PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 42:e07047, 2022
DOI: 10.1590/1678-5150-PVB-7047

Original Article
Livestock Diseases

Susceptibility of goats to poisoning by *Tephrosia cinerea*[1]

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ABSTRACT.- Brito Junior J.R.C., Soares Y.G.S., Nascimento M.J.R., Ferreira J.S., Alves R.C., Dantas A.F.M., Riet-Correa F. & Galiza G.J.N. 2022**. Susceptibility of goats to poisoning by *Tephrosia cinerea***. *Pesquisa Veterinária Brasileira 42:e07047, 2022*. Laboratório de Patologia Animal, Hospital Veterinário, Centro de Saúde e Tecnologia Rural, Universidade Federal de Campina Grande, Campus de Patos, Av. Universitária s/n, Bairro Santa Cecília, Patos, PB 58708-110, Brazil. E-mail: joaoricardo055@hotmail.com

Spontaneous and experimental poisoning by *Tephrosia cinerea* in the northeastern semiarid region of Brazil has only been described in sheep. Pathologically, such poisoning leads to ascites and centrilobular liver fibrosis. However, these effects require an experimental study in goats. This study aimed to determine the goats' susceptibility to the ingestion of *T. cinerea* and the minimum toxic dose, describing the main clinical and anatomopathological findings. Poisoning was reproduced experimentally in one sheep that received 10g/kg of the ground plant and in two goats, the first receiving a dose of 5g/kg and the second receiving 10g/kg of the ground plant. The sheep presented abdominal distension 34 days after beginning the ingestion of the plant, developing sternal decubitus, breathing difficulty, opisthotonos, mandibular trismus, salivation, dysphagia, vocalization, and pedaling movements on the 50th day of the experiment. Fluid accumulation was observed in the abdominal cavity and liver via necropsy, with an irregular, slightly whitish capsular surface. Histologically, the main lesions observed in the liver were moderate fibrosis, marked sinusoidal distension, accompanied by marked hemorrhage, sometimes forming bridges between the centrilobular regions, associated with a dissociation of hepatocyte cords. There were discrete Alzheimer's type II astrocytes in the gray matter in the region of the occipital cortex in the nervous system. Goat 2 showed apathy, drowsiness, and weight loss; on the 62th day, lateral decubitus evolved to sternal decubitus, with a rotation of the neck towards the flank. At necropsy, marked edema was observed on the face and dewlap, and a slight accumulation of liquid; slightly yellowish material was observed in the abdominal cavity. There were discrete blackened areas on the capsular surface in the liver. Histologically, the liver showed mild centrilobular fibrosis associated with mild dissociation of hepatocyte cords and mild vacuolar degeneration of the hepatocyte cytoplasm. Goat 1 showed no clinical signs; at necropsy, discrete multifocal areas were observed in the liver on the capsular surface. Histologically, diffuse intracytoplasmic vacuolar degeneration of hepatocytes was detected. The clinical picture and anatomopathological findings differ between the species, proving the lower susceptibility of goats to *Tephrosia cinerea* ingestion (compared to sheep), with differences in the pathogenesis and epidemiological aspects of poisoning.

INDEX TERMS: Experimental poisoning, *Tephrosia cinerea*, sheep, goats, toxic plants, hepatotoxic plants, centrilobular fibrosis.

[1] Received on March 5, 2022.
Accepted for publication on March 28, 2022.

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RESUMO.- [**Suscetibilidade de caprinos à intoxicação por *Tephrosia cinerea*.**] A intoxicação espontânea e experimental por *Tephrosia cinerea* no semiárido nordestino só foi descrita em ovinos. Patologicamente, tal intoxicação leva a ascite e fibrose hepática centrolobular. No entanto, esses efeitos requerem um estudo experimental em caprinos. Este estudo teve como objetivo determinar a suscetibilidade de caprinos à ingestão de *T. cinerea* e a dose tóxica mínima, descrevendo os principais achados clínicos e anatomopatológicos. A intoxicação foi reproduzida experimentalmente em um ovino que recebeu 10g/kg da planta moída e em dois caprinos, o primeiro recebendo a dose de 5g/kg e o segundo recebendo 10g/kg da planta moída. O ovino apresentou distensão abdominal 34 dias após o início da ingestão da planta, desenvolvendo decúbito esternal, dificuldade respiratória, opistótono, trismo mandibular, salivação, disfagia, vocalização e movimentos de pedalada no 50º dia do experimento. Foi observado acúmulo de líquido na cavidade abdominal e fígado via necropsia, com superfície capsular irregular e levemente esbranquiçada. Histologicamente, as principais lesões observadas no fígado foram fibrose moderada, distensão sinusoidal acentuada, acompanhada de hemorragia acentuada, por vezes formando pontes entre as regiões centrolobulares, associada à dissociação dos cordões de hepatócitos. Havia discretos astrócitos de Alzheimer tipo II na substância cinzenta na região do córtex occipital no sistema nervoso. A cabra 2 apresentou apatia, sonolência e perda de peso; no 62º dia, decúbito lateral evoluiu para decúbito esternal, com rotação do pescoço em direção ao flanco. Na necropsia, observou-se edema acentuado na face e barbela, e leve acúmulo de líquido; foi observado material levemente amarelado na cavidade abdominal. Havia áreas enegrecidas discretas na superfície capsular no fígado. Histologicamente, o fígado apresentava leve fibrose centrolobular associada à discreta dissociação dos cordões de hepatócitos e leve degeneração vacuolar do citoplasma dos hepatócitos. A cabra 1 não apresentou sinais clínicos; na necropsia, discretas áreas multifocais foram observadas no fígado na superfície capsular. Histologicamente, foi detectada degeneração vacuolar intracitoplasmática difusa dos hepatócitos. O quadro clínico e os achados anatomopatológicos diferem entre as espécies, comprovando a menor suscetibilidade dos caprinos à ingestão de *Tephrosia cinerea* (em relação aos ovinos), com diferenças na patogênese e aspectos epidemiológicos da intoxicação.

