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POSSIBLE INTERACTION BETWEEN LAMENESS, FERTILITY, SOME MINERALS, AND VITAMIN E IN DAIRY COWS

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Abstract

Fifty-six cows that became lame within 60 d postpartum and 157 healthy cows (controls) were used in the study. The results from the following variables were analysed: calving to the first service interval, calving to conception interval, conception rate at the first service, and overall conception rate. Lameness was associated with a statistically significant 9 d increase in the interval from calving to the first service. In the lame cows, the interval from calving to conception was by 27 d longer ($P<0.05$) than those in the control cows. The conception rate at the first service was 41.4% (control - 54.6%). The serum vitamin E concentration was lower in the cows with lameness than that in the control cows: 2.31±0.24 µg/mL *versus* 2.44±0.12 µg/mL. However, the difference was not statistically significant. The serum P concentration in the lame cows (4.56±0.24 mg/dL) was significantly different from that in the controls (5.35±0.16 mg/dL). The mean Zn concentration was significantly lower one in the cows with lameness (57.81±3.8 µg/dL) compared with the control cows (85.51±6.01 µg/dL). These results suggest that there is a possible interaction between lameness, fertility, and serum P and Zn concentrations in dairy cows.

Key words: cows, lameness, fertility, minerals, vitamin E.

Lameness is one of the most important health problems that cause reproductive performance failure of dairy cows in the world. The incidence of lameness has been steadily increasing over the last years (7), possibly due to the intensification of production, genetic reasons, and changes in management practices. The prevalence of lameness in dairy herds was reported between 2% and 25% (3, 5). Lameness in dairy cattle, more likely occurs during the first 60-90 d of postpartum (9), and causes large economic losses. The economic importance of lameness is reported as attributable to impaired reproductive performance (12, 21), decreased milk production (8), culling from the herd (29), and cost of treatment and control methods (11, 23). The most important etiological factors of lameness are feeding management and nutrition, hygiene, housing systems, deficiency in vitamins and minerals, calving period, and general management, (2, 5, 16, 17).

In modern dairy herds, fertility is of major economic importance. In order to gain maximum profit, calving intervals should not exceed one year. The achievement of a 365-d calving interval requires an early resumption of ovarian activity and a high first service conception rate. Late resumption of ovarian activity postpartum has a detrimental effect on the reproductive performance in dairy cows (18). In addition, other reproductive problems such as cystic ovaries, repeat breeding, anoestrus, or silent heat, which develop in the post-partum period, cause impaired reproductive performance (28, 19).

Vitamins and minerals are necessary for animal biological development and reproduction. Despite their low requirements, severe or even marginal deficiencies of trace elements can cause substantial economical losses in animal productivity. A deficiency of vitamin E at the cellular level is generally accompanied by an increase in lipid peroxidation of cellular membranes. Therefore, cells exposed to oxidative stress (*i.e.* a laminitic insult) will show more rapid injury and necrosis by vitamin E deficiency.

The objective of this study was to investigate the relationship between lameness and impaired fertility (decrease in the conception rate, lengthening of the interval from calving to first service and the interval from calving to conception), and their association with some minerals and vitamin E.

Material and Methods

Animals. The study was carried out at a dairy farm in the Aydin Region of Turkey. Three hundred and eighteen 3–8-year-old Holstein dairy cows were used. Almost all the cows were housed in free-stall barns. The cows were milked twice daily, and were fed a total mixed ration three times a day. The mean lactation yields of the cows ranged from 5 247 kg to 7 015 kg. The average amount of daily feed intake consisted of 8–

10 kg of concentrated feed and 18–22 kg of maize silage per cow. Hay and clover were provided *ad libitum*. After calving, the calves were separated from their mothers and cared for in special compartments. All the cows following parturition were examined for reproductive tract status including retained foetal membranes. The type of lesions was identified in lame cattle and their etiology and treatment options were investigated. It was reported by the resident veterinarians that the reproductive problems in animals such as cystic ovaries, impaired fertility and uterine infections were diagnosed, treated, and recorded consistently. A programme was developed for the treatment of foot diseases and the resident veterinarians were instructed over the telephone when necessary. The reproductive management consisted of a voluntary waiting period of 60 d and oestrus in cows was detected by visual observation. The cows were artificially inseminated at the first observed oestrus following completion of a 60-d voluntary waiting period. Pregnancy was diagnosed by rectum palpation 42–60 d following artificial insemination.

