Toxicity of Signal Grass (Brachiaria Decumbens): a Review Article

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ABSTRACT

Signal grass (Brachiaria decumbens) is important forage from tropical regions such as Africa, Asia, Australia and South America. However, a limiting factor for the use of this grass is its toxicity. Sporadic outbreaks and spontaneous cases of hepatogenous photosensitisation in ruminants grazing on this grass have been reported. B. decumbens toxicity affects all farm ruminants. Sheep are more susceptible than other animal species and the young are more susceptible than adults. The potential importance of steroidal saponins present in B. decumbens has been established suggesting that photosensitisation by this grass belongs to the group of plant-induced hepatogenous photosensitisation. This review article will be critically shades lights on the B. decumbens profile, its toxic principle, pathology, clinical pathology and the basis of oxidative stress dependant pathogenesis of signal grass intoxication. Furthermore, it will integrate findings on the research progress on B. decumbens intoxication and the previous preventive and/or therapeutic trials planned to minimizing or removing its deleterious toxic effects.

Keywords: Brachiaria decumbens, toxicity, ruminants, pathology, therapy

1. Introduction

Globally, photosensitisation in ruminants is of economic and welfare importance. It refers to the development of skin lesions caused by the interaction between sunlight with exogenous photodynamic substances that are capable activating free radicals. Photosensitisation diseases are divided into three categories. Type I: primary photosensitisation which is caused by dietary substances that are absorbed and subsequently react with sunlight in the integument. Examples include hypericin in St. John’s wort (Hypericum perforatum and Hypericum crispmum) commonly called Aran poisoning (Saleem et al.,1999; Shindala et al.,1998;Youkhana et al.,1996;Kako and Al-Sultan,1992;Kako et al.,1993) , fagopyrin in buckwheat (Fagopyrum esculentum) and furocoumarins in various plants including parsnips, parsley and celery (Araya and Ford, 1981). Type II: photosensitisation due to an excessive aberrant photosensitising pigment synthesis, the only example is inherited congenital porphyrias in domestic animals due to excessive production of prophyrsins substance, which is the photo dynamic agent (Clare, 1952). Type III: Secondary or hepatogenous (ictrogenous) photo-sensitisation in grazing livestock occurs as a result of liver damage. The damaged liver...
is unable to adequately remove a chlorophyll metabolite, phytoporphyrin (phylloerythrin), from the blood for excretion in the bile. Phylloerythrin, a photodynamic agent is produced by rumen micro-organisms. Obviously, all economically important photosensitisation diseases of ruminants are of this type. Thus, secondary photosensitisation may occur with a wide variety of hepatotoxic agents and it is most severe when large amounts of lush, green forage are ingested, which produce a large chlorophyll intake (Cheeke, 1995). It has been noticed that hepatogenous photosensitisation syndrome (HPS) is most often associated with the grazing of particular stressed hepatotoxic grasses. This may occur in late spring and summer when good rain is followed by a period of hot conditions.

*Brachiaria decumbens* is a stoloniferous, high yielding grass well adapted to a wide range of soils in the humid and sub-humid (down to 1000 mm annual rainfall) tropics, but also grows well in the coastal subtropics showing some tolerance of drought and cold. Furthermore, it exhibits tolerance of low soil fertility, drought resistance and is relatively free from pests and diseases (Loch, 1977). It is one of the most important improved grass species and in recent years an increasing interest in developing *B. decumbens* pasture has been shown by various countries in Asia, Africa, South and Central America. *B. decumbens* could provide all the forage requirement of ruminant in the tropics and this has helped to promote the growth of this livestock sector.

In Malaysia, this grass has been shown to have an agronomic potential as an excellent pasture species under local climatic condition. It has been extensively planted in most livestock farms in Malaysia and by smallholding livestock farmers since it grows well even with substandard management practices and gives impressive yields of both green and dry matter (Wong, 1980).

The capacity of *B. decumbens* to release allelopathic substances to the environment is well established. Allelopathy, simply defined as "the release of phytotoxins by plants" (Bais et al., 2003). Unfortunately, numerous reports have shown that *B. decumbens* is hepatotoxic to sheep, goats and cattle (Abas et al., 1983; Salam Abdullah, 1987; Meagher et al., 1996; Lemos et al., 1997) and can develop hepatogenous photosensitisation in animals when consumed as a sole diet for long periods.

Obviously, the plant factors responsible for liver damage characterized by deposition of crystalloid substances in the parenchymatous cells of liver and biliary ductal system are lithogenic steroidal saponins (Bridges et al., 1987). These crystalloid materials have been identified as mainly the insoluble salts of episarsasapogenin β-D-glucuronide and/or epismilagenin β-D-glucuronide (Flaøyen, 1996, 2000).

