HEPATIC LESIONS IN CATTLE GRAZING ON Brachiaria Decumbens IN MESETAS, META (COLOMBIA)

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ABSTRACT
Brachiaria spp. is the predominate pasture grass for cattle grazing in the Orinoco watershed in Colombia. However, it has been recognized that this grass can cause liver damage, leading to photosensitization in ruminants; such injury is caused by the steroidal saponins found in this plant. Liver samples taken from five clinically-healthy bulls’ left and right liver lobes and portal vein entrance were processed by routine histological techniques to evaluate liver lesions caused by Brachiaria decumbens in cattle grazing on Colombia’s Eastern plains. The main lesions observed in these tissues were mononuclear cell cholangiohepatitis, foamy macrophages, moderate bile pigment accumulation, hepatocyte death, binucleated hepatocytes, moderate bile duct hyperplasia and multiple foci of mild fibrosis in portal areas; these were corroborated by Masson’s trichrome staining. Such lesions were predominantly distributed at the portal vein entrance, frequently being located in the periportal region. This type of lesion has usually been attributed to Brachiaria decumbens consumption, and was present in clinically healthy animals exclusively feeding on this type of grass. Thus we conclude that the tissue alterations found herein were caused by Brachiaria decumbens. This research should be expanded to involve a larger selection of cattle populations, throughout a broader geographical region.

Key words: Brachiaria, cattle, liver, foamy macrophage, fibrosis.

LESIONES HEPÁTICAS EN BOVINOS MANTENIDOS EN PASTURAS DE Brachiaria decumbens EN MESETAS, META (COLOMBIA)

RESUMEN
En la Orinoquía colombiana hay una considerable cantidad de ganado bovino que se alimenta predominantemente de Brachiaria spp. Sin embargo, se ha reconocido que esta pastura puede ocasionar daño hepático y posterior fotosensibilización en rumiantes, por las saponinas esteroidales que contiene. Con el propósito de evaluar las lesiones hepáticas en ganado bovino procedente de los Llanos Orientales colombianos, se tomaron y procesaron, mediante técnicas histológicas de rutina, muestras de hígado de los lóbulos derecho e izquierdo, así como de la entrada de la vena porta de cinco toros clínicamente sanos. Las principales lesiones observadas en estos tejidos fueron colangio-
hepatitis mononuclear, macrófagos espumosos, acumulación moderada de pigmentos biliares, muerte de hepatocitos, hepatocitos binucleados, moderada hiperplasia de ductos biliares y múltiples focos de fibrosis leve en áreas periportales, que fueron corroborados por tinción tricrómica de Masson. Estas lesiones se distribuyeron predominantemente en la entrada de la vena porta y se localizaron con frecuencia en la entrada de la región periportal. Dado que estas lesiones se atribuyen comúnmente al consumo de *Brachiaria decumbens*, y estaban presentes en animales clínicamente sanos exclusivamente alimentados con esta pastura, se concluye que las lesiones aquí encontradas fueron causadas por la *Brachiaria decumbens*. Se sugiere ampliar este estudio involucrando más bovinos, granjas y municipios.

**Palabras clave:** *Brachiaria*, ganado bovino, hígado, macrófagos espumosos, fibrosis.

**INTRODUCTION**

Colombia has the ninth largest cattle population in the world (FAO 2010). Many of these cattle graze on the Orinoco watershed in eastern and central Colombia where *Brachiaria* grasslands predominate (Rincón and Jaramillo 2010). A significant number of livestock graze on *Brachiaria* pasture in Mesetas, a town located in the Meta department.

Studies from different parts of the world have associated *B. decumbens*, *B. brizantha* and *B. humidicola* consumption with pathogenic photosensitization in ruminants (Cruz et al. 2001; Meagher et al. 1996); such condition was initially attributed to sporidesmin (a toxin produced by the fungus *Pithomyces chartarum*, which colonizes forage in certain environmental conditions (Tokarnia et al. 2000). Nevertheless, it is now known that steroidal saponins, specifically protodioscin, found in *Brachiaria* spp. can cause liver damage leading to photosensitization (Castro et al. 2011; Meagher et al. 1996). Diosgenin and yamogenin, (aglycone portions of principal saponins present in *Brachiaria*), turn into β-D-glucuronide in the rumen; this precipitates upon contact with biliary calcium, thereby forming crystals. Deposition of such crystal obstructs the biliary system, thus compromising phylloerythrin excretion, a photoactive metabolite from chlorophyll which, in turn, promotes photosensitization in animals (Flaoyen and Wilkins 1997; Lajis et al. 1993).

