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PERIPARTURIENT RESPONSES OF MULTIPAROUS HOLSTEIN COWS TO VARYING PREPARTUM DIETARY PHOSPHORUS CONCENTRATIONS

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PERIPARTURIENT RESPONSES OF MULTIPAROUS HOLSTEIN COWS TO VARYING PREPARTUM DIETARY PHOSPHORUS CONCENTRATIONS

By

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ABSTRACT

PERIPARTURIENT RESPONSES OF MULTIPAROUS HOLSTEIN COWS TO VARYING PREPARTUM DIETARY PHOSPHORUS CONCENTRATIONS

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Diets containing 0.21, 0.31, and 0.44% P, dry basis, were fed for 28 d before parturition to 15, 13, and 14 multiparous Holstein cows, respectively. Cows fed 0.21% P had lower prepartum serum P concentrations than cows fed 0.31 or 0.44% P. Neither total serum Ca nor plasma ionized Ca was altered by prepartum dietary P concentrations. Cows fed 0.21% P tended to have lower prepartum osteocalcin concentrations than cows fed 0.31 or 0.44% P, suggesting less bone formation. Neither pre- nor postpartum serum deoxypyridinoline concentrations were influenced by dietary treatment, suggesting no effect on bone resorption around parturition. Energy-corrected milk yield during the first 28 d of lactation was not affected by prepartum dietary P treatments. We conclude that a prepartum dietary P concentration of 0.44% was too high because it resulted in lower total serum Ca concentrations around parturition compared with 0.21 and 0.31% P. Cows fed 0.21% P consumed slightly above NRC 2001 requirements at 34 g of P/d. These cows fed 0.21% P prepartum had higher total serum Ca and plasma ionized Ca concentrations around parturition compared with 0.31 and 0.44% P. Energy-corrected milk yield during the first 28 d of lactation was not altered. We conclude that consuming 34 g of P/d (0.21% P in this experiment) was adequate to meet the needs of the periparturient multiparous Holstein cow.

I dedicate this thesis to my Mom, Carolyn Peterson and my Sister, Leah Peterson. They have both given me the confidence to stand up tall, the endurance to keep on trying and enough love to fill an ocean. They have kept me smiling when things got difficult and kept me on the right path when I tended to falter.

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LIST OF ABBREVIATIONS

AC	absorption coefficient
ADF	acid detergent fiber
AIC	Akaike's Information Criterion
BCS	body condition score
BEB	base excess
BW	body weight
CP	crude protein
DM	dry matter
DMI	dry matter intake
DPD	deoxypyridinoline
ECD	expected calving date
ECM	energy-corrected milk
HYP	hydroxyproline
iCa	ionized Ca
NDF	neutral detergent fiber
NE_L	net energy of lactation
NRC	National Research Council
OC	osteocalcin
PTH	parathyroid hormone
P_i	inorganic phosphorus
PYD	pyridinoline
SBC	Schwarz's Bayesian Criterion

SCC	somatic cell count
SE	standard error
SNF	solids-not fat
Trt	treatment
$1,25(OH)_2D_3$	1,25 dihydroxyvitamin D_3
$25\text{-}OHD_3$	25 hydroxyvitamin D_3

CHAPTER 1

INTRODUCTION

Of the mineral elements, phosphorus (P) has the most biological diversity. For example, it is located in every cell of the body and is found in nucleic acids, phospholipids, cell signaling enzymes, and energy-transferring molecules such as adenosine triphosphate (ATP). Normal plasma inorganic P (P_i) concentration is between 4 and 8 mg/dl in the mature ruminant animal (Goff, 1998).

In some circumstances, dairy farmers add P to diets of dry and lactating cows to meet NRC (2001) P requirements. In many cases, P is fed above requirements, which may lead to environmental problems through increased P excretion and subsequent application to the soil and runoff into surface waters. Feeding dietary P to match the cow's requirement can result in 25 to 30% less manure P and decrease P supplementation cost by up to $15/cow per yr compared with feeding above NRC (2001) requirements (Wu et al., 2000).

One reason P is overfed is because of the potential occurrence of hypophosphatemia around parturition. Chronic hypophosphatemia is defined as plasma P_i between 2 and 3.5 mg/dl whereas acute hypophosphatemia usually is accompanied with plasma P_i below 2 mg/dl (Goff, 1998). Some dairy nutritionists and veterinary practitioners believe that increasing P concentrations in the diet above requirements may decrease the incidence of periparturient hypophosphatemia (Beede and Davidson, 1999). However, I can find no evidence in the scientific literature to support this supposition. Instead, I

hypothesize that feeding in excess of requirements during the dry period may predispose cows to metabolic problems such as hypophosphatemia or hypocalcemia.

The current NRC (2001) re-evaluated the values used for calculating total dietary P requirements. NRC (1978, 1989) reported absorption coefficients (**AC**) for dietary P of 0.55 and 0.50, respectively, regardless of P source. In contrast, NRC (2001) used an AC for dietary P of 0.64 for forages and 0.70 for concentrates, and supplemental mineral sources have been assigned individual AC (NRC, 2001). The requirement for absorbed P reported in NRC (2001) for a 765-kg Holstein 250 d into gestation is 21 g/d. In NRC (1989) a comparable value was 15 g/d during the last 2 mo of gestation. If we assume an AC of 0.68, dividing the requirement for absorbed P by 0.68, the requirement for total dietary P in late gestation is 31 g/cow per d (NRC, 2001). In contrast, using an AC of 0.50, the total dietary P requirement in late pregnancy equals 30 g/cow per d (NRC, 1989).

Only two research papers have been published describing the effects of prepartum dietary P concentrations on responses of plasma P, Ca, Mg, and vitamin D metabolites (Barton et al., 1987; Kichura et al., 1982). In general, decreasing prepartum dietary P led to a decrease in prepartum plasma P and a tendency to increase plasma Ca around parturition. However, results of both of these studies are difficult to interpret because DMI (Kichura et al., 1982; Barton et al., 1987), prepartum dietary P concentrations (Barton et al., 1987), or postpartum diet composition were not reported (Kichura et al., 1982). Also, the predominant breed in the dairy industry, the Holstein, was not used in the

experiments (Kichura et al., 1982; Barton et al., 1987). No research has adequately evaluated the optimal dietary P concentration for late pregnant dairy cows.

Late stages of gestation and early stages of lactation contribute to changes in both Ca and P metabolism (Liesegang et al., 2000a). Increased bone resorption occurs because of skeletal mineralization of the fetus in late gestation and milk production during lactation (Brommage and DeLuca, 1985; Fukuda and Iida, 1993). In the cow, milk production requires an available supply of P, and bone resorption has been estimated to supply 500 to 600 g of P during the first few weeks of lactation (Wu et al., 2000). A large portion of P mobilized from bone tissue may be a direct consequence of Ca mobilization in early lactation (Wu et al., 2000). Two biochemical bone markers, osteocalcin (**OC**) and deoxypyridinoline (**DPD**), markers of bone formation and resorption, respectively, have been evaluated in multiparous cows. Liesegang et al. (2000a) reported no effect of milk yield on serum OC and urinary DPD concentrations. Cows with hypocalcemia at parturition had similar urinary DPD concentrations compared with cows without hypocalcemia at parturition (Liesegang et al., 1998). Plasma OC concentrations were reduced around the time of parturition in multiparous Holstein cows (Naito et al., 1990) and in primi- and multiparous cows (van Mosel and Corlett, 1990).

The effects of dietary P concentrations on plasma OC concentrations have been evaluated only in sheep (Corlett and Care, 1988; Scott et al., 1994). Both studies reported that sheep fed diets deficient in P had lower plasma OC concentrations than sheep fed an adequate P diet. The effects of prepartum

dietary P concentrations on serum OC and DPD concentrations around parturition in dairy cows have not been reported.

Therefore, I hypothesize that feeding 0.21% dietary P from 28 d prepartum until parturition is adequate to meet the requirements of the periparturient multiparous Holstein cow without adverse metabolic or production effects. The objective of this study is to evaluate the effects of varying prepartum dietary P concentrations on mineral metabolism and lactational performance of multiparous Holstein cows.

CHAPTER 2

REVIEW OF LITERATURE

The following review of literature covers the most important areas of P and Ca metabolism in the periparturient dairy cow. Information on how the cow regulates P and Ca to maintain homeostasis and how dietary P affects bone metabolism and P and Ca homeostasis during both pregnancy and lactation is presented.

REGULATION OF PHOSPHORUS AND CALCIUM

Understanding the regulatory mechanisms of both P and Ca is important for determining the optimum dietary P concentration for the late pregnant dry cow. Determining circulating concentrations of P, Ca, and the hormones that regulate them can help to determine if the cow is receiving adequate amounts of dietary P.

Phosphorus and Calcium Homeostasis. Phosphorus and Ca pools are maintained in the body through the regulation of intestinal absorption, kidney excretion, and bone resorption (Horst, 1986). Both of these mineral elements can leave the circulating pool by feces, urine and bone in the non-lactating, non-pregnant cow. In the lactating, pregnant cow, Ca and P also exit the circulating pool through the fetus and mammary gland. Phosphorus and Ca pools are under regulation of three hormones – parathyroid hormone (**PTH**), 1,25

dihydroxyvitamin D_3 [**1,25(OH)$_2$D$_3$**], and calcitonin. Parathyroid hormone is secreted from the parathyroid glands and regulates Ca homeostasis while 1,25(OH)$_2$D$_3$ is a metabolite of vitamin D produced in the kidney that regulates both Ca and P homeostasis (Horst, 1986). Calcitonin is a hormone that is primarily secreted from the thyroid glands and inhibits bone Ca resorption and stimulates urinary P loss (Reinhardt et al., 1988).

Vitamin D and Parathyroid Hormone. There are two natural sources of vitamin D for the ruminant. There is a photochemical conversion of 7-dehydrocholesterol to vitamin D_3 in the skin (Horst and Reinhardt, 1983). Ruminants can get vitamin D from plants as a result of a photochemical conversion of ergosterol to vitamin D_2 (Horst and Reinhardt, 1983). Additionally, crystalline forms of vitamin D_2 and D_3 can be supplemented in the diet. Sommerfeldt et al. (1979, 1981) showed that ruminal microorganisms convert vitamin D_2 and D_3 into four unidentified metabolites. They also found that up to 80% of vitamin D disappears from rumen media during a 24 h incubation. Rumen microorganisms may serve as detoxifiers for ruminants consuming large does of vitamin D (Horst and Reinhardt, 1983).

When vitamin D_3 enters the blood stream, it circulates at low concentrations in the cow (Horst et al., 1981), probably due to its rapid uptake by the liver (DeLuca, 1981). Once vitamin D_3 is in the liver, it is converted to 25 hydroxyvitamin D_3 (**25-OHD$_3$**) in the microsomes and the mitochondria by vitamin D-25-hydroxylase (Madhok and DeLuca, 1979; Jones et al., 1976). In ruminants, this enzyme preferentially hydroxylates vitamin D_3 (Sommerfeldt et al., 1981).

Parathyroid hormone, Ca, P and vitamin D are all regulators of 25-OHD_3 metabolism (DeLuca, 1981). Hydroxylation of 25-OHD_3 in the kidney by 1α–hydroxylase results in the formation of 1,25$(OH)_2D_3$ (Reinhardt et al., 1988; Hove et al., 1984), the most biologically active form of vitamin D_3 (Horst and Reinhardt, 1983). The main functions of 1,25$(OH)_2D_3$ are to stimulate active transport of Ca in the small intestine, activate bone resorption, and decrease urinary Ca loss to maintain positive Ca balance (Horst and Reinhardt, 1983). In the ruminant, PTH acts as the primary control for 1,25$(OH)_2D_3$ production (Engstrom et al., 1987; Goff et al., 1986; Hove et al., 1984). When the parathyroid gland senses negative Ca balance, it secretes PTH that stimulates 1α–hydroxylase in the kidney (Reinhardt et al., 1988). DeLuca (1981) postulated that Ca, through high concentrations of PTH, indirectly affects 1α-hydroxylase activity, whereas P directly affects 1α-hydroxylase activity. These results were not supported, however, when Kichura et al. (1982) showed no elevation in plasma 1,25$(OH)_2D_3$ concentrations during dietary-induced hypophosphatemia in cows.

Model of Phosphorus Regulation. Figure 1 is a schematic of P regulation adapted from Horst (1986). Effects of increased and decreased P intake are indicated in this model. As P intake decreases, the subsequent decrease in P absorption and in plasma P concentration results in a pituitary-mediated increase in 1α–hydroxylase activity. Increased kidney 1α–hydroxylase activity increases 1,25$(OH)_2D_3$ production, which, in turn, increases P absorption from the gut. Conversely, when P intake increases, P absorption and plasma P concentrations increase. This increase in plasma P concentrations result in a decrease in 1α–hydroxylase activity that lowers plasma 1,25$(OH)_2D_3$

concentrations. As plasma $1,25(OH)_2D_3$ concentrations decrease, gut absorption of P decreases (Horst, 1986).

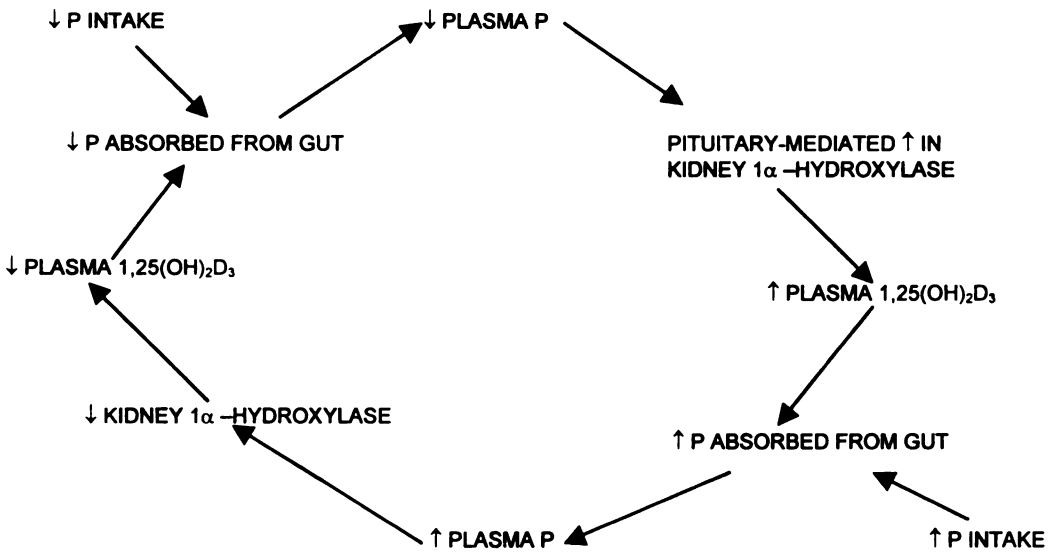


FIGURE 1. Effects of dietary P on P regulation.

