropsy findings in animals intoxicated by *Brachiaria* spp. Grasses commonly include dermatitis, widespread jaundice, and liver atrophy or hepatomegaly with distinct lobular patterns (Diamantino *et al*., 2020; Carmo *et al*., 2021; Kono *et al*., 2022). In the present study, the preservation of *B. decumbens* positively influenced the overall morphological and morphometric performance of T3 sheep. The absence of toxic compounds in the preserved grass likely facilitated optimal nutrient absorption and utilization, contributing to improved growth and health. The liver size and relative weight in the T3 group were the second highest, averaging 1.3% of total body weight, a general indicator of the physical performance of young, growing animals. The average liver weight of sheep in this study remained within the typical range of 1.3% to 1.9%, as reported by Li *et al*. (2021). Montanholi *et al*. (2017) noted a positive correlation between liver weight, nutritional status, and physiological stage. Young, growing sheep typically exhibit a higher liver-to-body weight ratio than adult sheep, reflecting a healthier overall condition. Therefore, the improved morphological and morphometric performance observed in T3 sheep is likely attributable to the elimination of saponins from *Brachiaria* spp. through grass preservation.

In contrast, T2 sheep fed fresh *B. decumbens* exhibited signs of saponin toxicity, including enlargement of the liver, lungs, kidneys, and spleen. The ingestion of toxic compounds such as steroidal saponins can induce hepatic reactions, including hepatocyte proliferation or hypertrophy (Pupin *et al*., 2016). In response to saponin exposure, the liver may undergo adaptive changes to cope with the altered physiological conditions. During liver regeneration, injuries caused by infections or toxic damage can activate mature hepatocytes, biliary epithelium, and endothelial cells, triggering proliferation until the normal functional mass is restored. While many adaptive responses of hepatocytes are primarily metabolic, morphological changes such as the enlargement of other organs can also occur (de Oliveira *et al*., 2013). The increased cell proliferation and macrophage infiltration associated with the inflammatory response to saponins likely contributed to the organ enlargement observed in T2 sheep.

Gross lung lesions were seen in T2 sheep, including congestion in both lung lobes, pus in the caudal lobes, and visible hemorrhages when the lungs were cut. Liver injury likely contributed to these changes, as the liver plays a central role in immunity and inflammation. While the liver is the main site for bioactivating steroidal saponins through cytochrome P450 enzymes, lung cells, such as club cells, also take part in this process, which can worsen pulmonary inflammation. Rosa *et al*. (2016) reported similar findings, where goats fed *B. decumbens* developed bronchopneumonia with consolidation, hyperemia, emphysema, and lung abscesses. A weakened immune system increases the risk of secondary infections like pasteurellosis, which can progress to pneumonia (Rosa *et al*., 2016). The higher respiratory rate in T2 sheep may be a result of liver dysfunction caused by saponins. Liver damage disrupts bile acid metabolism, leading to the buildup of toxic metabolites that interfere with normal respiratory function. Cholestasis from bile duct blockage triggers systemic inflammation and oxidative stress, causing the body to increase respiration to restore balance (Brum *et al*., 2007). Additionally, saponin-induced liver damage can reduce ammonia clearance, which may lead to hepatic encephalopathy and further affect breathing patterns.

Histopathology

Examining both macroscopic and microscopic changes helps identify the causes of disease and confirm tissue damage. *B. decumbens* contains toxic compounds that can trigger histological changes, especially in the liver, leading to impaired organ function and poor health (Castro *et al*., 2018). In this study, sheep fed ensiled *B. decumbens* (T3) showed healthy tissue structures, similar to the control group, with no signs of cytoplasmic crystal accumulation or other lesions. This indicates that ensiling effectively removed steroidal saponins, preserving the structure and function of vital organs. The absence of inflammatory cell infiltration, necrosis, and hemorrhage in the T3 group suggests that ensiling prevents hepatogenous intoxication and protects against liver damage. This process breaks down saponin sugar chains and chlorophyll, reducing the risk of hepatogenous photosensitization while maintaining the grass's nutritional quality (Ayemele *et al*., 2024). These results show that ensiled *B. decumbens* not only eliminates harmful metabolites but also provides a safer and more nutritious feed compared to fresh grass.