TERMOS DE INDEXAÇÃO: Intoxicação experimental, *Tephrosia cinerea*, ovinos, caprinos, plantas tóxicas, plantas hepatotóxicas, fibrose centrolobular.

INTRODUCTION

More than 130 toxic plants are recognized in Brazil to directly affect the health of animals (Tokarnia et al. 2012). Some plant species that cause hepatic alterations such as fibrosis, necrosis, and secondary photosensitization in production animals are significant (Santos et al. 2008). In the northeastern semiarid region, the significant hepatotoxic plants in the spontaneous poisoning of small ruminants are *Crotalaria retusa*, *Tephrosia cinerea*, *Panicum dichotomiflorum*, the species of the genus *Urochloa* spp. (*Brachiaria* spp.) (Assis et al. 2010) and the species *Chamaecrista serpens* (L.) Greene (Mendonça et al. 2021).

Tephrosia cinerea is described as a shrub or sub-shrubs that belong to the family *Fabaceae* Lindl. (Queiroz & Tozzi 2015), popularly known as "falso anil." It is an invasive plant widespread in semiarid regions, resistant to drought, and it mainly affects sheep, causing a disease known as "barriga d'água" (water belly) (Santos et al. 2007).

T. cinerea poisoning results in clinical signs of abdominal distention (ascites), weight loss, depression, dyspnea, and exercise intolerance that can appear weeks or months before the sheep die (Santos et al. 2007, Riet-Correa et al. 2013, Silveira et al. 2018, Câmara et al. 2019). At necropsy, the lesions found in the affected animals are observed in the liver, having an uneven surface and hard consistency, ascites, hydrothorax, and hydropericardium. Histologically, periacinar fibrosis is observed in the liver, associated with hemorrhaging. In some cases, in the central nervous system, there is vacuolization of white matter and presence of Alzheimer type II astrocytes, characterizing hepatic encephalopathy (Santos et al. 2007, Riet-Correa et al. 2013, Silveira et al. 2018, Câmara et al. 2019). The toxicity mechanism of the plant is still elusive, and a study performed with samples of leaves and seeds of *T. cinerea* did not detect the presence of pyrrolizidine alkaloids (Santos et al. 2007).

Spontaneous and experimental poisoning by *T. cinerea* has only been described in sheep (Santos et al. 2007, Riet-Correa et al. 2013, Silveira et al. 2018, Câmara et al. 2019). There are no reports of spontaneous poisoning in goat species, and experimental studies are necessary to describe the clinical signs and anatomopathological findings (Geraldo Neto et al. 2013).

The objective of this study was to determine the susceptibility of goats to the ingestion of *T. cinerea*, as well as the minimal toxic dose, describing the main clinical and anatomopathological findings.

MATERIALS AND METHODS

Tephrosia cinerea was administered to two goats and one sheep in the Laboratory of Animal Pathology University Veterinary Hospital of the "Universidade Federal de Campina Grande" (LPA/HVU/UFCG), Campus de Patos/PB. The plant was harvested in two rural properties, one in the municipality of Patos, Paraíba, in July 2019, and the other in the municipality of Jucurutu, Rio Grande do Norte, in October 2019. In both, there had been outbreaks of *T. cinerea* poisoning in sheep. After collection, the plant was dried in the shade for seven days, crushed, ground, and stored in plastic bags at room temperature. Three Moxotó goats and two Mestizo sheep (Santa Inês) were selected; males, six months old, from the Jatobá Experimental Farm, "Núcleo de Pesquisa para o Desenvolvimento do Trópico Semi-Árido" (Research Center for the Development of the Semi-Arid Tropic – Nupeárido), UFCG, Patos/PB, reared in areas free of *T. cinerea*. All animals were housed in individual pens, were dewormed, and underwent ten days of adaptation, receiving concentrate (corn and wheat bran) at 1% of live weight, Tifton grass hay (*Cynodon dactylon*), and water at will.

