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The effect of transient, moderate dietary phosphorus deprivation on phosphorus metabolism, muscle content of different phosphorus-containing compounds, and muscle function in dairy cows

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ABSTRACT

Hypophosphatemia is a common finding in periparturient and anorectic cattle. Although the clinical relevance of hypophosphatemia in cattle is uncertain, it has been empirically associated with persistent recumbency, specifically in periparturient dairy cows. The objective of the present study was to determine if transient dietary phosphorus (P) deprivation over a course of 5 wk, by feeding an approximately 40% P-deficient ration to lactating dairy cows, would result in altered muscle function or muscle P metabolism severe enough to present a risk for animal health and well-being. In addition, we wanted to determine the association between the plasma phosphate concentration ([Pi]) and muscle tissue P content to assess to what extent intracellular P deprivation of muscle cells could be extrapolated from subnormal plasma [Pi]. Ten healthy multiparous, mid-lactating dairy cows received a ration with a P content of 0.18% over a period of 5 wk. Following the P-deprivation phase, the same ration supplemented with P to obtain a dietary P content of 0.43% was fed for 2 wk. Blood and urine samples were collected regularly and muscle biopsies were obtained repeatedly to determine the P content in muscle tissue. Function of skeletal and heart muscles was evaluated by electrocardiography and electromyography conducted repeatedly throughout the study. Feeding the P-deficient ration resulted in the rapid development of marked hypophosphatemia. The lowest plasma [Pi] were measured after 9 d of P depletion and were, on average, 60% below predepletion values. Plasma [Pi] increased thereafter, despite ongoing dietary P depletion. None of the animals developed clinical signs commonly associated with hypophosphatemia or any other health issues. Urine analysis revealed increasing renal calcium, pyridinoline, and hydroxypyridinoline excretion with ongoing P deprivation. Biochemical muscle tissue analysis showed that dietary P depletion and hypophosphatemia were not associated with a decline in muscle tissue P content. Electromyographic examination revealed increased occurrence of pathological spontaneous activity in striated muscles after 2 wk of dietary P depletion in several cows, which could be suggestive of neuromuscular membrane instability. No effect on heart muscle activity was identified electrocardiographically. These results suggest that counter-regulatory mechanisms were sufficient to maintain normal muscle tissue P content during transient and moderate P deprivation. Muscle function was not grossly affected, although the increased occurrence of pathological spontaneous activity suggests that subclinical neuropathy or myopathy, or both, may have occurred with ongoing P deprivation. The results presented here indicate that plasma [Pi] is unsuitable for assessing muscle tissue P content in cattle.

Key words: phosphorus, muscle, hypophosphatemia, dairy cattle, electromyography

INTRODUCTION

Hypophosphatemia is a common finding in the periparturient dairy cow and it has been associated empirically with diseases of fresh dairy cows such as postparturient hemoglobinuria and periparturient recumbency (Goff, 2004; Grünberg, 2014). The clinical relevance of hypophosphatemia in recumbent animals is contentious but an undisputed empirical observation is that hypophosphatemia is more common or more pronounced in recumbent periparturient cows that are unresponsive to parenteral calcium administration (Goff, 2000; Metzner and Klee, 2005; Ménard and Thompson, 2007; Grünberg, 2014). Pronounced hypophosphatemia has been convincingly associated with impaired muscle function of striated and cardiac muscle in several experimental and clinical studies conducted in humans and differ-

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ent animal species not including cattle, but the precise mechanism through which phosphorus depletion or hypophosphatemia may impair muscle function is not well understood (Lotz et al., 1968; Fuller et al., 1976; Montanari et al., 1984; Subramanian and Khardori, 2000; Amanzadeh and Reilly, 2006; Grünberg, 2014).

