

Review

Signal Grass (*Brachiaria decumbens*) Toxicity in Grazing Ruminants

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Abstract: Signal grass (*Brachiaria decumbens*) is a highly productive tropical grass that is widespread through South America, Australia, Indonesia, Vanuatu and Malaysia due to its adaptation to a wide range of soil types and environments. Animal production from these *B. decumbens* pastures is highly variable due to sporadic outbreaks of photosensitisation associated with low growth rates of young animals, anorexia and wasting. The identification of *B. decumbens* toxicity through clinical signs may grossly underestimate the impact and severity of the disease. Affected animals without clinical signs have elevated serum liver enzyme concentrations resulting from blockage of the bile ducts by birefringent crystals, identified as calcium salts of steroidal saponins found in leaves and stems. The concentrations of the steroidal saponins vary through the year and within the plant. Young, green leaves contain 5–10 times the saponin concentration of mature leaves indicating that *B. decumbens* pastures are likely to be more toxic during sprouting and early growth. Previous exposure, selective grazing, and avoiding toxic leaves may partly explain apparent resistance of some animals to *B. decumbens* toxicity. Further research is needed to define growing conditions that produce elevated saponin levels and to investigate the impact of *B. decumbens* on rumen function.

Keywords: *Brachiaria decumbens*; signal grass; toxicity; photosensitisation; steroidal saponins; ruminants

1. Introduction

Brachiaria spp., generally referred to as signal or palisade grasses based on the similarity of the flower head structure to a railway signal, originated in East Africa (Uganda, Kenya, Tanzania, Rwanda, Burundi and Zaire) as a pasture resource for grazing by cattle and wildlife, growing at a range of altitudes from 500 to 2300 m a.s.l. These species have since been replaced with other forage species [1] that are more suitable to the changing climate. There has been confusion with *Brachiaria* species names being interchangeable over the past 50 years with *B. eminii*, *B. decumbens*, and *B. ruziziensis* all being used for the same plant material [2,3]. The most widely used cultivar as a grazing pasture globally is *B. decumbens* cv. Basilisk which was bred from seed introduced into Australia in 1930 from Uganda [4] and approved for registration in 1973. Signal grass (*Brachiaria decumbens*) has been introduced into many tropical countries including tropical and sub-tropical areas of Australia [5–8], Vanuatu [9], Colombia [10,11], Indonesia, Fiji [12], Trinidad [13] and Venezuela [14] with Brazil currently having more than 50 million hectares of *Brachiaria* spp. pastures [4,15,16]. In Malaysia, *Brachiaria* species have been planted on more than 80% of improved farming pastures with *B. decumbens* as the most favoured species [17]. Livestock production from these pasture systems has been variable even when both pasture quality and quantity were high. This review will focus on constraints to the use of *B. decumbens* cv. Basilisk as a pasture for grazing ruminants.

2. Agronomy of *Brachiaria decumbens* (Signal Grass)

B. decumbens is a low-growing decumbent perennial grass with flowering stems up to 100 cm high originating from the prostrate, multi-noded stems; plants can spread by both rhizomes and stolons as well as through seed production. *B. decumbens* has been widely adopted as a pasture for grazing ruminants due to its high nutritive value and aggressive growth habit that provides a dense ground cover able to suppress weeds. However, the aggressive growth habit can result in pastures becoming monocultures [2,18] by suppressing companion plants such as legumes, thus reducing the potential nutritive value of the pastures for livestock. Legumes with stoloniferous growth habits, such as *Arachis pintoii* (pinto peanut) and *Desmodium heterophyllum* (creeping hetero) and plants with shrubby/tree growth habits (such as *Leucaena leucocephala* and *Stylosanthes guianensis*) have been used with some success as legume companion species *B. decumbens* pastures [18–21]. The ability of *B. decumbens* to withstand shading has made it an important companion to *Leucaena* [22], and it has been successfully grown as a pasture in coconut plantations [23,24].