The experiment started on September 26, 2020, and the animals received the ground plant, added concentrate at 1% live weight, Tifton grass hay (*Cynodon dactylon*) *ad libitum,* and water at will. Goat 3 and Sheep 2 were used as controls, consuming only concentrate at 1% of live weight, Tifton grass hay (*Cynodon dactylon*) *ad libitum,* and water at will. The weight of animals, amount of plant administered, experiment period, and type of death of the animals are presented in (Table 1).

Liver fragments were collected, including pre-stomachs, abomasum, intestines, kidney, adrenal, lymph nodes, lung, heart, and the central nervous system, and fixed in 10% buffered formalin; these were set in histological slides, using hematoxylin and eosin (HE) staining. Blades of liver fragments from all poisoned animals were stained by Masson's trichrome to show the fibrous connective tissue. This project is registered with the Research Ethics Committee (CEP) of the Rural Health and Technology Center (CSTR) from the Federal University of Campina Grande (UFCG). Process number (CEP 69-2013).

RESULTS

Sheep 1 totaled 50 days of experimental poisoning, consuming the mixture in the trough (10g/kg of ground plant + concentrate at 1% of live weight); forced administration of the plant was performed orally (Table 2). After 34 days from the beginning of the ingestion of the plant, the sheep presented abdominal distension with a positive fillip test (Fig.1). On the 50th day of the experiment, the sheep was found in the stall in sternal recumbency, unresponsive to external stimuli, breathing difficulty, opisthotonos, mandibular trismus, salivation, dysphagia, vocalization, and pedaling movements. The animal was euthanized. Sheep 1 lost 3.200kg of weight in 50 days of the experiment.

There were approximately 1.800mL of slightly yellowish fluid, sometimes gelatinous and easily clotted, in the abdominal cavity at necropsy (Fig.2). Portosystemic shunts were observed between the mesenteric veins (Fig.2). The liver had an irregular capsular surface, sometimes depressed by irregular striations, with capsule, slightly off-white, and fibrillar material adhered between the hepatic lobes (Fig.3); it was firm to the cut, with whitish areas mainly around the vessels (Fig.4). In the pericardial sac, 6mL of translucent fluid was observed.

Microscopically, the liver of Sheep 1 was observed on the external surface, exhibiting moderate, diffuse capsular thickening that protruded into the parenchyma. There was moderate fibrosis in the centrilobular region associated with hemorrhage that varied from moderate to severe (Fig.5). There was marked sinusoidal distension in the centrilobular region, accompanied by severe bleeding, sometimes forming bridges between centrilobular regions, associated with dissociation of hepatocyte cords (Fig.6). Moderate centrilobular fibrosis was evidenced by special staining by Masson's trichrome (Fig.7). Occasional Councilman-Rocha Lima bodies were also observed. In the nervous system, in the gray matter in the region of the occipital cortex, there were discrete Alzheimer's type II astrocytes, characterized by astrocytes in pairs or multiples with large oval cores with loosely arranged chromatin (Fig.8). No changes were observed in the other organs.

Goat 2 totaled 62 days of experimental poisoning, consuming the mixture in the trough (10g/kg of ground plant + concentrate at 1% of live weight), and forced administration of the plant was performed orally (Table 3). The animal showed apathy, drowsiness, and weight loss. After 62 days of the experiment, the animal was found in the stall in lateral recumbency, unable to remain stationary, yet it remained responsive to external stimuli. When kept in sternal recumbency, the same rotated the neck towards the flank (Fig.9). Goat 2 died, and a necropsy was performed. Goat 1 totaled 112 days of experimental

Table 1. Experimental intoxication of *Tephrosia cinerea* in small ruminants

Animal species	Live weight	Amount of plant administered	Period of plant consumption	Clinical outcome
Caprine 1	13,400 kg	5 g/kg	111 days	Euthanasia
Caprine 2	11,600 kg	10 g/kg	61 days	Natural death
Ovine 1	12,600 kg	10 g/kg	49 days	Euthanasia
Control				
Caprine 3	11,800 kg	-	-	Euthanasia*
Ovine 2	12,200 kg	-	-	Euthanasia*

* Control animals were euthanized 118 days after the start of the experiment.

Table 2. Experimental poisoning of *Tephrosia cinerea* in sheep

Days of consumption	Amount of plant consumed
1st to 34th day	7,682 kg (mixture consumed in the trough)
35th to 45th day	675 g (mixture consumed in the trough)
46th to 49th day	700 g (plant forced feeding - orally)

Table 3. Experimental poisoning by *Tephrosia cinerea* in goats

Days of consumption	Amount of plant consumed
Caprine 2	
1st to 25th day	1,938 kg (mixture consumed in the trough)
26th to 61st day	3,920 kg (plant forced feeding - orally)
Caprine 1	
1st to 25th day	1,845 kg (mixture consumed in the trough)
26th to 111st day	5,121 kg (plant forced feeding - orally)

poisoning, consuming the mixture in the trough (5g/kg of ground plant + concentrate at 1% of live weight), and forced administration of the plant was performed orally (Table 3). After 112 days of the experiment, the animal was active, with no clinical signs, with the abdominal region moderately bulging and slimming. The animal was euthanized. Goat 2 lost the equivalent of 1.800kg of weight during the 62 days of the experiment, and Goat 1 lost 3.400kg of weight during the 112 days of the experiment.

