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Research Article

Hypocalcaemia in Ossimi Sheep Associated with Feeding on Beet Tops (*Beta vulgaris*)

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Abstract: Hypocalcemia was diagnosed in 48 Ossimi ewes in a flock of 125 sheep. Clinical signs, feed analysis, biochemical findings, and response to treatment were used for diagnosis. Lactating ewes were more frequently affected (56.25%) than pregnant (33.33%) and dry non-pregnant (10.41%) ewes. Teeth grinding, voiding soft pellets, absence of recumbency, non-tympanic rumen, dry mouth, and thin body condition were atypical findings.

Ruminal fluid analysis showed significant increases of potassium and ammonia ($P < 0.05$), and a significant decrease of magnesium ($P < 0.05$) in diseased ewes, as compared with clinically normal ewes. Serum calcium, magnesium, and glucose in the diseased ewes were significantly lower ($P < 0.001$), ($P < 0.01$), ($P < 0.05$) than in clinically normal and control ewes, respectively; however, there was a significant increase of acetone ($P < 0.01$).

In all, 2 ewes were slaughtered due to the failure to respond to treatment and only the kidneys had gross abnormality. Histopathological examination showed degenerative changes in the renal tubules, with deposition of calcium oxalate crystals. The results of this study indicate that the clinical syndrome of hypocalcemia and renal failure occurred in Ossimi sheep that feed on beet tops. The observed atypical signs of hypocalcemia highlight the importance of examining serum and ruminal fluid to determine the proper intervention.

Key Words: Hypocalcemia, hypomagnesemia, beet tops, Ossimi sheep, Egypt

Introduction

Hypocalcemia is a metabolic disorder that occurs most commonly about the time of lambing or during early lactation. The disorder presents sporadically and generally affects less than 5% of a flock, though it occasionally occurs as an outbreak (1). Several reports describe the occurrence and outbreaks of hypocalcemia in intensively-housed ewes and in those fed quality concentrated rations (2,3). It occurs alone or can be associated with hypomagnesemia (4). Many stressors were found to predispose to the occurrence of hypocalcemia, including dietary factors (5).

Hypocalcemia syndrome occurs when pastures contain calcium chelating agents, such as oxalates (6). Clinical problems associated with feeding on plants with high oxalate content have been fully described in cattle and sheep (7). Sugar beet tops is a byproduct of an important crop worldwide and its dry form or silage is used as a component of ration formulations (8). Beet tops are reported to contain a significant amount of oxalate; the amount differing according to variety and soil characteristics (9), but its analysis in our locality has not been reported.

Although hypocalcemia is known to occur sporadically in sheep in Egypt, its frequency increases, especially in flocks with dietary mismanagement. To the best of our knowledge there are no published reports that describe the clinical syndrome associated with feeding on beet tops and the factors that influence its occurrence in sheep. Consequently, the aim of the present study was to describe the clinical and biochemical changes associated with hypocalcemia due to feeding on sugar beet tops in Ossimi sheep in middle Egypt.

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Materials and Methods

Animals

The study included 48 ewes in a flock of 125 Ossimi sheep in the Dakahlia governorate, Egypt suspected of having hypocalcemia. Additionally, 20 dry, non-pregnant clinically normal ewes from the same flock were also investigated (clinically normal group) and 20 clinically healthy ewes in the same locality were studied and used as a control group (ewes were fed a ration composed of hay (ad libitum), 0.5 kg concentrate/day, and a mineral mixture at 0.25%).

History and Clinical Examination

In February 2006 we were called to examine an Ossimi sheep flock with a history of the sudden appearance of tremors, teeth grinding, stumbling, tachypnea, tachycardia, and anorexia. Signs appeared 5 days after beginning to feed on sugar beet tops. The animals had mild diarrhea for 1-3 days after turning to graze beet tops, which resolved spontaneously. There was no other feed or additives given to the sheep flock. The animals under investigation underwent a thorough physical examination and 13 clinical signs were recorded in each case.

Feed Analysis

In total, 5 samples of beet tops were collected from farms in which the problem occurred, which were analyzed according to the Association of Official Analytical Chemists (10).