B. decumbens is resilient and is grown over a wide range of soil types including infertile acid soils with low pH (<3.5) and high aluminium levels [4,25] and climates ranging from tropical to sub-tropical. In Western Australia, *B. decumbens* was included in a mixed species pasture mix for the sub-tropics (sandy soils and a maximum annual rainfall of around 600 mm) [26]. This recommendation has now been withdrawn due to outbreaks of photosensitisation in grazing livestock on the mixed pastures. *B. decumbens* is able to withstand short-term drought conditions [2,27–29] by re-allocation of biomass and adjustment of growth rates resulting in no significant differences in biomass yield [29] but is highly susceptible to waterlogging [2] and can only tolerate flooding for a few days.

B. decumbens produces more dry matter than most tropical grasses during the dry season [30] and is capable of producing 15–27 mt (metric tonnes) dry matter (DM)/hectare/year [31–33]. In a study undertaken in South Sulawesi, Indonesia, *B. decumbens* produced more dry matter than other improved pasture species during the dry season (5700 kg/ha and 1910–3500 kg/ha respectively). It has been suggested that the ability to respond to small amounts of rainfall that occurred in the dry season was due to the extensive root system of *B. decumbens* [29,30]; plants produce new growth rapidly with out-of-season rain events during the dry season and with the break of season. Young vegetative material has been associated with reported outbreaks of photosensitisation among ruminants grazing *B. decumbens* pastures [34–37] (see Section 3).

B. decumbens responds well to defoliation either through grazing, harvesting or complete defoliation such as burning [23,38]. In Papua New Guinea, *B. decumbens* regrows readily after fire, a practice used by local villagers to hunt animals or as part of the “slash and burn” management of standing grass to prepare new food gardens [39–42]. *B. decumbens* has been shown to persist better than *Panicum maximum* (guinea grass) under high stocking rates in far north Queensland (Australia) [43].

Brachiaria species, including *B. decumbens*, utilise nitrogen efficiently [2] due to an ability to favour root growth in extreme conditions and to harvest biological nitrogen from bacterial species in the soil [44,45] equivalent to 30–50 kg N/hectare. *B. decumbens* cv. Basilisk has the ability to uptake higher levels of calcium, phosphorus and nitrogen than other *Brachiaria* species in both high and low soil fertility conditions [45]. Data from a number of experiments, extrapolated by Rao *et al.* [45] have indicated that 40% of plant nitrogen content in *B. decumbens* was derived from biologically fixed N₂. *B. decumbens* has shown, in plot trials, an increasing response to the addition of nitrogen fertiliser up to 400 kg N/hectare depending on nitrogen source [46,47], producing more than 40 mt DM/hectare/year at this level. In Brazil, the late summer application of nitrogen (100 kg N/ha) together with 95 days of deferred grazing, were found to produce the greatest biomass on an annual basis [48]. Split applications of nitrogen fertiliser have been recommended, as the growth response is greater in the period immediately following the application of nitrogen [49].

3. Animal Production on *B. decumbens* Pastures

Brachiaria species have been widely implemented as quality pastures for animal production. Nitrogen content in early regrowth periods (2–5 weeks) ranged from 10–28 g N/kg dry matter decreasing to 7–15 g N/kg DM for 10–12 weeks regrowth [2,21,40,50–52]. *In vitro* dry matter digestibility showed similar variation with early growth ranging from 56%–78% up to five weeks of age decreasing to 41.6% to 63.7% as the pasture matures [2,21,30,47,51,53,54]. The estimated nutritive value, and therefore the potential level of liveweight gain, of *B. decumbens* is similar to other tropical pasture species such as *Panicum maximum* (guinea grass), *Digitaria decumbens* (pangola grass) and *Setaria anceps* (setaria) [20,30,51,54,55]. Analytical data on chemical composition and potential digestibility (*in vitro* DM digestibility) of *B. decumbens* have shown high variability between data sets sampled through the growing period. This variability may be due to one or more factors such as season (wet or dry), fertiliser applications (timing and level), species combinations (e.g., presence of

legumes), growing conditions (trial plot vs. grazing pasture), sampling procedures (plot harvest or picked selection) and analysis methods (*in vivo* vs. *in vitro* digestibility determination).