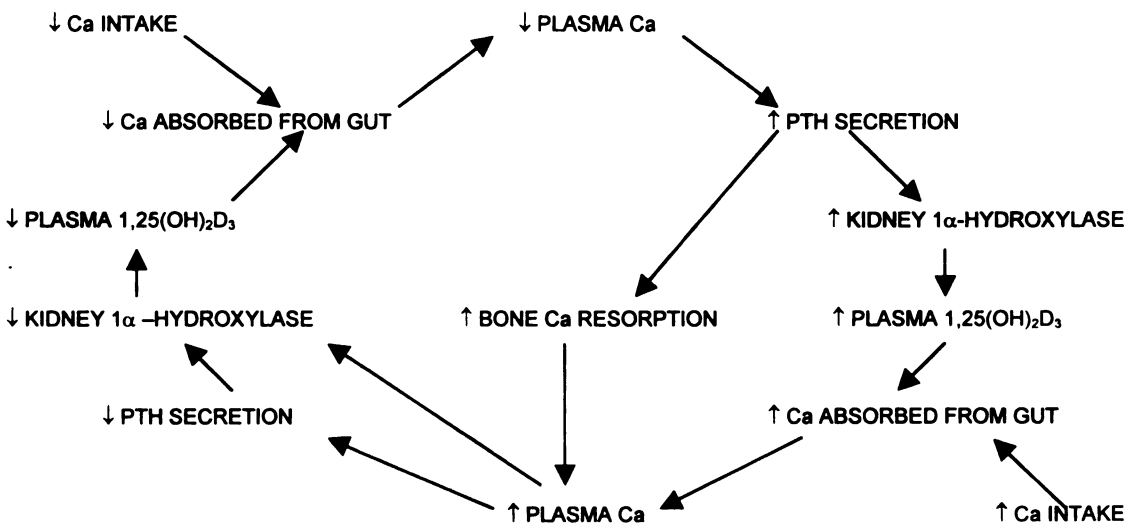


FIGURE 2. Effects of dietary Ca on Ca regulation.

Model of Calcium Regulation. Figure 2 is a schematic of Ca regulation adapted from Horst (1986). Effects of increased and decreased Ca intake are indicated in this model.

As Ca intake decreases, Ca absorption and plasma Ca concentrations decrease. This decrease in plasma Ca concentrations results in an increase in PTH secretion from the parathyroid glands. The increase in circulating PTH increases 1α-hydroxylase activity. Plasma $1,25(OH)_2D_3$ production increases as a direct result of the increase in 1α-hydroxylase activity. Both plasma $1,25(OH)_2D_3$ and PTH increase bone resorption while $1,25(OH)_2D_3$ increases Ca absorption from the gut. Conversely, when Ca intake or bone resorption increases, an increase in plasma Ca also is noted. Increasing plasma Ca concentrations result in a decrease in both PTH secretion and 1α-hydroxylase activity. Finally, the decrease in 1α-hydroxylase activity results in less $1,25(OH)_2D_3$ production that decreases Ca absorption from the gut (Horst, 1986).

Phosphorus Absorption. In general, absorbed dietary P is in direct relationship to phosphorus intake in ruminants (Care, 1994). The efficiency of P absorption is low when dietary intake of P is deficient and increases with the intake of P until the requirements are met. A further increase in intake is associated with a reduction in absorption efficiency (Challa et al., 1989). A net absorption of P from the reticulo-rumen occurred in sheep (Breves et al., 1988; Beardsworth et al., 1989). Additionally, Edrise and Smith (1986) found a net absorption of P from the bovine omasum. However, the major site of P absorption is the small intestine (Grace et al., 1974).

Net absorption of P occurs in the small intestine until the pH becomes too high for P ions to remain in solution in high concentration (Shirazi-Beechey et al., 1989). In non-ruminants, P transport by the small intestine consists of both an active and passive process (Wasserman and Taylor, 1976). The active transport process is separate from that associated with Ca transport and is readily saturable, so that passive absorption predominates at high intestinal P concentrations (Reinhardt et al., 1988). The vitamin D pathway stimulated the active transport process when rats were fed a low P diet (Gray, 1987). Sheep can adapt their efficiency of P absorption from the small intestine in response to dietary P depletion (Care et al., 1980). To achieve this, the capacity of the brush border membrane to transport P ions is enhanced (Shirazi-Beechey et al., 1991). Unlike non-ruminants, however, low dietary P did not increase the production rate of $1,25(OH)_2D_3$ in sheep to achieve this adaptation (Maunder et al., 1986). Schroder et al. (1990) showed that dietary P deficiency in goats increased the efficiency of the intestinal receptor for $1,25(OH)_2D_3$, thus making the circulating $1,25(OH)_2D_3$ more effective at the gut level. However, when sheep were fed a very low Ca diet, there was an increase in the efficiency of absorption of both Ca and P in the small intestine. This change was accompanied by an increase in circulating $1,25(OH)_2D_3$ concentration due to the decrease in circulating Ca concentration (Abdel-Hafeez et al., 1982).

Calcium Absorption. Dietary Ca is absorbed according to requirement and when ruminants are fed a low Ca diet the efficiency of Ca absorption is increased (Braithwaite, 1974; Braithwaite, 1975). The percentage of Ca absorption from the small intestine of the dairy cow increased in response to a

reduced dietary Ca intake and Na_2EDTA infusion to induce hypocalcemia (van't Klooster, 1976). The mechanism of this adaptive response to hypocalcemia is thought to be via an increase in secretion of PTH that in turn causes an increase in plasma $1,25(OH)_2D_3$ concentration (Care et al., 1980). A comparable increase in circulating $1,25(OH)_2D_3$ concentrations were observed in normal parturient cows and even higher $1,25(OH)_2D_3$ concentrations were reported in cows with parturient paresis (Horst et al., 1977). A similar increase in PTH concentrations also was observed in paretic animals (Jorgensen, 1974). Alternatively, during hypercalcemia, the plasma PTH concentration is low and consequently, 1α–hydroxylase activity is depressed. Less efficient Ca absorption results from the decreased plasma $1,25(OH)_2D_3$ concentration (Horst, 1986). Dietary P concentration is another factor that affects intestinal Ca absorption in ruminants. In the non-ruminant, a low P diet increased the percentage of Ca absorbed (Fox et al., 1978). However, Young et al. (1966b) demonstrated that feeding a P-deficient diet to sheep reduced Ca absorption rate.

Bone Formation. The skeleton contains 99% of the total Ca and 80% of the total P in the body (Horst, 1986). In ruminants, the amount of Ca and P deposited into bone is lower in older animals than younger (Ramberg et al., 1975). The amount of Ca deposited in bone reaches a maximum at 1 yr of age, when deposition is reduced drastically, and levels off at about 9 yr of age in the dairy cow (Horst, 1986).

Bone formation occurs as a result of calcification of a specialized organic matrix in young animals. Vitamin D plays a primary role in the mineralization process. An insufficient supply of Ca and P to the chondroblasts and osteoblasts

that mediate bone formation is the major reason for a failure of mineralization. It is unclear whether the absence of vitamin D metabolites could result in additional failure of bone mineralization (Horst, 1986; Reinhardt et al., 1988). However, 24,25 dihydroxyvitamin D_3 or some metabolite of 25-OHD_3 other than 1,25$(OH)_2D_3$ may play a role in the mineralization process (Norman, 1980; Bordier et al., 1978).

Bone Resorption. Both PTH and 1,25$(OH)_2D_3$ influence bone Ca and P mobilization to supply circulating Ca and P concentrations (Reinhardt et al., 1988). For optimal bone resorption of Ca, both PTH and 1,25$(OH)_2D_3$ are needed (DeLuca, 1981). Skeletal reserves of Ca and P are diminished during lactation but are replenished during late lactation and the dry period (Braithwaite, 1976). In early lactation, the animal depends mostly on Ca absorption from the intestine, and bone resorption plays a minor role until 1 to 2 wk postpartum (Ramberg et al., 1975; Ramberg et al., 1984). In addition, bone Ca and P resorption response is somewhat resistant to PTH and 1,25$(OH)_2D_3$ stimulus during this time period. However, when cows are fed a low Ca diet prepartum, bone contributes to the Ca pool (Goings et al., 1974).

Usually, bone deposition of Ca equals the amount of Ca resorbed. However, there are cases where either deposition or resorption occurs at uneven rates. When bone deposition occurs more rapidly than bone resorption over an extended period of time, the animal may develop osteopetrosis (Horst, 1986; Reinhardt et al., 1988). Both congenital and nutritional ostepetrosis have been described in ruminants (Krook et al., 1971; Ojo et al., 1975).

Demand for Phosphorus and Calcium during Pregnancy and Lactation. Maternal demands for P and Ca are increased during pregnancy and lactation as a result of the additional requirements for the fetus and for milk production in the dairy cow (NRC, 2001). In sheep, the demand for Ca increases most rapidly in late gestation and reaches a maximum in early lactation with little change at parturition (Braithwaite, 1976). However, demands for Ca in the dairy cow change slowly during gestation but increase 2 to 3 times at parturition. Demands for P and Ca decrease slowly in both sheep and cattle as milk yields decrease throughout lactation (Braithwaite, 1976).

Phosphorus Excretion. Fecal excretion is by far the primary route of excretion in dairy cattle (NRC, 2001). Urinary excretion is secondary and typically less than 5% of the total P excretion. The rate of excretion of endogenous fecal P is related directly to P intake and rate of absorption and is related inversely to the rate of Ca absorption (Briathwaite, 1976). Fecal excretion of P also may be related to plasma P_i concentration and may play an important role in P homeostasis (Young et al., 1966b). Urinary P excretion is usually low, but there is a tendency for a higher amount of urinary P excretion with increased dietary P intake (Braithwaite, 1975). Urinary excretion is high when animals receive a Ca-deficient diet but decreases as Ca intake increases (Braithwaite, 1975). These changes in P excretion occur as a result of changes in bone metabolism (Braithwaite, 1975). An increased rate of bone resorption is needed to supply Ca during a Ca deficiency. As a result, there is a release of large amounts of phosphorus from bone into the blood stream that is presumably excreted in the urine. A subsequent increase in Ca intake results in increased

gut Ca absorption that leads to a decrease in bone resorption (Braithwaite, 1976).

Calcium Excretion. Urinary excretion of Ca by ruminants is very low and is largely unaltered by changes in the rate of Ca intake or absorption (Braithwaite, 1975). However, during P deficiency (Young et al., 1966a) or after the ingestion of acidic substances (Horst and Jorgensen, 1974) urinary excretion of Ca is increased, possibly because of the reduced secretion of PTH. However, the endogenous excretion of Ca in feces is higher in ruminants than in non-ruminants (Care et al., 1980).

In conclusion, when dietary P is fed below requirement there is a decrease in plasma P concentration. There is a pituitary-mediated increase in kidney 1α-hydroxylase activity that increases $1,25(OH)_2D_3$ production. Unlike regulation of Ca homeostasis, there is no change in PTH concentration. The increased $1,25(OH)_2D_3$ production increases P absorption from the gut, returning circulating P concentrations to normal (Horst, 1986). Additionally, plasma Ca concentrations increase as a result of low P diets (Kichura et al., 1982; Barton et al., 1987) that could be due to enhanced intestinal absorption of Ca by a vitamin D-mediated transport mechanism (Barton et al., 1987).

BIOCHEMICAL MARKERS OF BONE FORMATION AND RESORPTION

Because bone is the primary source of P and Ca in the body, it is important to understand the contribution that bone makes to the circulating pools of P and Ca. To date, four biological bone markers (indicators of bone formation

or resorption) have been evaluated in the dairy cow. Detailed information on biological bone markers and the effects of dietary P concentration on biological bone markers are presented in the following section.

Biochemical bone markers of bone metabolism are specific proteins that can be found in both blood and urine that indicate bone formation or resorption. Examples of biochemical bone markers include osteocalcin, hydroxyproline, deoxypyridinoline, and pyridinoline.

Late stages of gestation and early stages of lactation contribute to changes in both Ca and P metabolism (Liesegang et al., 2000a). Increased bone resorption occurs because of skeletal mineralization in the fetus during late gestation and milk production in mammals (Brommage and DeLuca, 1985; Fukudu and Iida, 1993). Milk production requires an available supply of P. Bone resorption can supply 500 to 600 g of P in early lactation (Wu et al., 2000). A large portion of P mobilized from bone tissue may be a direct consequence of Ca mobilization in early lactation (Wu et al., 2000). Ternouth and Sevilla (1990) suggested that up to 30% of bone P is removed during early lactation. Bone markers such as hydroxyproline (**HYP**), deoxypyridinoline (**DPD**), pyridinoline (**PYD**), and osteocalcin (**OC**) have been used to monitor bone resorption and formation in several species such as humans, monkeys, swine, rats, and lambs (Cross et al., 1995; Cahoon et al., 1996; Carter et al., 1996; Egger et al., 1994; Scott et al., 1994; Corlett et al., 1990).

Hydroxyproline, DPD and PYD are all biological markers of bone resorption whereas OC is a biological marker of bone formation. Because HYP is not only derived from bone, but from non-bone tissues (Uebelhart et al., 1990)

and only about 10% of it is excreted in urine (Klein and Yen, 1970), researchers recently have focused more on PYD and DPD concentrations in urine and blood, suggesting that they may be more accurate predictors of bone resorption. Plasma OC concentrations have been evaluated in pregnant, lactating and non-lactating sheep (Farrugia et al., 1989). In addition, several studies have reported urinary and serum concentrations of OC, DPD, PYD and HYP in dairy cows (Liesegang et al., 2000a; Liesegang et al., 2000b; Liesegang et al., 1998; Naito et al., 1990; van Mosel and Corlett, 1990). Two studies evaluated the effect of dietary P on plasma OC concentrations in sheep (Scott et al., 1994; Corlett and Care, 1988) and another study evaluated the effect of dietary P on serum OC concentrations in swine (Carter et al., 1996).

Bone Markers in Sheep. Farrugia et al. (1989) reported the temporal patterns of plasma OC concentrations in 36 pregnant ewes around parturition compared with 39 age-matched non-pregnant ewes. From d 35 of gestation until parturition, the pregnant ewes had lower plasma OC concentrations compared with the age-matched ewes. After parturition, age-matched ewes maintained plasma OC concentrations at 18 μg/L throughout the entire postpartum period while the ewes sampled during the postpartum period did not reach 18 μg/L until 14 d postpartum. Plasma OC concentrations increased to 40 μg/L at 24 d postpartum, at which point they gradually decreased until 64 d postpartum. The authors concluded that plasma OC concentrations appeared to be regulated by factors during and shortly after ovine pregnancy. These results are surprising since one would not think that plasma OC concentrations would rise so rapidly after parturition because of the potential demand on bone for Ca and P for milk

production. The temporal pattern established in this study is valuable information providing evidence that bone formation decreases around parturition resulting in less Ca and P being incorporated into bone.

Bone Markers in Dairy Cows. Several studies have evaluated the effects of hypocalcemia (Liesegang et al., 2000b; Liesegang et al., 1998), milk yield (Liesegang et al., 2000a), and dietary Mg concentrations (van Mosel and Corlett, 1990) on bone marker concentrations in dairy cows. Additionally, temporal patterns of blood OC (Liesegang et al., 2000a; Naito et al., 1990; van Mosel and Corlett, 1990) and urinary DPD, HYP and PYD (Liesegang et al., 2000a; Liesegang et al., 1998; van Mosel and Corlett, 1990) around parturition have been reported

Eighteen multiparous cows with hypocalcemia around parturition had similar urinary DPD concentrations compared with 19 multiparous cows with normal periparturient periods (Liesegang et al., 1998). However, cows with hypocalcemia at parturition had higher urinary HYP concentrations from 5 through 14 d postpartum compared with normal cows. In a related study, hypocalcemia and hypophosphatemia were induced in six multiparous Brown Swiss cows through EDTA (disodium ethylenediaminetetraacetic acid) infusion at a rate of 0.55 mg/kg per minute for 5 min. An increase in both urinary DPD and HYP was observed (Liesegang et al., 2000b).

Milk yield (4900 vs. 6500 kg/305d) did not alter urinary DPD, HYP, PYD or serum OC concentrations in multiparous purebred and crossbred Brown Swiss cows (Liesegang et al., 2000a). Additionally, neither 20 nor 74 g dietary Mg/cow per day fed from 7 wk prepartum through 1 wk postpartum changed plasma OC

or urinary HYP concentrations (van Mosel and Corlett, 1990). In this study, however, that cows in their first or second parity had higher plasma OC and urinary HYP concentrations as compared with cows in their third or greater parity. These results are supported by Braithwaite (1976) who concluded that multiparous dairy cows have a decrease in bone mobilization which could account for the increase incidence of hypocalcemia in aged cows compared with cows in their first or second parity.

