Research, Society and Development, v. 9, n. 7, e506974642, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i7.4642

Santos Júnior, DA, Olinda, RG, Oliveira Filho, EF, Soares, PC, Dantas, AFM, Simões, FVD & Miranda Neto, EG. (2020). Nutritional muscular dystrophy in lambs reared on pasture in semiarid region of Brazil. ***Research, Society and Development***, 9(7): 1-20, e506974642.

Distrofia muscular nutricional em cordeiros criados a pasto na região semiárida do Brasil

Nutritional muscular dystrophy in lambs reared on pasture in semiarid region of Brazil

Distrofia muscular nutricional en corderos criados en pastos en la región semiárida de Brasil

Recebido: 18/05/2020 | Revisado: 19/05/2020 | Aceito: 23/05/2020 | Publicado: 24/05/2020

Dinamérico de Alencar Santos Júnior
ORCID: https://orcid.org/0000-0002-7709-4629
Universidade Federal do Oeste da Bahia, Campus de Barra, Brasil
E-mail: juniordinamerico@yahoo.com.br
Robério Gomes Olinda
ORCID: https://orcid.org/0000-0002-7767-9571
Centro de Saúde e Tecnologia Rural, Campus de Patos, Universidade Federal de Campina Grande, Brasil
E-mail: rgumes@hotmail.com
Emanuel Felipe Oliveira Filho
ORCID: https://orcid.org/0000-0003-2836-5549
Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Brasil.
E-mail: felipe130188@gmail.com
Pierre Castro Soares
ORCID: https://orcid.org/0000-0002-5680-3940
Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Brasil
E-mail: pcastro.pe@gmail.com
Antônio Flávio Medeiros Dantas
ORCID: https://orcid.org/0000-0002-6123-2273
Centro de Saúde e Tecnologia Rural, Campus de Patos, Universidade Federal de Campina Grande, Brasil
E-mail: dantas.af@pq.cnpq.br

Sara Vilar Dantas Simões

ORCID: https://orcid.org/0000-0003-3692-9199

Centro de Saúde e Tecnologia Rural, Campus de Patos, Universidade Federal de Campina Grande, Brasil

E-mail: saravdsimoes@gmail.com

Eldinê Gomes de Miranda Neto

ORCID: https://orcid.org/0000-0002-9398-6539

Centro de Saúde e Tecnologia Rural, Campus de Patos, Universidade Federal de Campina Grande, Brasil

E-mail: eldinemneto@hotmail.com

Resumo

Este artigo apresenta dados importantes sobre o diagnóstico de distrofia muscular nutricional (DMN) em ovinos criados na região semiárida do Nordeste do Brasil, com foco em dados clínicos, manejo nutricional, aspectos anatomopatológicos e quantificação do selênio sérico por metodologia analítica altamente sensível. A região semiárida do Brasil é de grande importância na criação de pequenos ruminantes e muitas doenças que afetam os rebanhos estão relacionadas a falhas no manejo nutricional, medidas sanitárias, períodos de maior seca ou baixa pluviosidade, pouca disponibilidade de forragem nativa em determinados períodos do ano e falta de suplementação com nutrientes energéticos, proteínas e minerais. A falta de publicações sobre surtos de deficiência de selênio demonstra a importância do diagnóstico nessa área. A concentração média de Se no soro sanguíneo dos cordeiros foi muito baixa (2,52±1,02µg/L). Os achados nos músculos esqueléticos são compatíveis com as características observadas na DMN, caracterizadas por necrose e calcificação de miofibrilas. Além de vasculite necrosante nas arteríolas hepáticas e necrose da coagulação hepatocelular nos hepatócitos foram observadas, provavelmente desencadeadas pela peroxidação lipídica das membranas celulares. Os cordeiros deste surto, criados em pastagem na região semiárida, apresentaram baixa concentração sérica de Se, com manifestação clínica e anatomopatológica da NMD. Assim, a suplementação com Se na dieta deve ser recomendada regularmente para evitar o aparecimento de novos casos. Os níveis séricos de Se são indicadores úteis para o diagnóstico de distrofia muscular nutricional em cordeiros, associados a dados clínicos e anatomopatológicos.

Palavras-chave: Mineralização; Miopatia nutricional; Nutrição mineral; Oligoelementos; Ruminantes.

Abstract

This article presents important data on the diagnosis of nutritional muscular dystrophy (NMD) in sheep reared in the semiarid region of Northeast Brazil, with a focus on medical clinic data, nutritional management, anatomopathological aspects and quantification of serum selenium by highly sensitive analytical methodology. The semiarid region of Brazil is of great importance in the creation of small ruminants and many diseases that affect herds are related to failures in nutritional management, sanitary measures, periods of greater drought or low rainfall, little availability of native forage at certain times of the year and lack of supplementation with energy nutrients, proteins and minerals. The lack of publications on outbreaks of selenium deficiency demonstrates the importance of diagnosing in this area. The mean concentration of Se in the serum from the lambs was very low (2.52±1.02µg/L). The findings in the skeletal muscles are compatible with the characteristics observed in NMD, characterized by necrosis and calcification of myofibrils. In addition, necrotizing vasculitis in hepatic arterioles and hepatocellular coagulation necrosis in hepatocytes were observed, probably triggered by lipid peroxidation of cell membranes. The lambs from this outbreak, reared on pasture in the semiarid region, presented low serum Se concentration, with clinical and anatomopathological manifestation of NMD. Thus, supplementation with Se in the diet should be recommended regularly to avoid the appearance of new cases. Serum Se levels are useful indicators for the diagnosis of nutritional muscular dystrophy in lambs, associated with clinical and anatomopathological data.

