

Serum Calcium Concentration and Some Biochemical Parameters in Lambs with White Muscle Disease

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Abstract: White Muscle Disease (WMD), also called muscular dystrophy, is a nutritional and enzootic disease of lambs characterized by muscle degenerations. The aim of WMD this study was to determine if WMD in lambs is associated with changes in serum calcium concentrations. Fifteen lambs with WMD and ten healthy lambs of Akkaraman breed were the subject of the study. Prior to treatment (at day 0) with a mixture of sodium selenit, vitamin E and vitamin B₁, serum calcium concentration in lambs with WMD was higher than that in healthy lambs ($p < 0.05$). Similarly, prior to the treatment, blood Creatine Kinase (CK), Aspartate Aminotranferase (AST), Lactate Dehydrogenase (LDH) and Alkaline Phosphatase (ALP) concentrations in lambs with WMD were significantly higher compared to healthy lambs ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively). Thirty days after treatment, none of the parameters were significantly different between treated lambs and healthy controls ($p > 0.05$). As a result, in addition to well known biochemical parameters including CK, LDH, AST, ALT and ALP, serum calcium concentration is also elevated in lambs with WMD.

Key words: Lamb, white muscle disease, calcium, biochemical parameters, LDK, CK

INTRODUCTION

White Muscle Disease (WMD), also called muscular dystrophy, is a nutritional and enzootic disease of lambs characterized by muscle degenerations (Kozat, 2007; Or *et al.*, 2003). The disease primarily affects the musculature, including muscles of the back, extremities, pectoral, intercostals, heart and diaphragm (Imren and Sahal, 1991). Clinical signs of the disease occur depending on the muscles affected and the severity of muscle degeneration. As the skeletal muscles are affected, clinical signs vary from mild stiffness to an inability to stand. Lambs may tremble due to pain, when held in a standing position. A stiff gait and hunched appearance are common in lambs with WMD. Diseased lambs may have normal appetites; however they eventually become too weak to nurse the ewe. When, the disease affects the heart, the affected lambs show clinical signs similar to pneumonia, including difficulty in breathing, nasal discharge and fever. Skeletal and cardiac muscle may be affected independently; however the disease may affect both skeletal and cardiac muscle simultaneously (Kennedy *et al.*, 1987; Kozat, 2007; Sekin *et al.*, 1996).

Vitamin E and selenium deficiencies play a significant role in etiology of the disease (Imren and Sahal, 1991; Kozat, 2007; Or *et al.*, 2003; Osame *et al.*, 1990). As Se and vitamin E deficiencies induce lipoperoxidation in tissues, which results in muscle degeneration and calcification (Imren and Sahal, 1991). Prior to the first clinical signs of

WMD in lambs, there is a certain increase in blood Creatine Kinase (CK), Aspartate Aminotranferase (AST), Lactate Dehydrogenase (LDH) and Alkaline Phosphatase (ALP) concentrations, indicatives of muscle degeneration (Camas *et al.*, 1997; Chariot and Bignani, 2003; Imren and Sahal, 1991; Sekin *et al.*, 1996). On the other hand, GSH-Px enzyme activity is slightly decreased (Pamukeu *et al.*, 2001). It reported that serum Calcium (Ca) level in muscle damage in (dystrophic) mice is significantly higher compared to that of healthy ones (Whitehead *et al.*, 2006).

The aim of the present study was to determine whether muscle degenerations in lambs with WMD is associated with changes in serum Ca levels.

MATERIALS AND METHODS

In the present study, 15 lambs with WMD and 10 healthy lambs were used. The cases were diagnosed based on clinical examinations (hind leg stiffness, hunching, stilted gait and inability to stand) and laboratory findings. The lambs were of Akkaraman breed and at 3-6 weeks of age. Upon diagnosis, a commercial mixture (Yeldif, injectable solution, Dif[®]) were administered subcutaneously twice with an interval of 2 weeks at a dose of 1 mg sodium selenit, 60 mg vitamin E and 40 mg vitamin B₁, per lamb. Lambs were clinically examined daily for a period of 30 days. All lambs were nourished only by their mother's milk.

Blood samples were collected from the jugular vein into tubes at days 0 (prior to treatment) and 30. Upon collection, blood samples were centrifuged at 3 000 rpm for 15 min. Subsequently, sera were collected and stored at -20°C, until analyses. Serum CK, LDH, AST, ALT and ALP concentrations were measured using the Roche-Cobas Integra 800 autoanalyzer. Serum Ca concentration was measured using the Hitachi automatic analyzer (Hitachi, Ltd, Tokyo-Japan). The results were statistically analyzed by One Way (ANOVA).