Based on estimated nutritive values, animal production from *B. decumbens* pastures would be expected to be comparable to production from other commonly used tropical grass species and from medium quality temperate pastures. This expectation is supported by grazing trials that compare pasture species/composition, stocking rate and growth rates of sheep, goats and cattle; these comparisons have shown that daily and annual live weight gains from grazing *B. decumbens* is comparable to or may exceed growth rates on *P. maximum* pastures—0.46 to 0.78 kg/head/day and 0.49 to 0.61 kg/head/day, respectively [10,18,21,55,56]. Cattle in North Queensland, aged 15 months to three years of age, grazed tropical pastures including *B. decumbens* under a rotational grazing system at stocking rates that ranged from 0.7 to 5 head/ha [47]; annual weight gains were 211 to 950 kg/ha/year [43,47,55,57] with the highest live weight gains achieved on *B. decumbens* pastures. *B. decumbens* persist under high stocking rates and continuous grazing [55,58].

However, conflicting data from trials and from on-farm records indicate that nutritive value, as determined by chemical analysis, may not be a true indicator of potential animal performance. Analysis of growth rates for Brahman cattle in the Markham and Ramu Valleys of Papua New Guinea and in Queensland, Australia showed lower growth rates on *B. decumbens* pastures (0.29–0.37 kg/head/day) in the late wet season/early dry season pastures following rapid pasture growth compared to growth rates during the late dry season (0.57–0.65 kg/head/day) [47,59,60]. Since pastures in the early dry season would be considered to have a high nutritive value following the seasonal rains, these data conflict with performance expectations. Growth rates calculated from farm records over five years, from two different production systems in the Ramu Valley in Papua New Guinea, showed that Brahman weaner cattle on *B. decumbens* pastures (with supplements) averaged 0.05 kg/head/day and 0.2 kg/head/day for 1–100 days and 100–200 days post weaning, respectively [59] irrespective of the production system. The two production systems were (i) born, reared and weaned onto *B. decumbens* pastures or (ii) born onto native pastures and weaned onto *B. decumbens* pastures. Studies in Brazil, however, have shown that sheep adapted to *B. decumbens* pastures gained weight at a similar rate to animals on *P. maximum* but had evidence of liver dysfunction indicated by elevated serum enzyme levels [16]. Naïve sheep (*i.e.*, sheep which had no previous exposure to *B. decumbens* pastures) lost weight rapidly, had elevated serum liver enzyme levels and a 50% mortality rate.

Grazing trials report rates of weight gain and persistence of the pasture under grazing for *B. decumbens* pastures. No reports of production problems, photosensitization or deaths have been found in grazing trial data. However production/disease issues on these pastures have appeared as disease outbreak reports, generally from commercial farms rather than from trials. Recent studies in Brazil have attempted to link live weight gain and grazing history of the livestock species to clinical and sub-clinical disease occurrences [16,41].

4. Toxicity of *B. decumbens* Pastures

Although analyses and animal production studies indicate that *B. decumbens* pastures can produce high live weight gains in grazing livestock, there are many reports of low weight gains,

general ill-thrift and sporadic outbreaks of photosensitivity in goats [36,61], sheep [34,62–65], llama [66], buffaloes [67], deer [68] and cattle [69] across Colombia, Brazil, Australia, Papua New Guinea [59,70], Malaysia and Sri Lanka.

4.1. Photosensitisation and Clinical Biochemistry

Photosensitisation outbreaks are often reported when the pasture is young and actively growing. This includes growth after cultivation or slashing [62], in the early wet season or during the dry season following an out of season rain event [34,36,61]. Photosensitisation outbreaks have often been observed in sheep and goats within 10 days of grazing established *B. decumbens* pastures [61–63] and after three weeks in new growth pastures following out of season rainfall events [34,37,40,71,72]. Clinical signs of photosensitisation include progressive weight loss and anorexia, oedema and necrotic tissue, crusting and sloughing of skin in non-pigmented and exposed areas such as ears, face, rump, flank and vulva regions in females as well as visible jaundice [40,62]. Affected animals seek shade, grazing in the evening or early mornings or remaining under trees and shrubs rather than actively grazing [36,40,63]. Data from reported outbreaks indicate that young animals (sheep <12 months and cattle <2 years) are most likely to show clinical signs [41,42,65,73,74] with older animals not exhibiting clinical signs. In these outbreaks, morbidity in sheep varied from 0.42% to 100% and 0.08% to 7.9% for cattle [40–42,63,74–76].