Keywords: Mineral nutrition; Mineralization; Nutritional myopathy; Ruminants; Trace elements.

Resumen

Este artículo presenta datos importantes sobre el diagnóstico de distrofia muscular nutricional (NMD) en ovejas criadas en la región semiárida del Brasil, con un enfoque en datos de clínicas médicas, manejo nutricional, aspectos anatomopatológicos y cuantificación de selenio en suero mediante metodología analítica altamente sensible. La región semiárida de Brasil es de gran importancia en la creación de pequeños rumiantes y muchas enfermedades que afectan a los rebaños están relacionadas con fallas en el manejo nutricional, medidas sanitarias, períodos de mayor sequía o poca lluvia, poca disponibilidad de forraje nativo en ciertos momentos del año y falta de suplementación con nutrientes energéticos, proteínas y minerales. La falta de publicaciones sobre brotes de deficiencia de selenio demuestra la importancia del diagnóstico en esta área. La concentración media de Se en el suero de los

corderos fue muy baja (2.52±1.02 µg/L). Los hallazgos en los músculos esqueléticos son compatibles con las características observadas en NMD, caracterizadas por necrosis y calcificación de miofibrillas. Se observaron vasculitis necrosante en arteriolas hepáticas y necrosis de coagulación hepatocelular en hepatocitos, probablemente desencadenada por la peroxidación lipídica de las membranas celulares. Los corderos de este brote, criados en pasturas en la región semiárida, presentaron una baja concentración de Se en suero, con manifestación clínica y anatomopatológica de NMD. Por lo tanto, la suplementación con Se en la dieta debe recomendarse regularmente para evitar la aparición de nuevos casos. Los niveles séricos de Se son indicadores útiles para el diagnóstico de distrofia muscular nutricional en corderos, asociados con datos clínicos y anatomopatológicos.

Palabras-clave: Mineralización; Miopatía nutricional; Nutrición mineral; Oligoelementos; Ruminantes.

1. Introduction

Nutritional muscular dystrophy (NMD) is a degenerative muscle disease seen in many animals, especially sheep, goats, and calves, causing a decrease in the animals' resistance to diseases, delayed growth and development, infertility, and a decrease in the production of meat, milk, and wool. NMD develops due to insufficiency of Se and/or vitamin E in food during fetal and neonatal life and is seen in animals worldwide. An Se deficiency occurs when the soil contains less than 0.5 mg Se/kg and locally harvested food contains less than 0.1 mg Se/kg (Hefnawy & Tórtora Pérez, 2010; Suttle, 2010; Ghanem et al., 2016; Rodriguez et al., 2018; Yildirim et al., 2019).

Selenium (Se) is an essential trace element present in the tissues of animals, and its importance for health and productivity has been well established (Xun et al., 2012), as it protects organisms from oxidative damage. There are many biological functions of this element, which is present in several selenoproteins (Pavlata et al., 2012). A deficiency in Se seems to be common in the diets of farm animals, possibly because there are multiple risk factors that precipitate the appearance of this disease, such as rapid growth or dietary factors, including an excess of unsaturated fatty acids in the feed, stressful factors, such as keeping the animals in the open after periods of stabling during the winter, long-distance walking, and vaccination and de-worming management of the flock. On the other hand, pastures poor in Se and Vitamin E or animals that receive feed deficient in these elements may also be predisposed to the disease (Maas et al., 1994; Amorim et al., 2005; Rodriguez et al., 2018).

Animals with NMD present symptoms such as hyporexia, weight loss, difficulty standing, lordosis, tachycardia, dyspnea, tachypnea, ataxia, and hardening of the muscles. In young animals, death occurs due to myocardial degeneration (Ataollahi et al., 2013; Yildirim et al., 2019). Several clinical manifestations are associated with Se deficiency in farm animals. Among the reported syndromes caused by the deficiency, we can include nutritional myopathy characterized by segmental necrosis, and mineralization and regeneration of muscle fibers. In animals that present lesions with subacute evolution, all stages of segmental necrosis and regeneration (polyphasic and multifocal lesions) are observed. The success of muscle regeneration depends on the integrity of the sarcolemmal tubules. Therefore, if the animals are treated with Se in the early stages of nutritional myopathy, the muscles are able to regenerate properly, returning to normal (Hefnawy & Tórtora Pérez, 2010; Beytut et al., 2002; Ramírez et al., 2004; Contreras et al., 2005; Yavuz et al., 2017).

Nutritional muscular dystrophy (NMD), also known as nutritional myodegeneration, is associated with Se deficiency. NMD lesions are mediated by oxidative damage to cell membranes, which allows extracellular calcium to flow to damaged cells, causing activation of calcium-dependent proteases that degrade myofibrils and other cellular components, leading to myodegeneration and myonecrosis (Maas et al., 1984; Amorim et al., 2005; Rodriguez et al., 2018).

There are few reports of cases of NMD in lambs in Brazil, with only two cases published in sheep and one in goats. An outbreak of NMD in sheep was reported in the city of Campina Grande, state of Paraíba, Brazil (Amorim et al., 2005); another outbreak was reported in sheep raised in the rural area of the municipality of Mossoró, in the state of Rio Grande do Norte, Brazil (Bezerra et al., 2007); the animals were fed a diet rich in protein and carbohydrates. More recently an outbreak of NMD in goats was recorded in the Central region of Rio Grande do Sul, Brazil (Rosa et al., 2013). Se was not quantified in animal serum in any of these reports and the GSH-Px activity, which has a high relationship with the serum Se concentration, was not evaluated; the diagnosis was based on clinical and epidemiological information and anatomopathological analysis of different tissues. There is no record of NMD in other regions of the state of Paraíba, such as in the agreste region, which is an important breeding region for small ruminants, with a strong influence on the agribusiness of goats and sheep.