Temporal patterns of bone markers have been reported in the multiparous dairy cow. A decrease in plasma OC concentrations (Naito et al., 1990; van Mosel and Corlett, 1990) and serum OC concentrations (Liesegang et al., 2000a) was reported around parturition. Additionally, an increase in urinary DPD, HYP and PYD was reported around parturition (Liesegang et al., 2000a; Liesegang et al., 1998; van Mosel and Corlett, 1990).

These results provide temporal patterns of OC, DPD, PYD and HYP around parturition, during hypocalcemia, and during hypophosphatemia. Understanding the effect of diet and physiological status (i.e., milk production, hypocalcemia) on bone formation and resorption is imperative when evaluating dietary P concentrations.

Effect of Dietary P Concentrations on Bone Metabolism. To date, no research has been published evaluating the effects of dietary P concentrations on serum OC or DPD concentrations in dairy cattle. Two studies in sheep, however, have evaluated the effects of dietary P concentrations on plasma OC concentrations (Corlett and Care, 1988; Scott et al., 1994) and urinary DPD concentrations (Scott et al., 1994). Additionally, the effect dietary P

concentration on serum OC concentrations in growing pigs has been reported (Carter et al., 1994).

Scott et al. (1994) evaluated the effects of two dietary P concentrations, 0.09 and 0.27% P, on bone and mineral metabolism in eight crossbred male lambs for 6 wk. During the control period, the plasma OC concentration did not differ. When dietary treatments were applied, the four lambs on the low P diet had lower plasma OC concentrations as compared with the four lambs on the normal P diet, however treatment did not alter urinary PYD or DPD concentrations. In a related study, six crossbred sheep were fed a 0.167% P diet for one month and then fed a diet containing 0.036% P for an additional month (Corlett and Care, 1988). Both plasma P_i and OC concentrations were reduced during the second month as compared with the first. Authors from both studies concluded that the decrease in plasma OC concentrations were associated with a reduction in bone formation rate that may account for the slight increase in plasma Ca concentrations seen in both studies. Finally, Carter et al. (1996) evaluated the effects of four dietary P treatments (0.35, 0.55, 0.75, and 0.95% P) on serum OC concentrations in thirty-six growing pigs. Serum OC concentrations decreased linearly with increasing dietary P concentrations.

In conclusion, little is known about the effects of dietary P on bone metabolism in sheep or pigs. No research has evaluated the effects of dietary P concentrations on bone metabolites in cows. Serum concentrations of OC and DPD seem to be reliable markers of bone formation and resorption, respectively. These two markers should be used not only to evaluate the effects of dietary P

concentrations on them, but also to establish temporal patterns around parturition.

EFFECTS OF DIETARY PHOSPHORUS CONCENTRATIONS ON DRY MATTER INTAKE, BLOOD METABOLITES AND MILK YIELD

Understanding the regulation of P and Ca homeostasis is important when evaluating different dietary P concentrations. However, both parity and dietary P concentrations also will influence DMI, milk production, and circulating P, Ca, Mg concentrations. The following section includes information on the influences of parity and prepartum dietary P concentrations on peripartum responses in the dairy cow. Additionally, the effects of feeding various concentrations of dietary P during lactation are also reported. This information may help with the overall understanding of P homeostasis and how it may be altered through feeding different dietary P concentrations.

Effect of Parity on Blood Metabolites. Temporal patterns of plasma P, Ca, PTH and $1,25(OH)_2D_3$ (Barton et al., 1981; Horst et al., 1978) were reported during periparturient period in young and aged cows. Aged paretic cows (≥ third parity) exhibited lower plasma P and Ca concentrations at parturition than either young (≤ second parity) or aged nonparetic (≥ third parity) cows (Barton et al., 1981; Horst et al., 1978). These cows also showed the most dramatic increase in plasma $1,25(OH)_2D_3$ around parturition as compared with cows in the other two groups (Barton et al., 1981; Horst et al., 1978). Plasma PTH concentrations increased in both groups of aged cows at 0.5 d prepartum. Paretic aged cows

had higher PTH concentrations from 1.5 d prepartum through 2 d postpartum as compared with aged non-paretic cows (Horst et al., 1978).

Effects of Prepartum Dietary Phosphorus Concentrations. Because only two studies have evaluated the effects of prepartum dietary P concentrations on peripartum responses in the multiparous dairy cow, results of these studies will be discussed in detail.

Kichura et al. (1982) investigated the influences of prepartum dietary P and Ca concentrations on vitamin D metabolism and milk fever in multiparous Jersey cows. Twenty multiparous Jersey cows were fed one of four diets from 4 wk prior to expected calving date (**EDC**) until parturition. The four diets contained low Ca, low P (**LCLP**); low Ca, high P (**LCHP**); high Ca, low P (**HCLP**); or high Ca, high P (**HCHP**). The basal ration, LCLP, was formulated to contain 9.5 g Ca and 10 g P/cow per day. The high Ca and P diets contained 86 g Ca and 82 g P/cow per day, respectively. At parturition, cows were fed a lactation ration containing NRC (1978) recommendations for P though these values were not specified. Neither prepartum nor postpartum DMI was reported. Temporal patterns of plasma Ca, P, $1,25(OH)_2D_3$ and HYP concentrations were reported around parturition.

Cows on the low Ca diets had lower plasma Ca concentrations than cows on the high Ca diets only 2 d after the start of the trial. At parturition, cows fed the HCLP diet had greater plasma Ca concentrations than cows fed the HCHP diet. One day after parturition, cows fed high Ca diets had lower plasma Ca concentrations compared with cows fed low Ca diets. Additionally, mean plasma Ca concentrations were lower in cows fed the HCHP diets 1 d postpartum than

cows on the other three treatment diets. Mean plasma P concentrations were lower in cows fed low P diets compared with cows fed high P diets during the entire prepartum period. There was a tendency for cows fed the LCLP diet to have greater mean plasma P concentrations compared with cows on the HCLP diet. From 4 to 1 d prepartum, cows on the low P diets had lower plasma P concentrations than cows on the high P diet. However, there was no difference in postpartum plasma P concentrations among the four treatment groups.

From 16 to 1 d prepartum, cows on the low Ca diets had higher plasma HYP concentrations compared with cows on the high Ca diets. Concentrations of plasma HYP tended to increase after parturition in all groups. Authors concluded that there was no indication that low prepartum dietary P increased bone resorption. Prepartum mean plasma $1,25(OH)_2D_3$ concentrations were higher for cows fed low Ca diets compared with cows fed high Ca diets. Dietary P did not influence mean plasma $1,25(OH)_2D_3$ concentrations. Four cases of parturient paresis occurred only in cows fed the HCHP diet. Researchers concluded that by feeding low Ca or low P diets, plasma Ca concentrations dropped less around the time of parturition compared with feeding a high Ca or P diet.

Barton et al. (1987) fed 30 multiparous Holstein, Ayrshire, Guernsey and Jersey cows three different P concentrations from 28 d prior to ECD until parturition. Dietary P concentrations were 0.7, 1, and 3 times the daily maintenance requirement for dietary P and dietary Ca concentrations were 3 times the maintenance requirement for Ca. However, the maintenance requirement of neither P nor Ca was not specified though NRC (1978) was cited

as the source. Samples were collected 7, 5, 3, 1 d prepartum, at parturition and 1, 3, 5, 7 d postpartum. Temporal patterns of plasma Ca, P, Mg, HYP, 24,25 dihydroxyvitamin D_3 and 1,25$(OH)_2D_3$ were reported. Neither prepartum nor postpartum DMI was reported.

Cows fed 0.7 times maintenance requirement for dietary P had lower mean plasma P concentrations prepartum, at parturition and until 1 d postpartum than cows fed 3 times maintenance requirement for dietary P. Prepartum dietary P had no effect on prepartum plasma Ca concentrations. However, cows fed 0.7 times maintenance requirement for dietary P had higher mean plasma Ca concentrations at 3 and 5 d postpartum than cows on the other two treatments. Prepartum dietary P did not alter prepartum or postpartum plasma Mg concentrations. Plasma HYP was not influenced by dietary P concentrations though plasma HYP increased in all treatment groups after parturition. Mean plasma concentrations of 24,25 dihydroxyvitamin D_3 and 1,25$(OH)_2D_3$ were not affected by prepartum dietary P concentrations. Authors concluded that it is beneficial to feed a low dietary P concentrations prepartum to have metabolites that regulate Ca homeostasis in an active state.

Effect of Dietary Phosphorus Concentrations During Lactation. Several studies evaluated the effects of varying dietary P concentrations on dry matter intake (**DMI**), body weight (**BW**), body condition score (**BCS**), serum P and serum Ca concentrations during the lactation cycle. Additionally, milk yield and components have been quantified in many of these studies.

Varying dietary P concentrations from 0.31 to 0.49% had no affect on DMI during an entire lactation (Wu and Satter, 2000; Wu et al., 2001; Wu et al., 2000).

However, Valk and Sebek (1999) reported that 0.24% dietary P decreased DMI after 20 wk compared to cows fed 0.28 or 0.33% dietary P. In a similar study, Call et al. (1987) observed a reduction in DMI of cows fed 0.24% dietary P after 6 wk compared to cows consuming 0.32 or 0.42% dietary P. Therefore, the results of these studies are inconclusive.

The effect of dietary P concentrations on BW has been reported in several studies. Call et al. (1987) reported that cows fed 0.24% dietary P had lower BW after 14 wk than cows fed 0.32 or 0.42% dietary P. Additionally, cows fed 0.24% dietary P lost more weight during the second lactation in a two lactation study compared with cows fed 0.28 or 0.33% dietary P (Valk and Sebek, 1999). In both of these studies, the decrease in DMI could have resulted in the increased BW loss. However, BW change was not affected by dietary P concentrations ranging from 0.31 to 0.49% (Wu and Satter; Wu et al., 2001; Wu et al., 2000). Additionally, BCS change was not altered when cows were fed diets containing 0.31 to 0.49% dietary P (Wu and Satter, 2000; Wu et al., 2000).

Serum P concentrations were lower when cows were fed 0.31% dietary P compared with cows fed 0.39 to 0.49% dietary P over an entire lactation (Wu et al., 2001; Wu et al., 2000). Additionally, cows fed 0.24% dietary P had lower serum P concentrations from parturition through 44 wk of lactation compared with cows fed 0.32 or 0.42% dietary P (Call et al., 1987). Only one study has evaluated the effect of feeding varying dietary P concentrations on serum Ca concentrations where cows fed 0.31% dietary P had higher serum Ca concentrations compared with cows fed 0.40 or 0.49% dietary P over an entire lactation (Wu et al., 2000).

The effects of dietary P concentrations on milk yield and milk components has also been evaluated. Milk yield was unaffected by feeding diets containing 0.31 to 0.49% dietary P over a lactation cycle (Wu and Satter, 2000; Wu et al., 2000). However, cows fed 0.24% dietary P had lower milk yields around peak lactation compared with cows fed 0.28 or 0.33% dietary P (Valk and Sebek, 2000). In a related study, cows fed 0.24% dietary P had lower fat corrected milk from 18 to 42 weeks of lactation compared with cows fed 0.32 or 0.42% dietary P (Call et al., 1987). Therefore, the results from these studies are inconsistent.

Dietary P concentrations ranging from 0.24 to 0.49% had no affect on milk fat percentage (Call et al., 1987; Wu and Satter, 2000; Wu et al., 2001; Wu et al., 2000). Additionally, milk lactose, solids-not fat, and SCC concentrations were unaltered by dietary P concentrations ranging from 0.31 to 0.49% (Wu and Satter, 2000; Wu et al., 2001; Wu et al., 2000). The effect of dietary P concentrations on protein percentage is inconsistent. Two papers have reported no effect of diets containing 0.31 to 0.49% P on milk protein percentage (Wu et al., 2001; Wu et al., 2000). However, feeding 0.24% dietary P decreased milk protein percentage compared with feeding 0.32 or 0.42% dietary P (Call et al., 1987). Additionally, dietary P concentration has had no effect on milk P percentage (Call et al., 1987; Forar et al., 1982; Wu et al., 2001).

In conclusion, the effects of parity on plasma concentrations of P and Ca around parturition have been established. Additionally, the effects of feeding various dietary P concentrations during lactation have been established. However, the effect of prepartum dietary P concentration on the peripartum cow is less well-understood, evaluated in only two studies (Barton et al., 1987;

Kichura et al., 1982). Therefore, more research should be done to evaluate the effect of prepartum dietary P concentrations on peripartum responses in the multiparous Holstein cow.

We hypothesize that feeding 0.21% dietary P from 28 d prepartum until parturition is adequate to meet the requirements of the periparturient multiparous Holstein cow without adverse metabolic or production effects. The objective of this study is to evaluate the effects of varying prepartum dietary P concentrations on mineral metabolism and lactational performance of multiparous Holstein cows.

CHAPTER 3

PERIPARTURIENT RESPONSES OF MULTIPAROUS HOLSTEIN COWS TO VARYING PREPARTUM DIETARY PHOSPHORUS CONCENTRATIONS

ABSTRACT

Diets containing 0.21, 0.31, and 0.44% P, dry basis, were fed for 28 d before parturition to 15, 13, and 14 multiparous Holstein cows, respectively. Cows fed 0.21% P had lower prepartum serum P concentrations than cows fed 0.31 or 0.44% P. Cows fed 0.31% P had lower prepartum serum P concentrations than cows fed 0.44%. Neither total serum Ca nor plasma ionized Ca was altered by prepartum dietary P concentrations. Cows fed 0.21% P tended to have lower prepartum osteocalcin concentrations than cows fed 0.31 or 0.44% P, suggesting less bone formation. Neither pre- nor postpartum serum deoxypyridinoline concentrations were influenced by dietary treatment, suggesting no effect on bone resorption around parturition. Energy-corrected milk yield during the first 28 d of lactation was not affected by prepartum dietary P treatments. We conclude that a prepartum dietary P concentration of 0.44% was too high because it resulted in lower total serum Ca concentrations around parturition compared with 0.21 and 0.31% P. Cows fed 0.21% P consumed slightly above NRC 2001 requirements at 34 g of P/d. These cows fed 0.21% P prepartum had higher total serum Ca and plasma ionized Ca concentrations around parturition compared with 0.31 and 0.44% P. Energy-corrected milk yield

during the first 28 d of lactation was not altered. We conclude that consuming 34 g of P/d (0.21% P in this experiment) was adequate to meet the needs of the periparturient multiparous Holstein cow.

INTRODUCTION

Few research results have been published on the effects of varying prepartum dietary P concentrations on cows during the periparturient period. In the current edition of "Nutrient Requirements of Dairy Cattle," values used for calculating total dietary P requirements were re-evaluated (NRC, 2001). Previous editions (NRC, 1978; NRC, 1989) reported absorption coefficients (AC) for dietary P of 0.55 and 0.50, respectively, regardless of P source. In contrast, NRC (2001) used an AC for dietary P of 0.64 for forages and 0.70 for concentrates, and supplemental mineral sources were assigned different individual AC (NRC, 2001). The requirement for absorbed P reported in NRC (2001) for a 765-kg Holstein cow 250 d into gestation is 21 g/d compared with 15 g/d during the last 2 mo of gestation (NRC, 1989). If we assume an AC of 0.68, dividing the requirement for absorbed P by the AC, the requirement for total dietary P in late gestation is 31 g/cow per d. The value is similar to that predicted by NRC (1989); 15 g/d divided by 0.50 equals 30 g/cow per d. The optimal dietary P concentration for late pregnant dairy cows has not been evaluated adequately.