There was marked edema in the face and submandibular region, with a positive Godet test at the necropsy of Goat 2. There was gelatinous and translucent material when retracting the skin. Approximately 34mL of slightly yellowish fluid was observed in the abdominal cavity. Gelatinous and translucent material was visualized around the viscera and in the mesentery (Fig.10). There were discrete, blackened areas on the capsular surface of the liver (Fig.11). Sectioning indicated reddish, punctate areas distributed throughout the parenchyma (Fig.12) and a moderately thickened gallbladder wall. There was marked edema in the abomasal folds. There were 13mL of translucent fluid in the pericardial sac. Additionally, there was marked gelatinous atrophy of the fat in the coronary sulcus and evidence of lymphatic vessels in the pericardium. When disarticulating the atlanto-occipital joint, gelatinous and translucent material was seen in the medullary canal. At the necropsy of Goat 1, the liver showed depressed areas on the capsular surface, which were multifocal and discrete. There were 5mL of translucent fluid in the pericardial sac.

Histologically, the liver of Goat 2 showed mild centrilobular fibrosis (Fig.13) associated with discrete dissociation of hepatocyte cords and discrete vacuolar degeneration of the hepatocyte cytoplasm. No changes were observed in the central nervous system and other organs. Mild centrilobular fibrosis in Goat 2 was evidenced by special staining by Masson's trichrome (Fig.14). There was diffuse intracytoplasmic vacuolar degeneration of hepatocytes in the liver of Goat 1 (Fig.15). Mild centrilobular fibrosis in Goat 1 was evidenced by special staining by Masson's trichrome (Fig.16). No changes were observed in the central nervous system and other organs.

