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Clinico-Pathological Responses of Sheep to Graded Levels of *Brachiaria decumbens* Diets

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ABSTRACT

Brachiaria decumbens is a highly productive tropical grass used for ruminant production. However, it can cause hepatogenous photosensitization, general ill-thrift, and deaths in ruminants due to the presence of steroidal saponins. This study determined the acute and chronic clinico-pathological responses of sheep to graded levels of *B. decumbens* diet. A total of 30 male crossbred Dorper sheep (six-month-old) used in the study were randomly divided into three treatment groups of 10 sheep each. Treatment 1 (T1), which served as the control group, was fed with *Pennisetum purpureum* and concentrates, whereas Treatments 2 (T2) and 3 (T3) were fed low (10%) and high (60%) levels of *B. decumbens*, respectively. The study period was divided into acute (7 days) and chronic (90 days) phases. The rectal temperature, respiratory rate, heart rate, and mucous membrane color were monitored throughout the experiment. At the end of each stage, five animals were selected from each treatment group and euthanized by slaughter to collect organs for gross pathology, organ morphometric and histopathological evaluations. The treated animals did not show significant changes ($p>0.05$) in rectal temperature, pulse, and respiration rates throughout the study period. However, the mucous membranes were noticeably becoming pale starting from day 60 to 90 in T3 and during the chronic phase (day 90) in T2. There was no lesion or morphometric change in any organ at postmortem examination. At histopathology, both T2 and T3 exhibited mild to moderate necrosis, hemorrhage, congestion, hydropic degeneration and edema ($p<0.05$) in the liver and brain. In summary, this study has established that both low and high levels of *B. decumbens* diets cause chronic brain and liver damages in the sheep model. This study can be used for future research on the effects of *B. decumbens* on grazing animals.

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Introduction

Brachiaria decumbens is one of the most favored pasture species in the tropics due to its adaptation to low soil fertility, drought, and freedom from pests and diseases (Faccin *et al*., 2014; Low, 2015; Chung *et al*., 2018). It has a high nutritive value in terms of dry matter digestibility and crude protein which are essential for animal growth and production (Low, 2015). Although *B. decumbens* is an important source of forage for ruminant production, there are many reports on its toxicity in sheep due to the presence of naturally occurring toxic compounds (Graydon *et al*., 1991; Brum *et al*., 2007). Protodioscin, the main steroidal saponins found in *B. decumbens*, is a common cause of liver injury in grazing animals (Muniandy *et al*., 2020).

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The clinical signs of *B. decumbens* intoxication includes jaundice, hepatogenous photosensitization, anorexia, and neurological signs at the later stage of the disease (Muniandy *et al*., 2020). Secondary or hepatogenous photosensitization occurs in ruminants due to liver damage caused by the toxicity of *B. decumbens* (Knight and Walter, 2003). The damaged liver fails to metabolize chlorophyll (phylloerythrin) for excretion in the bile leading to the development of skin lesions when exposed to sunlight (De Oliveira *et al*., 2013). At postmortem, the liver and kidney are the main vital organs affected by *B. decumbens* toxicity (Riet-Correa *et al*., 2011). Grossly, the liver of affected animals is enlarged and mottled with a brownish discoloration and the gall bladder is distended with bile (De Oliveira *et al*., 2013; Faccin *et al*., 2014) while the kidney is swollen and greyish yellowish in color (Assumaidee and Mustapha, 2012). Even at histopathological level, liver and kidneys are the major sites of cellular damage in *B. decumbens* toxicity. The liver histopathology is characterized by hydropic degeneration of hepatocytes, hyperplasia of bile duct epithe-

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lium, and mononuclear inflammatory cells infiltration (Graydon *et al*., 1991; Faccin *et al*., 2014). Birefringent crystals may be present within the bile ducts along with the accumulations of foamy macrophages during the chronic stage (Driemeier *et al*., 2002; Riet-Correa *et al*., 2011). Degenerative and necrotic changes may also be observed in the renal epithelial cells of the kidneys in the later stage of intoxication (Graydon *et al*., 1991).

