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Hypocalcemia in Dairy Cows: Meta-analysis and Dietary Cation Anion Difference Theory Revisited

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ABSTRACT

Data from 137 published trials involving 2,545 calvings were analyzed using random effects normal logistic regression models to identify risk factors for clinical hypocalcemia in dairy cows. The aim of the study was to examine which form, if any, of the dietary cation anion difference (DCAD) equation provided the best estimate of milk fever risk and to clarify roles of calcium, magnesium, and phosphorus concentrations of prepartum diets in the pathogenesis of milk fever. Two statistically equivalent and biologically plausible models were developed that predict incidence of milk fever. These models were validated using data from 37 trials excluded from the original data used to generate the models; missing variables were replaced with mean values from the analyzed data. The preferred models differed slightly; Model 1 included prepartum DCAD, and Model 2 included prepartum dietary concentrations of potassium and sulfur alone, but not sodium and chloride. Other factors, included in both models were prepartum dietary concentrations of calcium, magnesium, phosphorus; days exposed to the prepartum diet; and breed. Jersey cows were at 2.25 times higher risk of milk fever than Holstein cows in Model 1. The results support the DCAD theory of greater risk of milk fever with higher prepartum dietary DCAD (odds ratio = 1.015). The only DCAD equation supported in statistical analyses was $(Na^+ + K^+) - (Cl^- + S^{2-})$. This finding highlights the difference between developing equations to predict DCAD and those to predict milk fever. The results support a hypothesis of a quadratic role for Ca in the pathogenesis of milk fever (model 1, odds ratio = 0.131; Model 2, odds ratio = 0.115). Milk fever risk was highest with a prepartum dietary concentration of 1.35% calcium. Increasing prepartum dietary magnesium concentrations had the largest effect on decreasing incidence of milk fever in both Model 1 (odds ratio = 0.006) and Model 2 (odds ratio = 0.001). Increasing dietary phosphorus concentrations prepartum increased the risk of milk fever (Model 1, odds ratio = 6.376; Model 2, odds ratio = 9.872). The models presented provide the basis for the formulation of diets to reduce the risk of milk fever and strongly support the need to evaluate macro mineral nutrition apart from DCAD of the diet.

Key words: milk fever, meta-analysis, dairy cow, dietary cation-anion difference

INTRODUCTION

Metabolic factors that influence the risk of hypocalcemia of the dairy cow remain a matter of debate. Recently, there have been several comprehensive reviews of hypocalcemia in dairy cows (Horst et al., 1997; Goff, 2000; Oetzel, 2000; McNeill et al., 2002; Thilsing-Hansen et al., 2002; Lean et al., 2003). Areas of contention identified from these reviews include the most appropriate equation for use in predicting DCAD of the prepartum diet and the roles of dietary magnesium, phosphorus and calcium concentrations in the pathogenesis of hypocalcemia.

Meta-analysis, the quantitative analysis of previous studies, provides opportunities to investigate previously proposed hypotheses and to develop new hypotheses from large databases. In the study of disorders of relatively low incidence, such as milk fever, the availability of large numbers of observations provided by meta-analysis is useful for evaluating the effect on disease of factors that have physiologic effect. One such factor is DCAD. The DCAD equation cited by Ender et al. (1962) and used by Block (1984) {DCAD = $(Na^+ + K^+) - (Cl^- + S^{2-})$ [Equation 1]} is the most commonly used form of the equation. Horst et al. (1997) recommended that other anions and cations be included in the equation and proposed DCAD = $(0.38\ Ca^{2+} + 0.3\ Mg^{2+} + Na^+ + K^+) - (Cl^- + S^{2-})$ [Equation 2]. Goff (2000) proposed a variation of this equation based on the capacity of different salts to acidify urine and recommended DCAD = $(0.15\ Ca^{2+} + 0.15\ Mg^{2+} + Na^+ + K^+) - (Cl^- + 0.25\ S^{2-} + 0.5\ P^{3-})$ [Equation 3]. Following research of Spears et al. (1985), who estimated that the

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absorption of sulfur from the gastrointestinal tract was 60% of dietary intake, Tucker et al. (1991) suggested that DCAD = $(0.38\ Ca^{2+} + 0.3\ Mg^{2+} + Na^{+} + K^{+}) - (Cl^{-} + 0.6\ S^{2-} + 0.5\ P^{3-})$ [Equation 4]. One goal of the study was to determine, which, if any of these equations most accurately predicted risk of milk fever.