Se has significant influence on the musculoskeletal, reproductive, and immune systems, as well as on the productivity of the herd. Research on the dynamics of antioxidant nutrients, such as Se, has attracted the interest of numerous researchers, verifying the

beneficial effects that this trace element has on the body of ruminants (Guimarães et al., 2011). In this context, no publications have been recorded with lambs with NMD in other regions of Northeast Brazil, in which a diagnosis was made through a set of data related to the precise quantification of Se in biological materials, such as whole blood, serum, or even muscle tissues, such as muscles and liver, enabling diagnosis with a wider range of data, so that the pathophysiology of this important nutritional disease in livestock animals can be better understood. Given the above, the objective of the current work was to describe the epidemiological and clinical-pathological findings and the quantification of Se in serum from lambs reared on pasture in Paraíba State, Northeast Brazil with Se deficiency.

2. Material and Methods

2.1. Characterization of the sample space

The outbreak occurred in a property in the city of Queimadas, in the agreste region of Paraíba, between August and October, a period characterized by the end of the rains in the region. The herd consisted of 30 goats and 120 sheep of the Dorper breed; of this total, 50 were young animals, below 6 months of age. The animals were bred with the purpose of producing lambs for slaughter, in a pasture rearing system, in an area with predominantly native vegetation and African star grass (*Cynodon niemfuensis* Vanderyst), and with a topography of steep terrains. The water offered was from a reservoir located on the property. At night, the animals were housed and received mineral supplementation, in covered, suspended troughs, formulated for cattle (Suprafós 500g ®), in the proportion of 3 packages (1.5 kg) of the product for each 25 kg of common salt.

A visit to the farm was carried out to record epidemiological data and perform a clinical examination of the sick animals, evaluating attitude, posture, mucous color, degree of dehydration, physiological parameters, such as heart and respiratory rates, rectal temperature, ruminal movements, and a neurological examination.

2.2. Sample collection, preparation and analysis

Blood samples were collected from 16 young sheep, corresponding to 32% of the total young sheep. The blood was obtained by jugular venipuncture, in vacutainer® siliconized tubes, without anticoagulant, to obtain serum. Blood samples without anticoagulant were kept at room temperature, for blood clot retraction and obtention of serum. All tubes were

subjected to centrifugation for 15 minutes at 500 G. The serum aliquots were subsequently conditioned in Eppendorf tubes and stored at –20ºC.

One sheep that was very weak was euthanized *in extremis* and submitted to necropsy. Fragments of organs of the thoracic and abdominal cavities and central nervous system were collected, as well as fragments of various muscle groups of the thoracic, pelvic, and cervical region, and all were fixed in 10% buffered formaldehyde. The collected tissues were processed for histopathology, cut into 5 µm sections, and stained using the hematoxylin-eosin (HE) technique.

To quantify serum Se, serum samples were processed by microwave assisted digestion (MARS - Microwave System®). All the reagents used in this work were of analytical grade, from the Merck brand, and all solutions were prepared in ultrapure water, obtained through an ultra purifying system (Milli-Q System, Millipore, Bedford, MD, USA). All glassware used in the preparation of the samples was washed first with Extran detergent and then kept in a 5% HNO3 bath for a minimum period of 24 hours. The standard Se stock solution, used in the preparation of the calibration curve, was prepared using $NaSeO_3$ and Milli-Q water.

2.3. Digestion and quantification of Se

For the digestion process, a volume of 1.0 mL of blood serum was allocated in digestion flasks and added to 5 mL of concentrated HNO_3. The heating schedule for the microwave oven was carried out using a 35-minute ramp, using the power of 800 W, 160°C (step 1: 120ºC - 10 min, step 2: 160ºC - 15 min, step 3: cooling - 10 min). After completing the program, the tubes were left to cool in the oven for 15 minutes. After the digestion stage, the pressure in the flasks was relieved in an exhaust hood. The digested contents were transferred to conical tubes, and then Milli-Q water was added to complete the tubes to a final volume of 15 mL.

For the quantification of Se, an atomic absorption spectrometer with hydride generator - HGAAS, model (Varian/Spectr AA-220) was used. The results were expressed in µmol/L. The analyses were performed at the Analytical Chemistry Laboratory of the Research Support Center (CENAPESQ) of the Federal Rural University of Pernambuco (UFRPE).

3. Results and Discussion

3.1. Clinical-epidemiological aspects

Clinical signs were observed in five animals of the Dorper breed, aged around 60 days, which fell ill in a period ranging from 8-25 days. One sheep died 25 days after the onset of clinical signs. Three sheep that presented clinical signs of the disease were treated with 20% oxytetracycline (Terramicina LA®), with 4 applications of 3ml every 48 hours; 5% dexamethasone (Biodex®), with 5 applications of 1.5 ml/day; Vallée® organic modifier and SM® antitoxic (with four applications of 2ml/day). The medication was initiated soon after the onset of clinical signs, concomitant with the provision of specific mineral salt for sheep (Suprafós® 70). After medication, the animals evolved satisfactorily, with observed recovery. On a second visit to the property, another animal, 60 days old, with clinical signs, was identified, in permanent decubitus for approximately 48 hours. The physical examination of the animals identified difficulty in getting up, although presenting an alert sensory state and appetite present, congested mucous membranes, engorged episcleral vessels, and moderate dehydration. In the assessment of physiological parameters, tachycardia, tachypnea, hyperthermia, and ruminal hypotonia were identified.

