CHRONIC EFFECTS OF FLUORIDE IN TUJ SHEEP ON SERUM LEVELS OF TOTAL PROTEIN, ALBUMIN, URIC ACID, AND NITRIC OXIDE AND ACTIVITIES OF LACTATE DEHYDROGENASE AND LEUCINE AMINOPEPTIDASE

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SUMMARY: Twenty healthy yearling Tuj ewe-lambs with a mean body weight of 31±2 kg were divided into two equal groups. Each group of ten sheep was provided with hay and water *ad libitum* throughout the study. The water for the control group contained 0.49 mg F/L, whereas the water for the experimental group contained 13.8 mg F/L, equivalent for the latter group to an intake of *ca.* 1.8 mg F/kg bw/day. By the 12th week the urinary fluoride concentration in the experimental group had increased from 0.9 ppm at the start to 1.78 ppm, and by the 24th week it reached 8 ppm, at which point various serum determinations were made and the study was terminated. In the experimental vs. the control groups the results were as follows: total protein 7.41±0.03 vs. 7.67±0.07 g/dL (p<0.01), albumin 3.97±0.36 vs. 4.72±0.28 g/dL, nitric oxide 49.90±3.1 vs. 35.75±2.7 µmol/L (p<0.01), uric acid 0.49±0.03 vs. 0.29±0.02 mg/dL (p<0.001), lactate dehydrogenase (LDH) 676.7±21 vs. 518±35 U/L (p<0.01), and leucine aminopeptidase (LAP) 7.28±0.5 vs. 6.18±0.53 U/L. Changes in the albumin level and the LAP activity were not statistically significant. During and at the end of the study the urine fluoride concentration of the control group was unchanged.

Keywords: Albumin; Lactate dehydrogenase (LDH); Leucine aminopeptidase (LAP); Nitric oxide; Protein; Sheep serum; Tuj sheep; Uric acid.

INTRODUCTION

Volcanic and industrial fluoride emissions can cause increased intake of F from food, air, or drinking water that can harm human and animal health.[1,2] Following ingestion, F accumulates in bones and teeth and disperses toward cardiac muscle, liver, skin, and erythrocytes.[3] In hard tissues it can cause skeletal and dental fluorosis, and in soft tissues it can produce metabolic disturbances and increased free radical activity.[4,5]

Among livestock animals, cows and sheep are especially sensitive to the toxic effects of F. Endemic fluorosis has often been observed in these ruminants.[1] The aim of this study was to determine the effect of experimentally induced chronic fluorosis on serum levels in sheep of protein, albumin, uric acid, and nitric oxide and also the activities of serum leucine aminopeptidase (LAP) and lactate dehydrogenase (LDH). In a parallel study, two of our colleagues also determined plasma malondialdehyde (MDA) and red blood cell reduced glutathione (GSH) levels in the same sheep.[6]

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MATERIALS AND METHODS

Twenty healthy, yearling Tuj ewe-lambs with a mean body weight of 31±2 kg were divided into two equal groups (n = 10) and placed in separate sections of the study farm. Ear tags were applied to keep track of each animal. Before beginning the study, the experimental ewes were kept in their section for 30 days to adapt to the farm conditions. The experimental and control groups were provided hay and water *ad libitum* throughout the study. The local water of the control group contained 0.49±0.03 ppm F. For the experimental group the F concentration of 42-L batches of this water was raised to 13.8 ppm by addition of 1.24 g NaF, giving an estimated NaF daily intake of *ca*. 4.0 mg NaF (= 1.8 mg F)/kg bw from drinking an average of 4.0 L of water/day. Urinary F concentrations were determined weekly and then fortnightly with an EDT Micro 2 pH/ION meter to determine the timing of chronic fluorosis.[8] When the urinary F level reached 8 ppm the experiment was terminated. At this point the blood samples were taken from the *V. Jugularis*, and sera were separated and stored at –24 ºC.

Total serum protein, albumin, uric acid, and LDH were analysed with diagnostic commercial kits (Chema). Nitric oxide (NO) was analysed by the method of Cortas and Wakid.[9] LAP was analysed as described by Goldbarg-Rutenburg (G-R).[10] Data were analysed with Minitab statistical software.[11] Student's *t* test was used to compare the differences between control and experimental groups. Statements of significance were based on $p<0.05$ unless otherwise noted.