There is a strong evidence pointing that *B. decumbens* significantly affects liver and kidney functions and ultimately cause poor animal health and production. However, there is a dearth of published data on the gross and histopathological features of *B. decumbens* toxicity in the brain and other vital organs of small ruminants. To the best of the authors' knowledge, no study has evaluated the changes in morphometric features of the lungs, heart, liver, spleen, kidneys, and brain in response to *B. decumbens* toxicity. Furthermore, there is no information on the actual amount of the grass that can cause intoxication in small ruminants. It is therefore necessary to conduct further investigation to evaluate the acute and chronic effects of ingesting specific amounts of *B. decumbens* in animal model. This study was conducted to determine the acute and chronic clinical signs, gross pathology, organ morphometric, and histopathology in sheep fed with low and high levels of *B. decumbens* diets.

Materials and methods

Animals

The design and experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee (Approval number: UPM/IACUC/AUP-R046/2019). A total of 30 six-month-old male crossed breed Dorper sheep were purchased and randomly divided into three treatment groups of 10 sheep each. All sheep were dewormed using oral Albendazole (2.5%) suspension during two weeks acclimatization before the start of the experiment. During the experiment, the sheep were kept in individual metabolic pens and fed with freshly cut grass supplemented with pellets at the rate of 70:30 ration/animal/day. The feed requirement of each sheep was calculated based on their bodyweight and the amount was increased weekly. Drinking water was provided to the animals *ad libitum* throughout the study period.

Measurement of saponins in B. decumbens

The level of *B. decumbens* in each treatment was determined by evaluating the saponins concentration of five-week-old freshly harvested grass. The saponins concentration (low and high levels) of *B. decumbens* was determined using the method established by Yuliana *et al*. (2014). In this study, different percentages of *B. decumbens* (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%) were mixed with *Pennisetum purpureum*. Firstly, the mixtures were oven-dried at 50 °C for 12 hours before being ground to pass a 0.5 mm sieve. Then, 10ml of 100% methanol was added to 0.5 g of sample and placed in a water bath for 20min at room temperature. Each sample was centrifuged twice at 4 ^{0}C for 10 min and the supernatants were collected. The supernatants were mixed with 0.2 mL vanillin, 0.25 mL ethanol, and 2.5 mL 72% H2SO4, and vortexed. After that, the samples were heated in the water bath at 60^{0}C for 10 minutes. After cooling to room temperature, the absorbance of those samples was read in the UV-Vis spectrophotometer at a wavelength of 544 nm. The concentration of saponins in each sample was derived standard curve linear regression according to the standards' concentration and the corresponding absorbance (Fig. 1). The sample with 10% of *B. decumbens* mixture was selected as the low level while 60% of *B. decumbens* mixture was selected as the high level because the level of saponins present was peaked at 60% and maintained high throughout the 70, 80, 90, and 100% of *B. decumbens* mixture. The required level of *B. decumbens* was mixed with different percentages of *P. purpureum* before feeding sheep in the treatment groups.

Experimental design

After acclimatization period, Treatment 1 (T1, control) sheep were fed with *P. purpureum* and concentrates as the basal diet, whereas Treatment 2 (T2) and 3 (T3) sheep were fed with low (10%) and high (60%) levels of *B. decumbens* and observed for 90 days. The study period was divided into acute (7days) and chronic (90days) stages. The responses of rectal temperature, respiratory rate, heart rate, and mucous membrane colour were monitored and recorded throughout the study period. At the end of both acute and chronic stages, five animals from each treatment group were slaughtered for gross pathology, organ morphometric, and histopathology evaluations.

Postmortem and organ morphometric analysis

A standard postmortem examination was conducted on slaughtered animals at the research abattoir in the Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia. The lungs, heart, liver, spleen, kidneys, and brain were grossly examined intact to record the lesions. The length and width of each organ were also measured using a measuring tape to determine the organ morphometric.

