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Influence of feeding a low-phosphorus diet on leucocyte function in dairy cows

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ABSTRACT

Phosphorus depletion and hypophosphatemia have been described to interfere with immune function in rats and humans. In dairy cows, hypophosphatemia has been associated with muscle weakness and recumbency as well as with intravascular hemolysis resulting from increased osmotic fragility of erythrocytes, but so far, the influence of P depletion and hypophosphatemia on immune function has not been studied. Therefore, the aim of this study was to investigate whether P depletion and ensuing hypophosphatemia are associated with impaired granulocyte and lymphocyte function. Eight mid-lactation dairy cows were fed a P-deficient ration (0.2% P/kg of DM) for a period of 4 wk. The depletion phase was preceded by a 2-wk acclimatization period and followed by a 2-wk repletion phase, during which the same ration was supplemented with P to meet or exceed daily requirements. Blood samples were collected at the end of the acclimatization period, after 2 and 4 wk of P depletion, and at the end of the repletion phase. Plasma phosphate concentrations ([Pi]) were determined and white blood cells were counted and isolated. General immune function was investigated by performing a phagocytosis assay with *Staphylococcus aureus* and a lymphocyte stimulation test (LST) with concanavalin A and pokeweed mitogen. The plasma [Pi] decreased significantly, with the lowest values (mean 0.7 ± 0.2 mmol/L) occurring after 2 wk of depletion, although depletion was continued for another 2 wk. During repletion, plasma [Pi] increased above baseline concentrations. Granulocyte counts changed in parallel with plasma [Pi] over time, decreasing significantly at 2 wk after P depletion and increasing again thereafter. Granulocyte survival after phagocytosis was lowest after 4 wk of P depletion. Phagocytosis activity of surviving granulocytes determined by mean fluorescence intensity was higher, indicating that phagocytosis was not negatively influenced by P depletion. Lymphocyte stimulation showed a similar trend, with a decreasing stimulation index at the end of P depletion, but differences were not statistically significant. Data presented in this study indicate that hypophosphatemia leads to a decrease in granulocyte counts. Chronic P depletion impairs granulocyte survival during phagocytosis but not phagocytosis activity. Lymphocyte function is not influenced by P depletion.
Key words: dairy cow, phosphorus depletion, hypophosphatemia, leucocyte function

INTRODUCTION

Phosphorus in dairy cows has received increased attention over the past decades because of environmental concerns with excessive fecal P excretion and also because of empirical associations that were made between P depletion and clinical signs such as muscle weakness and recumbency or postparturient hemoglobinuria, a condition associated with severe intravascular hemolysis (Gerloff and Swensen, 1996; Stockdale et al., 2005). Legal regulations limiting the P content in soil have led to incentives to reduce the dietary P content of ruminant feed to reduce the P content in manure. Current estimates for dietary P requirements for dairy cows have been shown to be adequate, although the adequacy of currently used P requirements has been studied mainly in the light of reproductive performance, milk production, and bone stability (Valk and Šebek, 1999; Wu and Satter, 2000; Wu et al., 2000; Valk et al., 2002). Notwithstanding, concerns have been raised for periparturient dairy cows, where P requirements for milk production increase with continuously increasing milk yields, whereas the dietary P content is restricted and feed intake is at its nadir around parturition. Hypophosphatemia, which is widely used as indicator for P depletion, was observed in over 10% of dairy cows in early lactation in one study (Macrae et al., 2006). Although the clinical relevance of subnormal serum phosphate concentrations ([**Pi**]) is currently poorly understood, hypophosphatemia has been incriminated

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as a potential cause for the downer cow syndrome and postparturient hemoglobinuria (Gerloff and Swensen, 1996; Stockdale et al., 2005).

In other species, P status has been shown to influence cellular immune function (Craddock et al., 1974; Fuller et al., 1976; Brautbar et al., 1982; Kiersztejn et al., 1992; Kegley et al., 2001). In piglets, a high-P diet led to an increase of blastogenic responses of lymphocytes, whereas a low-P diet in rats, dogs, and humans impaired phagocytosis of PMNL (Craddock et al., 1974; Kegley et al., 2001). A reduction in ATP concentration and an increase in intracellular Ca concentration have been proposed to be the cause of this phenomenon (Kiersztejn et al., 1992).