Biochemical Analyses

Blood samples were collected from each animal into a plain tube and stored at 4 ºC overnight, and then the serum was separated. Ruminal fluid was collected from 26 diseased ewes, 12 clinically normal ewes, and 14 control ewes. Serum was examined for calcium, inorganic phosphorus, magnesium, glucose, and acetone. Ruminal fluid was examined for Ca, Mg, P, K, Na, Cl, and ammonia. Na and K were estimated using a flame photometer (Sherwood model 410, UK). Acetone was estimated using 2% vanillin reagent in freshly prepared 4N KOH and was analyzed spectrophotometrically at 415 nm. Ruminal fluid ammonia was assayed by the phenol-hypochlorite nitropruside method (11). The remaining parameters were estimated following standard methods using commercial test kits (Spinreact, Spain).

Treatment Protocol

Diseased sheep received an intravenous dose (100 cc) of a calcium magnesium combination (Cal-D-Mag®, Pfizer, Egypt). All animals were also subcutaneously injected with 25 cc of 20% magnesium sulphate. All the animals were treatment 3 times at 24-h intervals.

Pathological Examination

Ewes that were slaughtered due to poor treatment response were subjected to gross postmortem examination and specimens were obtained from kidneys for histopathological examination. Tissue sections were prepared and stained with Van Kossa, and hematoxylin and eosin.

Statistical Analysis

A mean and standard deviation was calculated for each variable. Differences between means were calculated by one way ANOVA, followed by a post hoc LSD multiple comparison test, using SPSS v.11.01 for Windows (SPSS, Chicago, USA).

Results

In all, 48 ewes showed the clinical signs of hypocalcemia. As shown in Table 1, depression, anorexia, tachypnea, tachycardia, hypothermia, tremors, and ruminal hypomotility were the most commonly observed typical signs. However, teeth grinding, voiding of soft pellets, absence of recumbency, non-tympanic rumen, dry mouth, and thin body condition were the most prevalent atypical findings.

Analysis of beet tops showed that oxalates (4.12% ± 0.42%), K (4.12% ± 0.51%), and crude protein (16.2% ± 2.85%) content were beyond the normal limits; however, other nutrients in the beet tops showed no obvious abnormality (Table 2).

Ruminal fluid analysis showed a significant increase ($P < 0.05$) of K in diseased ewes (33.98 ± 4.67 mmol/l) in comparison to clinically normal (29.09 ± 2.28 mmol/l) and control ewes (25.61 ± 1.93 mmol/l). Ammonia also significantly increased in the diseased ewes, as compared to the control group (Table 3).

The results of biochemical examination (Table 4) showed that serum calcium was significantly lower ($P < 0.01$) in the diseased ewes than in the clinically normal and control ewes. Moreover, serum magnesium in the

Table 1. Clinical findings of hypocalcaemia in sheep fed on beet tops.

Variable	Clinical Finding	Number of Ewes	%
Depression	Varying degrees	48	100
Body condition	Thin	44	91.66
Appetite	Anorexia	42	87.50
Ruminal examination	Hypomotile rumen	41	85.41
	Tympany	10	20.83
	Ruminal stasis	7	14.58
Recumbency	Non-recumbent	40	83.33
	Recumbent	8	16.66
Respiration	Tachypnoea > 40/min.	39	81.25
Teeth grinding	Positive	39	81.25
Heart rate	Tachycardia > 100/min.	38	79.16
Defecation	Soft pellets	38	79.17
	Constipation	10	20.83
Hypothermia	(36.9 ºC-37.9 ºC)	36	75.00
Muscular tremors	Mild	35	72.91
	Moderate	13	27.08
Salivation	Dry mouth	30	62.50
	Frothiness	18	37.5
Stumbling	Frequent	29	60.42

Table 2. Results of chemical analysis of beet tops.

Parameter	Minimum	Maximum	Mean	SD
DM (%)	8.90	18.00	12.62	3.53
CP (%)	11.76	19.30	16.21	3.19
NE (Mcal/kg)	2.40	2.90	2.64	0.20
CF (%)	10.00	11.80	10.93	0.67
EE (%)	1.50	3.70	2.79	0.82
NFE (%)	47.80	55.00	51.88	2.79
Ash (%)	14.50	24.00	19.00	3.70
Ca (%)	0.65	1.20	0.80	0.22
P (%)	0.18	0.23	0.21	0.20
Mg %	0.19	0.23	0.20	0.01
Na (%)	0.40	0.75	0.49	0.14
K (%)	3.70	0.00	4.10	0.52
Oxalate (%)	3.50	4.60	4.12	0.42
Nitrate (%)	0.69	0.94	0.85	0.10

Table 3. Results of ruminal fluid analysis (Mean ± SD) in sheep with hypocalcaemia associated with feeding on beet tops.

