

Effect of excess nitrate on Xalda sheep

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Abstract

This study analyzed potential causes of a 40% mortality rate observed in a flock of endangered Xalda sheep, indigenous to the province of Asturias in northern Spain. We found that productive and reproductive parameters of the flock were also severely affected. In order to diagnose the cause of mortality and illness in the sheep, we examined the animals and their living conditions. We found high concentrations of nitrate in animal serum, well water and the surrounding pastures. Based on these findings and the absence of a geological source for nitrogen in the paddock, we believe that inappropriate management of manure in neighboring dairy farms resulted in nitrate toxicity in the Xalda sheep. This study highlights the importance of good practices in manure management and the need to monitor nitrate toxicity in pastures. It also highlights the importance of anthropogenic factors on the conservation of animal genetic resources.

Case report

The Xalda is an endangered breed of sheep indigenous to the Asturias province in northern Spain. Xalda sheep belongs to *Ovis aries celticus* trunk, presenting a small size, and black, white or greyish or even spotted coat colour. It represents the only sheep in Spain with small head always straight, or even slightly hollowed, profile on females and sub-convex in males.

In this study, we focused on a flock containing 87 sheep located in the coastal region of the Asturias with a temperate oceanic climate. The animals were distributed across two independent paddocks located 1 km apart: 42 sheep grazed in paddock A (1.12 ha), while 45 sheep grazed in paddock B (1.62 ha). The flock in both paddocks consisted of adult and juvenile female and male sheep in good body condition. The same deworming protocols were consistently applied in both paddocks. During the period of nine months of observation 22% of animals in paddock B died due to unknown causes, with most animals showing symptoms of diarrhea and very poor body condition. During the breeding season, only 9 of 35 (27%) sheep in paddock B gave birth, compared to 39 of 42 (93%) sheep in paddock A. Of 8 of the lambs born in paddock B, 4 (50%) died before being three months old. After weaning, 27 lambs from paddock A were transported to paddock B, of which 11 (41%) died within two months after transfer. Lambs in paddock B that survived until the age of five months were put down in a slaughterhouse, and their carcass weight was about 4.5 kg, which is half the normal carcass weight for their age and breed. In summary, the mortality rate in paddock B was 40%, while that in paddock A was 6%. Paddock B also showed much lower rates of fertility (27 vs 97%) and weaning (44 vs 100%). All data was provided by the breeder.

To understand the potential causes of poor sheep health and productivity, paddocks and animals were examined between September and November 2018. Paddock A is an apple orchard practically flat and features a natural meadow. Paddock B is an apple orchard on a 7%

slope, with a well that acts as a water source, and the entire paddock is covered with thick green pasture with minimal floristic variability. Grass samples (300 g) were collected from three locations in paddock B (upper, central and lower subplot) to estimate the nitrate/nitrite ($\text{NO}_3^-/\text{NO}_2^-$) content in grass juice, determine their species composition [1], and carry out a complete nutritional analysis. Nitrate/nitrite content in grass was roughly estimated using a colorimetric method (MQuantTM Nitrat-Test, Merck), and more precisely determined in well water using a photometric method (Alce Calidad internal normalized work procedure). Even though the grass in paddock B was lush and green (Figure 1), it had very low species diversity (Table 1). Only two species were found in the central subplot (*Agropyrum sp.* and *Avenula sp.*) and lower subplot (*Agropyrum sp.* and *Trifolium repens*). In the central subplot, *Avenula sp.* represented more than 80% of the grass species. *Agropyrum sp.* accounted for 39.56% of species in the upper subplot and 51.13% of the species in the lower subplot. Nearly half the grass samples collected from the lower subplot were dead. All species found are considered to be nitrophilic invasive plants [2] that can grow rapidly in nitrate-rich soils and selectively push out other species. Indeed, our analyses indicated high concentrations of nitrate in all three subplots of paddock B. The central subplot, where the well is located, showed the highest concentration of nitrate (25-50 mg/l) in grass, while 10 mg/l was found in upper and lower subplot grass. Nitrite was not detected in any of the three subplots grass samples. Using a more accurate photometric method, we determined nitrite levels in well water to be below 0.03 mg/l and nitrate levels to be 57 mg/l (Alce Calidad, assay report:

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Table 1. Species composition analysis