B. decumbens or signal grass toxicity outbreaks are reported as clinical signs (predominantly secondary hepatogenous photosensitisation) and descriptions of behavioural changes. Secondary photosensitisation is a result of the build-up of phylloerythrin in the skin. Phylloerythrin is a photodynamic molecule produced from the metabolism of plant chlorophyll [77] by microorganisms and normally removed from blood for excretion in bile. Circulating phylloerythrin levels are increased due to hepatic dysfunction and/or bile duct lesions [78]. Skin cells become highly reactive in the presence of visible and UV radiation through increased photoexcitation of these photodynamic molecules [79]. Animals with pigmented skin are less likely to show clinical signs [34,40,63,79,80]; hair and skin pigments protect animals from the impacts of UV exposure. For this reason, areas most affected on animals displaying signs of photosensitivity tend to be those that have minimal hair cover and are unpigmented [79] such as ears, muzzle, vulva and areas around the tail. Clinical signs (*i.e.*, photosensitisation), as the indicator of animals affected by *B. decumbens* toxicity, is likely to be a gross underestimate of the number of animals affected, especially in a pigmented flock or herd.

Biochemical data from clinical and experimentally induced cases suggest that liver dysfunction is a major component of observed subclinical and clinical disease signs. Clinical outbreaks are accompanied by changes in liver enzyme concentrations, particularly serum AST (aspartate aminotransferase) and serum GGT (gamma glutamyltransferase); increased concentrations of these enzymes indicate impaired liver function and have been recorded with other grass species that have also been associated with hepatogenous photosensitisation [16,40,64,66–68,72,76,80]. Clinical biochemistry collected from reported outbreaks and from feeding/grazing trials indicates that serum GGT levels are likely to be more highly elevated (compared to normal range information) than serum AST levels. Recorded serum GGT levels of affected animals ranged from 100 U/L to 978 U/L (normal range 20–52 U/L) and serum AST levels ranged from 88.8 U/L to 1810 U/L (normal range

60–300 U/L) [40,64,68,76,80,81]. Similar serum concentrations have been recorded for animals in the same grazing groups but not displaying clinical signs. The correlation between serum GGT levels and possible *B. decumbens* toxicity is higher than for serum AST levels possibly due to the narrow normal range associated with serum GGT levels. GGT is an enzyme located in the cell membranes in the bile ducts and hepatocytes [82]; serum levels increase with membrane damage due to accumulation of bile salts resulting in oxidative stress and decreased antioxidant potential [82,83]. Changes in serum AST could be confirmed only when initial values for each animal or herd/flock had been determined. Animals with dark wool and/or pigmented skin are less likely to display clinical signs but are as likely to have elevated liver enzymes [40,63,80] suggesting that clinical signs are not suitable indicators of the number of animals affected by *B. decumbens* toxicity.

Comparison of serum biochemistry of clinically affected and non-affected animals indicated that, in many cases, serum AST and GGT levels were elevated to the same extent in both groups [64,68,84]. The proportions of affected naïve sheep and goats in three grazing trials on *B. decumbens* pastures in Papua New Guinea determined as clinical signs or through serum biochemistry was 38% and 75%, 15% and 42% and 27% and 82%, respectively [40]. These data suggest that the use of clinical signs to define outbreaks may be flawed, severely underestimating the number of affected animals. Weight loss (with or without clinical signs) of grazing livestock has often been associated with *B. decumbens* pastures where outbreaks have occurred and with animals in experimental feeding trials [16,40,42,80,84]; monitoring of condition score and live weight may be better tools for identifying affected animals.