Study design. Cows that displayed lameness within the first 60 d of postpartum, with the lesions localised in the claw, were assigned to the lame cows group (n = 56). The control cows (n = 137), without a history of lameness at least within the first 120 d of lactation, were selected at random as the control group. The cows that did not exhibit oestrus within 60 to 80 d after calving, received progesterone (Crestar®, Intertvet), prostaglandin $F_{2\alpha}$ (Dalmazin®, Vetas). GnRH (Ovarelin® DIF), and prostaglandin $F_{2\alpha}$ (Dalmazin®, Vetas).

Individual reproductive data was recorded on the computerised dairy-management system. The individual cow reproductive data included a lactation number, calving date, the types of lesions in lame cows, date of occurring lameness, conception rate, first service date, last service date, number of services, and reproductive status (not serviced, recently bred, pregnant, and culled after the voluntary waiting period). Calculated reproductive indices included the number of days to the first service (d), conception rate at the first service (%), overall pregnancy rate (%), and calving to conception interval (d).

Blood samples (10 ml) were collected from the jugular vein of each animal. The blood samples were allowed to stand for 2 h at room temperature to allow proper clotting. Then the samples were centrifuged at 5 000 rpm for 10 m and the serum samples were stored at -20°C until analyses.

Reagents and chemicals. The δ-, γ- and α-tocopherols and α-tocopherol acetate were purchased from Sigma (St. Louis, MO, USA). HPLC-grade methanol and acetonitrile were obtained from Riedel-deHaen (Germany). All other reagents and chemicals were of an analytical grade. To prevent the degradation of tocopherols, the experiment was performed in a dark room.

Analytical procedures. The serum concentrations of Ca, P, Zn, Fe, and Cu were determined using the commercially available kits (Spinreact, Santa Coloma, Spain). The analyses were carried out according to the manufacturer's instructions. The serum δ-, γ- and α-tocopherols were analysed using a validated and modified high performance liquid chromatography method (HPLC) of Ruperez *et al.* (27). The analytes were identified with the retention times of pure reference standards. The recoveries of the molecules under study were calculated by internal standard with a comparison of the peak areas resulting from direct injections of standards prepared in acetonitrile. The calibration graphs for the tocopherols were prepared (linear range 0.01-5 μg/mL). The limit of quantification (0.01 μg/mL) of the assay was determined by diluting serum containing endogenous tocopherols. The mean extraction recoveries were 96.4% for γ-tocopherol and 98.29% for α-tocopherol.

The results were presented as mean values with standard errors (±SE). Comparisons of all parameters between the cows with and without lameness were made by unpaired *t* test with Welch's correction. Differences were considered statistically significant when $P<0.05$ was against the control group.

Results

The number of cows with lameness during the first 60 d postpartum was 56 out of 308 animals (18.2%). Among the lame cows; 38/56 (67.8%) were diagnosed with lesions related to the hoof horn quality or subclinical laminitis (white line disease, sole ulcer and sole abscess), 11/56 (19.6 %) showed interdigital dermatitis, and 7/56 (12.5%) had digital dermatitis. Five (8.9%) lame cows were removed from the herd due to untreatable lameness. All the cows participated in a regular reproductive-management programme conducted by veterinarians involving pregnancy diagnosis by rectal palpation in the inseminated cows, 42–75 d after the last recorded insemination and treatment of non-cycling cows. Seven (12.5%) lame cows and 13 (9.5%) non-lame ones (control), were used for a timed insemination protocol, respectively. Therefore, 44 lame cows and 124 non-lame ones were inseminated and were used for the analysis.

The serum levels of vitamin E, Ca, P, Zn, Fe, and Cu in the lame and non-lame cows are given in Table 1 and descriptive data for reproductive records for both groups are shown in Table 2. The serum vitamin E concentration was lower in the cows with lameness than in the control cows: 2.31±0.24 μg/mL *versus* 2.44±0.12 μg/mL. However, the difference was not statistically significant. The serum P concentration in the lame cows (4.56±0.24 mg/dL) was significantly different from that in the control cows (5.35±0.16 mg/dL, $P<0.05$). Mean Zn concentration of the cows with lameness was significantly lower (57.81±3.8 μg/dL) than that of the control cows (85.51±6.01 μg/dL, $P<0.001$). The blood Ca, Fe, and Cu levels were similar in both groups, with no significant differences between the cows with lameness and healthy ones (Table 1).