Two research papers described the effects of varying prepartum dietary P concentrations on responses of plasma P, Ca, Mg, and vitamin D metabolites around parturition (Barton et al., 1987; Kichura et al., 1982). However, results of both of these studies are somewhat difficult to interpret because DMI (Barton et al., 1987; Kichura et al., 1982), prepartum dietary P concentrations (Barton et al., 1987), or postpartum diet composition were not reported (Kichura et al., 1982). Additionally, the predominate breed in the dairy industry, the Holstein, was not represented in these studies (Barton et al., 1987; Kichura et al., 1982).

Late stages of gestation and early stages of lactation contribute to changes in both Ca and P metabolism (Liesegang et al., 2000a). Increased bone resorption occurs because of skeletal mineralization of the fetus in late gestation and milk production during lactation (Brommage and DeLuca, 1985; Fukuda and Iida, 1993). Milk production requires an available supply of P and bone resorption has been estimated to supply 500 to 600 g of P during the first few weeks of lactation (Wu et al., 2000). A large portion of P mobilized from bone tissue may be a direct consequence of Ca homeostasis and mobilization in early lactation (Horst, 1986; Wu et al., 2000). Two biochemical bone markers, osteocalcin (**OC**) and deoxypyridinoline (**DPD**), markers of bone formation and resorption, respectively, recently have been evaluated in multiparous cows. Liesegang et al. (2000a) reported no effect of level of milk yield on serum OC and urinary DPD concentrations. Cows with hypocalcemia at parturition had similar urinary DPD concentrations compared with cows without hypocalcemia at parturition (Liesegang et al., 1998). Plasma OC concentrations were reduced

around the time of parturition in multiparous Holstein cows (Naito et al., 1990) and in primi- and multiparous cows (van Mosel and Corlett, 1990).

The effects of dietary P concentrations on plasma OC concentrations have been evaluated only in sheep (Corlett and Care, 1988; Scott et al., 1994). Both studies showed that sheep fed diets deficient in P had lower plasma OC concentrations than sheep fed a diet adequate in P. The effects of prepartum dietary P concentrations on serum OC and DPD concentrations around parturition in dairy cows have not been reported.

We hypothesize that feeding 0.21% dietary P from 28 d prepartum until parturition is adequate to meet the requirements of the periparturient multiparous Holstein cow without adverse metabolic or production effects. The objective of this study is to compare the effects of varying prepartum dietary P concentrations on peripartum mineral element metabolism and lactational performance of multiparous Holstein cows.

MATERIALS AND METHODS

Cows and Experimental Design

Forty-five nonlactating, pregnant multiparous Holstein cows were used in a randomized block design. Cows were blocked according to parity and expected calving date (ECD) to one of three prepartum dietary treatments. On Treatment 1 there were 6, 6, and 3 cows in parities 2, 3, and 4+, respectively.

On Treatment 2 there were 5, 5, and 3 cows in parities 2, 3, and 4+, respectively. On Treatment 3 there were 6, 5, and 3 cows in parities 2, 3, and 4+, respectively. Cows were dried-off and placed in tie-stalls approximately 60 d before ECD. They remained on study through 28 d postpartum.

Treatments and Diets

Each cow was fed a standardization diet from 60 to 28 d before ECD (Treatment 2; Table 1). From 28 d before ECD to parturition, each cow was fed a different dietary treatment formulated to contain either 0.18, 0.30 or 0.42% P, dry basis, designated throughout as Treatment 1 (**Trt 1**), Treatment 2 (**Trt 2**), and Treatment 3 (**Trt 3**), respectively. Average days prior to parturition in which dietary treatments actually were fed were 28, 30, and 28 d for Trt 1, 2, and 3, respectively (SEM = 2.4). Dietary P concentrations were chosen so that the middle concentration would supply the dietary requirement for a 765-kg cow at 250 d of gestation (approximately 0.31%, depending upon dry matter intake (**DMI**)). Treatments 1 (0.18% P) and 3 (0.42% P) were to provide equal increments below and above the dietary requirement. All dietary treatments contained the same amount of corn silage, alfalfa silage, beet pulp pellets, corn starch, ground corn, blood meal, biuret, ammonium chloride, and HCl-treated soybean meal (SoyChlor 16-7™, West Central Soy, Ralston, IA). Different amounts of monoammonium phosphate [$(NH_4)H_2PO_4$] in Trt 2 and 3 replaced rice hulls of Trt 1 to increase dietary P concentrations whereas urea was

removed to keep the diets isonitrogenous (Table 1). Corn starch and beet pulp pellets were used because of their high NE_L content and low P concentrations. Blood meal was used because it is a high protein, low P feed source. Prepartum diets contained 1.59 Mcal NE_L/kg and 14.8% CP, dry basis (Table 1).

When signs of parturition were evident, cows were moved to maternity pens and fed treatment diets until parturition (typically less than 24 h). After parturition, all cows were fed the same diet formulated to have 1.72 Mcal NE_L/kg and 18.7% CP, DM basis (Table 2).

Feeding and Analysis

Diets and Orts. The experiment lasted from September 2000 through February 2001. Cows were fed individually once daily at 0900 h. Orts were weighed once daily at 0800 h from 60 d before ECD through 28 days in milk (**DIM**) to determine feed intake. Samples of each individual forage and concentrate ingredient were taken bi-monthly, dried at 60°C for 48 h, ground first through a 5-mm screen, and then through a 2-mm screen (Thomas-Wiley mill; Aurthur Thomas Company, Philadelphia, PA). Samples were composited and subsampled after grinding. Composite feed samples of each individual ingredient were sent to a laboratory (Dairy One, Inc., Ithaca, NY) for CP, NDF, ADF, and mineral element analyses. Analyses of P concentration for each forage and concentrate ingredient were done monthly throughout the experiment to verify P concentrations throughout.

Body Weights and BCS. Cows were weighed weekly from 60 d before ECD through 28 d postpartum. Three independent technicians assigned body condition scores (**BCS**) to each cow weekly from 60 d before ECD through 28 d postpartum (Wildman et al., 1982). The BCS values of each technician were averaged for each week for each cow for statistical analyses.

Sampling and Analytical Methods

Blood. Blood samples (30 ml) were taken from the coccygeal vessels using three, 10-ml evacuated glass tubes and one Li-heparin coated glass tube (Fisher Scientific, Chicago, IL) at 0630 h (prior to daily feeding) on d 28, 25, 22, 19, 16, and 13 prior to ECD. Samples were taken daily from 10 d prior to ECD until parturition. Postpartum samples were taken at the time of calving (0 h), 6, 12, 18, 24, 36, 48, 60, and 72 h, as well as 4, 5, 6, 7, 14, 21, and 28 d postpartum. One blood sample in an evacuated glass tube from each cow was placed immediately in a water bath (32°C for 2 h after collection). This was done to hasten clot retraction so that adequate serum yields could be obtained with minimum exposure of the serum to the clot. It also was important to limit clot exposure to serum to minimize artificial elevation of P. After 2 h, the tube was removed from the water bath and centrifuged at 3000 x *g* for 20 min. Serum was harvested and divided into four equal parts in 1.5-ml plastic microcentrifuge tubes and immediately frozen at –20°C. This serum later was used for analyses of serum P, Ca and Mg. After collection, the other two evacuated glass tubes were

placed immediately on ice and allowed to clot at 10°C for 1 h. At this time, the tubes were centrifuged at 3000 x *g* for 20 min. The serum from one tube was divided into four equal parts in 1.5-ml plastic microcentrifuge tubes and immediately frozen at –80°C for analyses of serum osteocalcin (**OC**) and deoxypyridinoline (**DPD**). After collection, the tube with Li-heparin was placed immediately on ice, and plasma was analyzed within 30 min after collection using a Stat Profile 4 blood gas and mineral element analyzer (Nova Biomedical, Walthman, MA) to determine pH, pCO_2, hematocrit and anion gap and concentrations of base excess (**BEB**), HCO_3^-, ionized Ca (**iCa**), Na, K, and Cl.

Serum OC was determined using a competitive immunoassay (Novocalcin; Quidel Corporation, San Diego, CA). Samples were run in duplicate in nine, 96-well microtiter plates. All samples were randomized without regard to cow number or sampling time. The assay was validated in our laboratory for bovine blood (Appendix 1). The inter- and intra-assay variation was calculated using a pooled serum sample run in duplicate in every plate. The inter-assay variation was 20.0% and the intra-assay variation was 4.7%. Serum DPD was analyzed using a competitive immunoassay (Total DPD; Quidel Corporation, San Diego, CA). Samples were run in duplicate in nine, 96-well microtiter plates. This assay was validated in our laboratory for bovine blood (Appendix 2). The inter- and intra-assay variation was calculated using a pooled serum sample run in duplicate in every plate. The inter-assay variation was 23.6% and the intra-assay variation was 8.1%.

Additionally, 125 μl of serum were deproteinized with 875 μl of 20% trichloroacetic acid and analyzed for Ca, Mg, and P concentrations. Calcium and Mg concentrations were analyzed by a flame atomic absorption spectrometer (Varian SpectrAA 220; Mulgrave Victoria, Australia) using a certified Mg and Ca reference standard (1 mg/ml) (Fisher Scientific, Chicago, IL). Phosphorus concentrations were determined by colorimetric assay (Friske and Subbarow, 1925) adapted to a microplate reader (SpectraMax 190). Samples were run in 49, 96-well microtiter plates. The inter- and intra-assay variation was calculated using a certified P reference standard (1 mg/ml) run in duplicate in every plate (Spex CertiPrep, Metuchen, NJ). The inter-assay variation was 5.4% and the intra-assay variation was 1.1%.

Milk. Cows were milked twice daily at 0500 and 1500 h. Milk yield was measured at each milking using a Perfection 3000 Boumatic weigh meter system (Boumatic, Madison, WI) and milk samples were taken during the morning and afternoon milkings on d 7, 14, 21, and 28 postpartum using a proportional sampler. Morning and afternoon milk samples were kept separate and approximately 100 ml were transferred into plastic vials and stored at 10°C for no longer than 12 h. These morning and afternoon milk samples were analyzed separately by Michigan DHIA (East Lansing, MI) for fat, protein, lactose, solids not fat (**SNF**), and somatic cell count (**SCC**) concentrations. A weighted milk composition for the daily yield of each cow was computed based on the relative yields of the morning and afternoon milking and the respective composition values from each milking. Energy-corrected milk (**ECM**) was calculated with the

equation: ECM (lb) = 0.3246 x milk yield (lb) + 12.86 x fat yield (lb) + 7.04 x protein yield (lb) (Dairy Records Management Systems, 1999). An additional 5 ml of milk were taken from each milking (morning and afternoon) and frozen (-20°C) for later analysis of P content.

Phosphorus content of milk was analyzed in the following manner. Milk from morning and afternoon samples within day was composited by volume in proportion to milk yield at each milking. Five ml of the composited milk were placed in a 100-ml volumetric flask and ashed with 4 ml of sulfuric acid using a hot plate at 440°C. The samples were evaporated to dryness when the contents of the flasks turned black. To dried samples, 5 ml of hydrogen peroxide were added slowly drop-wise until the solution cleared. This procedure of sequential acid and hydrogen peroxide addition was repeated as needed and samples were considered digested when all black residue disappeared. Digested samples were brought to 100-ml volume with deionized distilled water. Samples were analyzed colorimetrically for P content (Friske and Subbarow, 1925) as adapted to a microplate reader (SpectraMax 190, Sunnyvale, CA). Samples were run in four, 96-well microtiter plates. The inter- and intra-assay variation was calculated using certified P reference standard (1 mg/ml) run in duplicate in every plate (Spex CertiPrep, Metuchen, NJ). The inter-assay variation was 8.2% and the intra-assay variation was 3.7%.

Statistical Analyses

Data were analyzed as a randomized block design with repeated measures using PROC MIXED procedures of SAS (1999). Five different covariate structures were examined for each independent variable. The five covariate structures examined included CS and AR(1) for variables with evenly spaced data and SP(POW), SP(EXP) and SP(GAU) for variables with unevenly spaced data. The SP(POW) structure was determined to be the most appropriate covariate structure for the variables with unevenly spaced data as indicated by Akaike's Information Criterion (**AIC**) and Schwarz's Bayesian Criterion (**SBC**) values (SAS, 1999). The AR(1) structure was determined to be the most appropriate covariate structure for variables with evenly spaced data as indicated by AIC and SBC values (SAS, 1999). The statistical model consisted of treatment, cow, parity and time and all two-way and three-way interactions. The model was: $Y_{ijkl} = \mu + \alpha_i + \beta_j + \chi_k + D_{l(ij)} + (\alpha\beta)_{ij} + (\beta\chi)_{jk} + (\alpha\chi)_{ik} + (\alpha\beta\chi)_{ijk} + \varepsilon_{ijkl}$, where μ = overall mean, α_i = fixed effect of treatment, β_j = fixed effect of parity, χ_k = fixed effect of time, $D_{l(ij)}$ = random effect of cow within the ith treatment and jth parity, $(\alpha\beta)_{ij}$ = interaction between the ith treatment and jth parity, $(\beta\chi)_{jk}$ = interaction between the jth parity and kth time, $(\alpha\chi)_{ik}$ = interaction between the ith treatment and kth time, $(\alpha\beta\chi)_{ijk}$ = interaction among the ith treatment, jth parity and kth time, and ε_{ijkl} = random error. The test term was cow (parity by trt). For analyses of body weight (**BW**) and BCS change, ECM yield and milk composition, the model was similar except without the time variable and interactions with time. Data are presented as least-squares means. Differences among means were declared at $P < 0.05$, and trends were noted ($0.05 < P <$

0.10. The effects of treatments (Trt 1 vs. Trt 2 and Trt 3, Trt 2 vs. Trt 3), day (linear, quadratic and cubic) and treatment by day interactions were compared using orthogonal contrasts.

RESULTS AND DISCUSSION

The presentation is divided into three parts: prepartum (28 actual d prepartum until parturition); peripartum (7 d prepartum through 7 d postpartum); and, postpartum (first 28 d of lactation). Pre-experiment data were collected from 42 and 35 d prior to actual parturition when all cows were fed Trt 2. Least-squares treatment means (pooled across parity and time) for dependent variables in the pre-experiment, prepartum, peripartum, and postpartum periods are in Tables 2, 3, 4, and 5, respectively. The treatment by time interaction plots for each variable are in Appendix C. However, if the three-way interaction was significant (trt by parity by time), the three-way interaction is presented. Additionally, tables presenting probability values from ANOVA for each variable are in Appendix D.

Prepartum Period

Diet Composition, Intake, Body Condition Score and Body Weight Change. The analyzed dietary P concentrations of Trt 1, Trt 2, and Trt 3 were 0.21, 0.31, and 0.44% P, dry basis, respectively (Table 1). Chemical

composition of prepartum dietary treatments was similar except for P concentrations (Table 1).

Pre-experiment DMI tended to be higher for cows on Trt 1 than cows on Trt 2 and 3 ($P = 0.06$; Table 3). However, pre-experiment DMI as a percent of body weight was not different among treatment groups. Because all cows did not calve on their ECD, cows were on treatment diets 28, 30, and 28 d for Trt 1, 2 and 3, respectively (SEM = 2.4).