Typically, hepatogenous photosensitisation outbreaks due to steroidal saponins containing plants occur sporadically and are difficult to reproduce experimentally. Jaundice and photosensitivity are the main consistent clinical signs of these outbreaks, while oedema, haemoglobinuria and nervous signs after prolonged exposure are observed occasionally. Biochemically, a marked increase in icterus index and significant elevation of aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT) activities are diagnosed in affected animals. At necropsy, varying degree of jaundice, subcutaneous oedema and an enlarged, firm and icteric liver with enlarged gall bladder are usually noticed. Histopathologically, centrilobular hepatocellular necrosis with or without portal fibroplasia, bile duct hyperplasia and cholangitis are mostly seen (Abas et al., 1983; Noordin, 1988; Noordin et al., 1989;
Graydon et al., 1991; Flaøyen, 1992; Zhang, 2000; Wisløff et al., 2002; Assumaidaee et al., 2010).

2. General Profile of Brachiaria decumbens

2.1 Botanical Description

The genus Brachiaria, family Panicea includes about 100 species, which occur in tropical and subtropical regions of both eastern and western hemispheres, but mostly in Africa. Six major perennial species namely, B. decumbens, B. brizantha, B. dictyoneura, B. humidicola, B. mutica and B. ruziziensis have been used as pastures with varying degree of success (Loch, 1977).

B. decumbens is a low to moderately high growing trailing perennial with upright, sword-shaped leaves. Its hairy leaves are a key distinguishing feature. New shoots and roots develop from each node of its stoloniferous base. The flowering stem terminates in three or four spike-like seed stalks. It is a bunching grass that can also spread from runners, giving dense soil cover, with a canopy usually under 40 cm when grazed. Furthermore, it spreads rapidly from stolons which root readily from the nodes, and has a distinctive inflorescence a panicle of 2-3 racemes attached at right angles, thus the common name signal grass.

2.2 Distribution

Pantropical signal grass is native of the African savannas, with its centre of diversity in the surroundings of Lake Victoria, eastern Africa (Keller-Grein et al., 1996). It is one of the more widely used grasses in multiple tropical countries (Brazil, Colombia, Jamaica, Venezuela, Peru, Northern Australia and Malaysia). In the Brazilian savanna alone, there are around 15 million hectares of B. decumbens (Macedo, 2005). On Malaysian farms, Brachiaria species are planted on 80% of the total improved pasture land and 70% is B. decumbens alone (Chin, 1989).

2.3 Adaptability

B. decumbens is the most widely grown pastures in humid and sub-humid tropics. Some of the attributes that enable it to adapt to low-fertility acid soils are (a) maintenance of root growth at the expense of shoot growth, (b) acquisition of biological nitrogen (N) fixation predominantly by bacteria from at least three species of the genus Azospirillum: A. amazonense, A. brasilense and A. lipoferum. This fixation is particularly important in B. decumbens cultivated in N poor soils. (c) Acquisition of phosphorus through an extensive root systems and association with vesicular-arbuscular mycorrhizae and (d) acquisition of calcium through an extensively branched root system with large numbers of root tips (Miranda and Boddey, 1987; Rao, et al., 1996). The grass responds to low fertility by increasing the root to shoot ratio up to 30% in experimental conditions (Rao et al., 1996).

Unlike most food crops, it is directly derived from a wild apomictic germplasm accession that is highly resistant to aluminum (Al) and well adapted to infertile acid soils (Keller-Grein et al., 1996; Rao et al., 1996). Biophysiological, the tolerance to Al toxicity is due to the
absorption of more calcium (Ca) and magnesium and less Al in root tip cells under Al stress environment.

2.4 Forage Production

Different accessions of *B. decumbens* have been reported to produce 11.4 tonne dry matter ha$^{-1}$ yr$^{-1}$ in Brazil, with 26% of the total biomass production during the dry season and leaf to stem ratio of 1.07 to 1.51 (Valle et al., 1993). In Malaysia, the average dry matter yield is 25.5 tonne dry matter ha$^{-1}$ yr$^{-1}$. When fertilized with nitrogen and well managed, *Brachiaria* pastures give good quality, palatable forage enabling good animal performance (Valle et al., 1993). More specifically, *B. decumbens* produced more leaf area and finer roots (higher values of specific root length, SRL) under lower P growing conditions, presenting *B. decumbens* as a species with better low phosphorus (P) adaptation. Furthermore, according to Santos et al. (2004) signal grass harvested from the 50th day had adequate lactic acid bacteria populations which guarantee good fermentation, resulting in good quality silage, considering pH, NH$_3$ and organic acid values. Finally, *B. decumbens* a fast-growing and high biomass grass has a potential to clean-up of heavy metal polluted soils since it fulfills most of the requirements for chemically-induced phyto-extraction (Fabiana et al., 2006).

The dense cover of signal grass gives relatively weed-free pastures, but also prevents good compatibility with twining or erect legumes. Intensive grazing gives the best performance with high animal output as old leaf is not allowed to accumulate. Macfarlane and Shelton (1986) recommended that signal grass pastures should not be grazed shorter than 20 cm.

2.5 Nutrient Requirements

*Brachiaria* species have much lower requirements, especially of P and Ca, than other grasses such as *Panicum maximum*. Nitrogen is the most commonly limiting nutrient for the productive life of mono-specific swards of *Brachiaria* (Rao, et al., 1996). However, *B. decumbens* performs better than other *Brachiaria* species in unfertilised experimental conditions (Alvim et al., 1990).