Variable	Control (n = 13)	Clinically Normal (n =12)	Diseased (n = 26)
Ca (mmol/l)	2.10 ± 0.21	2.06 ± 0.24	1.96 ± 0.31
Mg (mmol/l)	2.27 ± 0.15[a]	2.07 ± 0.12[a]	1.75 ± 0.19[b]
P (mmol/l)	1.31 ± 0.15	1.33 ± 0.16	1.35 ± 0.16
K (mmol/l)	25.61 ± 1.93[a]	29.09 ± 2.28[b]	33.98 ± 4.67[c]
Na (mmol/l)	117.32 ± 3.66	118.36 ± 6.42	117.65 ± 3.42
Cl (mmol/l)	31.79 ± 2.02	31.28 ± 1.85	31.29 ± 1.79
Ammonia (mmol/l)	4.86 ± 0.71[a]	11.88 ± 2.61[b]	19.24 ± 4.54[c]

[a,b,c]Values in the same row with different superscript letters are significantly different.

Table 4. Results of biochemical examination (Ca, Mg, P, glucose, and acetone) in normal and hypocalcemic sheep.

Variable	Control (n = 20)	Clinically Normal (n =20)	Diseased (n = 48)
Ca (mmol/l)	3.18 ± 0.12[a]	2.90 ± 0.17[a]	1.35 ± 0.41[b]
Mg (mmol/l)	1.15 ± 0.30[a]	1.01 ± 0.10[a]	0.72 ± 0.08[b]
P (mmol/l)	2.10 ± 0.09	2.20 ± 0.27	2.10 ± 0.14
Glucose (mmol/l)	3.72 ± 0.12[a]	3.69 ± 0.13[a]	3.17 ± .13[b]
Acetone (mmol/l)	0.10 ± 0.01[a]	0.13 ± 0.02[b]	0.24 ± 0.04[b]

[a,b]Values in the same row with different superscript letters are significantly different.

diseased ewes was significantly lower ($P < 0.01$) than in the clinically normal and control ewes. Clinically normal ewes had significant lower magnesium ($P < 0.05$) than the controls. In contrast, there was a significant decrease in serum glucose ($P < 0.05$) and an increase in acetone ($P < 0.01$) levels in the diseased ewes, as compared to the clinically normal and control ewes.

The 48 diseased animals included 27 lactating, 14 pregnant, and 7 dry, non-pregnant ewes. There was only a significant decrease in serum calcium in the lactating (1.26 ± 0.37 mmol/l) and pregnant ewes (1.28 ± 0.41 mmol/l), as compared to the dry non-pregnant ewes (1.80 ± 0.21 mmol/l); however, the remaining parameters weren't significantly different.

Of the 48 diseased ewes, 46 responded dramatically to intravenous infusion of Cal-D-Mag, and magnesium sulphate; 41 of which (85.41%) completely recovered 2 days post treatment and 5 (10.41%) after 5 days. In total, 2 ewes failed to recover and gross postmortem examination revealed only congestion in both kidneys. Microscopically, there were cloudy swelling and degenerative changes of the renal tubules, indicating acute tubular necrosis; deposition of calcium oxalate crystals was also recorded (Figure).