Histopathology analysis and lesion scoring

Tissue samples were taken from the lungs, heart, liver, spleen, kidneys, and brain for histopathology analysis. Fragments of tissues were fixed in neutrally buffered 10% formalin for 14days. The tissues were then dehydrated in ascending grades of alcohol; cleared in xylene; embedded in paraffin wax; sectioned with a microtome and stained with hematoxylin and eosin (H&E). The slides were examined at various magnifications of a light microscope to study the cellular changes such as degeneration, necrosis, hemorrhages and congestion, and oedema. These cellular changes were then scored according to the established scoring method (Chung *et al*., 2017). The scores include score 0: normal (normal tissue); score 1: mild (less than 25% tissue affected); score 2: moderate (less than 50% tissue affected); and score 3: severe (more than 50% tissue affected).

Statistical analysis

All data collected were analyzed using Statistical Analysis Software version 9.4. One-way analysis of variance (ANOVA) and Tukey's multiple comparison post-hoc test was used to compare the means among experimental groups. The data were considered significant at $p<0.05$.

Results

Clinical responses

The mean rectal temperature, heart rate, and respiratory rate in all the treatment groups were within normal range throughout the 90 days of experiment. There were no signs of edema, jaundice, photosensitization, and neurological signs observed in all treatment groups.

There was effect of different levels of *B. decumbens* diets on the color of ocular mucous membrane in treated sheep throughout the 90 days study period. There was no obvious change in the ocular mucous membrane color among the treated sheep during the acute phase. Nevertheless, sheep from T3 started exhibiting paleness of the mucous membrane from day 60 until the end of study period but T2 sheep only exhibited pale mucous membrane at the chronic phase of study on day 90.

Gross lesion and organ morphometric

At necropsy, no apparent lesions were observed in all the vital organs including the lung, heart, liver, spleen, kidneys, and brain. There were also no significant differences ($p<0.05$) among the organs' length and width among the treatment groups during both acute and chronic phases (Table 1).

Histopathological findings

Histologically, there were no significant lesions ($p>0.05$) among the treatment groups during the acute phase. However, significant lesions ($p<0.05$) were observed among treatment groups during the chronic phase in which both T2 and T3 sheep showed mild and moderate cellular changes, respectively (Figs. 2-7). T2 sheep demonstrated mild necrosis and degeneration (1.06±0.15); hemorrhages and congestion (0.5±0.12); and hydropic degeneration (0.67±0.18) lesions in the liver. Besides, the brain tissues also presented mild neuronal degeneration and atrophy (0.89±0.16); hemorrhages and congestion (0.44±0.12); and edema (0.44±0.12) lesions. On the other hand, T3 sheep revealed moderate necrosis and degeneration (1.83±0.19); hemorrhages and congestion (1.11±0.21); and hydropic degeneration (1.72±0.16) lesions in the liver. The brain tissues indicated moderate neuronal degeneration and atrophy (1.72±0.14); hemorrhages and congestion (1.00±0.16); and edema (1.33±0.21) lesions.

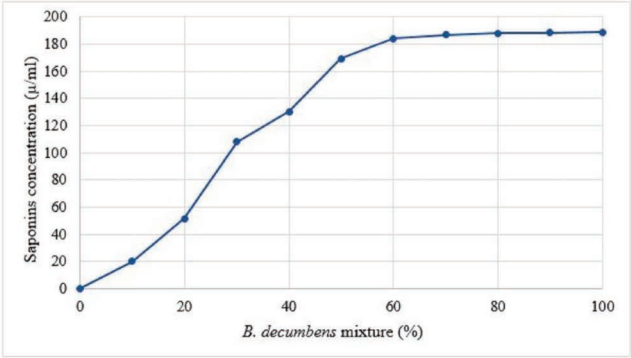

Fig. 1. The concentration of saponins in different proportions of *B. decumbens* and *P. purpureum* mixture.

Discussion

The rectal temperature, heart rate, and respiratory rate are the main vital signs which are used for diagnosing disease in animals (Menkes *et al*., 2017). Nonetheless, previous studies did not report the vital signs during *B. decumbens* toxicity in ruminants. In the present study, both groups of sheep fed with

Table 1. Organs' length and width of sheep fed with low and high levels of *B. decumbens* diets at different time phases.