Histologically, liver samples from cattle grazing on *Brachiaria* spp. are characterized by bile duct hyperplasia, fibrosis in the periportal region, hepatocyte degeneration and necrosis, the presence of birefringent crystals within macrophages, hepatocytes and bile ducts, as well as randomly distributed foamy macrophages in the liver. Such birefringent crystals have been detected in the renal tubules while foamy macrophages have been found in local lymph nodes (Riet-Correia et al. 2009).

Similar *Brachiaria*-attributed hepatic lesions in Colombian cattle were reported in 1976 and 1982; however, neither foamy macrophages nor birefringent crystals were observed (Barrera 1976; García et al. 1982). In 1976 Barrera advanced the hypothesis that saponins could be responsible for *Brachiaria*-related toxicosis; unfortunately, there has been no continuity in researching this topic in Colombia. It has also been reported that there is a negative correlation between weight gain and the number of foamy macrophages in the livers of cattle grazing on *B. decumbens*, thereby suggesting a subclinical condition with an important economical impact (Nunes et al. 2009).
Considering the cattle industry’s importance in Colombia (particularly regarding the Eastern plains where 19% of the country’s cattle is concentrated), it is important to understand factors influencing *Brachiaria* spp. toxicosis in cattle to provide management solutions. This study was thus aimed at assessing lesions in liver samples taken from cattle which had been grazing on *Brachiaria decumbens* near Mesetas (Meta department, Colombia) and were slaughtered in an approved abattoir.

**MATERIALS AND METHODS**

**Animals**

Cattle came from La Cristalina rural area in Mesetas (coordinates: N 03° 18’ 20.8” W 074° 01’ 58.8”; 749 masl) in the Meta department where they were born, raised and fed exclusively on *B. decumbens* pasture until being slaughtered for meat products. The farm was visited by veterinarians who confirmed that *B. decumbens* was the sole *Brachiaria* species, there was no macroscop ic evidence of fungi contamination and that there were no other poisonous plants.

**The animals’ characteristics**

The study involved five, clinically healthy and white skin color bulls (*Bos taurus indicus* × *Bos taurus taurus*) at 3.5 years of age, in good body condition and weighing 630 kg on average. The animals were sampled in a slaughterhouse located in Bogotá where cattle are processed for human consumption.

**Sampling**

Three tissue samples (portal vein entrance and left and right liver lobes) were taken from each of the five livers. Samples were processed by routine hematoxylin and eosin staining and Masson’s trichrome staining for visualizing connective tissue. Microcirculatory, inflammatory, degenerative and proliferative changes and cell death were assessed in samples.

**RESULTS**

Microscopic hepatic lesions were revealed in liver samples taken from clinically healthy cattle raised in Mesetas, Meta department, and grazed on *B. decumbens* throughout their life-cycle before being slaughtered; such lesions were more extensive and severe at the portal vein entrance and frequently being located in the periportal region. The lesions and percentages of animals affected by them were as follows: 100% moderate congestion in periportal and midzone areas, 100% leukocyte kidnapping or retention, 100% Kupffer cell activation and mixed cell infiltrate in the periportal area, with moderate mononuclear cell presence (mononuclear cell cholangiohepatitis), 80% foamy macrophages, 100% moderate hydropic change, 80% mild widespread micro-vacuolar fatty change, 20% moderate bile pigment accumulation, 100% cell individualization and rounding, 100% hepatocyte death, 80% binucleated hepatocytes, 80% moderate bile duct hyperplasia and 100% multiple foci of mild fibrosis in portal areas, corroborated by Masson’s trichrome staining. Figures 1 to 4 show the main lesions.