When being placed standing, generalized muscle tremors were observed, more evident in the hind limbs, in addition to tetraparesis. In the neurological examination of the sick animals, no sensory and motor deficits were observed that suggested injury to the brain, brain stem, or cerebellum. This was confirmed by the absence of morphological alterations in the histological study of serial cuts that covered segments of the entire nervous system of the sheep that was submitted to necropsy. However, the need to evaluate all the diagnostic possibilities, in order to increase accuracy and rule out nervous diseases that affect the central nervous system of sheep raised in the Northeast region, is emphasized. We included rabies, polioencephalomalacia, bacterial meningitis, listeriosis, and brain and spinal abscesses as a differential diagnosis (Guedes et al., 2007).

The clinical observations of these animals are similar to those observed by some authors who recorded case analyses of NMD in adult sheep and lambs. Lambs with NMD presented weakness, stiffness, difficulty standing, and dorsal arching, considering that Se is undoubtedly an essential trace element for nutrition and its deficiency is a risk factor for the development of NMD in many species (Beytut et al., 2002). An outbreak of NMD in Dorper sheep which presented hyperthermia, muscle weakness, difficulty in standing, muscle tremors, incoordination with hind limb paralysis, decubitus, depression, and decreased

pupillary and menace reflexes, as well as flaccid tongue and mandible, salivation, submandibular edema, dyspnea, and tachycardia (Amorim et al., 2005). Death occurred after a 6-12 hour clinical course. The animals in this outbreak were also from the state of Paraíba. The observations of another author also coincide with the present outbreak of NMD, in which the authors recorded an outbreak of NMD in Santa Inês sheep and found clinical signs of weakness, muscle tremors, incoordination, reluctance to walk, sternal decubitus, and dyspnea (Bezerra et al., 2007).

Were found lambs with difficulty walking and abnormal postural positions, characteristic of NMD, relating these clinical signs of degenerative malformations in skeletal muscle fibers with greater metabolic activity and reporting that NMD remains as a central component in suspected Se deficiency, where Se levels or GSH-Px activity cannot be determined (Hefnawy & Tórtora Pérez, 2010; Yildirim et al., 2019). In another report, lambs with NMD presented sternal decubitus and the inability to remain standing, limb stiffness; muscle tremors were observed when lambs were forced to stand, as also observed in the current case (Ghanem et al., 2016). The same authors found that the shoulder and gluteal muscles were stiff and swollen and verified tachypnea and tachycardia. These clinical alterations can be attributed to the Se deficiency resulting in widespread tissue lipoperoxidation which, consequently, led to hyaline degeneration of the muscle fiber, causing the lambs to assume abnormal postures and present severe difficulty in standing. The increase in respiratory and pulse rates can be attributed to alterations in respiratory muscles and the heart.

The following are considered probable NMD risk factors: fast-growing animals, with exuberant musculature, reared on pasture in predominantly native vegetation (Caatinga), and supplemented with mineral salt formulated to meet the nutritional requirements of cattle (Amorim et al., 2005). According to these authors, mineral salts formulated for other species, such as salts for cattle, contain concentrations of trace elements that can exert an antagonistic action to Se, compromising its metabolism. Dorper sheep seem to be more susceptible to the occurrence of white muscle disease or nutritional myopathy; this hypothesis is suggested based on the outbreaks reported in Dorper sheep in the Northeast region (Amorim et al., 2005). It is probable the edaphoclimatic conditions of the semiarid region, the breeding system adopted by the producers, and the inappropriate supplementation for the animal species favored the appearance of NMD, resulting from the lack of Se in these animals.

The animals were reared in a pasture farming system, in an area with predominately native vegetation and African star grass (*Cynodon niemfuensis* Vanderyst). What differs from

the sheep mentioned by a report that they were fed on native pasture and supplemented with wheat bran, corn bran, elephant grass, melon, and watermelon, in addition to commercial feed containing 23% protein and protein mineral salt; it having been postulated by the authors that a possible predisposing factor for the occurrence of the disease was overfeeding, through providing young animals with a diet rich in carbohydrates and proteins (Bezerra et al., 2007). Researchers also cited some factors predisposing sheep to the development of NMD, such as an excess of unsaturated fatty acids in the diet, prolonged or unusual muscle exercise for the animal, and adverse climatic exposure (Maas et al., 1994; Rodriguez et al., 2018). Poor quality food, such as straw or crop remains, often predispose to Se deficiencies (Maas et al., 1994). Ruminants appear to be more susceptible to the disease, with greater severity in small ruminants (sheep and goats), and that lambs aged 2-4 months are generally more affected and may demonstrate difficulty walking due to muscle damage, a fact which is strongly related to the conditions of the animals in this case (Ghanem et al., 2016).

In the therapeutic protocol established in the current case, the replacement of the mineral salt of cattle with one appropriate for the sheep species was recommended, in addition to supplementation with vitamin E. After these measures, the appearance of new sick animals and mortality ceased. The success of the treatment was probably due to the integrity of the sarcolemmal tubules, which in the early stages of nutritional myopathy are preserved so that the muscles regenerate properly, returning to normal. However, in chronic cases, regeneration can occur through budding and diffuse fibrosis of the myofibers (Barros, 2010)

Due care should be taken in guiding nutritional management, not offering minerals from other species. Geographical aspects of the region were also taken into account, which were related to the lack of Se due to the poor availability of the element in pastures. In this context, mineral supplementation specific to the sheep species was essential and it was recommended that it be offered throughout the year, particularly in young animals. According different authors, animal diets are usually supplemented with Se in order to provide adequate amounts of different essential trace elements and to prevent Se deficiency disorders (Xun et al., 2012; Hefnawy et al., 2014; Paiva et al., 2019; Nedelkov et al., 2020). Considering the serious impact on the productive efficiency of the affected animals and the death of animals in the herd, deficiency should be avoided, with mineral supplementation in regions with Se deficiency, as well as in animals with deficiency.