2.6 Pitfalls

Signal grass is highly susceptible to spittlebug (several genera and species of *Homoptera: Cercopidae*) attack. *B. decumbens* reproduction is apomictic (asexual seeds), which until recently, complicated its genetic manipulation so as to combine its broad edaphic adaptation with tolerance to such infestation (Miles and do Valle, 1997).

2.7 Genetic Resistance

Baber (1989) noted that the *B. decumbens* outbreaks are sporadic and large areas of *Brachiaria* pastures are being grazed without problems. Likewise, a few of the experimental sheep did not develop toxic signs at all after grazing *B. decumbens* which suggests strongly that there may be individual or genetical resistance towards signal grass toxicity (Salam Abdullah et al., 1990).
2.8 Toxic Compounds

Saponins are naturally occurring low molecular weight secondary plant glycosides which are found mainly but not exclusively in more than 100 plant families (Sparg et al., 2004). In general, saponins are well known toxic compounds due to their ability in lowering surface tension and their membranolytic activity. This is attributed either to their hexagonal structure allowing them to selectively remove lipid membrane or to their permanent interaction with cholesterol to form micelle-like aggregations within the plane of the biomembrane (Bangham and Horne, 1962). Furthermore, Flåøyen (1993) suggested that the surface activity of saponins might facilitate the uptake of other toxic substances from the gut. Obviously, it is impossible to ascertain this suggestion, since the role of saponins is related to the liver rather than to the intestine. Saponins are also known to influence both ruminal bacterial species composition and number through specific inhibition, or selective enhancement of growth of individual species (Sen et al., 1998). On the other hand, saponins in rumen can inhibit absorption of various micronutrients by forming insoluble complexes (Cheeke, 1998). Unfortunately, the prolonged exposure of human beings to excessive amounts of saponins has been known to produce hypertension, flaccid quadriplegia and hypokalemia (Izzo et al., 2005).

Saponins and in particular steroidal saponins from B. decumbens and at least nine other plants have been suggested to cause hepatogenous photosensitisation disease of sheep (Holland et al., 1991; Salam Abdullah et al., 1992; Miles et al., 1993; Flåøyen, 2000; Zhang, 2000). Steroidal saponins are naturally occurring toxic glycosides with core type spirostanes or furostanes bearing one or more sugar chains, usually at the C-3 carbon (spirostanes) or at the C-3 and C-26 carbons (furostanes) (Lacaille-Dubois and Wagner, 2000). Chemical analyses of plant species involved in HPS outbreaks has shown that the saponins of P. dichotomiflorum (Holland et al., 1991) and T. terrestris (Kellerman et al., 1991; Miles et al., 1993) are derived mainly from diosgenin, the saponins of B. decumbens (Meagher et al., 1996; Cruz et al., 2000) are derived from diosgenin (25R-isomer) and yamogenin (25S-isomer) and the saponins of N. ossifragum (Miles et al., 1993) are derived from sarsasapogenin and smilagenin (Figure 1). These findings are consistent with saturation of the 5,6-double bond, followed by epimerization at C-3 in ruminants and leads to the saturated analogue epismilagenin (25R-isomer) or episarsasapogenin (25S-isomer) with the 3α-OH, 5β-H stereochemistry.
Fig. 1. Structures of the steroidal sapogenins from plant sources and ruminal metabolism.

Steroidal saponins, as a mixture of sapogenin, 3-spirostanols have been isolated from the rumen contents of intoxicated sheep fed signal grass (Salam Abdullah et al., 1992) which were identified later as episarsasapogenin and epismilagenin by Lajis et al. (1993). In addition, diosgenin [(25R)-spiorost-5-en-3β-ol] was isolated from B. decumbens, the grass implicated in the outbreaks of hepatogenous photosensitivity in Malaysia (Zhang, 2000).

Pires et al. (2002) identified four steroidal saponins (3β-methoxy-lanost-9 (11)-ene, 3-O-β-D-glucopyranosyl-24 (S)-ethyl-22 E-dihydrocholesterol, 3-O-β-D glucopyra-nosyl-24(R)-ethyl-22 E-dihydrocholesterol and 3-O-α-L-rhamnopyranosyl-(1→4)-[L-rhamnopyranosyl (1-2)]-D-glucopyranosyl]-25 (S)-spirost-5-en-3-ol) and three sapogenins (diosgenin, Dioscin and yamogenin) from aerial parts of Brachiaria decumbens grass (Figure 2). Furthermore, a furostanol-like steroidal saponin known as 25R- and 25S- protodioscin isomers in B. decumbens leaves were isolated by Haraguchi et al. (2003).