The proportion of animals clinically affected does, however, appear to be greater for naïve livestock and for young and/or newly weaned livestock [64,74]. Naïve sheep introduced to *B. decumbens* pastures in Brazil had increased morbidity and mortality compared to experienced sheep (33.3% and 11.1% compared to 8.69% and 4.34% respectively) [42,76,80]. Data from reports for other pasture species associated with secondary hepatogenous photosensitisation found that 30%–60% of lambs less than 12 months of age were most affected resulting in case fatalities of up to 90% [64,85,86]. In Papua New Guinea, cattle born and weaned onto *B. decumbens* pastures had low numbers of clinically affected cattle compared to cattle born onto native pastures and weaned onto *B. decumbens* pastures [40] where the annual mortality rate was between 5% and 10%. However, based on farm records, the growth rates recorded in the two management systems were the same suggesting that the observed poor growth rates for livestock grazing *B. decumbens* pastures may be due either to *B. decumbens* toxicity *per se* or to extremely low intakes of pasture, possibly based on previous negative experience for those animals reared on *B. decumbens* pastures. Animals that were born and weaned onto *B. decumbens* pastures camped near the molasses/copra meal supplement and pastures have very limited evidence of grazing suggesting that cattle have developed avoidance behaviour [40].

4.2. Histopathology

Post-mortem examination of animals with *B. decumbens* toxicity indicates that the liver and kidneys are the major sites of cellular damage. Blockage of bile ducts is associated with thickening of bile canaliculi and the presence of birefringent crystals [40,64,65,67] within the bile ducts. Hydropic degeneration of hepatocytes, necrotic cells and the presence of foamy macrophages were found in

association with degenerated hepatocytes [42,64,65,67,74,87]. Multifocal cholangitis was common to these cases. Microscopic examination of liver tissue has provided evidence of bile duct proliferation and blockages with birefringent crystals often found in the bile ducts [40,41,64–67,69,80,87,88]. The severity of damage to hepatocytes is closely linked with the clinical symptom of jaundice [83]. Physical appearance of the liver shows brown/yellow discolouration, a high degree of mottling and an increased lobular pattern [40,42]. The link between *B. decumbens* pastures and the apparent toxicity recorded has been verified through trials where animals have been dosed with rumen contents from affected animals; clinical, biochemical and histopathological patterns were similar to those found in outbreaks and grazing trials [88–90]. Although these experiments did not identify the compound(s) responsible for elevated liver enzymes, histopathological changes and photosensitisation, a link between *B. decumbens* consumption and physical/physiological/biochemical observations was confirmed. Similar histopathological signs and the presence of birefringent crystals have been associated with pasture species such as *Panicum schinzii* [85,91,92], *Panicum coloratum* [93,94], *Panicum dichotomiflorum* [95,96], *Panicum miliaceum* [97] and *Tribulus terrestris* [98,99]. As a consequence, identification of possible toxic compounds has been focused across these pasture species as a group. Initial focus was on the possible role of sporidesmin, a toxin produced by the fungus *Pithomyces chartarum* as the physical signs were similar to those observed in animals suffering from facial eczema [100,101].

4.3. The Role of *Pithomyces chartarum*

Clinical signs of animals affected by *B. decumbens* toxicity are similar to those of animals with facial eczema. This disease affects sheep, deer, alpacas and cattle in New Zealand particularly in late summer/autumn [75,102]. Facial eczema is a secondary photosensitisation disease caused by the mycotoxin sporidesmin produced by the saprophytic fungus *Pithomyces chartarum* and was initially thought to be contributing factor to *B. decumbens* toxicity [100,101]. Early studies attempted to reproduce the symptoms observed in animals affected by *Panicum* toxicoses by dosing lambs with commercially available saponins with or without sporidesmin; the saponins used had been identified as components of birefringent bile crystals that are commonly associated with secondary photosensitisation. The dosed saponins did not produce liver lesions and sporidesmin produced a weak response only [103]. Further studies on the possible role of sporidesmin in *B. decumbens* toxicity have shown that there are negligible numbers of spores on pastures associated with either outbreaks or grazing studies, thus discounting any role of sporidesmin in this disease [42,64,72,76,104–106].