	T1 (Control)	T2	T3
Organ length (cm)			
Acute			
Lung	18.67±0.67[a]	18.60±0.76[a]	18.60±0.59[a]
Heart	9.87±0.37[a]	8.87±0.27[a]	9.07±0.22[a]
Liver	20.33±0.12[a]	20.00±0.12[a]	20.10±0.60[a]
Spleen	8.43±0.15[a]	8.10±0.15[a]	8.50±0.15[a]
Kidney	5.37±0.12[a]	5.27±0.15[a]	5.47±0.12[a]
Brain	9.23±0.15[a]	9.87±0.23[a]	9.90±0.40[a]
Chronic			
Lung	20.13±0.72[a]	20.13±0.33[a]	19.27±0.79[a]
Heart	10.03±0.82[a]	9.37±0.32[a]	9.53±0.37[a]
Liver	21.47±0.78[a]	20.33±0.77[a]	21.27±0.95[a]
Spleen	9.13±0.61[a]	8.47±0.12[a]	9.07±0.67[a]
Kidney	5.53±0.09[a]	5.43±0.12[a]	5.80±0.36[a]
Brain	9.57±0.46[a]	10.03±0.27[a]	10.70±0.10[a]
Organ width (cm)			
Acute			
Lung	15.00±0.15[a]	14.00±0.25[a]	15.17±0.44[a]
Heart	6.20±0.21[a]	6.17±0.29[a]	5.87±0.89[a]
Liver	11.63±0.52[a]	11.53±0.75[a]	12.33±0.90[a]
Spleen	5.27±0.20[a]	4.90±0.15[a]	5.37±0.17[a]
Kidney	3.57±0.23[a]	3.40±0.27[a]	3.23±0.52[a]
Brain	5.10±0.30[a]	5.33±0.42[a]	5.03±0.12[a]
Chronic			
Lung	15.90±0.46[a]	14.40±0.91[a]	15.67±0.84[a]
Heart	6.20±0.21[a]	6.17±0.29[a]	6.33±0.38[a]
Liver	11.80±0.68[a]	12.27±0.50[a]	13.33±0.69[a]
Spleen	5.90±0.15[a]	5.53±0.23[a]	6.03±0.32[a]
Kidney	3.90±0.06[a]	3.73±0.03[a]	3.90±0.23[a]
Brain	5.77±0.58[a]	6.33±0.19[a]	6.90±0.10[a]

Note: All values were expressed as mean ± SEM; all values are followed with the same superscript ([a]) within same row, which indicate nonsignificant differences ($p>0.05$).

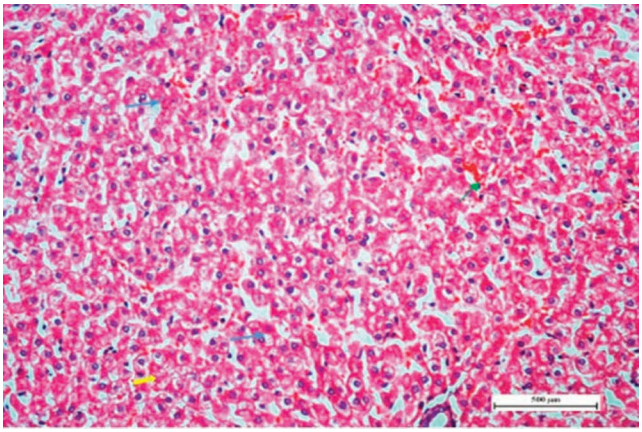

Fig. 2. A photomicrograph of the liver section of sheep from T3 group showing severe hydropic degeneration (yellow arrow) and hepatocyte necrosis (blue arrow) and focal hemorrhages in the liver parenchyma (green arrow). H&E stain, 200x magnification.