Table 1
Mean vitamin E, Ca, P, Zn, Fe, and Cu values in lame and non-lame (control) cows

	Vit E (µg/mL)	Ca (mg/dL)	P (mg/dL)	Fe (µg/dL)	Zn (µg/dL)	Cu (µgdL)
Lame (n=44)	2.31 ±0.24	8.19 ±0.78	4.56 ±0.24	221.41 ±42.7	57.81 ±3.8	65.53 ±2.29
Control (n=124)	2.44 ±0.12	9.87 ±0.41	5.35 ±0.16[a]	241.06 ±19.6	85.51 ±6.0[b]	73.54 ±4.38

[a]P<0.05; [b]P<0.001; ± SE.

Table 2
Mean fertility parameters of lame and non-lame cows

	Lame cows (n = 44)	Controls (n = 124)
Days to first service	91.7 ±6.7[a]	82.4 ±5.1[b]
Calving to conception interval (d)	133.8 ±14.3[a]	106.2 ±10.8[b]
Conception rate at first service (%)	41.4[a]	54.6[b]
Overall pregnancy rate (%)	83.9[a]	93.4[b]

Columns with different superscripts (a, b) differ P<0.05; ± SE.

The fertility records of lame cows were compared with those of the non-lame ones. In the lame cows, there was a longer interval from calving to first service (91.7 d *versus* 82.4 d) and a longer interval from calving to conception (133.8 d *versus* 104.3 d) than in non-lame cows (controls) (P<0.05). The conception rate at the first service and the overall conception rate in the lame cows were lower than in controls (41.4% *versus* 54.6% and 83.9% *versus* 93.4%, respectively) (P<0.05).

Discussion

In this study, 67.8% of the lesions in lame cows were related to white line disease, sole ulcer, and sole abscess. These conditions are recognised as horn growth abnormalities, associated with subclinical laminitis, which is considered to be of multifactorial etiology with several risk factors varying from farm to farm (13, 15, 31).

The serum vitamin E concentration was slightly lower in the cows with lameness than in the control cows. Most infectious and metabolic diseases in dairy cows occur in early lactation and is related to physiologic and management changes that occur during the transition period. One critical event is a significant suppression of the immune function in the peripartum period (7, 20). Peripartum immunosuppression is multifactorial but is associated with endocrine changes and decreased intake of critical nutrients (7). In particular, decreased phagocytosis and intracellular killing by neutrophils occurs in parallel with decreased circulating vitamin E (α-tocopherol) concentration (14). Neutrophils take part in the primary mechanism of uterine immune defence (4), as well as playing an important role in mammary defence (20). Vitamin E is a fat-soluble membrane antioxidant that enhances the functional efficiency of neutrophils by protecting them from oxidative damage following intracellular killing of ingested bacteria (10). Several studies (32, 33) have shown that dietary supplementation with 1000 IU of vitamin E/cow per day in the late dry period, mitigates the peripartum drop in circulating α- tocopherol, but this does not necessarily decrease the incidence of disease (1, 34).

Mean Zn concentration in the cows with lameness was significantly lower than in the control cows. It is known that a Zn deficiency can cause parakeratosis, hyperkeratosis, and hoof epidermal deformities. Baggott *et al.* (2) found that the concentration of Zn in the hoof horn of lame cows was lower than in healthy animals. They also noted that the harder keratin of the hoof wall contained a greater Zn concentration than the softer keratin of the heel. As described previously, Zn has an important role in keratinisation. Mulling (24) also indicated that organic Zn may have an important part in the regulation and activation of keratin protein production by the horn tissue. Stern *et al.* (30) investigated the effect of the form of Zn supplementation on clinical symptoms, microscopic coronary horn quality, and traction resistance. The animals receiving Zn organically bound to amino acids and peptides tended towards better clinical scores for microscopic horn quality and traction resistance. Both treatments improved macroscopic clinical evaluation scores. This was thought to be due to better absorption and utilisation of the organic forms of the mineral. Dairy cows fed additional organic Zn for 12-months, exhibited fewer cases of heel horn erosion, foot rot, inter-digital dermatitis and laminitis with a lower incidence of sole ulcers and white line separation (22).