High levels of protodioscin isomers in B. brizantha and B. decumbens were detected during the maturation phase suggesting that these grasses are more toxic during this stage (Brum et al., 2009). Contrarily, Meagher et al. (1996) showed higher quantities of diosgenin and yamogenin sapogenins in younger B. decumbens when compared with the mature grass. Sapogenins content can vary in the same species when they are cultivated in different sites (Meagher et al., 1996) due to several factors, such as environmental stress, plant age and developmental stage (Oleszek, 2002).
The experiments of feeding sheep with different forms of *B. decumbens* indicated that liver damage occurred much earlier in sheep fed with *B. decumbens* hay than those fed with the fresh grass. This accelerated effect was postulated to be due to hepatotoxic substances in *B. decumbens* becoming more concentrated during the drying process of the grass (Abas and Sharif, 1986).

Several workers have suggested that saponins may cause the diseases (Ender, 1955; Abdelkader et al., 1984; Lancaster et al., 1991), while others have debated whether saponins or sapogenins are the sole cause of it (Flaøyen et al., 1991b; Flaøyen, 1993). However histological alterations of cholangiohepatopathy associated with the presence of optically active, birefringent crystals in liver, biliary system and cells of renal tubules (Miles et al., 1991; Cruz et al., 2001), similar to those found in poisonings by other lithogenic hepatotoxic grasses suggested that *Brachiaria* species intoxication was due to lithogenic saponins contained in the grass (Lemos et al., 1997). Crystal-associated cholangiopathy was reproduced by supplying sheep with *B. decumbens* and its fractionated extract containing...
sapogenins found in pasture samples, ruminal content and bile (Cruz et al., 2000; Cruz et al., 2001). These crystals are the calcium insoluble salts of ß-D-glucuronides of sapogenin conjugates of either episarsasapogenin or epismilagenin only (Holland et al., 1991; Miles et al., 1991, 1992a,b, 1993, 1994b).

New Zealand researchers (Miles et al., 1991, 1992b; Munday et al., 1993) cautioned that although the bile crystals are saponin derivatives, plant saponins alone are unlikely to cause sufficient liver damage to result in photosensitisation. Miles et al. (1992a) suggest that other hepatotoxins such as mycotoxins (e.g., sporidesmin) may have a synergistic effect with saponins. This interaction between saponins and other hepatotoxic agents might explain the sporadic incidence of photosensitisation.

In fact, the disease initially was attributed to sporidesmin produces by *Phitomyces chartarum* spores in the pastures (Nobre and Andrade, 1976; Mullenax, 1991). However, this postulation was dismissed by Smith and Miles (1993) based on the findings of anti-nutritional factors namely steroidal saponins in the rumen liquor of *B. decumbens* intoxicated sheep (Salam Abdullah et al., 1992; Nordin Lajis et al., 1993). Subsequently, *B. decumbens* was declared hepatotoxic and regarded the main cause of the syndrome. This belief was supported by experimental trials in sheep grazing on pure stands of *B. decumbens* (Abas et al., 1983; Abas and Sharif, 1988; Salam Abdullah et al., 1989), feeding cattle and rats with rumen liquor from intoxicated sheep (Noordin et al., 1989) and by failure of isolation of suspected fungus from affected areas (Abas et al., 1983; Opasina, 1985).

3. Mechanism of *B. decumbens* Toxicity

Neither *B. decumbens* grass nor its sapogenin are toxic *per se* but certain compounds, as a result of microfloral activities in the rumen, or the interaction between saponins with other compounds in the gastrointestinal tract such as cholesterol or bile salt may contribute in the course of this grass toxicity (Gee and Johnson, 1988; Noordin et al., 1989). These suggestions were strongly confirmed by clinical observations among which the ethanolic extract of rumen liquor from *B. decumbens* intoxicated sheep caused a marked enlargement of the liver and severe necrosis of hepatocytes of rats (Salam Abdullah, 1987). Furthermore, even though cattle fed this grass developed no toxicity signs, the infusion of rumen liquor from *B. decumbens* intoxicated sheep into the rumen of cattle caused hepatic and renal dysfunction (Noordin et al., 1989).

Likely mechanism for the formation of biliary crystals involves the hydrolysis of sugars from saponins by ruminal metabolism, followed by reduction of double bond (C5-C6), radical epimerization of 3ß-OH to 3α-OH and finally conjugation with glucuronic acid. The glucuronides of epismilagenin and episarsasapogenin will bind with calcium ions and form insoluble salts that are deposited in the form of crystals. A further consideration is that diosgenin, smilagenin and epismilagenin, each of which are (25R)-sapogenins, have been shown to be 2 to 3 folds more lithogenic than the corresponding (25S)-isomers (yamogenin, sarsasapogenin and episarsa-sapogenin), respectively (Miles et al., 1991, 1993, 1994b). Flaøyen and Wilkins (1997) hypothesized that the greater the level of free and conjugated...
episapogenin isomers which are transported from the fore stomach to the duodenum and jejunum, the greater will be the liver's exposure to lithogenic (crystal-forming) episapogenins. These crystals cause inflammation and obstruction of the biliary system, and necrosis of periportal hepatocytes, resulting in jaundice, photosensitisation and hepatitis. According to Cruz et al. (2000) the crystalloid material can cause jaundice and photosensitisation by physical blockage of bile flow or the metabolites of saponins can cause specifies cholestasis with action similar to Lantadene A, a polymorphic crystal form that plays a pivotal role in Lantana camara-induced intrahepatic cholestasis and ictericity (Sharma et al., 1988). It may be possible that other plant or fungal toxins also affect the liver function leading to an abnormal metabolism of the ingested plant saponins and thus to the bile crystal formation.