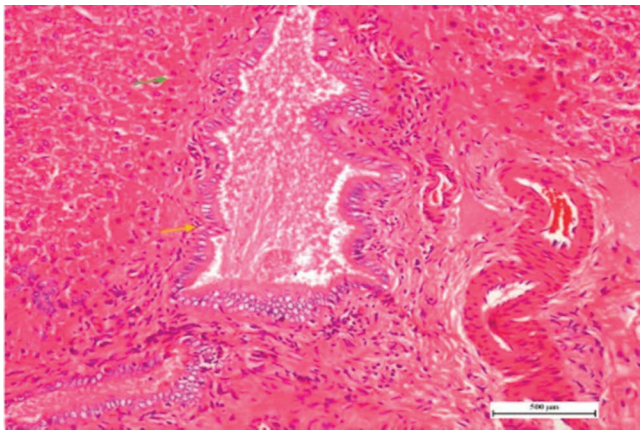

Fig. 3. A photomicrograph of the liver section of sheep from T3 group showing hyperplasia of bile duct epithelium (yellow arrow) with periportal necrosis (green arrow). H&E stain, 200x magnification.

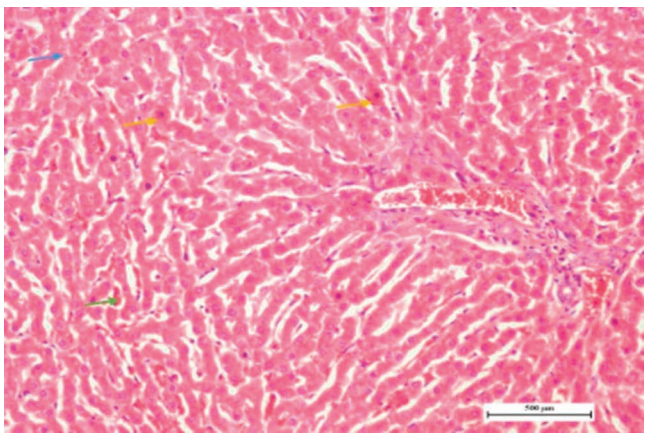

Fig. 4. A photomicrograph of the liver section of sheep from T2 group showing necrotic hepatocytes (yellow arrow), hydropic degeneration (blue arrow) and focal hemorrhages of the liver (green arrow). H&E stain, 200x magnification.

low and high levels of *B. decumbens* showed normal rectal temperature, heart rate, and respiratory rate throughout the study period. In different studies, experiments involving *Tribulus terrestris* and *Panicum miliaceum*, which are rich in saponins, influenced the body temperature, heart rate, and respiratory rate of some ruminants. Aslani *et al.* (2003) reported no significant changes in the body temperature, heart rate, or respiratory rates of sheep poisoned with 80% of *T. terrestris*, which was fed *ad libitum* for 42 days. Nevertheless, the same authors reported that there were elevations in the body temperature, tachycardia, and tachypnea in goats poisoned with the same grass (Aslani *et al*., 2004). On the other hand, Badiei *et al.* (2009) stated there were non-significant decreases in the body temperature, heart rate, and respiratory rate of experimental sheep intoxicated with *P. miliaceum* fed *ad libitum* for 35 days. These differences may be related to the susceptibility of different species towards saponins toxicity, the difference in dosage and time of exposure, as well as the type of saponins presence in the plant. Dichotomin and dioscin/protodioscin have been identified as the main steroidal saponins found in *B. decumbens* (Chung *et al*., 2018). Miles *et al.* (1993) have reported that *P. miliaceum* is composed of diosgenin and yamogenin, while *T. terrestris* yielded diosgenin and tigogenin. According to Opasina, (1985), sheep are more susceptible to *B. decumbens* intoxication than goats and cattle because the clinical incidence affecting sheep was more severe in comparison to other ruminants. Nevertheless, the absence of significant changes in the present study may be due to shorter duration of exposure to *B. decumbens* saponins.

