Ciência Rural
ISSN: 0103-8478
cienciarural@mail.ufsm.br
Universidade Federal de Santa Maria
Brasil

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Ciência Rural, vol. 39, núm. 1, enero-febrero, 2009, pp. 279-281
Universidade Federal de Santa Maria
Santa Maria, Brasil

Ciência Rural, Santa Maria, v.39, n.1, p.279-281, jan-fev, 2009
ISSN 0103-8478

Steroidal saponin concentrations in *Brachiaria decumbens* and *B. brizantha* at different developmental stages

Concentrações de saponina esteroidal em diferentes fases do desenvolvimento de *Brachiaria decumbens* e *B. brizantha*

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- NOTE -

ABSTRACT

Brachiaria *species contain steroidal saponins and are involved in outbreaks of hepatogenous photosensitization. This research presents the levels of a steroidal saponin, protodioscin, in the seeds and aerial parts of* ***B. brizantha*** *and* ***B. decumbens*** *during different developmental stages (growth, bloom, fructification and seed fall). The butanolic fraction of the ethanolic extract of each stage was submitted to thin layer chromatography (TLC) and spectrophotometric analysis through the Ehrlich reagent in 515nm. The chromatograms in TLC of the butanolic fraction of* ***B. brizantha*** *and* ***B. decumbens*** *showed similar spots as the protodioscin standard. The estimated level of protodioscin isomers in* ***B. brizantha*** *and* ***B. decumbens*** *ranged from 0.5% to 2.1%, having the highest level at the end of their developmental stages during seed falling comparison with the previous one. Protodioscin was not detected in the seeds. Outbreaks of* ***Brachiaria*** *spp. poisoning in central Western Brazil are frequently observed in pastures that had been more than 30 days without animals grazing, and also during the growing or blooming stage of the pastures. Other saponin determinations in toxic and non toxic pastures are necessary to determine the saponin concentrations that cause intoxication.*

Key words: ***Brachiaria*** *spp., photosensitization, protodioscin, spectrophotometry, steroidal saponins*

RESUMO

As ***Brachiaria*** *spp contêm saponinas esteroidais envolvidas no desenvolvimento de fotossensibilizacão hepatógena. No presente trabalho foram determinados os teores da saponina esteroidal protodioscina nas partes aéreas de* ***B. brizantha*** *e* ***B. decumbens,*** *durante as diferentes fases do desenvolvimento (crescimento, floração, frutificação e queda das sementes) e nas sementes. A fração butanólica do extrato etanólico de cada fase foi submetida à cromatografia de camada delgada (CCD) e à análise espectrofotométrica por meio do reagente de Ehrlich em 515nm. Os cromatogramas em CCD da fração butanólica de* ***B. brizantha*** *e* ***B. decumbens,*** *de cada fase do desenvolvimento, apresentaram manchas similares ao padrão de protodioscina. Por meio da análise de protodioscina por espectrofotometria, os teores de protodioscina em* ***B. brizantha*** *e* ***B. decumbens*** *variaram entre 0.5% e 2.1%, sendo mais altos na fase de queda das sementes. Nas sementes não foi encontrada protodioscina. Surtos de intoxicação por* ***Brachiaria*** *spp. no Centro-Oeste brasileiro são freqüentemente observados em pastagens diferidas por mais de 30 dias e também durante as fases de crescimento e florescimento. São necessárias outras dosagens de saponinas em pastagens tóxicas e não-tóxicas para determinar as concentrações capazes de causar intoxicação.*

Palavras-chave: ***Brachiaria*** spp., fotossensibilização, protodioscina, espectrofotometria, saponina esteroidal

Brachiaria species are important forages in tropical regions as Africa, Asia, Australia and South America. In Brazil, there are approximately 95 million hectare (ha) cultivated with ***Brachiaria*** species, including ***Brachiaria brizantha*** (60 million ha), ***Brachiaria decumbens*** (25 million ha) and others (10 million ha) (FERRAZ, 2003). Outbreaks of

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Recebido para publicação 08.05.08 Aprovado em 25.06.08

hepatogenous photosensitization have been reported in cattle (FIORAVANTI 1999; LEMOS et al., 1996b; LEMOS et al., 1997; MEAGHER et al., 1996), sheep (GRAYDON et al.,1991; LEMOS et al., 1996a) and goats (LEMOS et al., 1998) grazing ***Brachiaria*** spp.

Ruminants which develop hepatogenous photosensitization due to ***Brachiaria*** spp. ingestion present histological lesions of cholangiohepatopathy characterized by birefringent crystals in bile ducts and hepatocytes. These crystals have been reported as being insoluble salts of sapogenin glucuronides originated from steroidal saponins present in the plant (MEAGHER et.al, 1996; CRUZ et al., 2000; CRUZ et al., 2001). 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). In addition, a furostanol-like steroidal saponin known as 25R- and 25S- protodioscin isomers in ***B. decumbens*** leaves were previously isolated (HARAGUCHI et al., 2003).