It is imperative that the isolated diosgenin from signal grass has similar alkyl group of C-17 as anabolic steroids that is probably responsible for the development of jaundice and hepatic dysfunction as reported to be induced by anabolic steroids (Meeks et al., 1991).

Interestingly, an oxidative stress dependant pathogenesis of B. decumbens toxicity in sheep was postulated by Zhang (2000). In this postulation, the ingested diosgenin contained in the B. decumbens will undergo microbiologic metabolism in the rumen (Noordin, 1988). The resultant metabolites as shown by Lajis et al. (1993) and Flaøyen and Wilkins (1997) will be formed at different regions. However, the most important effect is the process happening in the liver. After uptake by hepatocytes, diosgenin and its metabolites undergo biotransformation, which is catalysed by mixed function oxidase system, yielding corresponding intermediate free radicals. These radicals promote lipid peroxidation of cellular and organellar membrane. Lipid peroxidation will result in membrane damage at both cellular and subcellular levels leading to organelle damage and cell necrosis.

Elevated plasma concentrations of urea and creatinine, and the renal histopathological changes in sheep intoxicated by diosgenin and its metabolite in B. decumbens and other steroidal saponin containing plants suggested that affected animals had been through a phase of nephrotoxicity (Graydon et al., 1991; Flaøyen et al., 1995; Flaøyen et al., 1997; Zhang, 2000; Wisløff et al., 2002; Badiei et al., 2009). Generally, membrane-permeabilising effect of saponins owing to their water solubility may be detrimental to the renal epithelial cells and thus associated with renal damage.

4. Toxic Effects of B. decumbens

4.1 Historical Background

There are some strong indications that B. decumbens and other Brachiaria spp., under conditions that are yet to be completely understood, may become toxic and cause hepatogenous photosensitisation, without the participation of other agents. The first observation of hepatogenous photosensitisation in sheep after grazing on pure strands of Brachiaria brizantha was reported in Australia (Briton and Paltridge, 1940). In Malaysia the first incidence of B. decumbens toxicity was observed in cattle in 1975 (Abas and Sharif, 1986). Later, in 1979, multiple cases of hepatic jaundice and photosensitisation due to B. decumbens grazing were observed in sheep and goats at the Malaysian Agriculture Research and Development Institute (MARDI) (Suparjo and
Abdul Wahid, 1980). Abas et al. (1983) reported 41% mortality and 100% morbidity in a group of 12 ewes grazing *B. decumbens* in Malaysia. Again, Abas et al. (1985) reported sheep deaths after grazing of *B. decumbens*, but noted less severe effects on goats. Such incidence occurred quite extensively in the government farms affecting mainly sheep and goats with high mortalities (Abas and Sharif, 1986).

A similar spontaneous or experimental cases of hepatogenous photosensitisation in sheep, goats, cattle and buffaloes grazing on *B. decumbens* has been described in Brazil (Oliveira et al., 1979; Meagher et al., 1996; Tokarnia et al., 2002; Brume et al., 2007), New Zealand (Smith and Miles, 1993), South America (Anon, 1979), Southeastern Asia (Abas et al., 1983; Murdiati and Lowry, 1983; Zamri-Saad et al., 1987; Noordin, 1988; Zhang, 2000; Assumaidaee et al., 2010), and West Africa (Opasina, 1985).

### 4.2 Clinical Signs

Hepatogenous photosensitisation in animals kept on pastures of *B. decumbens* has been observed in sheep, goats and cattle. Nevertheless in sheep, there was a high incidence of this syndrome with typical signs and severe pathological changes (Abas et al., 1983). In these animals, sheep of any age, but mainly lambs are more susceptible than cattle less than 2 years of age to *B. decumbens* intoxication. It has been observed in 20–45 days old calves, suggesting that the toxin is eliminated through the milk. However, goats were found to be less affected and required a longer period of grazing to be intoxicated compared to sheep (Abas et al., 1985). The clinical incidence affecting goats and cattle was, however, very low (Opasina, 1985). Clinically, the toxic effects of *B. decumbens* in sheep and goats, characterised by jaundice and photosensitivity. Later, neurological disorders such stamping of forelegs, stargazing, incoordination, circling movements and head pressing against the fence, emaciation, corneal opacity and dehydration were seen. In severe cases, ulcerative dermatitis with exudation and sloughing were observed at the photosensitised region (Abas et al., 1983; Button et al., 1987; Salam Abdullah et al., 1987; Noordin et al., 1987; Graydon et al., 1991; Flaoyen et al., 1995; Cruz, 2000; Seitz et al., 2004; Brum et al., 2007; Assumaidaee et al., 2010). Occasionally, bilirubinuria could be seen (Tokarnia et al., 2002). Furthermore, Riet-Correa et al. (2002) attributed the wasting syndrome and death in cattle associated with chronic grazing of *B. decumbens* to the intestinal granulomatous lesions induced by this grass. The clinical signs developed as early as one week (Abas et al., 1983; Assumaidaee et al., 2010) or two weeks (Salam Abdullah et al., 1990) after grazing on *B. decumbens* and usually die four weeks after grazing depending on the severity of the intoxication.

