



J. Dairy Sci. 99:1–12

<http://dx.doi.org/10.3168/jds.2015-9664>

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Dietary cation-anion difference and day length have an effect on milk calcium content and bone accretion of dairy cows

A. Boudon,*†¹ M. Johan,*† A. Narcy,‡ M. Boutinaud,*† P. Lamberton,*† and C. Hurtaud*†

*INRA, UMR 1348 PEGASE (Physiologie Environnement et Génétique pour l'Animal et le Système d'Elevage), F-35590 Saint-Gilles, France

†Agrocampus Ouest, UMR 1348 PEGASE, F-35000 Rennes, France

‡INRA, UR83 Recherches Avicoles, F-37380, Nouzilly, France

ABSTRACT

Milk and dairy products are an important source of Ca for humans. Recent studies have shown fluctuations in cow milk Ca content during the year in France, with high values in winter and with corn silage diets, and a decrease during May and June and with grass diets. The aim of this study was to identify the reasons for this seasonal decrease in milk Ca content by testing the effect of 2 levels of dietary cation-anion differences (DCAD; 0 mEq/kg of dry matter for DCAD 0 and 400 mEq/kg for DCAD 400) and 2 day lengths (8 h of light/d for short days: SD; and 16 h/d for long days: LD) on the Ca balances of dairy cows. The DCAD treatments were designed to mimic diets based either on corn silage or on herbage. The cows were only illuminated by solarium lights providing UVA and UVB. The trial was conducted according to 2 simultaneous replicates of a 4 × 4 Latin square design using 8 dairy cows averaging 103 ± 44 d in milk with 4 periods of 14 d. Data were analyzed by ANOVA with a model including treatment, cow, and period effects. No significant interaction was found between day length and DCAD treatments. With DCAD 400 compared with DCAD 0, blood pH increased and plasma ionized Ca content decreased, whereas the plasma total Ca content did not differ between treatments. Milk Ca content, however, increased with DCAD 400 compared with DCAD 0, in relation to a decrease in the amount of Ca excreted in urine. The DCAD had no significant effect on protein and casein contents and DCAD 400 tended to decrease milk yield. This illustrates that the udder did not decrease Ca uptake from the blood at high DCAD even though DCAD 400 decreased the mammary availability of Ca by decreasing the proportion of blood ionized Ca. Milk Ca and casein contents were significantly lower

with LD compared with SD, whereas day length had no effect on milk yield after 14 d of treatment. Bone accretion of cows increased when the Ca content of milk increased (i.e., with DCAD 400 compared with DCAD 0 and with SD compared with LD). This work suggests that long and sunny days could explain part of the seasonal decrease in milk Ca content in summer and refutes the hypothesis that low milk Ca contents at grazing could be due to the high DCAD of herbage. **Key words:** dairy cow, milk composition, mineral metabolism, calcium

INTRODUCTION

Milk and dairy products in westernized diets represent an optimum source of Ca and of other limiting nutrients such as K and Mg involved in bone health (Caroli et al., 2011). In particular, Ca intake positively affects bone mass, ensuring adequate bone development in childhood and youth (Caroli et al., 2011). Milk Ca is also an important determinant of milk coagulation and cheese-making abilities (Malacarne et al., 2014). Recent studies in France have shown a fluctuation in cow milk total Ca content during the year with a decrease of 100 to 200 mg/kg during May and June and with grass diets compared with diets based on corn silage (Hurtaud et al., 2011, 2014). In these studies, milk Ca contents could be low enough to impair rennet coagulation ability of milk (Malacarne et al., 2014). It has been established that a genetic variation of milk Ca content exists (van Hulzen et al., 2009) and that milk Ca content is the lowest in mid-lactation (Guéguen and Journet, 1961; van Hulzen et al., 2009). The results obtained by Hurtaud et al. (2011, 2014) suggest that the decrease in milk Ca content during spring could also be related to other environmental factors such as cow diet and day length. After May, cow diets are based on grazing rather than corn silage in most French dairy systems and day length is perceptibly higher with an increased probability of sunny days compared with the preceding months. A question is if and how these

Received April 2, 2015.

Accepted October 23, 2015.

¹Corresponding author: anne.boudon@rennes.inra.fr

changes in environmental conditions could be related to the spring decrease in milk Ca content.