RESULTS AND DISCUSSION

Table 1 shows serum Ca and CK, AST, ALP, ALT and LDH concentrations. At day 0 (prior to treatment), serum Ca concentrations in lambs with WMD were higher compared with those of healthy lambs at day 0 (p<0.05). Similarly, CK, LDH, AST, ALT and ALP concentrations were significantly higher in lambs with WMD compared with those of healthy lambs (p<0.001, p<0.001, p<0.001, p<0.01 and p<0.05, respectively). However, none of the parameters were significantly different between treated WMD lambs and healthy controls 30 days after treatment (p>0.05).

Data are presented as mean±standard deviation. The different superscripts in the same row represent the statistical significance among groups ab: p<0.001, p<0.01, p<0.05.

Nutritional degenerative myopathy can occur in ruminants with vitamin E and Se deficiencies (Kennedy *et al.*, 1987; Norton and McCarthy, 1986; Walsh *et al.*, 1993). This disease is characterized by skeletal and cardiac myonecrosis and by an increase in plasma CK activity (Fry *et al.*, 1994; Walsh *et al.*, 1993) and may result in lameness or sudden death (Walsh *et al.*, 1993). Abnormally high serum AST, LDH and CK activities in WMD occur due to the leakage of these predominantly intracellular enzymes through damaged muscle membranes (Imren and Sahal, 1991; Or *et al.*, 2003; Sekin *et al.*, 1996; Underwood and Suttle, 2001). The increase is roughly proportional to the amount of muscle damage (Underwood and Suttle, 2001). Sekin *et al.* (1996) also, clarified that serum CK, AST and LDH activities were significantly higher in lambs with subclinical and clinical WMD compared to healthy lambs (p<0.01). In another study, Or *et al.* (2003) notified that serum ALT, AST, ALP, LDH and CPK parameters were significantly higher in lambs with WMD compared to healthy lambs (p<0.01). Similarly, the present study found that serum CK, LDH, AST, ALT and ALP concentrations in lambs with WMD were significantly higher compared to control lambs (p<0.001, p<0.001, p<0.001, p<0.01 and p<0.05, respectively) prior to treatment (Table 1).

Table 1: Serum calcium and biochemical parameters in healthy lambs and lambs with White Muscle Disease (WMD)

Parameters	Control lambs	Lambs with WMD (0 day; prior to treatment)	Lambs with WMD (30 days after treatment)
Ca (mg dL ⁻¹)	9.32±0.67 ^a	11.03±0.67 ^b	9.64±0.69 ^a
CK (U L ⁻¹)	217.40±55.67 ^a	3012.40±1080.32 ^b	243.00±104.35 ^a
LDH (U L ⁻¹)	846.34±95.13 ^a	1386.70±168.29 ^b	854.50±135.29 ^a
AST (U L ⁻¹)	76.40±11.03 ^a	280.70±91.73 ^b	78.30±29.12 ^a
ALT (U L ⁻¹)	15.80±5.80 ^a	290.40±150.37 ^b	17.20±13.30 ^a
ALP (U L ⁻¹)	755.60±170.30 ^a	1422.20±770.30 ^b	830.10±440.18 ^a

Calcium has many important and essential functions for skeletal muscle performance (Berchtold *et al.*, 2000). All muscles use Ca²⁺ as their main regulatory and signaling molecule and the function of all muscle types is controlled by Ca²⁺ as a second messenger (Gissel, 2005). Any alteration in Ca²⁺ handling can disturb muscular function (Berchtold *et al.*, 2000). Muscle damage may lead to loss of function and widespread damage to muscle may have serious systemic implications due to leakage of intracellular constituents to the circulation (Gissel, 2005). Calcification is a common feature of damaged muscle and may be caused by mitochondrial calcium overload, due to impaired uptake of calcium by vesicles of the sarcoplasmic reticulum.

This, in turn, is due to structural changes and diminished calcium-binding proteins (Underwood and Suttle, 2001). Another study (Glesby *et al.*, 1988) reported that calcium content of skeletal muscle and isolated mitochondria, as well as oxidative phosphorylation of X-linked muscular dystrophic (mdx) mice were compared with normal animals at ages of 5, 10 and 23 weeks. The Ca content of dystrophic skeletal muscle increased at all ages.

Turner *et al.* (1991) have reported that the absence of dystrophin in Duchenne muscular dystrophy and muscular dystrophy in mice results in the more frequent opening of calcium leak channels in the sarcolemma. It is this hypothesis that increased calcium influx via these channels results in the elevated (Ca²⁺) seen in dystrophic muscle. In present study, serum Ca concentration was significantly higher in lambs with WMD compared to healthy lambs. The increased serum calcium levels in lambs with WMD results most likely from muscular dystrophy during the course of the disease. Further studies, should be conducted if serum calcium concentration is a powerful parameter to estimate the severity of muscle degenerations.

CONCLUSION

As a result, serum calcium concentration is elevated in lambs with WMD in concurrence with elevated serum CK, LDH, AST, ALT and ALP concentrations.

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