3.2. Quantification of Se

Articles in the literature report cases of NMD in sheep, addressing different aspects of the diagnosis, such as clinical-epidemiological signs, biochemical enzyme activity analyzes, and anatomopathological aspects, however, few link the diagnosis with the quantification of Se concentration in the blood (Beytut et al., 2002; Amorim et al., 2005; Bezerra et al., 2007; Yavuz, 2017).

One should consider the importance of quantifying Se, both in the liver, which represents the stock 'pool', and in the serum, which represents the homeostatic 'pool', allowing better understanding of the dynamics of the element in the organism. When possible, the enzymatic activity of GSH-Px should also be verified as it represents the functional "pool" (Underwood & Suttle, 1999). In this case, the diagnosis of NMD with epidemiological and clinical data, mineral quantification, and histopathology, allowed better understanding of this disease related to the lack of Se in lambs, strengthening the diagnostic hypothesis. The concentration of Se in serum is the most reliable method to screen a herd for Se deficiency, as this trace element in the blood is more chemically stable than GSH-Px and its concentration reflects the long-term Se status of the animal (Ghanem et al., 2016). The most accurate method for diagnosing Se status includes direct determination of Se in blood and tissues and indirect assessment by measuring GSH-Px activity in whole blood (Pavlata et al., 2012). In this case, serum Se quantification was performed, which made it possible to define the diagnosis accurately, associated with clinical and anatomopathological data.

The mean concentration of Se in the lamb serum was 2.52±1.02µg/L (0.032±0.013µmol/L) with a median of 2.21µg/L (0.028µmol/L), a lower limit of 1.26 µg/L (0.016µmol/L), and upper limit of 4.41µg/L (0.056µmol/L). Different authors refer to normal, marginal, and deficient limits in different publications, making it possible to compare the data obtained in this case. Se concentration in sheep plasma should be 150 to 350µg/L (1.91 to 4.45µmol/L), while values of 25-50µg/L (0.32-0.64µmol/L) are considered deficient. NMD is observed when levels are below 100µg/L (1.27µmol/L) (Øvernes et al., 1985). Sheep with less than 0.05 mg/L (0.63µmol/L or 49.86µg/L) of Se in whole blood as deficient (Waldner et al., 1998). The ideal concentration of Se in the blood serum of sheep is 120-150µg/L (1.52-1.91µmol/L), while values of 25-50µg/L (0.32-0.64µmol/L) are considered deficient (Hefnawy et al., 2007). Se in serum of lambs with NMD was quantified and values of 56.57±4.88µg/L (0.71±0.006µmol/L) were found in lambs in the control group, and values of 26.67±4.31µg/L (0.34±0.05µmol/L) in lambs before treatment and 51.27±2.26µg/L (0.65±0.03µmol/L) after treatment with sodium selenite (Değer et al., 2008).

A concentration equal to or less than 39.37µg/L (0.5µmol/L) of Se in the serum is considered as a marginal limit to characterize Se deficiency in lambs (Suttle, 2010). Important consider that in assessing the status of Se in sheep, three stages should be used: adequate (greater than 100µg/L or 1.27µmol/L), marginal (70 to 100µg/L or 0.89-1.27µmol/L), and deficient (less than 70µg/L or 0.89µmol/L) (Pavlata et al., 2012), while lambs without NMD had a mean selenium concentration of 57.35±1.04µg/L (0.73±0.01µmol/L), and lambs with NMD presented 18.8±0.52µg/L (0.24±0.007µmol/L) (Ghanem et al., 2016). More recently, was observed sheep with and without NMD and found that animals in the control group showed a significant variation in serum Se concentration of 72.44±3.15µg/L (0.92±0.04µmol/L) and those with NMD 62.21±5.51µg/L (0.79±0.07µmol/L) (Yildirim et al., 2019).

The values found in all lambs were below these benchmarks, making the discussion about the serum quantification of Se in animals with suspected NMD relevant, even if there is important data on clinical signs and anatomopathological findings; considering, also, the importance of subsidizing the guidance for mandatory Se supplementation for sheep raised in the studied region.

It should be considered that lambs present digestive activity similar to that of non-ruminants, in which the use of Se in the diet is greater (Hefnawy & Tórtora Pérez, 2010). Was postulated that sheep supplemented with Se in the prepartum period had lambs with a higher serum Se concentration than those of sheep which were not supplemented (Jalilian et al., 2012; Erdoğan et al., 2017; Nedelkov et al., 2020).

In the differential diagnosis, myopathies of nutritional and toxic origin should be considered, such as those caused by *Senna occidentalis* and *Senna obtusifolia*, ionophore antibiotics, and mitochondrial myopathies. Toxic myopathies, caused by the ingestion of plants, or ionophore antibiotics, cause degenerative lesions in the skeletal striated muscles in ruminants. The findings from the necropsy are usually pale, focal, and/or coalescent areas in skeletal muscle. In addition, histologically there are varying degrees of degeneration, necrosis, and rupture of skeletal muscle fibers, sometimes associated with proliferative and regenerative processes, but without mineralized fibers (Barros, 2010). The mineralization of skeletal muscle fibers is compatible with nutritional myopathy, related to the nutritional deficiency of Se and vitamin E (Amorim et al., 2005), which is in accordance with the pathological characteristics reported in the current study.