The serum P concentration in the lame cows was significantly different from that in the control cows. The blood Ca, Fe, and Cu levels were similar in both groups. Macro- and trace elements may play an important role in minimising laminitis through their roles in the immune function, the production of horn tissue, and the maintenance of epithelial and connective tissue. Cu may also improve digital integrity and plays an important role in immune function, in the production of horn tissue, and maintenance of the epithelial and connective tissue.

In the presented study, the effect of lameness on reproduction has been examined. The cows with lameness had longer calving to the first service interval and calving to conception interval than control cows. In the lame cows, calving to the first service time was approximately 92 d, compared to 82 d in controls. Lame cows had a longer period of ovarian activity resumption than the non-lame ones. Additionally, a study by Garbarino *et al.* (6) showed that lameness is associated with delayed ovarian activity during the early post-partum period. Several studies have reported longer calving to the first service intervals in lame cows than in control ones (12, 18, 21). However, in those studies calving to the first service interval was between 70 and 80 postpartum days, while in the studies of ours and Melendez *et al.* (21) the interval was approximately 92 and 95 d, respectively. In the present study, calving to conception interval was found to be 133 d (controls 106 d). Lucey *et al.* (18) reported the conception interval as 100 d, while Hernandez *et al.* (12) found it to be 140 d. In our study, lame cows had a lower overall conception rate than controls. This may indicate that the conception rate at the first service was lower for lame cows with more services per conception. The overall decreased fertility in lame cows has been documented in other studies (12, 18, 21). This decreased fertility in lame cows may be related to the pain and the long-term negative effects on the energy balance and loss of body condition caused by lameness, possibly associated with a hormonal insufficiency and nutrition imbalance during the postpartum period (12, 25, 26).

In conclusion, these results suggest that lameness in dairy cows within the first 60 d postpartum is associated with a decreased fertility and serum P, Zn, and vitamin E concentrations.

References

1. Allison R. D., Laven R. A.: Effect of vitamin E supplementation on the health and fertility of dairy cows: a review. Vet Rec 2000, **147**, 703–708.
2. Baggott D.G., Bunch K.J., Gill K.R.: Variations in some inorganic components and physical properties of claw keratin associated with claw disease in the British Friesian cow. Br Vet J 1988, **144**, 534–542.
3. Bergsten C.: Effect of conformation and management system on hoof and leg diseases and lameness in dairy cows. Vet Clin North Am Food Anim Pract 2001, **17,** 1–23.
4. Bondurant R. H.: Inflammation in the bovine female reproductive tract. J Dairy Sci 1999, **82**, 101–110.
5. Espejo L. A., Endres M. I., Salfer J. A.: Prevalence of lameness in high-producing Holstein cows housed in freestall barns in Minnesota. J Dairy Sci 2006, **89**, 3052–3058.
6. Garbarino E.J., Hernandez J.A., Shearer J.K., Risco C.A., Thatcher W.W.: Effect of lameness on ovarian activity in postpartum Holstein cows. J Dairy Sci 2004, **87**, 4123–4131.
7. Goff J.P., Horst R.L.: Physiological changes at parturition and their relationship to metabolic disorders. J Dairy Sci 1997, **80**, 1260–1268.
8. Green L. E., Hedges V. J., Schukken Y. H., Blowey R. W., Packington A. J.: The impact of clinical lameness on the milk yield of dairy cows. J Dairy Sci 2002, **85**, 2250–2256.
9. Gröhn Y.T., Erb H.N., McCulloch C.E., Saloniemi H.S.: Epidemiology of reproductive disorders in dairy cattle: associations among host characteristics. Prev Vet Med 1990, **8**, 25–39.
10. Herdt T. H., Stowe H. D.: Fat-soluble vitamin nutrition for dairy cattle. Vet Clin North Am Food Anim Pract 1991, **7**, 391–415.
11. Hernandez J., Shearer J. K.: Efficacy of oxytetracycline for treatment of papillomatous digital dermatitis lesions on various anatomic locations in dairy cows. J Am Vet Med Assoc 2000, **216**, 1288–1290.
12. Hernandez J., Shearer J. K., Webb D. W.: Effect of lameness on the calving-to-conception interval in dairy cows. J Am Vet Met Assoc 2001, **218**, 1611–1614.
13. Hoblet K.H., Weiss W.: Metabolic hoof horn disease. Vet Clin North Am Food Anim Pract 2001, **17**, 111–127.
14. Hogan J. S., Weiss W. P., Todhunter D. A., Smith K. L., Schoenberger P. S.: Bovine neutrophil responses to parenteral vitamin E. J Dairy Sci 1992, **75**, 399–405.
15. Kilic N.: Hoof lesions of dairy cattle in Turkey. Indian Vet J 2004, **81**, 1053–1054.
16. Livesey C.T., Fleming F.L.: Nutritional influences on laminitis, sole ulcer and bruised sole in Friesian cows. Vet Rec 1984, **114**, 510-512.
17. Livesey C.T., Marsh C., Metcalf J.A., Laven R.A.: Hock injuries in cattle kept in straw yards or cubicles with rubber mats or mattresses. Vet Rec 2002, **150**, 677-679.
18. Lucey S., Rowlands G.J., Russeell A.M.: The association between lameness and fertility in dairy cows. Vet Rec 1986, **118**, 628–631.
19. Lopez-Gaitus F., Santaloria P., Yaniz J., Fenech M., Lopez-Bejar M.: Risk factors for postpartum ovarian cysts and their spontaneous recovery or persistence in lactating dairy cows. Theriogenology 2002, **58**, 1623-1632.
20. Mallard B.A., Dekkers J.C., Ireland M.J., Leslie K.E., Sharif S., Vankampen C.L., Wagter L., Wilkie B.N.: Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. J Dairy Sci 1998, **81**, 585–595.
21. Melendez P., Bartolome J., Archbald L. F., Donovan A.: The association between lameness, ovarian cysts and fertility in lactating dairy cows. Theriogenology 2003, **59**, 927–937.
22. Moore C.L., Walker P.M., Jones M.A., Webb J.M.: Zinc methionine supplementation for dairy cows. J Dairy Sci 1988, **71**, 152.
23. Moore D.A., Berry S.L., Truscott M.L., Koziy V.: Efficacy of a nonantimicrobial cream administered topically for treatment of digital dermatitis in dairy cattle. J Am Vet Med Assoc 2001, **19**, 1435–1438.