In recent years, concerns have been voiced within the dairy industry because of incentives to reduce the P content in manure of ruminants to reduce pollution of soils and surface waters with P. These incentives are believed to reduce the dietary P supply to dairy cattle and thereby present a risk for health and wellbeing, particularly in the periparturient period. Numerous studies have confirmed that current estimates of daily dietary P requirements of dairy cattle are adequate and do not jeopardize long-term health or productivity (Call et al., 1987; Valk and Sebek, 1999; Wu et al., 2000, 2001). Nevertheless, the transition from the dry phase to lactation presents several challenges for mechanisms regulating P homeostasis in cattle. These include the onset of lactation with rapidly increasing milk production associated with a rapid increase in P requirements for milk production, as well as transient feed intake depression during the periparturient period and an obviously altered equilibrium of the P distribution between intra- and extracellular space (Grünberg, 2014). Several field studies confirmed that hypophosphatemia is common not only in recumbent cattle but also in clinically healthy dairy cows (Metzner and Klee, 2005; Macrae et al., 2006; Ménard and Thompson, 2007).

Although the deleterious effect of severe hypophosphatemia and P depletion on muscle function is undisputed, the question remains whether hypophosphatemia or P depletion plays a role in the disease complex of the recumbent periparturient dairy cow. It is also unclear whether moderate and transient dietary P deprivation—as might occur in early lactating dairy cows fed a possibly P-deficient diet that is based on ration ingredients commonly used in Europe or North America—can become severe enough to result in clinically apparent disturbed muscle function. Indeed, little is known about the degree of P depletion or hypophosphatemia that is required to obtain disturbed muscle function in cattle. Another point of interest is the association between plasma inorganic phosphate concentration ([**Pi**]) and the P content in muscle tissue in cattle, because muscle tissue P depletion is often assumed based on an extrapolation from hypophosphatemia. Therefore, the objectives of the present study were to determine how far dietary P deprivation in dairy cows (achieved by feeding a P-deficient but otherwise balanced diet composed of common ration ingredients) alters the P content of muscle tissue or muscle function. We hypothesized that dietary P deprivation over a course of 5 wk would result in pronounced hypophosphatemia associated with a reduction of muscle tissue P content, which in turn may impair normal muscle function. We furthermore wanted to determine the association between plasma [Pi] and muscle tissue P content to assess to what extent intracellular P deprivation of muscle cells could be extrapolated from subnormal plasma [Pi].

MATERIALS AND METHODS

The national and institutional guidelines for the care and use of experimental animals were followed and all experimental procedures were approved by the Utrecht University Institutional Animal Care and Use Committee (DEC, permit no 2013.iii.03.033).

Animals and Housing

Ten healthy, lactating, nonpregnant Holstein-Friesian cows were used for this study. The required sample size for this study was calculated based on a crude but conservative estimate of the expected treatment effect on muscle tissue P content. The estimated effect was extrapolated from P-deprivation studies conducted in other species (Fuller et al., 1976). Cows were between 5 and 9 yr old (6.2 ± 1.3 yr, mean ± SD) and between 100 and 200 d in lactation. The mean BW was 615 ± 54 kg and the mean 305-d milk yield of the previous lactation was 9,920 ± 1,360 kg. All cows were healthy based on physical examination and hematological and blood biochemical examination. Cows were housed in individual tiestalls with rubber bedding, covered with sawdust, in a temperature-controlled facility.

Cows past peak lactation were selected for the present study in order to study the specific effect of P deprivation in lactating cows while avoiding the confounding effect of common metabolic alterations commonly occurring in the periparturient period and early lactation such as hypocalcemia, hypokalemia, or negative energy balance.

Study Design and Experimental Rations

After an acclimatization period of 2 wk (d 1 to 14), animals on study underwent dietary P deprivation for 5 wk (d 15 to 49). The P-deprivation phase was followed by a 2-wk period (d 50 to 65) during which P was supplemented in excess of requirements (repletion period). The cows received the same base ration offered as TMR throughout the entire study period. This ration was based on corn silage, grass seed straw, and beet pulp and was formulated to meet the current dietary recommendations for lactating cattle (Table 1), except for the P content (NRC, 2001). During the acclimatization

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