3.3. Anatomopathology

One animal was euthanized *in extremis* and submitted to necropsy. At necropsy, random pale areas were observed on the capsular surface of the liver parenchyma. In the histological examination, in multiple hepatic lobes, marked hypereosinophilia was observed in the hepatic artery wall in the portal space, characterizing a necrotizing vasculitis (fibrinoid necrosis). Multifocal areas of the periportal hepatocytes presented eosinophilic cytoplasm and were slightly retracted, but with maintenance of the general cellular architecture, although the nucleus was pyknotic, karyorrhexis, and/or karyolysis (hepatocellular coagulation necrosis) (Fig. 1A), in addition to diffuse thin vacuolization hepatocytes. The liver of lambs was often spotted and dark reddish purple, with occasional bleeding under the capsule (Beytut et al., 2002).

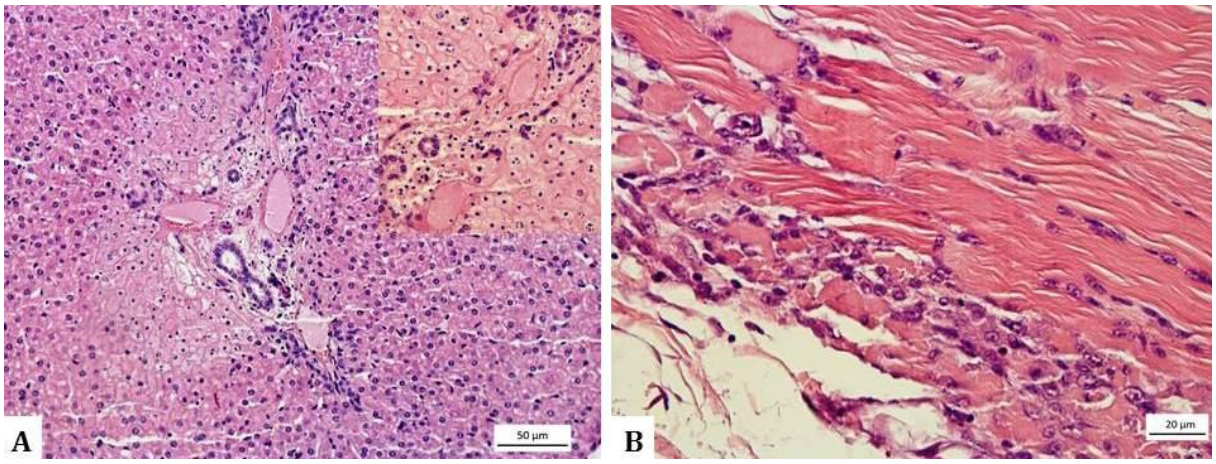
It is assumed that the genesis of these lesions was induced by lipid peroxidation, since the peroxides act on the lipid membranes and cause cell damage. The role played by Se as an integral part of the enzyme GSH-Px is known, acting in the reduction of lipid membrane peroxidation, caused by the action of toxic radicals on non-toxic compounds, and Vitamin E, which is an important fat-soluble antioxidant and is inserted in lipid membranes, acting synergistically with Se to protect biological membranes from high concentrations of lipoperoxidases (Suttle, 2010; Ataollahi et al., 2013). These discussions is reinforce, since Se is an essential trace element in animal nutrition and has multiple actions related to animal production, fertility, and disease prevention, as well as which GSH-Px is a selenoenzyme with a proven ability to prevent oxidative damage to cell membranes (Hefnawy & Tórtora Pérez, 2010). According to the same authors, NMD was the first recognized condition associated with Se deficiency and this causes mortality in newborns, especially in ruminants, and impaired production conditions in growing animals and adults.

In skeletal muscles, segmental necrosis was observed, characterized by retracted myofibrils, with loss of striations, eosinophilic cytoplasm, swollen and hyperplastic sarcolemmal nuclei, and, sometimes, pyknotic or fragmented nuclei (karyorrhexis). Moderate quantities of fibers were fragmented and there was a slight to moderate inflammatory infiltrate of mononuclear cells in all the examined muscles in the interstitium (Fig. 1B). The presence of mineralized fibers was also recorded. Different studies have reported significant and varied lesions found in the musculature of lambs affected by NMD, demonstrating great similarity with the observations found in the current work. In the anatomopathological diagnosis, the musculature is atrophied, friable, and with whitish bands, which correspond to the regions where tissue degeneration and mineralization occurred (Pugh & Baird, 2011).

Microscopically the alterations correspond to areas of hyaline degeneration, segmental necrosis, and mineralization, which lead to loss of function of the muscle involved (Contreras et al., 2005; Yavuz, 2017). In a study with lambs with NMD, was found that the main histopathological alterations found in skeletal and cardiac musculature consisted of hyaline, floccular, and segmental myofibril necrosis with marked eosinophilia and loss of transverse striations, accompanied by intense calcification and infiltration of mononuclear leukocyte cells (Bezerra et al., 2007). Other authors also registered fragmented muscle fibers, justifying that in the remaining unaffected fibers, the proliferation of nuclei could be observed as an attempt to repair the damage (Hefnawy & Tórtora Pérez, 2010). Proliferation of the muscular nucleus and mononuclear macrophages infiltrate in association with swollen muscle fibers (Hefnawy et al., 2014). Necrotic fibers are infiltrated with macrophages and fibroblasts, which in the histological analysis resulted in significant nuclear accumulation in the affected areas (Beytut et al., 2002).