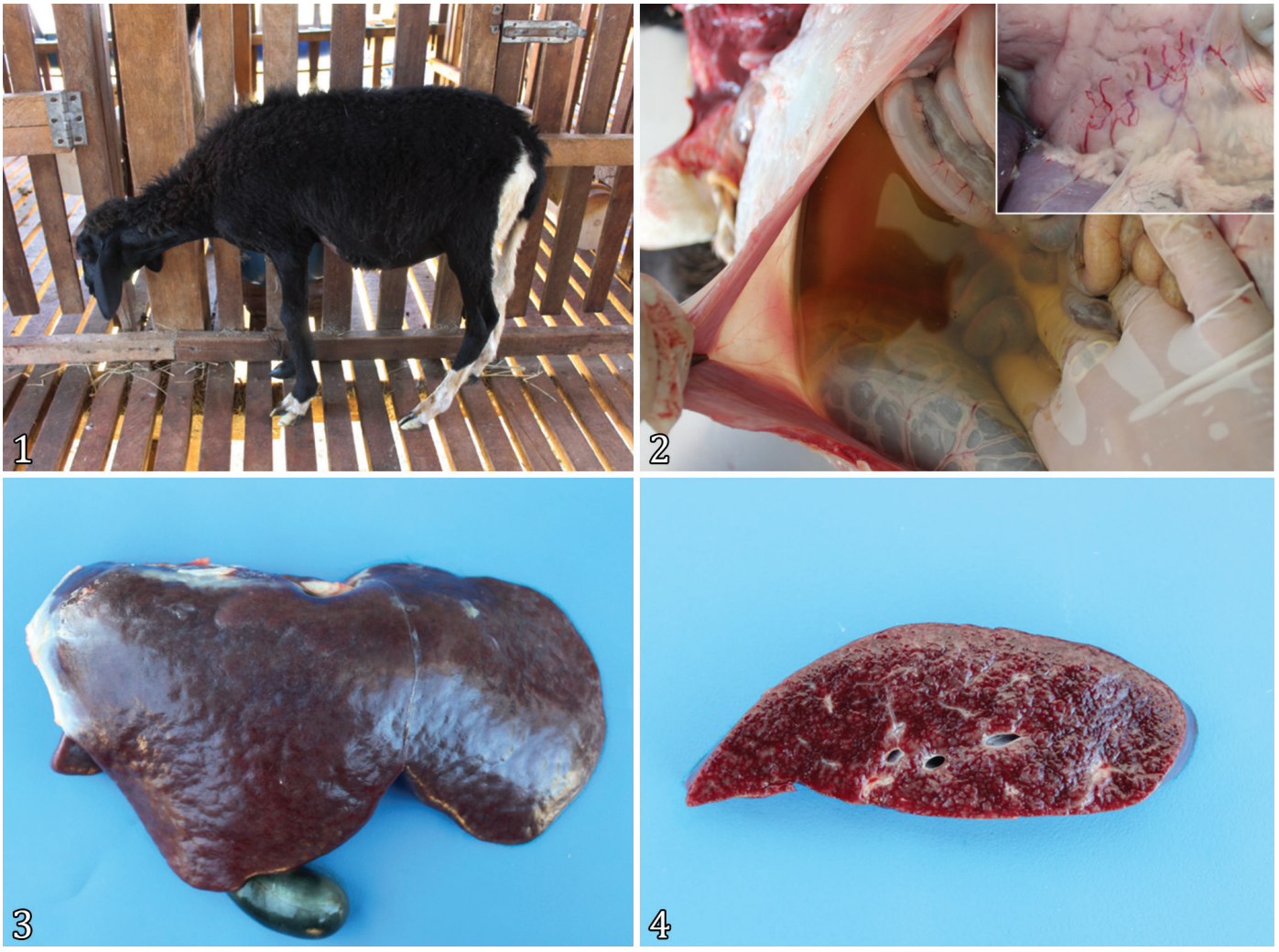

Fig.1-4. Sheep 1 experimentally poisoned by *Tephrosia cinerea*. (**1**) Animal showing abdominal distention and apathy. (**2**) Abdominal cavity with a presence of slightly yellowish free fluid. Highlight: portosystemic shunts between the mesenteric veins. (**3**) Liver with irregular capsular surface, sometimes depressed by irregular striations, with a slightly whitish capsule. (**4**) Liver, cut surface with whitish areas mainly around vessels.

DISCUSSION

Experimental poisoning with *Tephrosia cinerea* in small ruminants demonstrates the difference in plant palatability between species due to the greater consumption of the plant in the trough by the sheep compared to goats. In this study, Sheep 1 consumed a total of 8.357kg of the mixture in the trough and 700g of the plant by forced administration during the 50 days of the experiment. The animal presented abdominal distension and weight loss after 34 days of the total consumption of the mixture in the trough; on the 49th day, it presented apathy and mild ataxia, progressing to sternal recumbency, lack of response to external stimuli, breathing difficulty, opisthotonos, mandibular trismus, salivation, dysphagia, and later vocalization and pedaling movements; the animal was euthanized on the 50th day of the experiment. The clinical findings described in this experimental study corroborate the findings found by other authors (Santos et al. 2007, Riet-Correa et al. 2013, Silveira et al. 2018).

The necropsy findings are similar to those described in other studies with spontaneous and experimental poisoning by *T. cinerea* (Santos et al. 2007, Riet-Correa et al. 2013, Silveira et al. 2018, Câmara et al. 2019). The most evident histological findings in the liver of Sheep 1 diverge from those described in other studies of experimental poisoning in sheep by *T. cinerea*, which mainly highlight periacinar fibrosis, forming interlobular bridges and associated with moderate hemorrhage (Riet-Correa et al. 2013, Silveira et al. 2018). The differences in the histological findings in the present study, when compared to other authors, may be related to the determination of the dose of the plant administered to Sheep 1, corresponding to 10g/kg, as, in other experimental studies, the plant was administered *ad libitum* (Riet-Correa et al. 2013, Silveira et al. 2018). Marked hemorrhage in the centrilobular region results from distention and rupture of the sinusoids, occasionally leading to loss of cohesion of hepatocyte cords, degeneration, necrosis of hepatocytes, and consequently hepatic fibrosis (Riet-Correa et al. 2013).