### 4.3 Gross Pathology

General body condition of the affected animals characterized by varying degrees of icterus of subcutaneous and adipose tissue (especially the omental and mesenteric fat) as well as serous and mucous membranes. Furthermore, a prominence of subcutaneous gelatinous yellowish oedema was observed in the submandibular area. The liver was enlarged, firm, mottled, ochre or bronze-icteric in color with distended gall bladder and thick dark greenish
bile with evidence of a slight accentuation of lobular pattern (Abas et al., 1983; Button et al., 1987; Noordin, 1988; Graydon et al. 1991; Cruz et al., 2000, Driemeier et al., 2002; Brum et al., 2007). Yellow liver and whitish hard nodules in the mesenteric and hepatic lymph nodes were observed in cattle associated with chronic grazing of *B. decumbens* (Riet-Correa et al., 2002). Slightly swollen kidneys with grey-yellowish mottled appearance were mostly seen in *B. decumbens* intoxication (Graydon et al., 1991).

Photosensitisation results initially in swelling under the skin, which then sloughs leaving raw sores. This is overtly seen in lightly pigmented areas especially those directly exposed to sunlight like dorsum, face and ears.

### 4.4 Histopathology

Histopathological examinations of livers and kidneys of affected sheep suggest that *B. decumbens* toxin(s) directly damage the cells of the liver and kidney. The histological changes varied in severity according the time after the beginning of the signal grass ingestion.

Dominantly, liver damage characterized by parenchymatous changes ranging from diffuse patterns of zonal necrosis in single centrilobular hepatocyte with mononuclear inflammatory cell infiltration to diffuse parenchymatous hydropic and/or fatty changes. Furthermore, minor to moderate portal fibroplasia and bile duct proliferation were almost always present (Abas et al., 1983; Noordin, 1988; Zhang, 2000; Brum et al., 2007; Assumaïdaee et al., 2010). Occasionally, slight to moderate lesions were observed in the liver, as diffuse cloudy swelling, lysis of some liver cells, vacuolization, individual necrosis of hepatocytes with characteristic perinuclear cytoplasmic clearing, slight megalocytosis, proliferation of Kupffer cells, lymphocytic pericholangitis with proliferation of epithelial cells of the bile ducts. According to Zhang (2000), disarray of hepatic cell plate and dilatation of sinusoids in the liver of *B. decumbens* intoxicated sheep are also the features of cholestasis.

Ruminants which develop hepatogenous photosensitisation due to *Brachiaria* spp. ingestion present histological lesions of cholangiohepatopathy characterized by birefringent crystals in hepatocytes, swollen Kupffer cells and ductular epithelial cells of biliary duct system. Aggregates of acicular clefts and crystals were described in bile canaliculi, bile ducts, Kupffer cells, hepatocytes and in kidney tubules of sheep grazing *B. decumbens* by Graydon et al. (1991). The number of crystals increased with the length of exposure to the plant and sheep that died had the largest accumulations. Crystal-associated cholangiopathy was reproduced by supplying sheep with *B. decumbens* and its fractionated extract containing sapogenins found in pasture samples, ruminal content and bile (Cruz et al., 2000; Cruz et al., 2001).

Birefringent crystals have also been shown recently in the cytoplasm of periportal hepatocytes. Further crystal negative images were also found within cytoplasm of macrophages present inside the hepatic sinusoids and in the periportal spaces (Saturnino and Klause, 2010). They were more significant in the liver and incriminated to be the major cause of bile stasis, bile duct proliferation, swelling and vacuolation of both hepatocytes and foamy macrophages.

Multifocal accumulations of foamy macrophages, mainly in the liver, but also in hepatic and mesenteric lymph nodes, spleen and intestinal submucosa were observed in affected and
healthy animals grazing *Brachiaria* spp. (Driemeier et al., 1998, 1999; Riet-Correa, 2002; Brum et al., 2007). Histologically and ultrastructurally, these cells present negative crystals images (Driemeier et al., 1998) suggesting that they are formed in consequence of the crystal phagocytosis. These foamy macrophages have not been reported in poisoning by other plants containing lithogenic saponins, except for the report of hypertrophic Kupffer cells containing crystals observed in *Panicum coloratum* poisoning (Bridges et al., 1987).

The renal histopathological changes observed in several plants containing steroidal saponins showed marked dilatation or collapse and diffuse degenerative or necrotic changes of the epithelial cells in kidneys of sheep, goats, calves, lambs and cervids (Graydon et al., 1991; Flæøyen et al., 1995a,b; Wisløff et al., 2002). Besides, the tubular lumen filled with eosinophilic proteinaceous casts, crystalloid materials or acicular clefts in kidney tubules of sheep grazing *B. decumbens* were described by Graydon et al. (1991).