Overall DMI was 15.5 kg/cow per d (SEM = 0.7) during the 28 d before parturition and was not affected by dietary treatment (Table 4). This result is different from that of Valk and Sebek (1999), in which DMI decreased during the dry period when cows were fed 0.24% compared with 0.28 or 0.34% dietary P. However, in that study cows were fed the same dietary P concentration during the preceding lactation, and this may have affected DMI during the dry period. In the current study, DMI decreased as day of parturition approached ($P < 0.01$; Figure 1). All cows on all treatments consumed approximately 17 kg/cow per d from d 28 until d 13 prepartum. Subsequently, DMI gradually decreased from 17 kg/cow per d at d 13 to 9 kg/cow per d at 1 d prepartum. Prepartum DMI expressed as a percent of BW was not different among treatments (Table 4).

There was a treatment by time interaction on P intake (g/d) pooled across parity ($P < 0.01$; Table 4). Cows on Trt 1 had a more constant P intake (less relative absolute change) during the 28 d prepartum than cows fed Trt 2 and 3 (Trt 1 vs. 2, 3 by day [linear]; $P < 0.01$; Figure 2). Additionally, P intake

decreased more rapidly as parturition neared for cows fed Trt 3 than Trt 2 (Trt 2 vs. 3 by day [linear]; $P < 0.02$; Figure 2).

Originally, Trt 2 was formulated to supply the NRC (2001) dietary P requirement whereas Trt 1 and Trt 3 were to be below and above requirement. According to NRC (2001), a cow weighing 765 kg, consuming 16 kg DM/d, and 250 d into gestation should consume 31 g P/d. Because some dietary ingredients sampled throughout the study had higher P concentrations than originally analyzed, the actual P concentrations of the TMR were slightly higher than originally formulated. Additionally, because cows ate more dry matter than originally predicted (13.8 kg/d; NRC 2001), P consumptions of cows on Trt 1, 2 and 3 actually were 34, 48 and 68 g P/cow per d, respectively (SEM = 2; Table 4). As a result, cows on Trt 1 consumed nearest the NRC (2001) dietary P requirement of 31 g/d.

Pre-experiment BCS was lower for cows on Trt 1 than the average of Trt 2 and 3 ($P < 0.01$; Table 3). This resulted simply from the assignment of cows to treatments in which we did not consider BCS. This resulted in a tendency for cows on Trt 1 to have lower BCS than cows on Trt 2 and Trt 3 ($P = 0.08$; Table 4). During the prepartum period, BCS increased 0.35 units (time effect; $P < 0.01$). However, BCS change across the prepartum period was not different due to treatment or parity. No other results are available regarding the effect of prepartum dietary P concentration on prepartum BCS, however, Wu et al. (2000) and Wu and Satter (2000) reported no effect on BCS of varying dietary P treatments over the entire lactation cycle.

During the pre-experiment period BW was similar among treatment groups ($P > 0.10$; Table 3). The BW change across the prepartum period was not different due to treatment, parity, or time. In other studies, varying dietary P concentrations during the lactation cycle did not affect BW (Wu et al., 2000; Wu and Satter, 2000; Wu et al., 2001). In this experiment, the mean differences among the treatment groups for both BCS and BW most likely were due to assignment. Cows were blocked to treatments according to parity and ECD, but not according to BCS or BW.

Serum Phosphorus and Calcium. Pre-experiment serum P concentrations did not differ among treatment groups and averaged 6.63 mg/dl (SEM = 0.41; Table 3). Prepartum serum P was influenced by treatment with cows on Trt 1 having lower serum P concentrations than the average of cows on Trt 2 and 3 ($P < 0.01$; Table 4). The average prepartum serum P concentration for cows fed 0.21% dietary P was 5.06 mg/dl that is within the normal range for the mature ruminant animal (4 to 8 mg/dl; Goff, 1998). Additionally, cows on Trt 2 had lower serum P concentrations than cows on Trt 3 ($P < 0.01$; Table 4). The sample designated as 28 d before parturition was taken 1 to 3 d after dietary treatments were introduced (Figure 3). These results are similar to those of Kichura et al. (1982) in which cows fed 10 g P/d had lower plasma P concentrations than cows fed 80 g P/d from 4 wk prepartum until parturition. Barton et al. (1987) reported that cows fed 0.7 times maintenance requirement for P prepartum had lower prepartum plasma P concentrations than cows fed at either 1 or 3 times maintenance requirement for dietary P (NRC, 1978 [Table

2A]). In the current study, the main effect of parity tended to influence serum P concentrations with cows in parities 2, 3, and 4+ having serum P concentrations of 6.4, 6.0, and 5.7 mg/dl, respectively (SEM = 0.34; $P = 0.07$). Horst et al. (1978) reported no differences in prepartum plasma P concentrations between nonparetic aged (≥ third parity) and young (≤ second parity) cows.

Overall, prepartum dietary P did not affect prepartum total serum Ca concentrations ($P > 0.10$; Table 4). Barton et al. (1987) also reported no change in prepartum plasma Ca concentrations when cows were fed either 0.7, 1, or 3 times maintenance requirement for dietary P. Similarly, Kichura et al. (1982) reported no change in prepartum plasma Ca concentrations when cows were fed either 10 or 80 g P/d. In the current study, however, cows on Trt 1 maintained a more constant serum Ca concentration from 5 until 1 d prepartum while serum Ca concentrations from cows on Trt 2 and 3 decreased at a greater relative magnitude as parturition approached (Trt 1 vs. 2, 3 by time [cubic]; $P = 0.05$; Figure 4). The main effect of parity influenced serum Ca concentrations where cows in parities 2, 3, and 4+ had total serum Ca concentrations of 9.4, 8.5, and 9.5 mg/dl, respectively (SEM = 0.32; $P < 0.01$). Horst et al. (1978) and Barton et al. (1981) reported no difference in plasma Ca concentrations between aged nonparetic (≥ third parity) and young (≤ second parity) cows. However, aged paretic cows had lower plasma Ca concentrations as compared with aged nonparetic and young cows in both studies.

Blood Mineral Elements. Among all cows and across all days, prepartum serum Mg concentrations averaged 1.89 mg/dl (SEM = 0.05; Table 4).

Concentrations were not altered by dietary treatment, parity, or time ($P > 0.10$). Similarly, Barton et al. (1987) reported that prepartum dietary P intake of 0.7, 1, or 3 times maintenance requirement (NRC, 1978) for dietary P had no effect on prepartum plasma Mg concentrations.

There was a tendency for a three-way interaction of parity by treatment by time for prepartum plasma iCa ($P = 0.06$). This was due to greater day to day variation in iCa for cows in parity 4+ from d 10 until d 1 prepartum compared with cows in parities 2 and 3. Plasma iCa concentrations were not altered by dietary treatment pooled across parity and time (Figure 5). Though the effect of dietary P concentrations on iCa concentrations have not been reported previously, temporal patterns were similar to those observed by Rodriguez (1998) from 10 d prepartum until parturition when dietary Ca varied.

Plasma Na, K, and Cl concentrations also were evaluated during the prepartum period, however the numerical differences among treatments were very small (Table 4).

Plasma Acid-Base Variables. Plasma variables of pH, BEB, pCO_2, HCO_3, and anion gap were evaluated during the prepartum period (Table 4). Although there were a few treatment and treatment by time interactions among these variables, the overall differences were small.

There was a three-way interaction of parity by treatment by time on prepartum plasma hematocrit ($P < 0.01$). Cows in both parities 2 and 3 responded similarly to treatments across time. However, cows in parity 4+ had

greater day to day variation compared with cows in parities 2 and 3 especially from 20 to 1 d prepartum.

Serum Osteocalcin and Deoxypyridinoline. Osteocalcin is a biological bone marker that is in an indicator of bone formation. There was a tendency for cows on Trt 1 to have lower serum OC concentrations than cows on Trt 2 and 3 ($P = 0.06$; Table 4). There also was a tendency for cows on Trt 2 to have higher serum OC concentrations than cows on Trt 3 pooled across parity and time ($P = 0.07$; Table 4). There was no Trt 1 vs. 2, 3 by time interaction for prepartum serum OC concentration. However, the response of serum OC concentrations across sampling time did not have the same pattern (Trt 2 vs. 3 by time [quadratic]; $P < 0.05$; Figure 6). Corlett and Care (1988) and Scott et al. (1994) reported that decreasing dietary P concentrations were accompanied by a decrease in plasma OC concentrations in sheep. Naito et al. (1990) characterized temporal patterns of prepartum plasma OC in multiparous Holstein cows and found that plasma OC concentrations decreased from 22 ng/ml at 5 d prepartum to 16 ng/ml at parturition. Results of our study are similar. Finally in our study, there was a parity by time interaction pooled across dietary treatment ($P < 0.05$). Serum OC concentrations decreased over time for cows in parities 2 and 3. However, serum OC concentrations for cows in parity 4+ increased from 3 to 2 d prepartum. Results from van Mosel and Corlett (1990) showed that cows in first or second parities had higher plasma OC concentrations from 42 d prepartum until parturition than cows in third or greater parity.

Deoxypyridinoline is a biological bone marker that is an indicator of bone resorption. There were no effects of dietary treatment, parity or time on prepartum serum DPD concentrations (overall mean ± SEM = 2.68 ± 0.21 ng/ml; Table 4). Prepartum DPD concentrations plotted across time are shown in Figure 7. To date, there are no results in the literature on serum DPD concentrations in peripartum dairy cows. However, Scott et al. (1994) reported that neither 0.09 nor 0.27% dietary P fed to lambs altered urinary DPD concentrations.

Periparturient Period

In the current study, the peripartum period was defined as 7 d prepartum through 7 d postpartum. Results presented in this section are for only this time frame and statistical analyses were run accordingly.

Dry Matter Intake. Peripartum DMI was not influenced by the main effects of prepartum treatment or parity (Table 5). There was an effect of time ($P < 0.01$)

Serum Phosphorus and Calcium. Serum P concentrations were not influenced by the main effect of treatment ($P > 0.10$; Table 5). However, there was an interaction of dietary treatment by time. Cows on Trt 1 had the lowest serum P concentrations prepartum and the highest serum P concentrations postpartum, whereas serum P concentrations of cows fed Trt 2 and 3 followed more similar patterns across the peripartum period (1 vs. 2, 3 by time [cubic]; $P <$

0.01; Figure 3). Serum P concentrations of cows fed Trt 1 prepartum climbed above concentrations of cows on Trt 2 and 3 by 3 d postpartum and remained higher until d 20 postpartum. Barton et al. (1987) reported that cows fed 0.7 times P maintenance requirement (NRC, 1978) from 28 d prepartum until parturition had lower plasma P concentrations from 7 d prepartum until 1 d postpartum compared with cows fed at 1 or 3 times P maintenance requirement. Kichura et al. (1982) reported that cows fed 10 g P/d prepartum had lower plasma P concentrations from 4 d prepartum until 2 d postpartum compared with cows fed 80 g P/d.

Dietary treatments did not affect serum Ca concentrations pooled across parity and time ($P > 0.10$). Cows on Trt 1 maintained more constant serum Ca concentrations from 5 until 1 d prepartum whereas serum Ca concentrations of cows on Trt 2 and 3 decreased at a greater relative magnitude as parturition neared (Trt 1 vs. 2, 3 by time [quadratic]; $P < 0.05$; Figure 4). In addition, cows on Trt 2 had higher serum Ca concentrations than those on Trt 3 from 7 d prepartum until about 1 d postpartum; from 1 through 5 d postpartum Ca concentrations were similar (Trt 2 vs. 3 by time [cubic]; $P < 0.05$; Figure 4). Kichura et al. (1982) reported similar results with cows fed 10 g P/d having higher plasma Ca concentrations from 1 d prepartum through 4 d postpartum compared with cows fed 80 g P/d. Additionally, Barton et al. (1987) reported higher plasma Ca concentrations from 3 to 5 d postpartum for cows fed 0.7 times P maintenance requirement (NRC, 1978) as compared with cows fed at either 1 or 3 times maintenance requirement for dietary P. Barton et al. (1987) concluded

that the increase in plasma Ca when cows were fed 0.7 times maintenance requirement for P could have been due to enhanced intestinal absorption of Ca by a vitamin D-mediated transport mechanism. In our experiment, parity influenced serum Ca concentrations pooled across treatment and time. Cows in parities 2, 3, and 4+ had serum Ca concentrations of 9.0, 7.9, and 9.0 mg/dl, respectively (SEM = 0.32; $P < 0.05$). However, Horst et al. (1978) reported similar plasma Ca concentrations among aged nonparetic (≥ third parity) and young (≤ second parity) cows from 2 d prepartum until 2 d postpartum.

Hypophosphatemia is defined as abnormally low concentrations of phosphates in the circulating blood (Stedman's Medical Dictionary, 1961). Chronic hypophosphatemia is defined as plasma P_i between 2 and 3.5 mg/dl and acute hypophosphatemia is defined as plasma P_i below 2 mg/dl (Goff, 1998). Recumbency and paresis are associated with concentrations of plasma P of less than 1 mg/dl (Goff, 1998). In this experiment, neither recumbency nor paresis was observed. To calculate incidence rates of hypophosphatemia, hypophosphatemia was defined as serum P concentrations below 3.5 mg/dl (T. H. Herdt, personal communication). Incidence rates of hypophosphatemia immediately after parturition (0 h sample) were 64, 38, and 25% for cows on Trt 1, 2, and 3, respectively. Cows on Trt 1 had a higher incidence rate of hypophosphatemia by this definition than cows on Trt 2 or 3 immediately after parturition ($P < 0.04$). Six hours after parturition the incidence rates of hypophosphatemia for cows on Trt 1, 2, and 3 were 50, 15, and 17% (Trt 1 vs. 2 or 3; $P < 0.01$). Even though cows on Trt 1 had a higher incidence rate of

hypophosphatemia as defined, no cows exhibited recumbency, paresis, or acute hypophosphatemia (less than 2 mg/dl; Goff, 1998). Nonetheless, the number of cows with 0 h serum P concentrations of less than 2 mg/dl were 3, 1, and 3 for Trt 1, 2, and 3, respectively.

Hypocalcemia was defined as either total serum Ca concentrations below 8 mg/dl or plasma iCa concentrations less than 4 mg/dl (T. H. Herdt, personal communication). There was a tendency for cows on Trt 1 to have a lower incidence rate of hypocalcemia than cows on Trt 2 or 3 at parturition (0 h) when total serum Ca concentrations were evaluated ($P = 0.10$). Cows on Trt 1, 2, and 3 had incidence rates of 50, 69 and 75%. However, when plasma iCa concentrations were evaluated, dietary treatments did not affect incidence rates of hypocalcemia either at parturition (0 h) or 6 h postpartum ($P > 0.10$). We conclude that either 0.31 or 0.44% P fed 28 d prepartum until parturition may increase the incidence of hypocalcemia at parturition compared with feeding 0.21% P.

Blood Mineral Elements. Serum Mg concentrations were not influenced by dietary treatment from 7 d prepartum through 7 d postpartum pooled across parity and time (Table 5). Serum Mg concentrations remained fairly constant at an average of 1.86 mg/dl from 7 d prepartum until parturition for all cows on all treatments. After parturition, serum Mg concentrations decreased gradually until they reached 1.55 mg/dl 4 d postpartum and began to increase to an average value of 1.7 mg/dl by 7 d postpartum for all cows on all treatments. Barton et al.

(1987) reported no effect of dietary P concentrations on peripartum plasma Mg concentrations.

There was a three-way interaction of parity by treatment by time for plasma iCa ($P < 0.05$). Cows in parities 2 and 3 followed very similar patterns from 7 d prepartum through 7 d postpartum. Plasma iCa of cows in parity 4+ however, was more variable from 1 d prepartum until 3 d postpartum when compared with cows in parities 2 and 3. Temporal patterns during the periparturient period are similar to those reported by Rodriguez (1998) for multiparous Holstein cows fed varying dietary Ca concentrations.