On the other hand, significant histopathological lesions ($p<0.05$) were observed in multiple organs, including the lungs, liver, kidneys, jejunum, and ileum, in T2 sheep fed fresh *B. decumbens*. The present study demonstrated that steroidal saponins from *B. decumbens* initiate Kupffer cell phagocytosis of these toxins, acting as a final defense mechanism of the gut barrier, as evidenced by inflammatory cell infiltration in various organs (Chung *et al*., 2018). Continuous ingestion of *B. decumbens* containing saponins appears to disrupt the inflammatory response, leading to the accumulation of white blood cells in the lungs, liver, jejunum, and ileum. Compared to the lungs, the lower severity of microscopic liver lesions may be attributed to a relatively lower saponin concentration, which did not induce significant intoxication within the study duration (Muniandy *et al*., 2021). Additionally, prolonged intake of saponin-rich *B. decumbens* may interfere with cellular repair processes, contributing to necrosis and tissue degeneration, particularly in the liver and jejunum, as observed in this study.

Saponins are known to inhibit the growth of ciliated protozoa in the rumen, leading to increased volatile fatty acid production, which elevates oxidative stress and induces liver damage (Kono *et al*., 2022). Both arterial and venous structures may also be compromised, and necrosis is likely associated with vascular insufficiency and impaired biliary drainage (Montoya-Ménez, 2019). Furthermore, only T2 sheep fed fresh *B. decumbens* exhibited mild edematous fluid accumulation in the alveolar spaces of the lungs. This may have resulted from congestion and hemorrhagic lesions across multiple organs, including the lungs, liver, kidneys, and ileum, leading to fluid leakage into the interalveolar spaces. According to Sim *et al*. (2012), saponins are known to reduce nutrient utilization and conversion efficiency in ruminants by impairing protein digestion through the inhibition of digestive enzymes such as trypsin and chymotrypsin. Consequently, decreased oncotic pressure in capillaries can lead to peripheral edema due to fluid transudation from capillaries into subcutaneous tissues.

Overall, although histopathological lesions were observed in T2 sheep, the saponin concentration was insufficient to induce additional crystallization, a phenomenon commonly reported in previous studies (Diamantino *et al*., 2020; Melo *et al*., 2021; Muniandy *et al*., 2021). Several factors, including age, nutritional status, sex, diet, prior or concurrent exposure to environmental

substances, and underlying illnesses, influence the body's response to toxic damage. These variables likely contribute to the observed variations in species-specific adaptations to liver injury.

CONCLUSION

This study underscores the benefits of ensiled *B. decumbens* in improving nutrient availability within the silage, as demonstrated by clinical responses, organ morphometry, gross morphology, and histopathological findings. The ensiling process effectively reduces steroidal saponin compounds in *B. decumbens*, with the results showing undetectable levels of saponins by spectrophotometry, as evidenced by the absence of adverse clinical responses and the overall improvement in body weight gain and vital signs in T3 sheep. Furthermore, organ morphometry, gross morphology, and histopathology results showed no significant differences compared to the control group, indicating that ensiled *B. decumbens* is a superior forage option for sheep compared to its fresh counterpart. In conclusion, while the findings support the hypothesis that ensiling *B. decumbens* mitigates saponin-related toxicity and improves health performance, further studies incorporating blood indices, genetic profiling, and parasitological evaluation are necessary to validate these results and enhance the robustness of dietary recommendations for small ruminants in tropical systems.

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ETHICAL STATEMENT

The experimental protocols were approved by the International Animal Care and Use Committee of Universiti Putra Malaysia (UPM/IACUC/AUP-R003/2023).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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