5. Identification and Metabolism of Toxic Compounds

Potentially toxic compounds have been isolated from leaf and stem fractions of plants associated with secondary hepatogenous photosensitisation, including *B. decumbens*, and identified as steroidal saponins [8,40,93,96,106–113]. Saponins are surface-acting glycosides that have the ability to produce a stable foam in an aqueous solution [114] and are classed as either triterpenoid or steroid based on structure [114]. Triterpenoid saponins are commonly found in dicotyledons while steroidal saponins are found in monocotyledons [115]. The activity of a saponin is a function of the position and type of sugar component of the molecule [115] with many plant species containing multiple saponins—for

example lucerne (alfalfa) roots contain 29 identified saponins. Saponins create pores in cell membranes and are associated with haemolytic activity [114,116]—this has been used as a qualitative test for the presence of saponins. The ability to disrupt blood cells is a consequence of interactions between the saponin and the sterols present in the red blood cell membrane and can be used as an indicator of the presence of saponins [116].

Steroidal saponins are found in the plant as glycosides of sapogenins with the sugar fraction being composed of one or more sugars. Although saponins have been found in a wide range of plants and crops, their role in plant metabolism and function is poorly understood [114,115]. Steroidal saponins have been commercialised as supplements, health foods for human consumption and potential anti-cancer treatments [116–121].

Saponins are present in a number of forages that are important for livestock. These include fodder plants such as *Acacia* spp., *Gliricidia sepium* and *Sesbania sesban* as well as legumes including soybean (*Glycine max*), lupins (*Lupinus* spp), lucerne (*Medicago sativa*), red clover (*Trifolium pratense*) and ladino clover (*Trifolium repens*) [115]. Saponins commonly found in forages include soyasapogenin, soyasapogenol, diosgenin, dioscin/protodioscin, and yamogenin [115]. Concentrations of saponins in plant tissues are highest in those that are vulnerable to insect, fungal or bacterial attack [115] suggesting that these compounds may act as a plant defense mechanism. It would therefore be expected that saponin concentrations in plant tissues would be highest when the plant is most vulnerable; this could include early growth after prolonged dry spells and flowering and/or seed set. *B. decumbens* spreads by seed distribution and through vegetative growth (stolons). This is supported by studies that found higher protodioscin concentrations at seed set and seed fall [64,112] and other studies that showed highest concentrations in new leaf shoots [35,40,105,108,111,122]. Outbreaks tended to be associated with growth after rain, particularly at the end of the dry season or after prolonged dry periods [34] rather than total rainfall through the growing season [105] suggesting that saponin concentrations were high. However, de Oliveira *et al.* [15] found positive correlations between total saponin concentration and green leaf mass ($p < 0.01$) and total saponin concentration and rainfall ($p < 0.05$). Protodioscin concentration was elevated after the first rains of the season (32.4 to 35.4 mg/g DM) but decreased as the wet season continued (3.9 to 8 mg/g DM) suggesting that initial high concentration could act as a deterrent to grazing animals and attack by insects, bacteria or fungi ensuring survival and establishment.

The main steroidal saponins found in *B. decumbens*, as well as many of the other species associated with secondary hepatogenous photosensitization, have been identified as dichotomin, protodioscin and dioscin [108,110,112]. Diosgenin is produced from acid hydrolysis of either dioscin or protodioscin [123]. Prior to 2000, the saponin content of pasture species including *B. decumbens* was reported as diosgenin and/or yamogenin concentrations (mg/kg DM). As these compounds have been identified as derivatives of the parent saponin “dioscin”, through improved analytical methodology, diosgenin equivalents can be calculated from protodioscin concentration using the conversion “(protodioscin mg/kg)/2.5” [8] to allow data comparison. Protodioscin concentrations in pastures associated with outbreaks, expressed as mg/g plant dry matter ranged from 5.2 mg/g DM to 35.4 mg/g DM [64,67,74,76]. Moore *et al.* (2014) [8] used a protodioscin concentration of 2 mg/g or 0.8 mg/g diosgenin equivalent as the critical value for potential toxicity of pastures. From data from

planted plot and field pasture trials, protodioscin concentrations ranged from 0 mg/g to 60.25 mg/g plant DM [8,16,40,64,76,80,105,108,111,112,122,124].