Impaired immune function around parturition has been described in dairy cattle (Kehrli and Goff, 1989), presumably caused by hormonal and metabolic changes, including Ca depletion (Dosogne et al., 1999; Hammon et al., 2006; Martinez et al., 2014). No influence of P on immune function in lactating dairy cows was detected when feeding diets according to NRC (2001) guidelines or above the P requirements (Mullarky et al., 2009). However, feeding cows below currently recommended P requirements has, to our knowledge, not yet been studied. Therefore, the objective of the study presented here was to explore the effect of marked dietary P depletion over a course of 4 wk in lactating dairy cows by a general assessment of PMNL and lymphocyte function determined by a phagocytosis and lymphoproliferation assay, respectively. We hypothesized that chronic P depletion interferes with the cellular immune function in dairy cows.

MATERIALS AND METHODS

Experimental Design

All procedures and treatments were approved by the Ethical Committee for Animal Experiments of Utrecht University (Utrecht, the Netherlands) and performed according to their regulations.

Eight Holstein Friesian dairy cows (parity ≥3) in mid lactation were enrolled in this experiment. Cows were housed in tie-stalls on rubber mats covered with sawdust in a temperature-controlled building. Cows were clinically healthy based on physical, blood biochemical, and hematological examination. The study consisted of a 2-wk acclimatization, a 4-wk P depletion, and a 2-wk P repletion period (Figure 1). Cows were fed the same base TMR, based on corn silage, grass seed straw, and beet pulp for the entire study period. This base TMR was formulated to meet the dietary requirements for lactating dairy cows, with the exception of the dietary P content (NRC, 2001). The unsupplemented ration containing 0.2% P/kg of DM, was fed during the entire P-depletion phase, but was supplemented with NaH_2PO_4 to obtain a dietary P content of 0.36% P/kg of DM during the acclimatization period and 0.45% P/kg of DM during the repletion period. Cows were fed ad libitum at 0700 and 1900 h and had free access to water at all times. Cows were milked twice per day before feeding. The health status of the cows was monitored on a daily basis.

Blood Sampling

Blood samples were collected at the end of the acclimatization period, 2 and 4 wk after the onset of P depletion as well as at the end of the repletion period (Figure 1). Blood was collected by venipuncture of a jugular vein using a 20-G Vacuette needle (Greiner Bio-One GmbH, Kremsmünster, Austria) and EDTA, Li-heparin, and serum tubes (Greiner Bio-One GmbH). Blood with EDTA was collected for leucocyte isolation and for determination of the leucocyte count and heparinized blood was required for the determination of the plasma [Pi]. Serum samples were only collected at the first sampling time. Serum samples were pooled

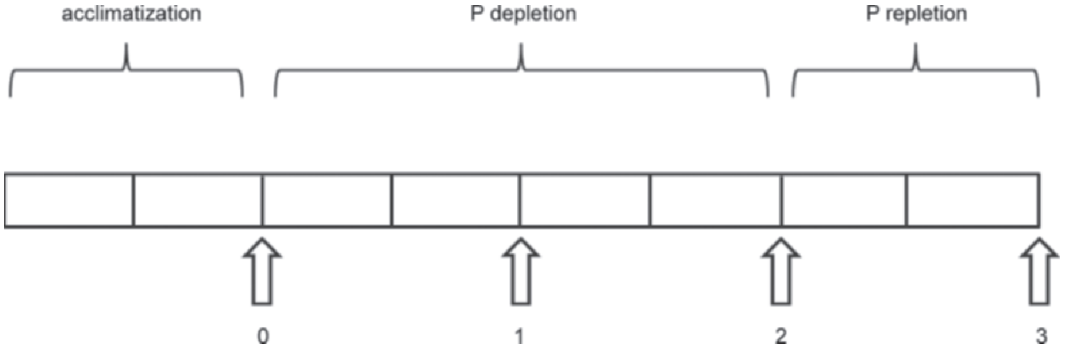

Figure 1. Timeline of the study. Each box represents 1 wk. Arrows indicate the time points of blood collection.