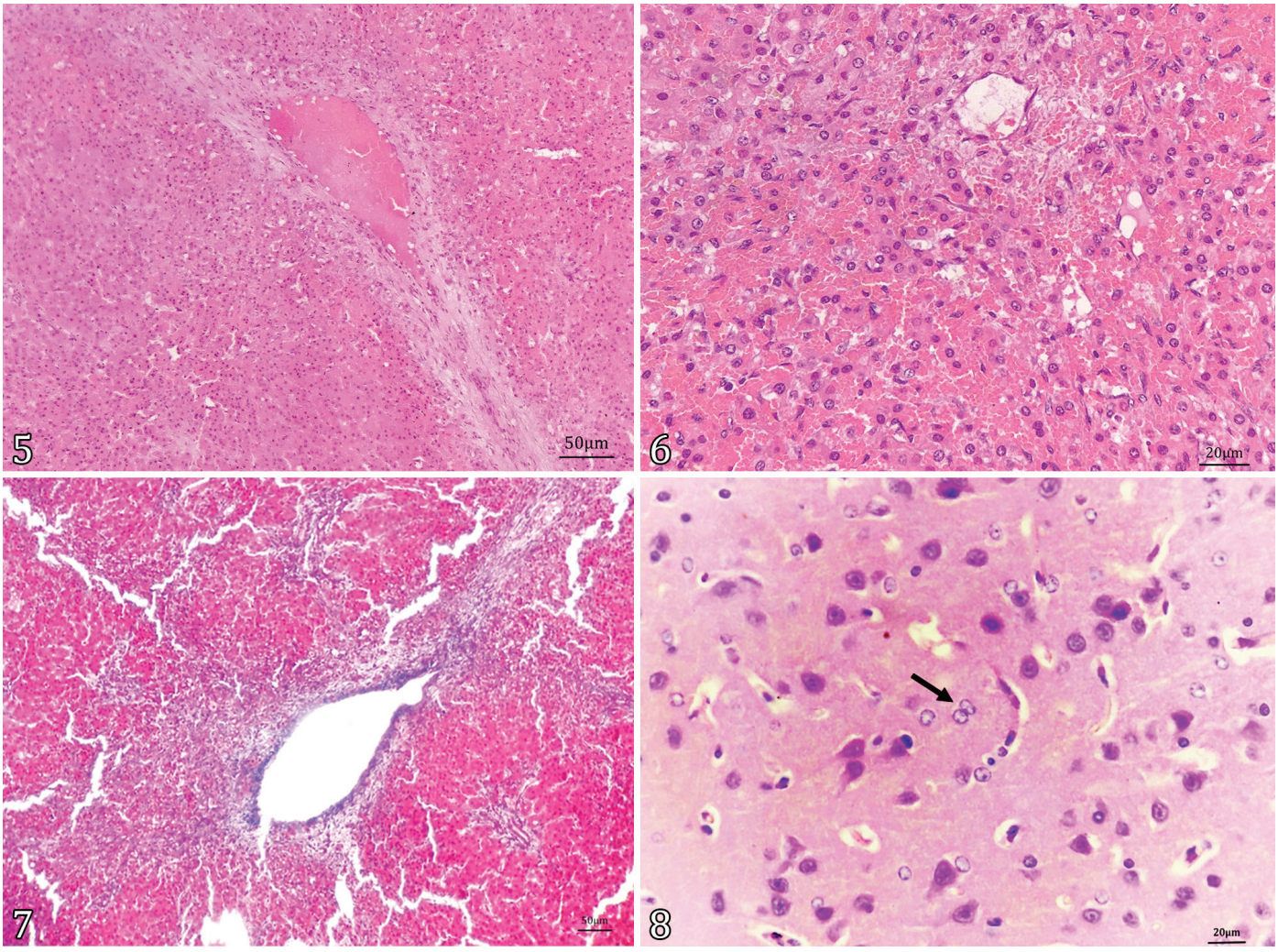

Fig.5-8. Sheep 1 experimentally poisoned by *Tephrosia cinerea*. (**5**) Liver with moderate fibrosis in the centrilobular region associated with hemorrhage. HE, obj.10x. (**6**) Liver with centrilobular region showing accentuated sinusoidal distension, accompanied by accentuated hemorrhage, sometimes forming bridges between centrilobular regions, associated with dissociation of hepatocyte cords. HE, obj.40x. (**7**) Liver with moderate centrilobular fibrosis. Masson's trichrome, obj.10x. (**8**) Gray matter of the occipital cortex, showing discrete Alzheimer type II astrocytes (arrow). HE, obj.40x.

The lesion in the central nervous system, although mild, is similar to that described in the work of Silveira et al. (2018), justifying the discrete neurological clinical signs present in the animal in this report.

The experimental poisoning with *T. cinerea* in goats demonstrates that even when the ground plant is supplied in the trough, adding concentrate at 1% of the live weight, the goats do not consume the plant spontaneously, demonstrating that they consumed 45.26% less of the mixture in the trough, compared to Sheep 1. In this way, it characterizes the difference between the species concerning the appearance of clinical signs, in which Sheep 1 and Goat 2 with the same dose of the plant, 10g/kg, presented a clinical picture 34 and 62 days, respectively, after the beginning of the experiment. Goat 1, at a dose of 5g/kg of the plant did not show characteristic clinical signs of poisoning. The anatomopathological findings of Goat 1 and Goat 2 were different from the findings found in Sheep 1 and sheep from other experimental studies (Santos et al. 2007, Riet-Correa et al. 2013, Silveira et al. 2018, Câmara et al. 2019). Macroscopic changes such as ascites and edema around the viscera, in the mesentery, and in the abomasal folds are described in cattle poisoned by plants that contain pyrrolizidine alkaloids (Lucena et al. 2010). On histology, the mild centrilobular fibrosis of Goat 2, the diffuse intracytoplasmic vacuolar degeneration of hepatocytes in Goat 1, and the absence of central nervous system lesions in both animals contrast with the histological findings found in sheep in other experimental studies (Riet-Correa et al. 2013, Silveira et al. 2018), evidencing the vulnerability of sheep to poisoning by *T. cinerea*.

In other studies of experimental poisoning in sheep where seeds, leaves, stems, and flowers were administered (Santos et al. 2007, Riet-Correa et al. 2013, Silveira et al. 2018) the authors suggested that the leaves are more toxic than the seeds. In the present work, in addition to grinding the whole plant, similar to the study developed by Silveira et al. (2018), the milling and weighing of the plant were carried out according to the weight of the animals, demonstrating the high toxicity of *T. cinerea*, at a dose of 10g/kg for Sheep 1 and low toxicity for Goat 2, which although the animal died spontaneously, showed discrete lesions.