Herbage is a highly cationic forage compared with corn silage, leading to high DCAD when diet is based on this forage (Roche et al., 2000; Apper-Bossard et al., 2006). It cannot be excluded that the high DCAD of herbage-based diet is responsible for the low milk Ca content on these diets. Plasma Ca can be either ionized, which is the biologically active form, or protein-bound. It has been shown that higher DCAD are associated with a lower proportion of plasma ionized Ca (Wang and Beede, 1992; Charbonneau et al., 2006), representing the form preferentially absorbed by the mammary gland. According to Moore (1970), high DCAD can increase blood pH, leading to a higher rate of Ca binding to serum proteins, likely due to a competition between H^+ and ionized Ca for binding sites. To our knowledge, the direct effect of DCAD on milk Ca contents has never been observed.

The data of Hurtaud et al. (2011, 2014) also suggested a strong correlation between day length and milk Ca content for a given breed, but this effect could not be fully dissociated from lactation stage. It can be hypothesized that day length could affect Ca metabolism either by UV-stimulated vitamin D synthesis at the skin level or by a photoperiodic effect in relation to melatonin secretion by the pineal gland (Dahl et al., 2000; Hymøller and Jensen, 2010). 1,25OH vitamin D is known to increase Ca intestinal absorption and to favor, with parathyroid hormone, bone resorption and Ca renal tubular reabsorption (Horst et al., 1994). Vitamin D may also play a role in initiating bone remodeling and modeling systems required for repairing bone (Jones et al., 1998; van Driel et al., 2006). However, the link between these effects and the amount of Ca available for milk synthesis remains to be elucidated. On the other hand, melatonin has been shown to be involved in bone turnover, increasing bone accretion in rodents (Ladizesky et al., 2006) and in sheep (Egermann et al., 2011). Lower circulating melatonin levels and long days have also been shown to increase milk yield in dairy cows (Dahl and Petitclerc, 2003). To our knowledge, however, the effect of melatonin and photoperiod on body flows of Ca and Ca balance and milk Ca content in cows remains to be characterized.

Our experiment aimed to explain the declining milk Ca content observed during spring. Our hypotheses were that (1) grazing could be a reason for this decrease because high DCAD of herbage could lead to a decrease in ionized Ca plasma content when cows are grazing, and (2) long days could also interfere with cow Ca metabolism. The aim of this study was thus to test the effect of 2 levels of DCAD calculated to simulate diets based either on corn silage or on herbage, and 2

day lengths, simulated with solarium lights providing UVA and UVB, on the Ca balances of dairy cows.

MATERIALS AND METHODS

Animals and Experimental Design

Four treatments, consisting of a factorial arrangement of 2 dietary cation-anion differences (0 mEq/kg of DM for DCAD 0 and 400 mEq/kg of DM for DCAD 400) and 2 day lengths (short days, **SD**, day length of 8 h vs. long days, **LD**, day length of 16 h/d) were compared on 8 lactating multiparous Holstein cows (103 ± 44 DIM; 31.5 ± 5.7 kg/d milk yield; 677 ± 57.6 kg of BW) over 4 periods of 14 d according to 2 simultaneous replicates of a 4×4 Latin square design. The DCAD was calculated as the difference between the dietary contents of cations (i.e., Na and K), and anions (i.e., Cl and S), expressed in milliequivalents per kilogram of DM. The 8 cows were kept in 2 chambers, one for each day length, and moved from one period to another to the treatment defined by the Latin square. The succession of treatments in the Latin square was balanced for the carryover effects. In each chamber, all the windows were covered with wood panels during the whole experiment, and special care was taken to switch off the corridor light when opening the doors. Animal feeding, milking, and care were performed under low-intensity red lights. During lightened periods of the day, light was only provided by reflectors containing bulbs with a sun-like radiation spectrum containing UVA and UVB (Ultra-Vitalux, Osram GmbH, München, Germany). For each cow, 4 bulbs were aligned from the head to the tail at a distance of 15 cm from the skin, the aim being to simulate an amount of radiation of 200 W/m^2 . This system was tested before the experiment to ascertain that the cows would not develop any skin burns. Cows were illuminated between 0700 and 1500 h for SD and between 0500 and 2100 h for LD. In each chamber, ambient temperature was maintained constant throughout the day at a target temperature of 17°C with a thermostatic convective heating system. In the chambers, cows were housed in individual tie stalls (1.4×2.0 m) with individual troughs and individual water bowls with free access to water. The four 14-d periods consisted of 9 d of adaptation to treatments and 5 d of measurements. The experiment was conducted at the INRA experimental farm of Mejustesse (longitude -1.71° , latitude $+48.11$, Brittany, France) from February 6, 2012, to April 6, 2012. Procedures relating to care and use of animals for the experiment were approved by an animal care committee of the French Ministry of Agriculture, in accordance with French regulations (decree-law 2001-464, May 29, 2001).

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