RESULTS

Horizontal lines on the surfaces of the teeth and a decrease of body weight gain ($p<0.001$) were observed in the experimental group as manifestations of fluorosis. By the 12^{th} week the urinary F concentration in the experimental group increased from 0.9 ppm at the start to 1.78 ppm, and by the 24^{th} week it reached 8 ppm. Over this period the urinary F concentration of the control group remained unchanged at 0.9 ppm.

As seen in the Table below, total protein levels were decreased ($p<0.01$) [$p = 0.0068$], whereas uric acid ($p<0.001$), NO ($p<0.01$) levels, and LDH ($p<0.01$) activities were increased in the experimental group compared to the control group. There was no significant change in the albumin values and LAP activities.

Table. Serum protein, albumin, nitric oxide, and uric acid levels and leucine aminopeptidase (LAP) and lactate dehydrogenase (LDH) activities in the control and experimental groups of Tuj sheep (mean±S.E.M)

	Control group (n =10)	Experimental group (n =10)	Literature values
Protein (g/dL)	7.67±0.07	7.41±0.03*	6–7.9[28]
Albumin (g/dL)	4.72±0.28	3.97±0.36	2.4–5.36[28,29]
LAP (U/L)	6.18±0.53	7.28±0.5	7.33±2.07[30]
NO (µmol/L)	35.75±2.7	49.90±3.1*	47.6±10.38[31]
Uric acid (mg/dL)	0.29±0.02	0.49±0.03†	0.2–2[28]
LDH (U/L)	518±35	676±21*	238–687[28]

*p<0.01; †p<0.001.

DISCUSSION

Natural or industrial chronic exposure of farm animals to F is known to cause chronic fluorosis resulting in significant economic losses.[12] Since urine is the main route of F excretion, increased urinary F is used as a specific and reliable diagnosis of fluorosis.[13]

In this study, the urinary F concentration of Tuj sheep exposed to 13.8 mg F/L in their drinking water reached 8 ppm by the 24th week, clearly indicative of chronic fluorosis.[12,13] Previous control studies on Tuj sheep in the region with 0.43±0.21 ppm F in the drinking water showed a urinary F concentration of 0.91±0.70 ppm,[14] similar to our results.

In agreement with our findings with fluorosed Tuj sheep, plasma albumin, and protein levels are reported to decrease in rats,[15] children,[3] and sheep[7,16] with chronic fluorosis. F inhibits protein synthesis by weakening the beginning of the peptide chain and by preventing the production of peptide chains in ribozomes.[17,18] F also increases the production of free radicals,[2] which ultimately react with sulphur radicals. The three-dimensional structure of albumin is then damaged due to the formation of disulphide bounds causing it to malfunction.[19,20]

Although serum uric acid levels were increased in our study, Shivashankara *et al*[3] found that plasma uric acid levels decreased in children[3] and in rats[21] with chronic fluorosis. Uric acid has a protective role in defence mechanisms of the body and is also a lipid peroxidation inhibitor and a radical scavenger.[22] It is tightly bound to copper and iron, both of which are known to have important roles in antioxidative defence mechanisms.[23] The increase of uric acid levels observed here might be the result of reactions removing radicals that are produced by F.

Here the serum NO concentration was also higher in the experimental group than in the control group. A positive correlation was observed between the concentration of F and NO synthesis in a study on fluorosed myocardial cells.[24] Superoxide anion increases in fluorosis.[2] In media which include superoxide anions, NO is transformed into peroxynitrite. Oxygen radicals generated from peroxynitrites are very active and can oxidize cell membrane lipids and finally cause cell death.[25]

Although not statistically significant, a rise was observed in LAP activity in the fluorosed sheep, which might be due to a moderate degree of cell damage. LAP is a cytosolic enzyme that is abundant in liver and pancreatic cells. Since it hydrolyses many amides and peptides containing free amino groups, its activity is commonly investigated in research on effects of toxic agents on serum and tissues.[26]

In our study, a significant increase ($p<0.01$) was observed in LDH activity. LDH is a cytosolic enzyme that is also used in the diagnosis of endemic fluorosis.[2,24] It is reported that serum LDH activity is increased in rabbits[27] and rats.[15] Similarly, the addition of F to culture media of myocardial cells increased LDH levels, evidently due to increased membrane permeability.[24]

Taken together, the cause of decreased serum total protein levels observed here might be due to inhibition of protein synthesis by F, and the increase in uric acid levels might be due to removal of free radicals. Additionally, the increase in serum NO might be due to the generation of peroxynitrite by fluoride, and the increase in LDH activities might be due to F-induced changes in cell the membrane permeability.

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Published by the International Society for Fluoride Research
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