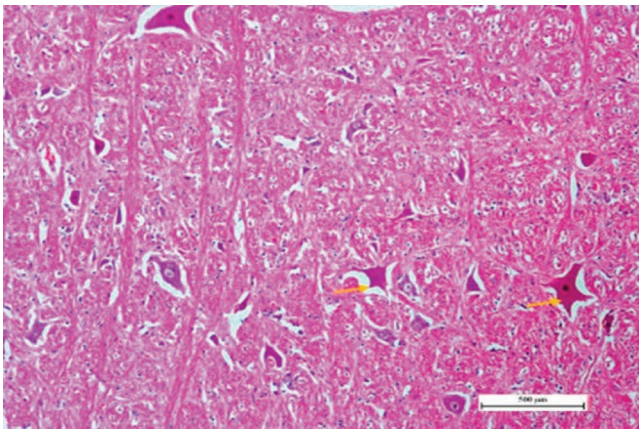

Fig. 5. A photomicrograph of the cerebrum section of sheep from T3 sheep showing prominent neuronal degeneration and atrophy (yellow arrow). H&E stain, 200x magnification.

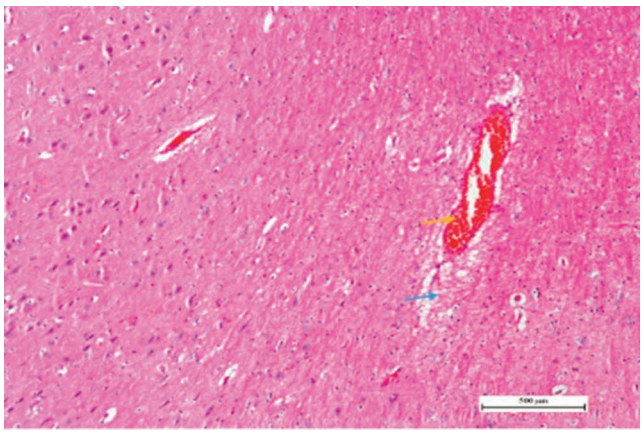

Fig. 6. A photomicrograph of the cerebrum section of sheep from T3 group showing severe congestion (yellow arrow) and perivascular edema (blue arrow). H&E stain, 200x magnification.

In most cases of *B. decumbens* toxicity, the clinical signs observed were jaundice, facial or submandibular edema, photosensitization, and neurological signs at the later stage (Graydon *et al*., 1991; Assumaidaee *et al*., 2010; Lelis *et al*., 2018). However, all sheep fed with *B. decumbens* did not develop any of these clinical signs in the present study. The only notable change was pale mucous membrane observed in T2 and T3

from day 90 and day 60, respectively. This finding could be an early indication of *B. decumbens* toxicity in sheep. The pale mucous membrane may be linked to decreasing number of erythrocytes due to intoxication of saponins, which may cause hemolysis of RBC (Cheeke, 1995; Sparg *et al*., 2004). Additionally, the higher level of intoxication used in this study decreased the time required for clinical response to develop indicating a more severe toxic effect. If the present study could last longer, the experimental sheep may develop jaundice due to inflammation and obstruction of the biliary system caused by the formation of biliary crystals (Cardona-Alvarez *et al*., 2016). The elevation of bilirubin due to cholestasis takes a longer period because of crystal formation (Lelis *et al*., 2018). Supporting this idea, De Oliveira *et al*. (2013) reported that buffaloes grazed on *B. decumbens* for two months also did not demonstrate jaundice, but the necropsy result revealed a slightly yellowish carcass that indicates jaundice would appear at the later stage of toxicity.

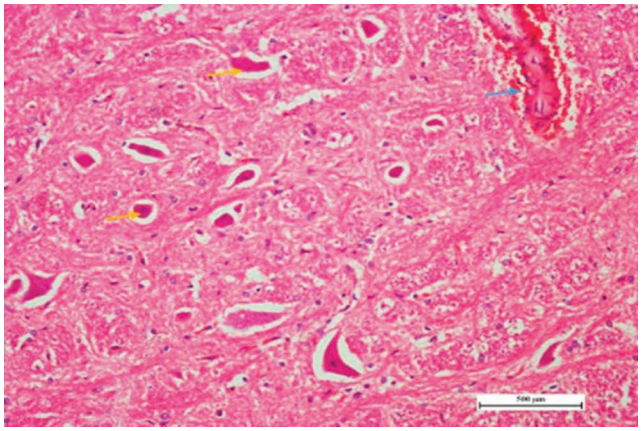

Fig. 7. A photomicrograph of a section of the cerebrum of sheep from T2 group showing neuronal degeneration and atrophy (yellow arrow) with perivascular hemorrhages (blue arrow). H&E stain, 200x magnification.