In other tissues, the affected areas of photosensitised skin initially developed erythema followed by oedema, serous exudation, scab formation and epidermal with dermal necrosis. The most dominant feature in most *B. decumbens* intoxications was the solar necrotizing vasculitis in the dermis (Graydon et al., 1991; Zhang, 2000; Assumaidaee et al., 2010). In the brain, numerous vacuolations of various sizes were observed in the white matter of the cerebellum giving rise to a status spongiosus. (Salam Abdullah et al., 1989). Interestingly, Riet-Correa et al. (2002) reported intestinal granulomatous lesions in cattle associated with chronic grazing of *B. decumbens*. Moreover, granulomatous cholangiohepatitis, full of multinucleated giant cells, centralized by birefringent crystalloid material was also observed by da Costa et al. (2006).

### 4.5 Clinical Biochemistry

Serum biochemistry of most *B. decumbens* intoxications suggested hepatobiliary and hepatoparenchymal damage with impaired renal function. Experimental *B. decumbens* toxicity in sheep increased the plasma icteric index almost 40 folds. Mostly, this increase was coincided with the appearance of jaundice. Other significant changes include increased in the total, direct and indirect bilirubin levels, concentrations of blood urea nitrogen (BUN) and creatinine and in the activities of the enzymes such as aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH) and gamma glutamyltransferase (GGT) (Abas et al., 1983; Noordin et al., 1989; Graydon et al., 1991; Zhang, 2000; Brum et al., 2007). Signal grass adversely affected the growth and activity of microorganisms in the rumen as reflected by greatly decreased concentrations of the volatile (acetic, propionic and butyric) fatty acids (Salam Abdullah and Rajion, 1990). Furthermore, *B. decumbens* toxicity in sheep induced changes in motility and pH environment of reticulo-rumen leading to ruminal stasis within 3 weeks of continuous grazing (Salam Abdullah et al., 1988).

All *B. decumbens* intoxicated sheep showed decreased in bromosulphophthalein (BSP) clearance. The progressive BSP retention was an indicative of immediate liver damage occurring in the affected sheep.

Grazing on signal grass significantly decreased the concentration of cytochrome P-450 and the activity of drug metabolizing enzymes, viz. aminopyrine-N-demethylase, aniline-4-
hydroxylase, UDP-glucuronyltransferase and glutathione S-transferase in liver and kidneys of intoxicated sheep (Khairi, 2003).

Brum et al. (2007) attributed the rise in GGT, direct and total bilirubin and cholesterol in intoxicated lambs but not the adult sheep grazing Brachiaria decumbens containing the saponin protodioscin to cholestasis. These results confirm previous reports of Button et al. (1987) that young sheep are more susceptible to the poisoning than adults. Biochemical analyses of llama (Lama glama) intoxicated with Brachiaria decumbens in Brazil revealed increased values of aspartate aminotransferase, gamma glutamyltransferase, direct and total bilirubin (Birgel et al., 2007).

Measurement of serum acute phase protein concentrations in cattle grazing Brachiaria decumbens pasture showed significant increase in the concentrations of haptoglobin, ceruloplasmin, antitrypsin and acid glycoprotein, respectively. These alterations may be useful in monitoring the progression of bovine HPS, including guide probable alteration on therapeutic procedures (Fagliari et al., 2007).

4.6 Histochemistry

The characterization of specific carbohydrate residues of storage material in foamy macrophages in tissues of animals exposed to Brachiaria spp. provides clues to understand the pathogenesis of their establishment. In cattle, these cells did not stain with Periodic acid-Schiff (PAS) but weakly with oil red (Driemeier et al., 1998). Furthermore, Gomar et al. (2005) found the lectin peanut agglutinin to be an excellent marker to differentiate and quantify these foam cells, and could be used as a specific biomarker.

4.7 Ultrastructural Changes

Ultrastructural manifestations of Brachiaria decumbens intoxication vary with the position of hepatocytes within the acinus. Cells in centrilobular areas may be necrotic, with progressively less severe degenerative changes as one progress along sinusoids back to the portal triad. These general ultrastructural features seen in the nuclei and subcellular organelles of the liver and kidney are non-specific alterations with respect to necrosis. Vacuolated cytoplasm with numerous small and dense bodies were seen, together with the markedly appearance of distorted small nuclei in severely affected hepatocytes. The mitochondria of less affected hepatocytes were markedly swollen and vacuolated.

One of the earliest consistent changes present in the hepatocytes of Brachiaria decumbens fed sheep was the proliferation of smooth endoplasmic reticulum (sER) leading to the dispersion of other organelles either peripherally or centrally (Zhang, 2000). Nevertheless, the periacinar distribution pattern of necrosis that appear as degradation of sER in the centrilobular zone is a response of lipid peroxidation induced by toxic intermediate molecules during late stages of Brachiaria decumbens toxicity. Furthermore, the presence of crystal-like clefts and/or presence of flocculent materials (bile plug) in and around the bile ductuli are usually accompanied by total or partial desquamation of microvilli (Zhang, 2000).