Due to of the great relevance of ***B. brizantha*** and ***B. decumbens*** as forages to Brazilian savannah regions, it is important to establish the saponins levels present in these plants during their developmental stages. Such data may help in pasture and animal management, as well as clarify the photosensitization etiopathogeny caused by ***Brachiaria*** spp.

B. brizantha and ***B. decumbens*** were sown, on 11/20/2002 and 01/15/2003, respectively, in two beds (2m x 3m), located at the Veterinary School at the Federal University of Goiás, Goiás State, Brazil. The seeds were produced by Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA Cerrados (Goiânia, Goiás State, Brazil). The pasture was weekly observed, and samples of leaves and stems were collected for saponin determination in four developmental stages (growth, blooming, fructification and after the seed fall) by cutting the plants at 3 cm from the soil. Seeds were also analyzed. Each sample was dried in oven at 50º C and crushed. Ten grams of powder of each growing stage were extracted with 96% ethanol. The ethanolic extract was concentrated under reduced pressure and successively partitioned in several immiscible solvents: water/ethylic ether, water/ethyl acetate and water/butanol saturated with water. The ether-soluble, ethyl acetate-soluble and butanol-soluble solutions were evaporated to dryness to obtain their respective residues. The butanol-soluble residue containing saponin was submitted to Thin Layer Chromatography (TLC) using silica gel 60 GF254 glass plate (Merck® S.A.), and developed in chloroform, methanol and water system (16:9:2). The spots were visualized by spraying 10% aqueous sulphuric acid and with Ehrlich reagent after heating to 110º C for 10 min and compared with protodioscin isomers isolated from ***B. decumbens*** (HARAGUCHI et al., 2003). Calibration curve and concentrations of protodioscin isomers in butanolic residues of both ***Brachiaria*** leaves were obtained by spectrophotometer method using Ehrlich reagent (Merck® S.A.). Reading was performed at 515 nm using spectrophotometer U-2001 (Shimadzu®) (GJULEMETOWA et al., 1982).

On TLC of the butanolic fraction, leaves in all developmental stages showed spots similar to the protodioscin isomers standard (R_f 0.28, R_f 0.34). However, the seeds did not contain protodioscin isomers. The absorbance measured in the calibration curve was linearly proportional to the saponin concentration, at interval between 0.1 and 0.4mg ml^{-1}, in accordance with the expression $A=0.2661c+0.0053$, being $R^2= 0.9999$ (A= absorbance; *c*= protodioscin isomers concentration). Thus, the estimated level of protodioscin isomers in ***B. brizantha*** and ***B. decumbens*** in different developmental stages ranged from 0.53% to 2.09%, having the highest level at the end of their developmental stages in comparison with the previous stages (Table 1).

Levels of protodioscin isomers in ***B. brizantha*** and ***B. decumbens*** detected in the present study showed a rise during the maturation of the plant suggesting that the plant is more toxic during this stage. On the contrary, MEAGHER et al. (1996) showed higher

Table 1 - Age of plants (days) and protodioscin isomers percentage (%) in ***Brachiaria brizantha*** and ***B. decumbens*** samples in different developmental stages

Developmental stages	***B. brizantha***		***B. decumbens***	
	Age of plants (days)	Protodioscin isomers (%)	Age of plants (days)	Protodioscin isomers (%)
Seeds	0	0	0	0
Growth	56	0.5	56	0.9
Bloom	96	1.0	62	1.0
Fructification	141	0.8	86	0.8
Seed fall	218	2.1	162	1.9

quantities of diosgenin and yamogenin, sapogenins in younger ***B. decumbens*** plants when compared with the mature ones. 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). Outbreaks of ***Brachiaria*** spp. poisoning in central western Brazil are frequently observed in pastures that had been more than 30 days without animals grazing. However, outbreaks also occur frequently during the growing stage of the pastures at the start of the rainy season (RIET-CORREA & MÉNDEZ, 2007). Saponin can vary in the same rangeland, e.g.: in an outbreak of poisoning by ***B. decumbens*** in sheep, the protodioscin isomers content in the pastures was 2.36%, in blooming stage. On the other hand, in a neighboring paddock grazed by cattle, the content was 1.63% (BRUM et al., 2007).

Few knowledgement exists about saponins from ***Brachiaria*** spp. and its related intoxication. Thus other determinations in toxic and non toxic pasture are necessary to determine the saponin concentrations capable to cause intoxication.

ACKNOWLEDGEMENTS

This work was financially supported by Institutos do Milénio, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Grant nº 420012/2005-2. To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for scholarship to the first author.

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