Species	Upper subplot			Central subplot			Lower subplot		
	Fresh mass	Dry mass	%	Fresh mass	Dry mass	%	Fresh mass	Dry mass	%
<i>Agropyron sp</i>	14.86	6.88	39.56	4.65	1.58	6.4	38.46	13.37	51.13
<i>Avenula sp</i>	14.81	4.39	25.25	123.79	20.74	83.96	-	-	-
<i>Medicago sp</i>	4.65	1.57	9.03	-	-	-	-	-	-
<i>Trifolium repens</i>	5.17	1.8	10.35	-	-	-	0.09	0.03	0.11
Dead material	3.51	2.75	15.81	15.59	2.38	9.64	21.42	12.35	47.23
Unidentified/Others	-	-	-	-	-	-	0.95	0.4	1.53



Figure 1. View of paddock B

46481). The latter exceeds the permissible limit of 50 mg/l for drinking water consumed by humans (Spanish Royal Decree RD 140/2003). The overall nutritional value and energy supply of paddock B grass samples were within normal ranges (SERIDA-Nutrition Laboratory assay report I17/21804-I17/21806).

Feces samples (5 g) were collected from four random animals in paddock B and analyzed for the presence of protozoa, nematodes, or cestodes based on fecal flotation in saturated saline solution. The results were within normal ranges for these parasites. Blood samples were obtained by jugular venipuncture in 5-ml EDTA-vacutainer tubes from eight randomly chosen adult sheep from each paddock. Serum samples were separated from the blood cells by centrifugation and analyzed using a nitrate/nitrite colorimetric assay (Sigma, Madrid, Spain). Serum from sheep in paddock B contained three times higher concentrations of nitrates (5.3 vs 1.7 ng/μl) and nitrites (1.8 vs 0.6 ng/μl) than serum from paddock A. Necropsies and histopathology were performed on two, 9-month-old affected animals from paddock B. Subcutaneous and pulmonary edema were observed, as well as generalized edema in the hydropericardium, ascites, and hydrothorax. Animals showed symptoms of cachexia, such as muscle and serous atrophy, as well as poor bone mineralization. The heart, kidney, liver, spleen, gut, and central nervous system did not show any relevant lesions. We did not observe evidence of infectious diseases caused by viruses, bacteria, or parasites.

Soil nitrate is the main source of nitrogen for plants. Under normal conditions, roots absorb soil nitrate while leaves fix the nitrate in proteins. When the natural balance is disrupted, and roots absorb nitrates faster than leaves can fix it in proteins, nitrates accumulate in plant tissue. Pastures, hay, and silage are the principal sources of nitrate in animal diets. Drinking water and accidental ingestion of

lubricants or fertilizers can also increase the concentration of nitrate in animals. In contrast, nitrite is rarely found in plants, and sources of nitrite are less common. Our results confirm the accumulation of high concentrations of nitrates in both the grass and well water in paddock B. A geological reconnaissance of paddocks A and B showed the underlying rock substrate to be formed by a stratigraphic formation including sandstones, siltstones, and pelites, all of which are lithologies of detritic origin, with high proportions of quartz and phyllosilicates. Nitrogen does not constitute neither a major nor secondary component of rock forming minerals in these types of rocks, thereby excluding the involvement of the substrate in paddock B nitrate accumulation.

We suggest that the cachexia, poor bone mineralization, reduced fertility and high mortality of Xalda sheep in paddock B is due to mineral imbalance caused by nitrate toxicity. Nitrates serve as a source of nitrites, which bind hemoglobin to form methemoglobin, reducing the blood's ability to transport oxygen from the lungs to the tissues. This reduces the energy status of the body and can lead to digestive system disorders. Depending on the percentage of methemoglobin present, symptoms range from lethargy, darkening of dark mucous membranes, slower growth and reduced fertility to diarrhea, weakness, ataxia, abdominal colic pain, and reproductive failure with fetal death or delivery of weak individuals. In ruminants, rumen microflora rapidly reduce nitrates to nitrites, making them very sensitive to poisoning [3]. Nitrites and nitrates pass directly from the rumen or intestine into the blood, so nitrate poisoning can be tested by analyzing plasma samples [4].

Our analysis suggests that chronic nitrate intoxication in grass and well water can lead to cachexia, reproductive failure, and death in adult sheep and lambs of the Xalda breed in Asturias. We believe that the accumulation of nitrates in paddock B was facilitated by anthropogenic factors, perhaps the inappropriate management of manure at a neighboring dairy farm located at the top of the slope. The Xalda breed are considered to be at risk of extinction, leading to the development of a breeding program [5]. Our results suggest that, despite efforts by public administrators and Xalda breeders, inappropriate management of dairy farm manure threaten indigenous genetic resources. Periodic monitoring of nitrate content in water sources and pastures is essential to prevent chronic nitrate toxicity [6].

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