Steroidal saponins, derived from the parent plant tissue saponins, have been isolated from rumen contents [125,126] and from bile in affected animals and identified primarily as metabolic derivatives of diosgenin [109,127] and yamogenin (the 25 S isomer of diosgenin) [93,108,109,126,128]. Lambs dosed with extracts from *B. decumbens* plant material developed liver lesions, bile duct proliferation with birefringent crystals found in the bile ducts [88,89,126]. Photosensitisation was not observed as the lambs were kept out of direct sunlight. Similarly, geeldikkop, the hepatogenous photosensitization disease associated with steroidal saponins found in *Tribulus terrestris*, was reproduced using crude plant extracts, producing similar liver lesions and birefringent crystals [90,129]. The extracts contained a mixture of saponins including diosgenin and yamogenin [129]. However, lambs dosed with the commercially available saponins diosgenin and sarsasapogenin into the rumen showed neither loss of appetite nor any evidence of liver damage [40,103] suggesting that either the individual saponins may not be hepatotoxic or that the doses were not high enough.

Metabolism of plant saponins found in *B. decumbens* and other species associated with hepatogenous photosensitisation has been elucidated using plant extracts; ingested diosgenin-derived saponins were rapidly hydrolysed by rumen microflora to parent saponins [127,129]; diosgenin was converted to epismilagenin, smilagenone, smilagenin and tigogenin and primarily absorbed from the jejunum [127,130,131]. However, these dosing trials did not produce either clinical or biochemical evidence of toxicity, the direct relationship between intake of saponin-containing plant material and clinical or subclinical disease [40,127,131]. The ruminal hydrolysis of plant saponins was confirmed through the analysis of rumen contents of sheep identified as suffering from *B. decumbens* toxicity [125,126]. Cattle dosed with rumen contents from affected sheep showed evidence of liver dysfunction through elevated serum liver enzyme concentrations, providing a further link between saponins in plant material, rumen metabolism and liver dysfunction [89].

Crystals found in the bile ducts of animals suffering from hepatogenous photosensitisation associated with *Panicum miliaceum* and *Tribulus terrestris* have been identified as insoluble calcium salts of the plant saponins [96,99,132,133]. These investigations have provided important information on the possible links between steroidal saponins in plant tissue, rumen metabolism and absorption of the derived compounds and the formation of crystals capable of blocking bile ducts. However, although the birefringent bile crystals have been shown to be calcium salts of saponins derived from diosgenin [90,132,133], dosing experiments using plant extracts or isolated saponins have provided limited evidence of the link between plant steroidal saponins, liver dysfunction, bile crystals and the clinical signs of this disease [40,103,127,129,130]. Similar results have been obtained for dosing experiment (rumen contents or extracted saponins) for the other species associated with secondary hepatogenous photosensitisation and containing steroidal saponins [88,90,99,129]. It is possible that the observed liver dysfunction and associated physical signs may be either the consequence of interactions between saponins or other chemical constituents in the plants or linked to the metabolic activity of rumen microorganisms. Dosing with rumen contents of affected animals has proved more successful in reproducing biochemical and clinical signs

Saponin (particularly protodioscin) concentration varies with plant and leaf age with senescing leaves generally containing lower concentration of saponins than younger leaves [40,111,134]. Data

from Brazil and Papua New Guinea have shown that there is a 5–10 fold increase in saponin concentration between new leaves and mature leaves [40,134]. This finding supports the observations that clinical outbreaks tend to be associated with new leaf growth. Ferreira *et al.* [134] found a strong relationship between saponin concentration on young leaves and hours of solar radiation. Ensiling *B. decumbens* has been shown to decrease protodioscin concentrations significantly (2.28 mg/g DM to 0 mg/g DM) over 20 days while conserving *B. decumbens* as hay only reduced protodioscin concentration by 48% [135]. There is no clear trend in saponin concentration with plant age *per se* [76,105,122], with higher concentrations found in plant material 60 days of age or younger but almost stable levels over the following 300 days [105,122]. Brum *et al.* [112] found that saponin concentrations varied with environmental factors, age, stage of growth and cultivation methods. There was no significant relationship in protodioscin concentrations in commercial paddocks sown with *B. decumbens* in Western Australia over a north to south range of 450 km even though some locations had consistently higher concentrations; protodioscin concentrations within sample dates over a two year period ranged from 15 mg/g DM to 60 mg/g DM [8]. Sample collection methods varied from selecting leaf only to whole plant and have been sampled by plucking, hand picking and plot harvest. Samples collected from outbreak sites have been obtained after the outbreak has been detected and may not be representative of plant material consumed by grazing livestock.