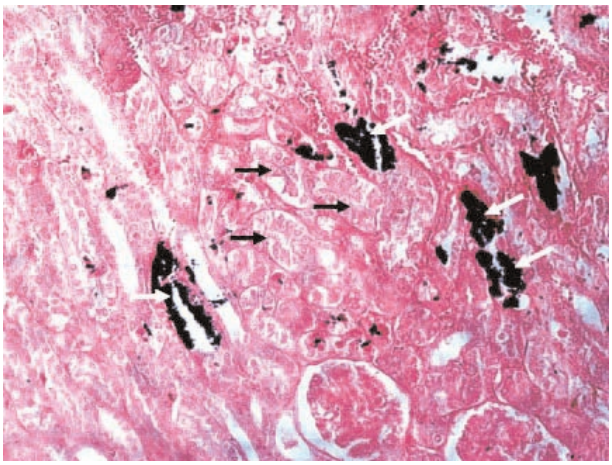

Figure. Photomicrograph of kidney of hypocalcaemic ewe showing deposition of calcium oxalate crystals in the renal tubules (white arrows), and degenerative changes in renal tubules (black arrows) (van Kossa X130).

Discussion

The aim of the present study was to describe the clinical and biochemical findings associated with hypocalcemia due to feeding on beet tops. Hypocalcemia was suspected based on clinical history and physical examination. Feed analysis, biochemical examination, and response to treatment confirmed our suspicion. The frequency of the disease (38.40%) was higher than previously described (3,4) and was classified as an outbreak. Lactating ewes were more frequently affected than pregnant and dry, non-pregnant ewes. Previous studies reported that hypocalcemia was more frequent in pregnant ewes, especially those fed concentrated rations (1,2,12).

Teeth grinding (81.25%), defecation of soft pellets (79.17%), absence of recumbency (83.33 %), dry mouth (62.50%), non-tympanic rumen (79.17%), and thin body condition (91.66 %) were atypical findings in the present study's hypocalcemic ewes, which is in contrast to previous reports (3-4). Depression, anorexia, tachypnea, tachycardia, hypothermia, stumbling, muscular tremors, and hypomotile rumen were the typical signs we observed, which were in accordance with previously described findings (4,13). The present study's findings might be attributable to depression of neuromuscular transmission and decreased muscle contraction.

Teeth grinding is not a common clinical finding in previous reports of hypocalcemia; however, in the present study it was observed at a high frequency. It was documented in ewes with combined hypocalcemia and hypomagnesemia. Teeth grinding is reported to be related more to hypomagnesemia than to hypocalcemia (14). Constipation was not a constant feature in the diseased ewes; most voided pasty soft pellets. The diseased ewes had mild diarrhea for 2 days following a sudden switch to feeding on beet tops. The diarrhea was probably due to the abrupt change of feeding pattern and the laxative effect of beet tops. The disease was more frequent in thin ewes. This result differs with that reported by Woldemeskel et al. (3), as most sheep in the flock were thin.

The chemical composition of beet tops varied according to variety and soil characteristics. The level of oxalates in the analyzed samples was sufficiently high to cause hypocalcemia. Oxalates at a level of 7%-16.6% is reported to be capable of producing acute poisoning and death (6). Sheep and cattle are better able to metabolize oxalates in the rumen, thereby reducing their effect on dietary calcium (15). They are able to tolerate small levels of excess oxalates in feed (6). Once absorbed from the gastrointestinal tract, soluble oxalates rapidly combine with serum calcium and magnesium, resulting in a sudden decrease in available serum calcium and magnesium (16), which results in renal failure (7). It is suggested that the oxalates level was sufficient to chelate calcium and magnesium; therefore, the clinical signs of hypocalcemia appeared. Crude protein content was 16.21%, which is enough to increase the level of ammonia in ruminal fluid. It was found that high crude protein intake and consequent high ruminal fluid ammonia could reduce Mg availability (16).

There was a significant increase ($P < 0.05$) of K in the ruminal fluid of diseased ewes, as compared to the clinically normal and control ewes. This could be attributed to excessive intake of beet tops, which have a high level of K. This finding also explains the presence of hypomagnesemia, which is in agreement with the well-known fact that high K intake and consequent high ruminal fluid level decreases Mg absorption (16-18). Moreover, hypomagnesemia was also recorded in animals fed a low sodium diet (19). Ruminal fluid ammonia also significantly increased in the diseased ewes, as compared to the control group, which was probably due to the high crude protein content of the beet tops and anorexia associated with the clinical signs. The sudden and acute increase of ruminal ammonia was reported to decrease Mg absorption from the temporarily isolated rumen of sheep (20).