Plasma Cl, Na, and K concentrations also were evaluated. However, there was no effect of treatment on these variables (Table 5).

Plasma Acid-Base Variables. Plasma variables of pH, BEB, pCO_2, HCO_3, anion gap, and hematocrit were evaluated during the peripartum period. However, no effect of treatment was observed for these variables (Table 5).

Postpartum Results

Intake, Body Condition Score and Body Weight Change. There was a three-way interaction of parity by treatment by time on postpartum DMI ($P < 0.04$; Figure 8). On average, cows in parity 3 had a higher DMI than cows in either parity 2 or 4+. In addition, cows in parities 3 and 4+ on Trt 1 had lower DMI from parturition until 7 d postpartum whereas cows on Trt 3 in parity 2 had lower overall DMI. There was more variation in DMI of cows in parities 3 and 4+ as

compared with cows in parity 2. Additionally, treatment did not alter DMI when expressed as a % of BW (Table 6). When varying dietary P concentrations were fed over the entire lactation cycle, no differences in DMI were detected (Wu et al., 2000; Wu and Satter, 2000; Wu et al., 2001).

There was no effect of treatment, parity or time on BCS or BW change over the 28 d after parturition (Table 6). Feeding different P concentrations over entire lactation cycles did not affect BCS or BW change (Wu et al., 2000; Wu and Satter, 2000; Wu et al., 2001).

Serum Phosphorus and Calcium. There was a treatment by time interaction for postparum serum P concentrations. Serum P concentrations of cows fed Trt 1 prepartum increased to greater magnitude during the 6 d postpartum compared with those of cows fed Trt 2 or 3 (Trt 1 vs. 2, 3 by day [quadratic]; $P < 0.01$; Figure 3). Kichura et al. (1982) reported lower plasma P concentrations from parturition until 2 d postpartum when cows were fed 10 g P/d as compared with 80 g P/d from 4 wk prepartum until parturition. However, Barton et al. (1987) reported no effect of prepartum dietary P concentrations on plasma P concentrations from 1 d through 7 d postpartum. In our experiment, there was a parity by time interaction pooled across dietary treatments ($P < 0.01$; Figure 9). Cows in parities 2 and 3 had similar serum P concentrations during the 28 d after parturition. However, while cows in parities 2 and 3 had increasing serum P concentrations from 7 through 28 d postpartum, serum P concentrations decreased from 6.2 to 4.9 mg/dl from 14 to 21 d postpartum for cows in parity 4+.

Dietary treatment did not affect postpartum serum Ca concentrations pooled across parity and time (Table 6). There was a interaction of treatment by time where cows on Trt 1 responded differently than cows on Trt 2 or 3 (Trt 1 vs. 2, 3 by day [quadratic]; $P < 0.05$; Figure 4). Note that the magnitude of difference for postpartum serum Ca concentrations from 6 to 28 d postpartum was small. In a related study, prepartum dietary P concentrations did alter postpartum Ca concentrations from 3 to 5 d postpartum where cows fed 0.7 times maintenance requirement for dietary P had higher plasma Ca concentrations compared with cows fed at 1 or 3 times maintenance requirement of dietary P (Barton et al., 1987).

Blood Mineral Elements. Serum Mg concentrations were not influenced by dietary treatment pooled across parity and time (Table 6). However, there was a tendency for an interaction of dietary treatment by time. Cows on Trt 2 and 3 had similar serum Mg concentrations at 4 d postparum, however, cows on Trt 3 increased at a greater rate than those on Trt 2 (Trt 2 vs. 3 by time [linear]; $P = 0.07$). However, the magnitude of the difference is only about 0.2 mg/dl. Barton et al. (1987) reported no difference in plasma Mg concentrations from parturition through 7 d postpartum when cows were fed 0.7, 1, or 3 times maintenance requirement for dietary P.

Plasma iCa, Na, K, and Cl concentrations also were evaluated during the postpartum period, however the numerical differences among treatment groups were small (Table 6).

Plasma Acid-Base Variables. Plasma variables of pH, BEB, pCO_2, HCO_3, and anion gap were evaluated during the prepartum period (Table 6). Overall differences among treatment means were small.

Serum Osteocalcin and Deoxypyridinoline. There was a three-way interaction of parity by treatment by time on postpartum OC concentration ($P < 0.05$; Figure 10). At parturition (0 d), cows in parities 2 and 3 had similar serum OC concentrations across the three treatments. However, cows in parity 4+ had increasing serum OC concentrations from Trt 1, 2 and 3 at parturition. Serum OC concentrations were similar on 1, 2, and 3 d postpartum. However, at 14 d postpartum serum OC concentrations ranked differently among treatments in the different parity categories. Results of van Mosel and Corlett (1990) indicated that young cows (first and second parities) had higher plasma OC concentrations from parturition until 7 d postpartum as compared with older cows (≥ third parity). Overall, our results do not support their findings. Naito et al. (1990) reported a gradual increase in plasma OC concentration from 8 ng/ml at 1 d postpartum to 17 ng/ml at 15 d postpartum in multiparous Holstein cows. Our results show a similar increase from 1 d to 14 d postpartum. However, our OC concentrations are nearly twice as high. Farrugia et al. (1989) reported an increase in plasma OC concentrations from parturition through 24 d of lactation in aged Merino ewes. Our data with cows, that of Naito et al. (1990) with cows and that of Farrugia et al. (1989) with ewes indicate that bone formation is depressed as parturition nears and increases after the onset of lactation. This could be due to changes in P and Ca homeostasis as a result of fetal demands, colostrum

production, and changes in DMI. The effects of dietary P on plasma OC concentrations were evaluated previously, though not in the postpartum dairy cow. Scott et al. (1994) showed that sheep fed a 0.09% P diet had lower plasma OC concentrations than those fed 0.27% dietary P. Also, Corlett and Care (1988) reported that plasma OC concentrations were reduced by 50% when sheep were fed 0.04% dietary P in contrast to 0.17% dietary P for 1 mo.

Postpartum serum DPD concentrations averaged 3.2 ng/ml across all cows on all treatments (SEM = 0.3; Table 6). Dietary treatment did not alter serum DPD concentrations pooled across parity and time. Postpartum DPD concentrations across time are shown in Figure 7. There was a tendency for an effect of parity pooled across dietary treatments and time. Cows in parities 2, 3, and 4+ had serum DPD concentrations of 3.7, 2.9, and 3.0 ng/ml, respectively (SEM = 0.30; $P = 0.09$). Though similar results are not present in the literature, Liesegang et al. (1998) characterized postpartum urinary DPD concentrations in multiparous cows from parturition until 14 d postpartum. They noted a gradual increase in urinary DPD concentration from d 1 through 9 postpartum. Additionally, Liesegang et al. (2000a) reported an increase in urinary DPD concentration from 14 d prepartum until 14 d postpartum in multiparous Brown Swiss cows. Urinary DPD concentrations decreased gradually from 14 d through 150 d postpartum.

Energy-Corrected Milk Yield and Milk Components. Average ECM yield (52.9 kg/d) was not affected by prepartum dietary P concentrations pooled across parity and time (Table 7). Milk somatic cell count was altered by

treatment with cows on Trt 1 prepartum having higher SCC than cows on Trt 2 or 3 ($P < 0.01$; Table 7). Additionally, cows on Trt 2 had higher SCC than cows on Trt 3 ($P < 0.04$; Table 7).

As a result of the different SCC among treatment groups, we included SCC in the statistical model as a covariate to account for any potential influence of SCC on ECM, fat, protein, lactose and SNF yields. Interpretation of results of fat, protein, lactose and SNF yields was not altered when SCC was included in the statistical model as a covariate. Therefore, results of the analysis of variance without SCC as a covariate are shown in Table 7.

Milk P concentrations were altered by the main effect of treatment ($P < 0.05$; Table 7). Cows on Trt 1 had higher milk P concentrations than cows on Trt 2 and 3 ($P < 0.01$). In other studies, milk P concentrations were not affected by varying dietary P concentrations (Forar et al., 1982; Call et al., 1987; Morse et al., 1992; Brintrup et al., 1993; Wu et al., 2000; Wu et al., 2001).

The main effect of treatment did not change milk fat percentage or yield (Table 7). When diets with different dietary P concentrations were fed during lactation, no effect on milk fat percentage was observed (Call et al., 1987; Wu and Satter, 2000; Wu et al., 2001; Wu et al., 2000). Additionally, when varying dietary P concentrations were fed over an entire lactation, no change in milk fat yield was reported (Wu and Satter, 1999). However, Wu et al. (2000) reported a decrease in milk fat yield when cows were fed 0.31% P during lactation compared with cows fed 0.40 or 0.49% P.

The average milk protein percentage across all cows on all treatments was 3.05% (SEM = 0.07; Table 7). Wu et al. (2001; 2000) fed varying amounts of dietary P during lactation and reported no change in milk protein percentage across lactation. However, feeding 0.24% dietary P during lactation decreased milk protein percentage compared with that of cows fed 0.32 or 0.42% dietary P (Call et al., 1987). Neither milk lactose nor SNF percentage or yield was affected by dietary treatment (Table 7).

CONCLUSIONS

Feeding 34 g of P/d (0.21% P in this experiment) during the last 4 wk of gestation is adequate for the multiparous Holstein cow resulting in no adverse effects on P and Ca metabolism or ECM yield (through 28 DIM). Feeding 0.44% P during the 28 d prepartum depressed total serum Ca concentrations around parturition compared with feeding either 0.21 or 0.31% P. Further research may be useful to evaluate the effects of prepartum dietary P concentrations between 0.21 and 0.31%.

Table 1. Ingredient composition and analyzed chemical composition[1] of prepartum dietary treatments with different P concentrations (% of dietary DM).

	Treatments[2]		
	1	2	3
Ingredients			
Alfalfa silage	17.5	17.5	17.5
Corn silage	27.0	27.0	27.0
Beet pulp pellets	27.0	27.0	27.0
Corn starch	9.14	9.14	9.14
Corn, ground	9.01	9.01	9.01
Mineral-vitamin mix[3]	3.28	3.28	3.28
Supplement[4]	2.11	2.11	2.11
Rice hulls	1.81	1.29	0.90
Blood meal	1.08	1.08	1.08
Biuret™ [5]	0.86	0.86	0.86
Urea	0.77	0.65	0.52
Ammonium chloride	0.39	0.39	0.39
Monoammonium phosphate	0.00	0.52	1.03
Chemical composition			
CP	15.0	15.1	14.5
ADF	25.3	24.9	24.8
NDF	38.6	38.4	38.1
Ca	0.78	0.80	0.79
P	0.21	0.31	0.44
Mg	0.36	0.37	0.37
K	0.93	0.97	0.96
Na	0.07	0.07	0.07
Cl	0.49	0.48	0.47
S	0.24	0.25	0.25
DCAD[6]	-2.00	-1.30	-1.30
NE_L[7]	1.58	1.58	1.58

[1]Each individual diet ingredient was sampled every other week, dried (60 °C), ground through a 2 mm screen and composited across the entire prepartum period.

[2]Dietary treatments: 1 = 0.21%; 2 = 0.31%; and 3 = 0.44% P, respectively.

[3]Composition (DM basis): soybean hulls = 91.4%; magnesium sulfate = 8%; manganese sulfate = 0.14%; zinc sulfate = 0.11%; copper sulfate = 0.06%; Se 0.99% = 0.06%; cobalt sulfate = 0.001%; ethylenediaminodihydroiodide (EDDI) = 0.001%; vitamin A, 488,000 IU/kg; vitamin D_3, 71,000 IU/kg; vitamin E, 2490 IU/kg.

[4]Supplement = SoyChlor 16-7™, West Central® Soy, Ralston, IA.

[5]Biuret™, Moorman's Manufacturing Company, Quincy, IL.

[6]DCAD = meq[(Na + K) - (Cl + S)]/100 g of dietary DM.

[7]NE_L (Mcal/kg of DM) = 0.0245 x TDN (% of DM) - 0.12; (NRC, 1989).

Table 2. Ingredient and chemical composition of postpartum diet.

Ingredient	% of DM
Corn silage	21.9
Alfalfa silage	21.5
Corn, high moisture	13.8
Mineral-vitamin mix	13.2
Soybean meal	9.41
Corn distillers grains	5.92
Protein supplement[1]	4.98
Beet pulp pellets	4.90
Liquid feed[2]	4.44
Chemical composition	
CP	18.7
ADF	17.1
NDF	30.0
Ca	0.80
P	0.40
Mg	0.25
K	1.19
Na	0.29
Cl	0.41
S	0.21
NE_L[3]	1.67

[1]Composition (DM basis): CP = 39.6%, Ca = 0.94%, P = 0.78%, Mg = 0.91%, K = 0.58%, Na = 0.95%, Cl = 0.30%, S = 0.15%, Se = 2 mg/kg, vitamin A = 11,000 IU/kg, vitamin D = 2,000 IU/kg, vitamin E = 40 IU/kg.

[2]Composition (DM basis): TDN = 89.4%, NE_L = 1.98 Mcal/lb, CP 30.3%, Ca = 0.9%, P = 1.2%, K = 3.0%, Mg = 0.5% and microminerals and vitamins.

[3]NE_L (Mcal/kg of DM) = 0.0245 x TDN (% of DM) - 0.12; (NRC, 1989).

Table 3. Least-squares means and orthogonal contrasts by prepartum dietary treatment assignment for pre-experiment variables (during 35 and 42 d before expected calving date; standardization period).

	Treatments[1]				Treatment	
Variable[2]	1	2	3	SE	1 vs. 2, 3	2 vs. 3
					P <	
DMI, kg/d	17.8	16.5	15.9	0.65	0.06	NS[3]
DMI, % BW	2.17	2.21	2.11	0.12	NS	NS
BCS	3.21	3.63	3.60	0.09	0.01	0.01
BW, kg	729	721	753	14.6	NS	NS
Serum variables						
Serum P, mg/dl	6.50	6.74	6.66	0.41	NS	NS
Serum Ca, mg/dl	9.87	9.40	9.51	0.37	NS	NS
Serum Mg, mg/dl	1.89	1.81	1.89	0.08	NS	NS
Plasma variables						
Ionized Ca, mg/dl	5.53	5.35	5.52	0.08	NS	NS
Na, mg/L	3306	3300	3301	9.99	NS	NS
K, mg/L	174	179	172	2.43	NS	NS
Cl, mg/L	3661	3655	3648	19.8	NS	NS
pH	7.41	7.42	7.42	0.01	NS	NS
BEB[4], mmol/L	3.29	3.12	3.13	0.42	NS	NS
pCO_2, mmHg	43.8	41.2	42.5	1.59	NS	NS
HCO_3, mmol/L	27.8	27.0	27.6	0.77	NS	NS
Anion gap, mM	17.1	18.2	17.4	0.55	NS	NS
Hematocrit, %	30.4	33.8	30.6	2.12	NS	NS

[1]Dietary treatments: 1 = 0.21; 2 = 0.31; 3 = 0.44% P, respectively.

[2]The means and SE for all variables are two single time points (35 d and 42 d). Both time points were used in statistical analysis.

[3]NS = not significant ($P > 0.10$).

[4]BEB = base excess.

Table 4. Least-squares means and orthogonal contrasts for treatment and treatment by day interactions for prepartum variables (28 through 1 d prepartum).