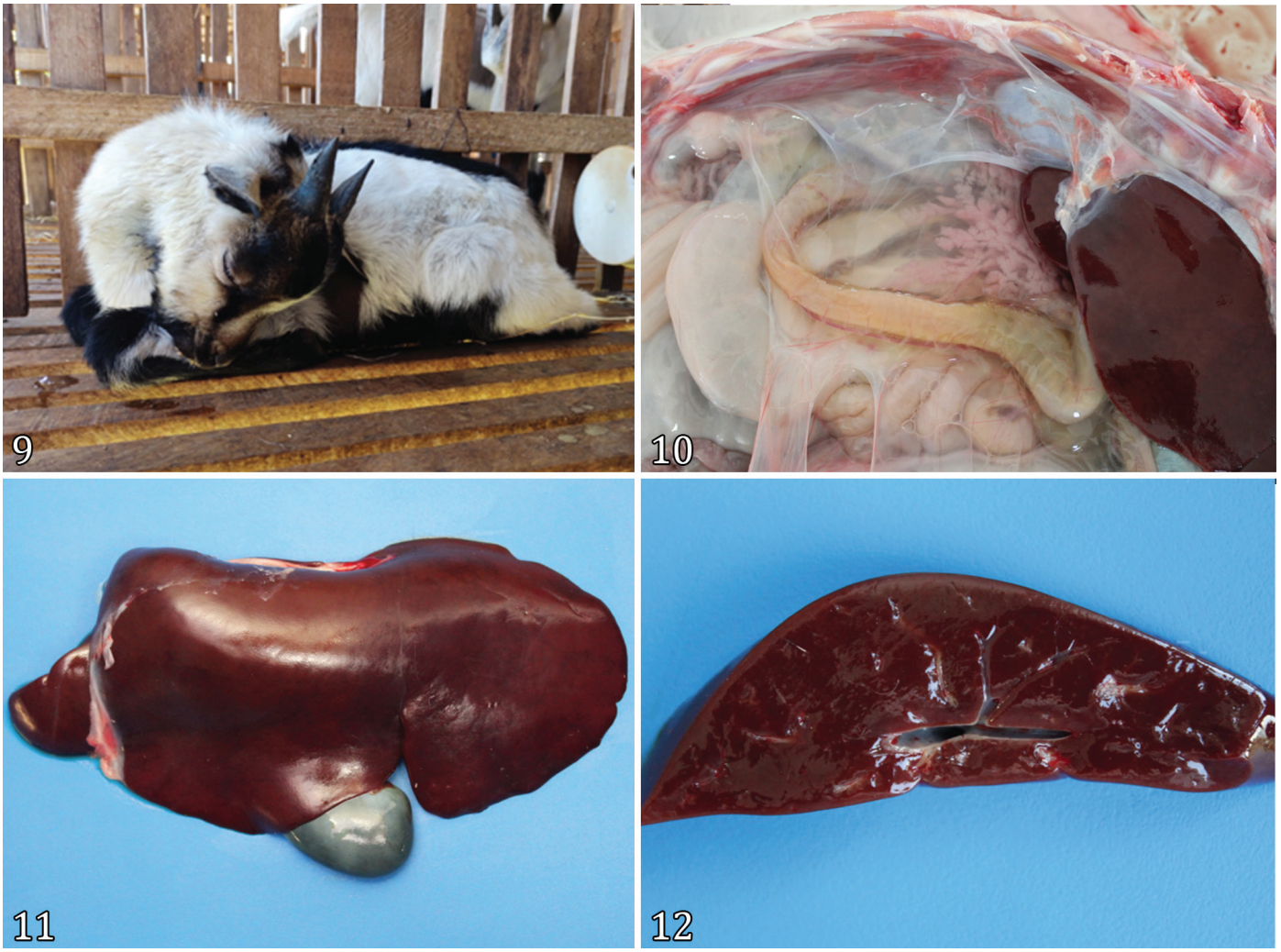

Fig.9-12. Goat 2 experimentally poisoned by *Tephrosia cinerea*. (**9**) Animal in sternal recumbency, rotating the neck towards the flank. (**10**) Abdominal cavity with gelatinous and translucent material around the viscera and in the mesentery. (**11**) Liver with discrete blackened areas on the capsular surface. (**12**) Liver, cut surface, with reddish punctate areas distributed throughout the parenchyma.