Experimental cholangiohepatopathy in sheep after chronic grazing on Brachiaria decumbens had been studied by Driemeier et al. (2002). Ultrastructurally, cleft-shaped structures, which represent the negative image of crystals, can be observed in hepatocyte and macrophage
cytoplasm. These structures are covered with membranes, resembling phagolysosomes. Lymph nodes and livers revealed hyperplasia of the sER with high electron dense granules in the lysosomes of affected hepatocytes.

In kidneys, the ultrastructural changes in tubular epithelial cells consisted of desquamation and alteration of relevant organelles representing degenerative changes. Severe cytosolic vacuolation or disintegration of organelar architecture of renal tubular cells was commonly observed. Besides, dilatation of protoplasmic feet and a deep enfolding embraced with marked condensed mitochondria are mostly predominant in the degenerative tubular cells. Abnormal mitochondria with disintegration of cristae and eccentrically placed rings are also seen (Noordin et al., 1987; Zhang, 2000).

5. Research Progress on B. decumbens Prevention and Treatment

Generally, the only known way to prevent most plant origin HPS in animals’ livestock is to keep them away from the dangerous photo-or hepatotoxic pastures. Additionally, protection from light, treatment and prevention of infection and fly strike and provision of nutritious feed are strongly recommended. Symptomatic treatment also should include a balanced high-energy, low-protein diet to avoid overloading the liver with nitrogenous compounds and intravenous polyionic fluids with glucose in severely affected animals.

Generally, animals suffering from HPS were treated symptomatically. In the early phase of the toxicity, glucocorticoids and antihistaminase will reduce the swelling of the head and facilitate healing. Antibiotics may be administered to avoid septicemia and severe secondary bacterial dermatitis.

Only sparse literature discusses B. decumbens intoxication therapy and to date there is no effective antidote for it. Salam Abdullah et al. (1994) carried out a trial in Malaysia to assess the effect of adding zinc sulphate to the drinking water at (1 g/liter) on the development of signal grass toxicity in sheep. This therapeutic trial provided a limited degree of protection only and through poorly interpretive mechanism. However, zinc was known to bind saponins in the gastrointestinal tract (Price et al., 1987) and perhaps renders them less toxic in the rumen of B. decumbens intoxicated sheep. Contrary, Zhang et al. (2001) found that Zn supplementation may depress antioxidant status and enhance lipid peroxidation during B. decumbens intoxication. In fact, excess Cu and Zn are detrimental since both minerals exacerbate the B. decumbens intoxication (Zhang, 2000).

Another chemoprotection or chemoprevention trials had been focused on certain compounds that have a potential to induce drug metabolizing enzymes which in turn can increase the effectiveness of xenobiotic detoxification. Oral administration of phenobarbitone (30 mg/kg bw) for five consecutive days before grazing on B. decumbens pasture, and thereafter, for three consecutive days every two weeks, resulted in significant increases in hepatic and renal activities of drug-metabolizing enzymes. The induction of drug metabolizing activity in sheep grazing on signal grass group was found to be lower than in animals given phenobarbitone alone. Phenobarbitone provided a degree of protection against the toxic effects of B. decumbens as indicated by the delay in the appearance of mild signs of toxicity (Hasiah, 2000). Seemingly, phenobarbitone-type cytochrome P-450 isoenzyme-induction may increase resistance against signal grass toxicity in sheep, even though this
protection is not fully successful. A significant concern with this compound lies in its actions as a bifunctional enzyme inducer that elevates the activities of both phase I (cytochrome P-450, aniline 4-hydroxylase and aminopyrine-N-demethylase) and phase II (UDP-glucuronyltransferase and glutathione S-transferase) enzymes (Khairi, 2003).

The administration of 5 mg/kg body weight griseofulvin orally for five consecutive days every other week for ten weeks did not protect sheep against B. decumbens toxicity as 5/7 animals treated with this compound and grazed on B. decumbens showed signs of toxicity (Hasiah, 2000). Unlike the use of phenobarbitone, griseofulvin as monofunctional inducer, only promoted phase II liver and kidney metabolizing enzymes and subsequently failed to protect these animals against signal grass toxicity.

On the other hand, the main preventive attempts in Brazil are based on the selection of resistant or resilient animals and on the development of Brachiaria species or varieties with low lithogenic saponin concentration (Riet-Correa et al., 2011).

6. Conclusion

Diosgenin and its metabolite in B. decumbens are hepatotoxic as well as nephrotoxic and to date there is no effective antidote for signal grass intoxication. The eradication of this rich grass to avoid its toxicity is rather impractical, since the available tropical feed resources were contributed to the poor production performance of small ruminants. Further molecular work should be conducted to confirm and clarify the role of oxidative stress pathway in the development of B. decumbens toxicity in ruminants and to develop pragmatic strategies to utilise this rich grass effectively without any toxic risk.

References


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