Differences in apparent susceptibility (or the possibility of resistance) to *B. decumbens* toxicity [16,64,80] may be due to selective grazing, with animals avoiding plants or plant parts that contain higher concentrations of saponins. Lambs dosed with plant secondary compounds avoided grazing pastures containing the dosed compound [136] and actively sought out species that did not contain the specific compound. Brahman cattle grazing drought stressed *B. decumbens* pastures in the Markham Valley in Papua New Guinea selected older and senescing leaves, leaving new leaves untouched; this suggests an active avoidance of plant components with high saponin concentration [40]. Brahman weaners born onto *B. decumbens* pastures avoided grazing *B. decumbens* pastures after weaning, relying on a molasses/copra meal/mineral supplement [40]; examination of the pasture revealed that very few plants had been consumed and showed evidence of individual leaf selection [40]. The apparent resistance of some animals to pastures containing high levels of saponins may therefore be a consequence of modified grazing behaviour, particularly where supplements or alternative species are available. However, since *B. decumbens* pastures tend towards monocultures by outcompeting companion species, avoidance of plants with high saponin concentrations may be difficult. Avoidance behaviour could provide a possible explanation, on predominantly *B. decumbens* pastures, for poor growth rates and wasting or weight loss often associated with livestock grazing *B. decumbens* pastures since minimising the intake of high risk pastures will reduce nutrient intake and thus reduce growth rates.

Low growth rates from animals consuming *B. decumbens* pastures could also be related to liver dysfunction (as indicated in elevated serum AST/GGT) or to changes in the rumen microbial population. Early studies showed reduced microbial population and cellulolytic activity of sheep grazing *B. decumbens* pastures, resulting in significantly lower levels of volatile fatty acid production [137]. Although saponins have been shown to reduce protozoal numbers in the rumen through rupture of the protozoal cell membrane and reduce methane production and to decrease the numbers of cellulolytic bacteria, the effects depend on the source of the saponins [115,138]. There is

some suggestion that rumen bacterial populations adapt to saponins under long term feeding, but this is yet to be confirmed [115]. Grazing trials with naïve and experienced sheep showed that naïve sheep had higher morbidity and mortality rates than experienced sheep [80]. Adaptation of rumen microorganisms may provide grazing livestock with an element of protection from *B. decumbens* toxicity.

6. Conclusions

B. decumbens is a tropical/sub-tropical pasture species that produces high quality feed for grazing ruminants. The suitability of *B. decumbens* as a quality pasture for ruminant livestock production is compromised by the presence of steroidal saponins including protodioscin, diosgenin and yamogenin. These compounds have been linked to sporadic outbreaks of secondary hepatogenous photosensitisation, particularly in young animals and have been implicated in sub-clinical disease leading to poor growth rates, wasting and anorexia. The occurrence of *B. decumbens* toxicity and its impact on animal productivity may be grossly underestimated since diagnosis is based solely on outbreaks of photosensitisation and recorded as such. Steroidal saponins have been linked to birefringent crystals that potentially block the bile ducts resulting in elevated liver enzymes. Assessment of liver dysfunction is not possible in a production system. Animal behavior changes (such as shelter seeking and grazing avoidance behaviours) may be indicative of toxic pastures. The concentration of these compounds is influenced by management, season, stage of growth and environmental conditions. Further research is needed to elucidate the factors influencing saponin concentrations in *B. decumbens*, to study the impact of the saponins from *B. decumbens* on rumen function, and to investigate apparent differences in susceptibility between individual animals.

Conflicts of Interest

The authors declare no conflict of interest.

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