Variable	Treatments[1]			SE	Treatment		Treatment by day[2]	
	1	2	3		1 vs. 2, 3	2 vs. 3	1 vs. 2,3	2 vs. 3
					P <			
DMI, kg/d	16.0	15.4	15.2	0.70	NS[3]	NS	NS	NS
DMI, % BW	2.07	2.08	2.01	0.09	NS	NS	NS	NS
P intake, g/d	33.7	47.7	66.7	2.00	0.01	0.01	0.01, L	0.02, L
BCS	3.52	3.77	3.74	0.11	0.08	NS	NS	NS
BCS change	+0.20	+0.05	+0.07	0.11	NS	NS	NS	NS
BW, kg	756	750	785	16.6	NS	NS	NS	NS
BW change, kg	+25.1	+22.3	+26.4	4.56	NS	NS	NS	NS
Serum minerals								
Serum P, mg/dl	5.06	6.41	6.60	0.24	0.01	0.01	NS	NS
Serum Ca, mg/dl	9.18	9.35	9.04	0.32	NS	NS	0.05, C	NS
Serum Mg, mg/dl	1.88	1.88	1.93	0.05	NS	NS	0.10, Q	NS
Plasma variables								
Ionized Ca, mg/dl	5.47	5.41	5.37	0.04	NS	NS	0.01, L	0.06, C
Na, mg/L	3338	3359	3352	6.38	0.03	0.03	NS	NS
K, mg/L	173	175	172	1.86	NS	NS	0.01, L	0.03, L
Cl, mg/L	3727	3693	3730	15.4	NS	NS	NS	NS
pH	7.44	7.43	7.44	0.01	NS	0.09	0.05, C	NS
BEB[4], mmol/L	4.07	4.75	3.67	0.42	NS	NS	NS	NS
pCO_2, mmHg	41.0	43.7	41.0	0.99	NS	0.07	NS	NS
HCO_3, mmol/L	27.8	28.8	27.5	0.49	NS	NS	NS	0.06, Q
Anion gap, mM	16.7	17.7	17.6	0.27	0.02	0.01	NS	NS
Hematocrit, %	30.3	31.1	30.8	0.33	NS	NS	0.01, C	0.01, C
Serum bone markers								
Serum OC[5], ng/ml	22.3	28.8	27.4	2.44	0.06	0.07	NS	0.02, Q
Serum DPD[6], ng/ml	2.57	2.73	2.75	0.21	NS	NS	NS	NS

[1]Dietary treatments: 1 = 0.21; 2 = 0.31; 3 = 0.44% P, respectively.

[2]L = linear, Q = quadratic, C = cubic effects of time.

[3]NS = not significant ($P > 0.10$).

[4]BEB = base excess.

[5]OC = osteocalcin.

[6]DPD = deoxypyridinoline.

Table 5. Least-squares means and orthogonal contrasts for treatment and treatment by day interactions for peripartum dry matter intake and blood variables (7 d prepartum through 7 d postpartum).

	Treatments[1]				Treatment		Treatment by day[2]	
Variable	1	2	3	SE	1 vs. 2, 3	2 vs. 3	1 vs. 2,3	2 vs. 3
						P <		
DMI, kg/d	13.4	14.3	13.7	0.97	NS[3]	NS	NS	NS
Serum minerals								
Serum P, mg/dl	5.71	6.02	6.24	0.26	NS	NS	0.01, C	NS
Serum Ca, mg/dl	8.68	8.91	8.40	0.32	NS	NS	0.02, Q	0.03, C
Serum Mg, mg/dl	1.74	1.77	1.81	0.06	NS	NS	NS	NS
Plasma variables								
Ionized Ca, mg/dl	5.13	5.08	5.08	0.06	NS	NS	0.03, Q	NS
Na, mg/L	3365	3382	3366	8.30	NS	NS	NS	NS
K, mg/L	174	174	173	1.90	NS	NS	0.04, C	NS
Cl, mg/L	3698	3680	3712	19.5	NS	NS	NS	NS
pH	7.43	7.43	7.44	0.01	NS	NS	0.01, C	NS
BEB[4], mmol/L	5.44	6.09	5.20	0.41	NS	NS	NS	0.02, Q
pCO_2, mmHg	44.1	45.7	42.5	1.04	NS	NS	0.06, C	0.02, Q
HCO_3, mmol/L	29.6	30.4	28.9	0.50	NS	NS	NS	0.02, Q
Hematocrit, %	30.5	31.1	30.5	0.35	NS	NS	0.02, C	0.01, Q
Anion gap, mM	17.1	17.3	16.9	0.37	NS	NS	0.04, Q	0.07, L

[1]Dietary treatments: 1 = 0.21; 2 = 0.31; 3 = 0.44% P, respectively.

[2]L = linear, Q = quadratic, C = cubic effects of time.

[3]NS = not significant (P > 0.10).

[4]BEB = base excess.

Table 6. Least-squares means and orthogonal contrasts for treatment and treatment by day interactions for postpartum dry matter intake and blood variables (from parturition through 28 d postpartum).

	Treatments[1]				Treatment		Treatment by day[2]	
Variables	1	2	3	SE	1 vs. 2, 3	2 vs. 3	1 vs. 2, 3	2 vs. 3
					P <			
DMI, kg/d	18.9	19.7	19.9	0.92	NS[3]	NS	NS	NS
DMI, % BW	2.88	3.05	3.00	0.14	NS	NS	NS	NS
BCS	2.99	3.20	3.16	0.39	0.08	0.09	NS	NS
BCS change	-0.11	-0.09	-0.12	0.04	NS	NS	NS	NS
BW, kg	657	665	695	11.0	NS	NS	NS	NS
BCS change, kg	-64.0	-59.0	-47.0	12.4	NS	NS	NS	NS
Serum minerals								
Serum P, mg/dl	6.33	6.09	6.13	0.25	NS	NS	0.01, Q	NS
Serum Ca, mg/dl	8.49	8.83	8.50	0.36	NS	NS	0.04, Q	NS
Serum Mg, mg/dl	1.73	1.73	1.79	0.06	NS	NS	NS	0.07, L
Plasma variables								
Ionized Ca, mg/dl	5.02	5.03	5.06	0.06	NS	NS	0.02, C	NS
Cl, mg/L	3648	3629	3659	17.7	NS	NS	NS	NS
Na, mg/L	3357	3365	3347	8.0	NS	NS	NS	NS
K, mg/L	174	173	174	1.98	NS	NS	NS	NS
pH	7.42	7.42	7.44	0.01	NS	NS	NS	NS
BEB[4], mmol/L	5.95	6.44	6.07	0.44	NS	NS	NS	NS
pCO_2, mmHg	45.8	46.7	44.5	1.01	NS	NS	NS	0.01, L
HCO_3, mmol/L	27.8	28.8	27.5	0.49	NS	NS	NS	NS
Anion gap, mM	17.7	17.4	16.6	0.39	NS	NS	NS	NS
Hematocrit, %	30.3	31.1	30.8	0.33	NS	0.10	0.03, L	0.08, L
Serum bone markers								
Serum OC[5], ng/ml	19.7	20.9	21.4	3.18	NS	NS	NS	0.04, Q
Serum DPD[6], ng/ml	2.96	3.00	3.56	0.30	NS	NS	NS	NS

[1]Dietary treatments: 1 = 0.21; 2 = 0.31; 3 = 0.44% P, respectively.

[2]L = linear, Q = quadratic, C = cubic effects of time.

[3]NS = not significant ($P > 0.10$).

[4]BEB = base excess.

[5]OC = osteocalcin.

[6]DPD = deoxypyridinoline.

Table 7. Least-squares means and orthogonal contrasts for treatment interactions for milk yield and composition variables (from parturition through 28 d postpartum).

	Treatments[1]				Treatment	
Milk variables	1	2	3	SE	1 vs. 2, 3	2 vs. 3
					P <	
ECM yield[2], kg/d	53.4	53.2	52.2	1.49	NS[3]	NS
Adjusted ECM[4], kg/d	54.4	53.2	51.7	1.58	NS	NS
Milk SCC, 1000/ml	1447	592	354	285	0.01	0.04
Milk P, mg/dl	76.9	70.6	65.5	2.25	0.01	0.06
Milk fat, %	5.44	5.32	5.04	0.23	NS	NS
Milk fat, kg/d	2.25	2.29	2.26	0.14	NS	NS
Milk protein, %	3.06	3.11	2.99	0.07	NS	NS
Milk protein, kg/d	1.23	1.29	1.30	0.05	NS	NS
Milk lactose, %	4.65	4.76	4.70	0.05	NS	0.09
Milk lactose, kg/d	1.89	2.00	2.04	0.18	NS	NS
Milk SNF, %	8.61	8.77	8.63	0.09	NS	NS
Milk SNF, kg/d	3.49	3.67	3.75	0.14	NS	NS

[1]Dietary treatments: 1 = 0.21; 2 = 0.31; 3 = 0.44% P, respectively.

[2]ECM yield (lb) = 0.3246 x milk yield (lb) + 12.86 x fat yield (lb) + 7.04 x protein yield (lb) (Dairy Records Management Systems, 1999).

[3]NS = not significant ($P > 0.10$).

[4]Adjusted ECM = ECM yield using SCC as a covariate in the statistical model.

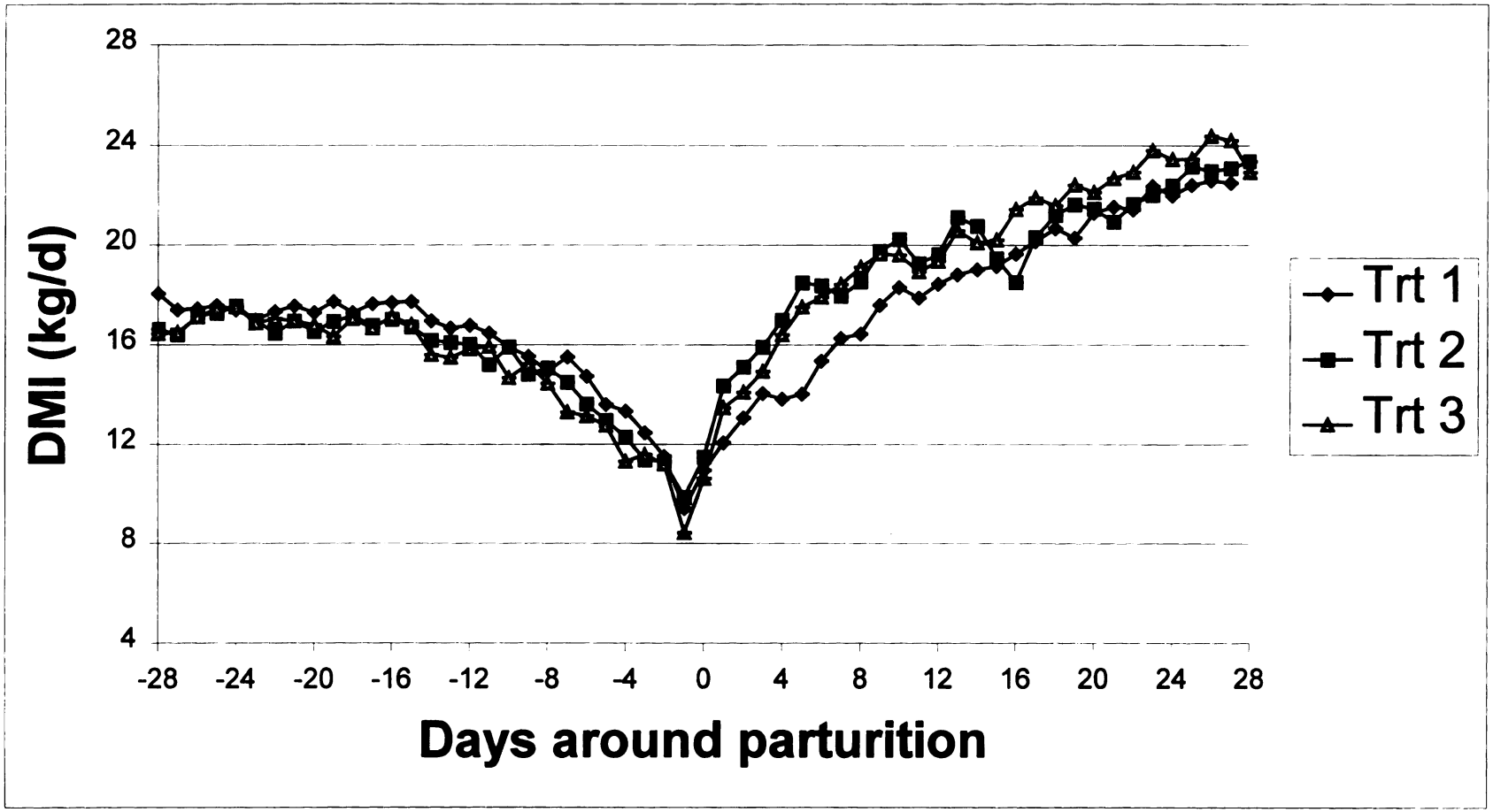


Figure 1. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on DMI from 28 d prepartum through 28 d postpartum (SEM = 1.2).

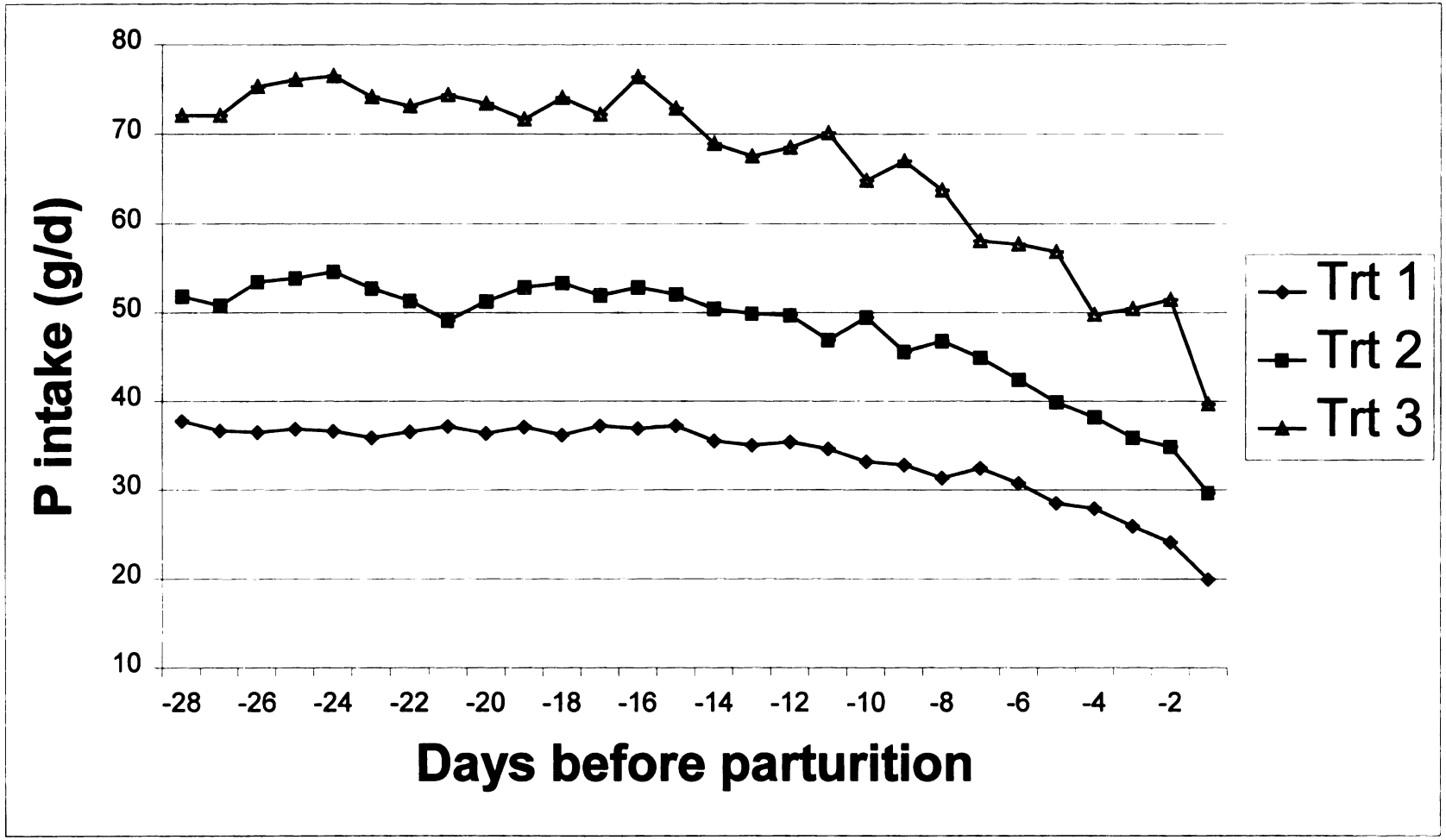


Figure 2. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on P intake during the 28 d before parturition (SEM = 3.0).