Furthermore, the damaged liver is unable to remove a chlorophyll metabolite known as phylloerythrin in the bile from the blood circulation. The accumulation of phylloerythrin under unpigmented skin areas such as the ears and muzzle cause dermatitis, a condition known as hepatogenous photosensitivity, when exposed to sunlight (De Oliveira *et al*., 2013; Cardona-Alvarez *et al*., 2016). Moreover, facial, and submandibular edema can occur during liver damage due to failure of the liver to produce albumin and other plasma proteins (Santos *et al*., 2008). Commonly, a high amount of ultraviolet (UV) rays are present under direct sunlight but, UV radiation is lower in shaded environments, which can delay the onset of photosensitization (Lelis *et al*., 2018). According to Lelis *et al*. (2018), *B. decumbens* grazing sheep managed under a silvopastoral system for 60 days also demonstrated some degree of photosensitization. The absence of skin lesions in the present study could be due to different management where those experimental sheep were placed under a shaded environment, which could delay the onset of photosensitization.

During the chronic stage of *B. decumbens* intoxication, the development of nervous signs such as ataxia, head shaking, circling movement, stamping of forelimbs, and reverse locomotion occurs due to hepatic encephalopathy (Cruz *et al*., 2001; Assumaidaee *et al*., 2010; Lelis *et al*., 2018). Generally, toxic substances such as ammonia and short-chain fatty acids will be eliminated when they pass through the liver, but this does not occur when there is severe liver damage. Consequently, the presence of these substances in the blood circulation will then cross the blood-brain barrier and reach the brain to cause neurological signs in affected animals (Santos *et al*., 2008; Albernaz *et al*., 2010). Conversely, neurological clinical signs were not exhibited throughout the present study which could be attributable to the short period of exposure to *B. decumbens* toxin. Similar findings were reported in other studies that reported the absence of neurological signs in *B. decumbens* toxicity (Graydon *et al*., 1991; Driemeier *et al*., 2002; Brum *et al*., 2007).

The gross lesion is one of the diagnostic workups which could describe the severity, duration, distribution, location, and nature of a disease (Chung *et al*., 2017). Previous studies have indicated that the liver and kidneys are the main organs affected during postmortem in *B. decumbens* intoxicated animals. The liver showed enlarged, firm, brown/yellow discoloration, mottled, and an increased lobular pattern with distended gall bladder (Riet-Correa *et al*., 2011; De Oliveira *et al*., 2013; Faccin *et al*., 2014). These happened because sapogenins from *B. decumbens* are mainly metabolized by the rumen microflora into epismilagenin and episarsasapogenin, which are then combined with glucuronic acid to produce b-D-glucuronides. The product will then bind with calcium ions and form insoluble biliary crystals as the final product. These crystals cause obstruction of biliary system and necrosis of hepatocytes eventually resulting in liver damage (Low, 2015; Cardona-Alvarez *et al*., 2016). Besides the liver, intoxicated animals also exhibited swollen with a grey-yellowish mottled appearance in the kidneys (Graydon *et al*., 1991). According to Assumaidee and Mustapha (2012), the membrane-permeabilizing effect of saponins attributable to their water solubility characteristic could be the main reason damaging the renal epithelial cells. Thus, other organ systems could be indirectly affected because of liver and kidney failures. For instance, pulmonary edema and hydropericardium were reported by Faccin *et al*. (2014) and Rosa *et al*. (2016), respectively. In contrast, there were no apparent lesions in all the vital organs such as the lung, heart, liver, spleen, kidneys, and brain in the present study. This could be due to the shorter duration of exposure to *B. decumbens* saponins.