Degenerated fibers can present an intense reaction to hematoxylin, with calcium deposition in the cells, which may justify mineralization (Hefnawy & Tórtora Pérez, 2010). Exceptionally, in chronic cases, the calcified muscles can be seen in the gross necropsy exam, which is why it is called NMD, however, the characteristic lesion is that the affected muscles are paler than the rest of the body musculature (Ramírez et al., 2004). Was reported that Zenker degeneration and fibrosis were evident in all samples of skeletal muscle examined (Beytut et al., 2002). The sarcoplasm of the affected fibers was filled with homogeneous eosinophilic granules and their crossed striations become obliterated. The necrotic areas appeared edematous and hypercellular, and many degenerate fibers were invaded by macrophages. The vascular lesions were prominent, with endothelial proliferation, mild fibrinoid necrosis, and infiltration of the adventitia with mononuclear cells of large arteries. In the liver, generalized periacinar congestion (Figure 1), hemorrhage, diffuse hepatocyte necrosis, and mononuclear cell infiltration were determined.

Figure 1. Se deficiency in sheep. (A) hepatocellular coagulation necrosis in hepatocytes in the periportal region, in addition to necrotizing vasculitis (fibrinoid necrosis) in the hepatic arteriole. (B) Skeletal muscle with necrotic fibers and inflammatory infiltrate of mononuclear cells.



4. Conclusions

The lambs from this outbreak, reared on pasture in the studied agreste region, presented low serum Se concentrations, with clinical manifestation of NMD, so that regular supplementation with Se in the diet was recommended to avoid the appearance of new cases. Serum Se levels are useful indicators for the diagnosis of NMD in lambs, associated with clinical and anatomopathological data. The findings with respect to skeletal muscles are compatible with those observed in nutritional myopathy, characterized by necrosis and calcification of myofibrils, necrotizing vasculitis in hepatic arterioles, and hepatocellular coagulation necrosis in hepatocytes, probably triggered by lipid peroxidation of cell membranes.

Funding

This research was funded by the Coordination for the Improvement of Higher Education Personnel (CAPES) and National Council for Scientific and Technological Development (CNPq).

Acknowledgments

PC Soares and AFM Dantas are grateful to the National Council for Scientific and Technological Development (CNPq) for their research productivity fellowship. Thanks to the Analytical Chemistry Center of the Research Support Center of the Federal Rural University of Pernambuco, for the serum selenium analyzes.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the research, publication or authorship of this article.

References

Amorim, S.L., Oliveira, A.C.P., Riet-Correa, F., Simões, S.V.D., Medeiros, R.M.T. & Clementino, I.J. (2005). Distrofia muscular nutricional em ovinos na Paraíba. *Pesquisa Veterinária Brasileira*, 25:120-124.

Ataollahi, F., Mohri, M., Seifi, H.A., Pingguan-Murphy, B., Abas, W.A.B.W. & Osman, N.A.A. (2013). Evaluation of copper concentration in subclinical cases of white muscle disease and its relationship with cardiac troponin I. *PloS One*, 8:1–5.

Barros, C.S.L. (2010). *Sistema muscular*. In Patologia Veterinária; Santos, R.L.; Alessi, A.C., Roca: São Paulo, SP, Brazil, pp.697-746.

Beytut, E., Karatas, F. & Beytut, E. (2002). Lambs with White Muscle Disease and Selenium Content of Soil and Meadow Hay in the Region of Kars, Turkey. *The Veterinary Journal*, 163:214-217.

Bezerra, F.S.B., Batista, J.S., Belém Melo, D.E., Lima Neto, E.S. & Farias, Y.J.M.D. (2007). Miopatia nutricional em ovinos no Rio Grande do Norte. *Acta Veterinaria Brasilica*, 1:60-63.

Contreras, P.A., Paredes, E., Wittwer, F. & Carrillo, S. (2005). Caso clínico: Brote de enfermedad del músculo blanco o miodegeneracíon nutricional em terneros. *Revista Científica-FCV-LUZ*, 15:401-405.

Değer, Y., Mert, H., Mert, N., Yur, F., Kozat, S., Yörük, I.H. & Sel, T. (2008). Serum Selenium, Vitamin E, and Sialic Acids Concentrations in Lambs with White Muscle Disease. *Biological Trace Element Research*, 121:39–43.

Erdoğan, S., Karadaş, F., Yilmaz, A. & Karaca, S. (2017). The effect of organic selenium in feeding of ewes in late pregnancy on selenium transfer to progeny. *Revista Brasileira de Zootecnia*, 46:147-155.

Ghanem, M.M., Anwar, M.R., Abd El-Raof, Y.M. & El-Attar, H.M. (2016). Clinical, hematological and biochemical changes in lambs suffered from nutritional muscular dystrophy. *Benha Veterinaty Medical Journal*, 30:61-66.

Guedes, K.M.R., Riet-Correa, F., Dantas, A.F.M., Simões, S.V.D., Miranda Neto, E.G., Nobre, V.M.T. & Medeiros, R.M.T. (2007). Doenças do sistema nervoso central em caprinos e ovinos no semiárido. *Pesquisa Veterinária Brasileira*, 27:29-38.

Guimarães, J.A., Xavier, G.C., Soares, P.C., Dantas, A.C., Soares, F.A.P., Mendonça, C.L., Guerra, M.M.P. & Silva Júnior, V.A. (2011). Oxidative stress profile in goats supplemented or not with selenium and vitamin E and submitted to scrotal insulation. *Semina: Ciências Agrárias*, 32:1955-1964.

Hefnawy, A.E.G., Lopez-Arellano, R., Revilla-Vazquez, A., Ramırez-Bribiesca, E. & Tortora-Perez, J. (2007). The relationship between fetal and maternal selenium concentrations in sheep and goats. *Small Rumimant Research*, 73:174–180.