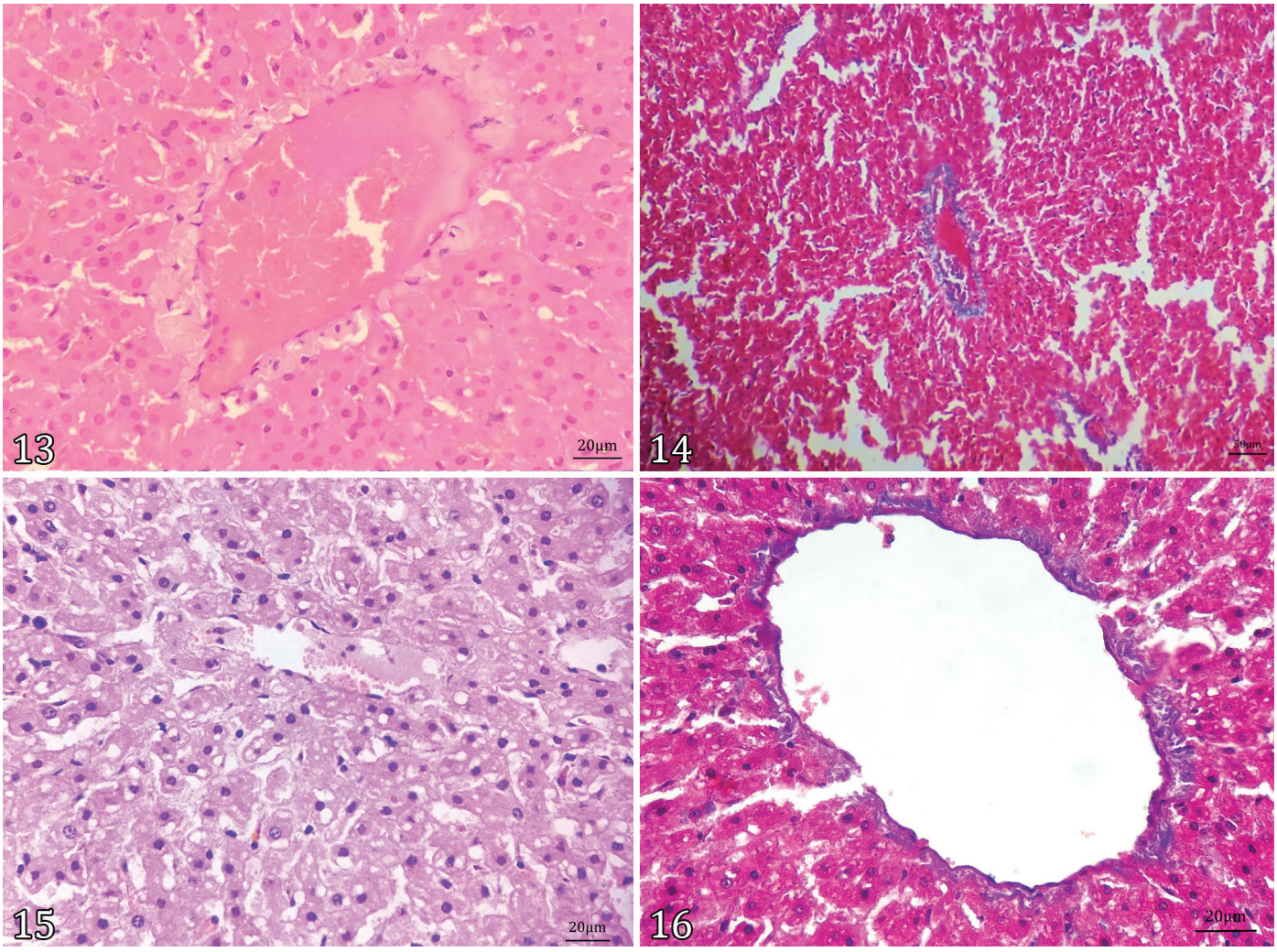

Fig.13-16. Goats 1 and 2 experimentally poisoned by *Tephrosia cinerea*. (**13**) Liver of Goat 2, with mild fibrosis in the centrilobular region. HE, obj.40x. (**14**) Liver with mild centrilobular fibrosis. Masson's trichrome, obj.10x. (**15**) Liver of Goat 1, with diffuse intracytoplasmic vacuolar degeneration of hepatocytes. HE, obj.40x. (**16**) Liver with mild centrilobular fibrosis. Masson's trichrome, obj.40x.

CONCLUSION

The experimental reproduction of poisoning by *Tephrosia cinerea* in goats shows the lower susceptibility of this species compared sheep, characterized by spontaneously reduced consumption of the plant, suggesting low palatability of the plant to goats. In goats, the toxic dose was 10g/kg, which resulted in a debilitating clinical outcome with death and mild histopathological changes.

Authors' contributions.- Brito Junior J.R.C., Soares Y.G.S., Nascimento M.J.R., Ferreira J.S., Alves R.C., Dantas A.F.M., Riet-Correa F. & Galiza G.J.N. contributed to the collection of epidemiological data, observation of clinical signs, experimental reproduction, and necropsy, in addition to contributing substantially to macroscopic and histopathological analysis, drafted and revised the manuscript and its final version.

Acknowledgments.- To the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES) for the scholarship of the first author.

Conflict of interest statement.- The authors declare having no conflicts of interest.

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