To further determine the toxic effect of *B. decumbens*, organ morphometric were conducted to suggest any enlargement or reduction in the size of organs. Organ morphometric parameters such as organ size and weight are some of the most important indicators of possible organ defects in pathology (Gholamzadeh *et al*., 2017). In the present study, organ sizes were calculated by measuring the length and width of an organ. There were no significant differences in the organs' length and width among the treatment groups during both acute and chronic phases. As mentioned earlier, both liver and kidneys will become enlarged in ruminants that ingested *B. decumbens* (Assumaidee & Mustapha, 2012; Faccin *et al*., 2014). Nonetheless, these lesions were absent in the current study most probably due to shorter exposures to *B. decumbens*.

The present study did not find any significant cellular changes in the vital organs of the treatments during the acute phase. On the contrary, Assumaidaee *et al*. (2010) reported the presence of lesions in the liver, kidney, and brain of intoxicated sheep seven days post-feeding with *B. decumbens*. This discrepancy in findings between these studies could be attributed to the varying levels of saponins in the diet as the previous study fed only *B. decumbens* continuously without mixing with other grasses. Also, the age of the grass used to feed sheep in the previous study might be younger compared to the present study. According to Muniandy *et al*. (2020), sprouting *Brachiaria* spp. (young leaves) contain higher concentrations of saponins and could be more toxic than the matured plants, hence leading to the development of histomorphological lesions during the acute phase.

In the present study, significant cellular lesions were only

observed among treatments during the chronic phase. Both T2 and T3 sheep showed mild and moderate necrosis and degeneration; hemorrhages, and congestion; and hydropic degeneration or edema in the liver and brain, respectively. The histopathological findings observed in this study corroborates the results of previous studies which reported that the liver is the target organ of *B. decumbens* toxicity, and shows hydropic degeneration, hepatocellular necrosis, focal hemorrhages, and hyperplasia of the bile duct epithelium (Graydon *et al*., 1991; Faccin *et al*., 2014). The present study did not find any birefringent crystals or foamy macrophages due to the shorter exposure of sheep to *B. decumbens*. This agrees with Gomar *et al*. (2005), who reported that the presence of these cells indicates a chronic condition because they do not appear during short periods or less than 150 days of intoxication. Moreover, there were no cellular lesions in the kidneys of sheep fed with both low and high levels of *B. decumbens*. Graydon *et al*. (1991) and Riet-Correa *et al*. (2011) stated that ruminants intoxicated with *B. decumbens* showed marked diffuse degenerative or necrotic changes of the epithelial cells in the kidneys as well as the presence of crystalloid materials in the tubular lumen. The time factor might be the main reason responsible for the negative findings in the kidneys because Driemeier *et al*. (2002) reported that sheep fed with *B. decumbens* for up to 150 days did not develop significant lesions in the kidneys.

In this present study, cellular lesions such as neuronal degeneration & atrophy, perivascular edema, hemorrhages and congestion were observed in the brain of T3 sheep during the chronic phase. However, Assumaidaee *et al*. (2010) reported the presence of spongy degeneration (status spongiosus) in the cerebellum with evidence of neurological signs which were absent in the current study because sheep were slaughtered before the development of lesion and manifestation of signs. Status spongiosus develops in the cerebellum due to hepatic encephalopathy induced by hyperammonemia because of hepatic failure (Assumaidaee *et al*., 2010). Status spongiosus occurs is caused by severe vacuolation in the white matter of the midbrain, brainstem, and cerebrum (Santos *et al*., 2008). The generalized congestion and hemorrhages reported in the present study could be an early sign of degeneration but not severe enough to cause neurological signs. As a result, only mild and moderate histopathology lesions were observed in T2 and T3 treatments, respectively.

Conclusion

In summary, *B. decumbens* is a forage species that produce high-quality feed for grazing animals, but the suitability is compromised by the presence of steroidal saponins. In the present study, only the mucous membrane color and histopathological results were significantly affected by the different levels of *B. decumbens* diets at different time phases. Therefore, this study has established the effects of low and high levels of *B. decumbens* diets and at different time phases in sheep.

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Conflict of interest

The authors declare that they have no conflict of interest.

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