24. Mulling C.: In: *The use of nutritional factors in prevention of claw diseases-biotin as an example for nutritional influences on formation and quality of hoof horn*. Mortellaro C.M., De Vecchis L., Brizzi A. (eds). Eleventh International Symposium on Disorders of the Ruminant Digit, Parma, Italy, 2000, pp. 78–80.
25. Pryce J.E., Coffey M.P., Simm G.: The relationship between body condition score and reproductive performance. J Dairy Sci 2001, **84**, 1508–1515.
26. Ruegg P.L., Milton R.L.: Body condition scores of Holstein cows on Prince Edward Island, Canada: relationships with yield, reproductive performance, and disease. J Dairy Sci 1995, **78**, 552-564.
27. Ruperez F.J., Mach M., Barbas C.: Direct liquid chromatography method for retinol, α- and γ-tocopherols in rat plasma. J Chromatogr B 2004, **800**, 225–230.
28. Schopper D., Schemer R., Weiler U., Claus R.: Einfluss der Milchleistung auf Fruchtbarkeitskriterien der Milchkuh post partum. Reprod Domest Anim 1993, **28**, 225-235.
29. Sprecher D.J., Hostetler D.E., Kaneene J.B.: A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology 1997, **47**, 1179–1187.
30. Stern A., Geyer H., Morel I., Kessler J.: Influence of inorganic and organic zinc compounds on claw quality of beef cattle. In: *Tenth International Symposium Lameness and Ruminants*, Lister, C.H.J., Ossent, P. (Eds.), Lucerne, Switzerland, 1998.
31. Van Amstel S.R., Shearer J.K.: Abnormalities of hoof growth and development. Vet Clin North Am Food Anim Pract 2001, **17**, 73–91.
32. Weiss W. P., Todhunter D. A., Hogan J. S., Smith K. L.: Effect of duration of supplementation of selenium and vitamin E on periparturient dairy cows. J Dairy Sci 1990, **73**, 3187–3194.
33. Weiss W.P., Hogan J.S., Smith K.L., Todhunter D.A., Williams S.N.: Effect of supplementing periparturient cows with vitamin E on distribution of α-tocopherol in blood. J Dairy Sci 1992, **75**, 3479–3485.
34. Yildiz H., Kaygusuzoglu E., Kizil Ö.: Concentrations of serum vitamins A, E, and C and β-carotene during pregnancy in cows. Bull Vet Inst Pulawy 2005, **49**, 199-202.