Hefnawy, A.E.G. & Tórtora Pérez, J.L. (2010). The importance of selenium and the effects of its deficiency in animal health. *Small Ruminant Research*, 89:185–192.

Hefnawy, A.E.G., Youssef, S., Aguilera, P.V., Rodríguez, C.V. & Tórtora Pérez, J.L. (2014). The Relationship between Selenium and T3 in Selenium Supplemented and Nonsupplemented Ewes and Their Lamb. *Veterinary Medicine International*, 1-6.

Jalilian, M.T., Moeini, M.M. & Karkodi, K. (2012). Effect of selenium and vitamin E supplementation during late pregnancy on colostrum and plasma Se, Cu, Zn and Fe concentrations of fat tail Sanjabi ewes and their lambs. *Acta Agriculturae Slovenica*, 100:123–129.

Maas, J., Parish, S.M., Hodgson, D.R. & Valberg, S.J. (1994). *Degeneração muscular nutricional*. In: Medicina Interna de Grandes Animais; Smith, B.P., Ed. Manole: São Paulo, SP, Brazil, pp. 1335-1351.

Maas, L., Bulgin, M.S., Anderson, B.C. & Frye, T.M. (1984). Nutritional myodegeneration associated with vitamin E deficiency and normal selenium status in lambs. *Journal of the American Veterinary Medical Association*, 184:201-204.

Nedelkov, K., Chen, C X., Martins, J.M.M.R., Melgar, A., Harper, M.T., Räisänen, S., OH, J.; Felix, T.L., Wall, E. & Hristov, A.N. (2020). Alternative selenium supplement for sheep. *Animal Feed Science and Technology*, 261:1-6.

Øvernes, G., Moksnes, K., Froslie, A., Gunnar, J. G. & Flaat, J. (1985). The effect of different levels of selenium in mineral mixtures and salt licks on selenium status in sheep. *Acta Veterinaria Scandinavica*, 26:405-416.

Paiva, F.A., Saran Netto, A., Corrêa, L.B., Silva, T.H., Guimarães, I.C.S.B., Del Claro, G.R., Cunha, J.A. & Zanetti, M.A. (2019). Organic selenium supplementation increases muscle selenium content in growing lambs compared to inorganic source. *Small Ruminant Research*, 175:57–64.

Pavlata, L., Misurova, L., Pechova, A., Husakova, T. & Dvorak, R. (2012). Direct and indirect assessment of selenium status in sheep – a comparison. *Acta Veterinaria Brunensis*, 57:219–223.

Pugh, D.G. & Baird, N. *Sheep and Goat Medicine*. Saunders: Philadelphia, USA, 2011; ISBN: 9781437723533.

Ramírez, B.E., Hernández, C.E., Hernández, C.L.M. & Tórtora, P.J.L. (2004). Effect of parenteral supplement with sodium selenite on lamb mortality and hematic values of selenium. *Agrociencia*, 38:43–51.

Rodriguez, A.M., Schild, C.O., Cantón, G.J., Riet-Correa, F., Armendano, J.I., Caffarena, R.D., Brambilla, E.C., García, J.A., Morrell, E.L., Poppenga, R. & Giannitti, F. (2018). White muscle disease in three selenium deficient beef and dairy calves in Argentina and Uruguay. *Ciência Rural*, Santa Maria, 48:e20170733.

Rosa, F.B., Caprioli, R.A., Silva, T.M., Galiza, G.J.N., Barros, C.S.L., Irigoyen, L.F., Fighera, R.A. & Kommers, G.D. (2013). Doenças de caprinos diagnosticadas na região Central no Rio Grande do Sul: 114 casos. *Pesquisa Veterinária Brasileira*, 33:199-204.

Suttle, N.F. (2010) *Mineral Nutrition of Livestock*, 4 ed.; CABI publishing: Wallingford, UK. ISBN 978-1-84593-472-9.

Underwood, E.J. & Suttle, N.F. (1999). *The Mineral Nutrition of Livestock*. 3 ed.; CABI Publishing: Wallingford, UK. ISBN 0 851991289.

Waldner, C., Campbell, J., Jim, G. E., Guichon, P. T. & Booker, C. (1998). Comparison of 3 methods of selenium assessment in cattle. *The Canadian Veterinary Journal*, 339:225-231.

Xun, W., Shi, L., Yue, W., Zhang, C., Ren, Y. & Liu, Q. (2012). Effect of High-Dose Nano-selenium and Selenium–Yeast on Feed Digestibility, Rumen Fermentation, and Purine Derivatives in Sheep. *Biological Trace Element Research*, 150:130–136.

Yavuz, O. (2017). The Pathological Investigations on Nutritional Myopathy Causing Lamb Deaths in Neonatal Period. *Journal of Bahri Dagdas Animal Research*, 6:1-8.

Yildirim, S., Ozkan, C., Huyut, Z. & Çinar, A. (2019). Detection of Se, Vit. E, Vit. A, MDA, 8-OHdG, and CoQ10 Levels and Histopathological Changes in Heart Tissue in Sheep with White Muscle Disease. *Biological Trace Element Research,* 188:419-423.

Percentage contribution of each author in the manuscript

Dinamérico de Alencar Santos Júnior – 40 %

Robério Gomes Olinda – 10%

Emanuel Felipe Oliveira Filho – 10%

Pierre Castro Soares – 10

Antônio Flávio Medeiros Dantas – 10%

Sara Vilar Dantas Simões – 10%

Eldinê Gomes de Miranda Neto – 10 %