It was evident that high levels of oxalates, K, and crude protein in beet tops played an important role in the occurrence of hypocalcemia and hypomagnesemia in the study ewes. Decreased energy intake due to the absence of other energy sources may have contributed to the decreased availability of magnesium. Giduck et al. (21) concluded that intraruminally infusing sheep with glucose stimulated magnesium absorption.

In the diseased ewes serum calcium was significantly lower ($P < 0.01$) than in clinically normal and control ewes. We think that the precipitous decrease of serum calcium was the main cause of the observed clinical signs. Lactating and pregnant ewes had significantly lower levels of calcium ($P < 0.01$) than dry, non-pregnant ewes. The serum magnesium level in diseased ewes was significantly lower ($P < 0.01$) than in clinically normal and control ewes. Clinically normal ewes had a significantly lower magnesium level ($P < 0.05$) than control ewes. We think that more than 1 factor interacted synergistically to decrease serum calcium and magnesium levels, in turn producing the observed clinical signs and that failure of homeostatic functioning may have aggravated the condition.

Unlike the results recorded by Woldemeskel et al. (3), all the ewes in the present study were hypoglycemic. Glucose levels were significantly lower ($P < 0.05$) in the diseased ewes than in the clinically normal and control ewes, probably due to anorexia and the effect of hypocalcemia. Oxalates are reported to have an irreversible effect on energy metabolism and plasma glucose level (9); however, experimental induction of oxalate poisoning by a lethal dose of Halogeton glomeratus in sheep resulted in hyperglycemia (22). Experimentally-induced hypocalcemia resulted in lower plasma glucose and lower endogenous glucose production in normo- and hyperketonemic ewes (23).

Acetone levels in the diseased ewes were significantly higher ($P < 0.01$) than in the clinically normal and control animals. Serum acetone has been used successfully for the diagnosis of ketosis in sheep (24), although 3-hydroxybutyrate was determined to be more reliable for diagnosing and monitoring the condition (1). Generally, the level of ketone bodies reflects the status of energy balance and their elevation indicates nutritional stress. In the present study, the sheep herders did not provide any feed to their animals other than beet tops. Hyperketonemia was reported to suppress endogenous glucose production (hepatic glucose production) and result in a significant reduction in the concentration of plasma glucose (25). We think that the combined effects of hypocalcemia and hyperketonemia played a role in precipitous decrease in plasma glucose observed in the present study.

The dramatic response to treatment observed in the present study supported our tentative diagnosis. The delayed recovery of 5 ewes may have been due to their poor body condition, which suggested they had severe energy deficit and supportive treatment might be required. Knight and Walter (9) proposed that intravenous calcium gluconate, magnesium sulfate, glucose, and a balanced electrolyte solution was the ideal treatment for cases with hypocalcemia due to oxalate poisoning. In humans, aluminum citrate was reported to protect against kidney tissue damage that occurs with high levels of oxalate accumulation, especially in cases of ethylene glycol poisoning (26).

Gross postmortem examination revealed only congestion of both kidneys. Microscopically, there was cloudy swelling and degenerative changes of the renal tubules, which indicated acute tubular necrosis. Deposition of calcium oxalate crystals was also a characteristic finding. Uremia and eventually renal failure caused by precipitation of calcium oxalate in the renal tubule might explain the failed response to treatment. Similar findings were previously recorded in cattle and sheep with Bassia hyssopifolia and halogeton poisoning (22,27). Moreover, the end metabolite of ethylene glycol, which precipitates in the kidneys in the form of calcium oxalate crystals, was believed to cause physical damage to the renal tubules (28). It was suggested that animals didn't die from the acute effects of low blood calcium levels and impaired cellular energy metabolism associated with oxalate poisoning, but from kidney failure (9).

On the basis of the clinical signs and the results of feed analysis, biochemical findings, treatment practice, and histopathological examination, the diagnosis of hypocalcemia syndrome due to feeding on beet tops was made. Many factors were shown to play a role in the condition, such as lactation, pregnancy, body condition, and dietary mismanagement. It is concluded that allowing sheep to graze only on sugar beet tops for more than 5 days resulted in hypocalcemia syndrome. The high frequency of the disease among the sheep and the presence of atypical signs are uncommon findings. Consequently, biochemical changes in both serum and ruminal fluids are of great importance for confirming the presumptive diagnosis of hypocalcemia syndrome.

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