The optimal dietary concentration of calcium in prepartum diets is also contentious. Goff (2000) concluded that calcium concentration in prepartum diets had little influence on the incidence of milk fever when fed at levels above the daily requirements, approximately 30 g of calcium/d. Oetzel (2000) and Thilsing-Hansen et al. (2002) noted that the practice of feeding very low levels of calcium prepartum, <20 g/d, is effective in controlling hypocalcemia. However, Oetzel (2000) recommended a daily intake of 150 g of calcium/d in the prepartum diet, a calcium concentration of between 1.1 and 1.5% of DM, in conjunction with a dietary DCAD (based on Equation 1) of approximately −15 mEq/100 g of DM. However, this recommendation was not supported by his meta-analysis, which showed that the highest milk fever risk occurred with a dietary calcium concentration of 1.16% (Oetzel, 1991). Lean et al. (2003) suggested a prepartum intake of 60 g of calcium/d based on the seminal studies of Boda and Cole (1954) and Ramberg et al. (1976, 1984).

To investigate and attempt to clarify these areas of contention, data from the meta-analysis of Oetzel (1991) were updated with relevant, subsequent trials and reanalyzed. Oetzel (1991) concluded that his results substantiated the DCAD theory of milk fever prevention and concluded that the role of calcium in milk fever was nonlinear; extremely low and extremely high concentrations reduced the relative risk of milk fever. It was concluded that sulfate and sodium were the dietary electrolytes most closely linked with milk fever and were more important than DCAD or calcium concentration. Enevoldsen (1993) expressed concern at the methods used by Oetzel (1991), including the sparseness of data in regard to the number of covariate patterns, and reanalyzed Oetzel's data. Enevoldsen (1993) similarly found that low dietary sulfur increased the risk of hypocalcemia, but disputed other conclusions of Oetzel (1991). In particular, calcium entered final models developed by Enevoldsen (1993) as a linear effect. Both Enevoldsen (1993) and Oetzel (1991) used fixed effects models for predicting outcomes, and it is now widely accepted that these models are vulnerable to overdispersion associated with clustering of effects in trials. Random effects models, for example logistic normal regression, are preferred for such analyses (Dohoo et al., 2003).

The aim of this study was to develop random effect, predictive models to test hypotheses arising from recent reviews using the much larger number of studies and improved statistical methods available since the meta-analyses of Oetzel (1991) and Enevlodsen (1993).

MATERIALS AND METHODS

The data set for this meta-analysis was developed using the original data set from Oetzel (1991) as published. Studies published since 1990 were added, as well as any studies originally excluded by Oetzel (1991), on the basis that cows were exposed to the transition diet for <14 d. Relevant papers were identified by computerized literature searches (CAB Abstracts, BA Abstracts, PubMed, Agricola, Medline, Biological Abstracts, Biosis Previews, Australian Bibliography of Agriculture and ScienceDirect), library searches of relevant journals, and a systematic review of citations in identified review papers. Contact was made with some workers in the field to identify studies that might have been missed through the literature review process.

In all, 48 English-language, published papers were reviewed. Of the papers that were assessed, only those that satisfied specific predetermined criteria were included in the analyses. These criteria included evidence 1) that the trial was randomized, 2) that the populations studied were pregnant dairy cows that calved during the trial period, and 3) that enough detail on dietary composition was provided to allow calculation of the DCAD of the transition diet without use of book values for mineral composition of feeds. Trials were ineligible for inclusion in the analysis 1) if there was insufficient evidence of randomization, 2) if the trial was confounded with supplementary treatments such as bovine somatotropin, 3) if the paper provided insufficient data on the variables being measured, or 4) if hypocalcemia was induced in the study animals by use of either feed restriction or calcium binding agents (such as NaEDTA given intravenously in cows or zeolites in feed). Length of exposure to the prepartum transition diet was determined for each trial that reported this variable. Breed classification was recorded, and lactation number was calculated using methods described by Oetzel (1991). The 4 variations of the DCAD equation detailed previously were calculated where possible in units of mEq/100 g of DM and were tested in statistical models developed. The final data set was derived from 35 papers detailing 137 individual trials involving 2,545 observed calvings. Details of the trials investigated are presented in Appendix A.

This data set contained a large number of variables that were not consistently reported in each study. There were, however, essentially 3 different subsets of data that could be evaluated. These contained information on dietary concentrations of calcium, magnesium, phos-