**DISCUSSION**

It is known that consumption of *B. decumbens* and *B. brizantha* by ruminants could produce clinical disease (hepatogenous photosensitization or progressive weight loss) joined to characteristic liver lesions (Riet-Correia et al. 2002; Riet-Correia et al. 2011, Souza et al. 2010); however it has
been reported that bovines and bubalines which consume *Brachiaria* could develop subclinical disease with hepatic lesions too (Nunes et al. 2009; Riet-Correa et al. 2010). Liver histologic examination of animals with clinical signs of *Brachiaria* poisoning shows vacuolation, necrosis hepatocytes, bilestasis, mononuclear cholangitis, and pericolangitis, periportal fibrosis and birefringent crystals in the bile ducts, macrophages and hepatocytes (Riet-Correa et al. 2011). Although, foamy macrophages have been observed in liver of poisoned animals with clinical signs, this finding together with vacuolar changes, mononuclear infiltrate, cholangiohepatitis and connective tissue proliferation, have been reported in slaughtered bovines and bubalines without clinical signs raised on *Brachiaria* pastures (Driemeier et al. 1999; Riet-Correa et al. 2010). The aforementio ned lesions were found in clinically healthy bovines from this study. The pathological lesions, considered in light of the animals’ good health, led to the conclusion that the liver changes were caused by consumption of *Brachiaria decumbens*.
The lesions found in this study were more extensive and severe at the portal vein entrance, as they were frequently located in the liver’s periportal region which is more exposed to toxins from the intestine (Haschek et al. 2010). There is widespread agreement that saponins present in Brachiaria spp. are the compounds which are responsible for liver injury. Saponins are hydrolyzed into sapogenins in the rumen and, once in the intestine, can reach the liver through the portal vein (Flaoyen and Wilkins 1997; Lajis et al. 1993), thereby primarily affecting the liver’s periportal area, which would explain why the most severe injuries were predominantly located in this area in the present study.

Hepatogenic photosensitization in ruminants grazing on B. decumbens has been attributed to accumulation of crystals in biliar canaliculi that leads to cholangiopathy. These crystals proceed from sapogenins that become lithogenic when combined with glucuronic acid in the liver upon exposure to calcium in the bile (Miles et al. 1992).

Although photosensitization has been a common finding amongst animals raised on the farm considered in this study, there were neither skin lesions nor crystals in the histological liver samples examined in this study. Photosensitivity may be determined by the amount of sapogenin-derived crystals obstructing biliary drainage (consequence of saponins quantity present in plants), thus leading to the circulation of the photoactive compound phylloerythrin. Brum et al. (2007) found that protodioscin’s levels of 2.36% in Brachiaria decumbens caused clinical and nonclinical lesions in sheep. We speculate that quantity of saponins present in Brachiaria ingested by animals tested was not sufficient to trigger crystal formation. Future studies should determine protodioscin concentration that produces clinical and nonclinical findings and severity in ruminants present in this region.

Foamy macrophages in the liver and lymph nodes are a common finding among ruminants grazing on Brachiaria spp., and may reflect clinical signs of the disease (Driemeier et al. 1999; Driemeier et al. 2002; Riet-Correa et al. 2002). Gomar et al. (2005) reported the presence of carbohydrates in foamy macrophages by using lectin histochemical techniques. However, other authors suggest that foamy macrophage content is abundant in lipid material (Russell et al. 2009); it has also been suggested that it is a product of lipid peroxidation in liver cells, having recently been established as being caused by Brachiaria grazing (Assumaidae et al. 2010). Regardless of foamy macrophage content, this type of cell was often found in the ruminants fed on Brachiaria spp. in this study.

Although B. decumbens is one of the most popular types of fodder on livestock farms in the Colombian Orinoco region, there has been a lack of reports regarding liver injury caused by its consumption in Colombia to date. Nunes et al. (2009) have established a negative correlation between foamy macrophages and weight gain in healthy cattle grazing on Brachiaria; as the animals evaluated in this study did not show any such clinical signs then this could have been indicative of economic loss attributed to lower production rates.

In contrast with other countries like Brazil where it has been clearly established that Brachiaria spp. consumption causes liver lesions in ruminants, this kind of injury has scarcely been studied in Colombia, a country having abundant Brachiaria grasslands. Since Brachiaria
spp. is a common pasture grass for cattle on Colombia’s Eastern plains the toxicological dynamics of metabolites from this grass should be evaluated in relation to geographical distribution of the grass.

Ascertaining saponin concentrations at various times of the year would be useful in order to make recommendations to improve pasture management. Further, studies about production losses caused when cattle consume *Brachiaria* are also needed, given that liver damage caused by the saponins contained in these plants could result in decreased beef or milk production and a concomitant increase in production costs from treating animals suffering severe liver disease. Similar research as the present study should be conducted over a larger area where cattle graze on *Brachiaria*-dominated pastures, as this type of study would provide a broader indication of the seriousness of the toxicity and the extent of the productive losses.

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