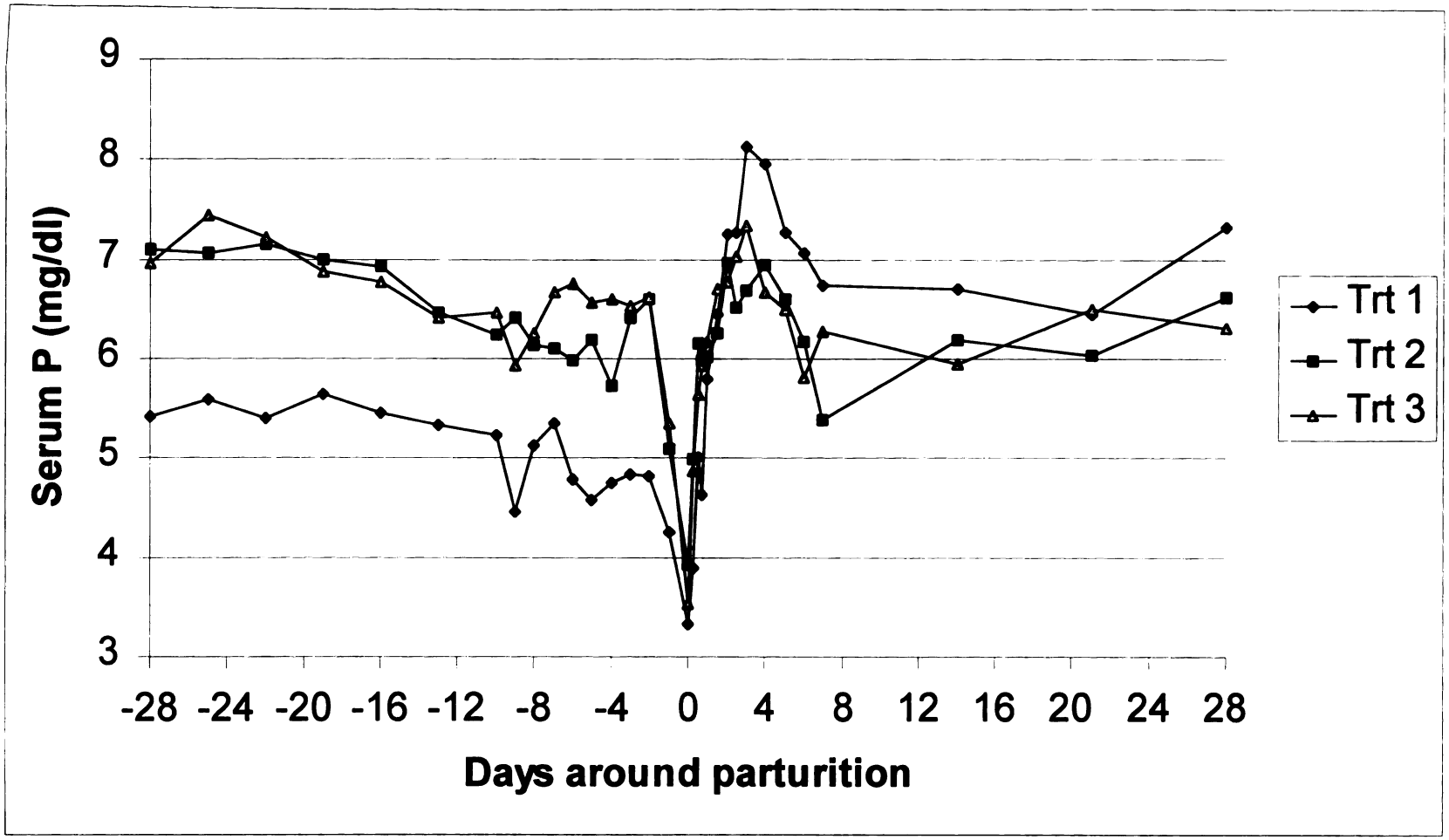


Figure 3. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on serum P concentrations from 28 d prepartum through 28 d postpartum (SEM = 0.38).

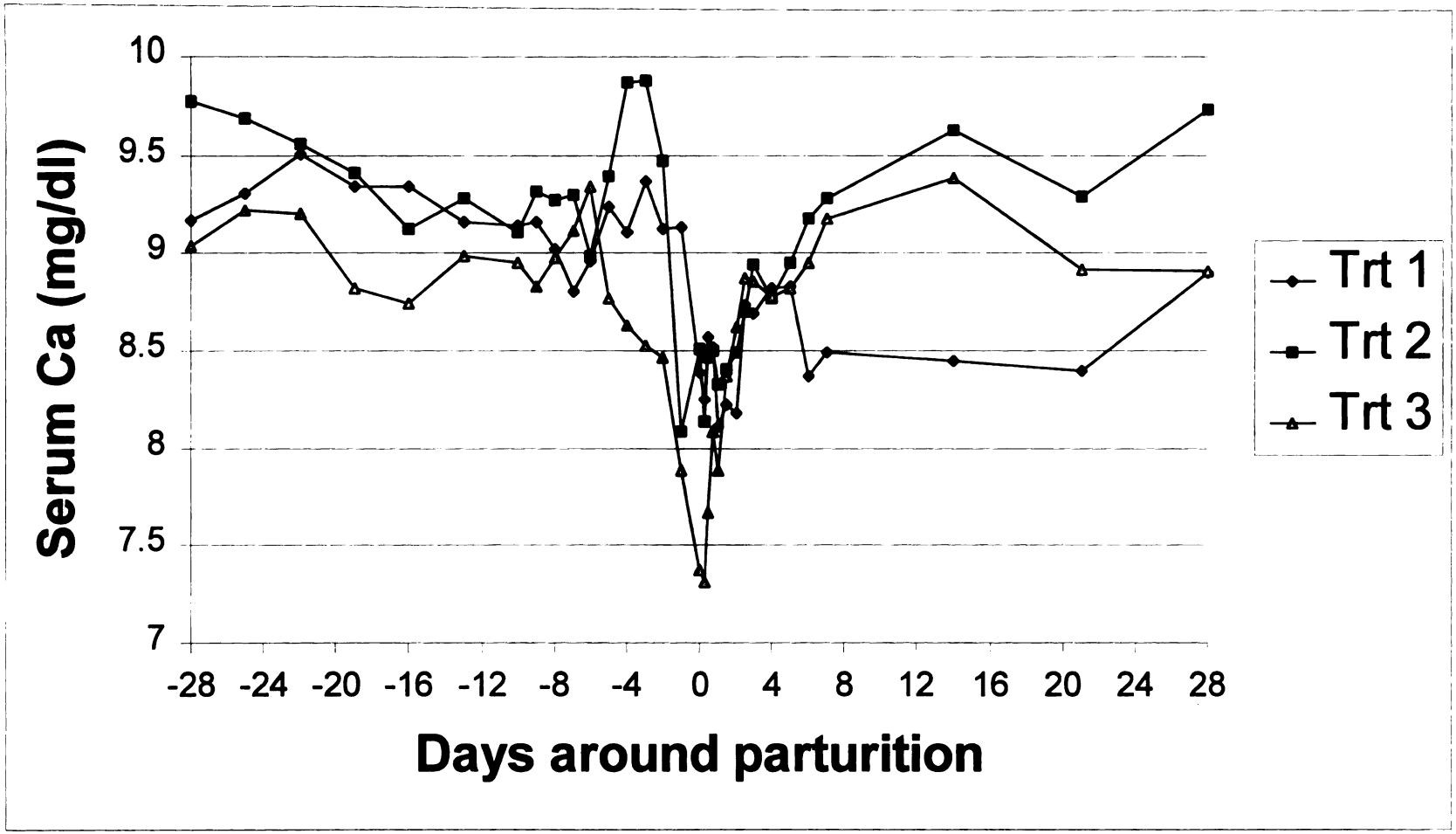


Figure 4. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on total serum Ca concentrations from 28 d prepartum through 28 d postpartum (SEM = 0.49).

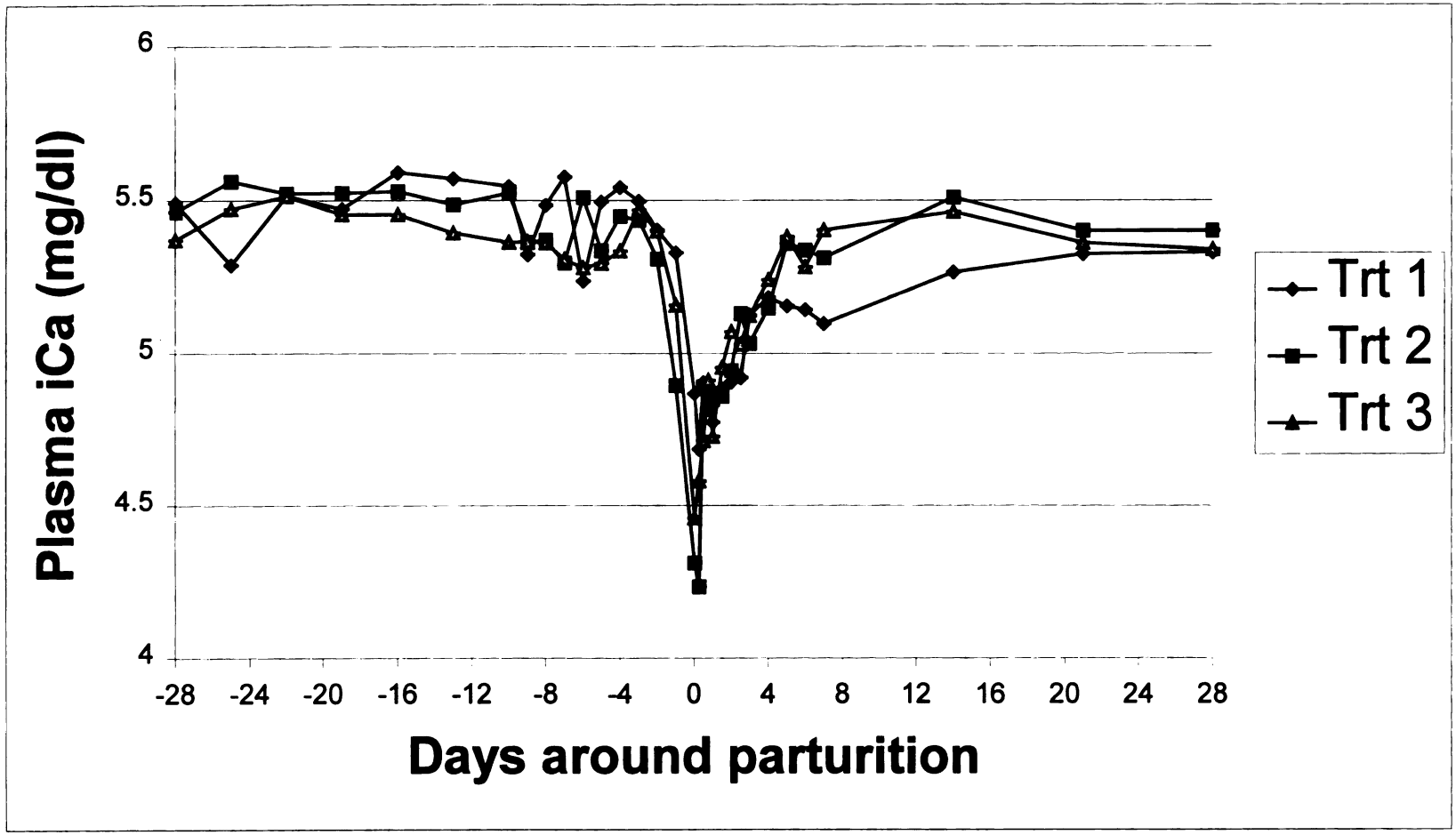


Figure 5. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on plasma ionized Ca concentrations from 28 d prepartum through 28 d postpartum (SEM = 0.07).

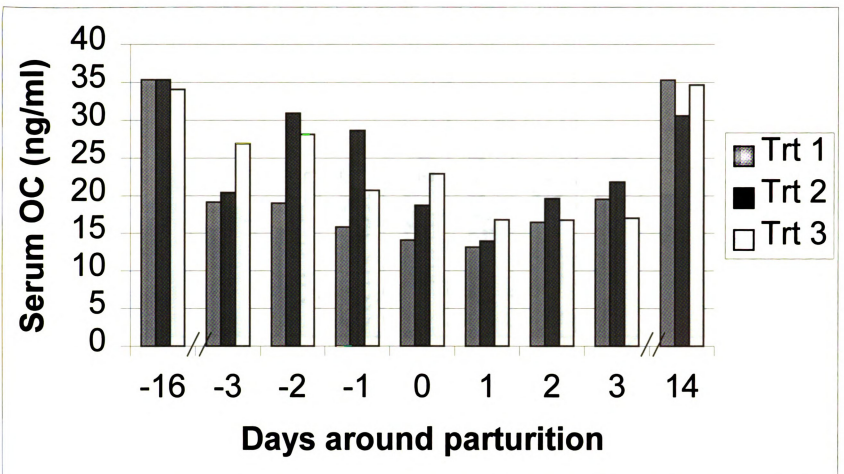


Figure 6. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on serum osteocalcin concentrations from 16 d prepartum through 14 d postpartum (SEM = 3.5).

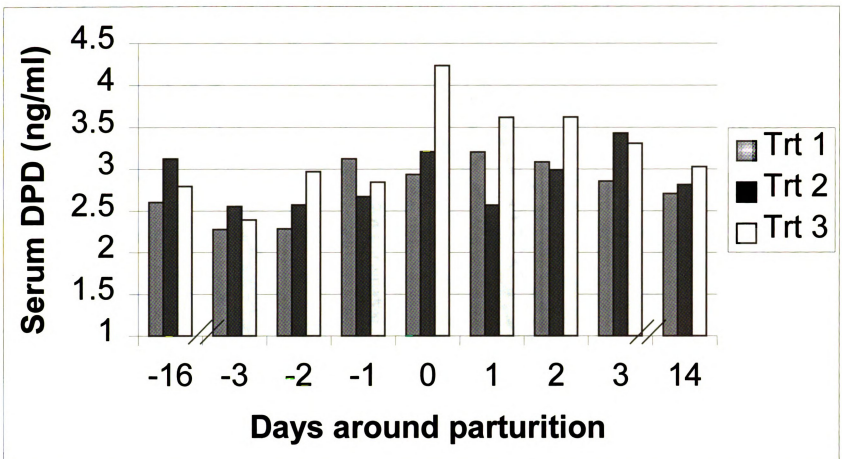


Figure 7. Serum deoxypyridinoline concentrations from 16 d prepartum through 14 d postpartum (SEM = 0.40). Values are least-squares means by treatment. There were no treatment (trt), time, or parity effects ($P > 0.10$).

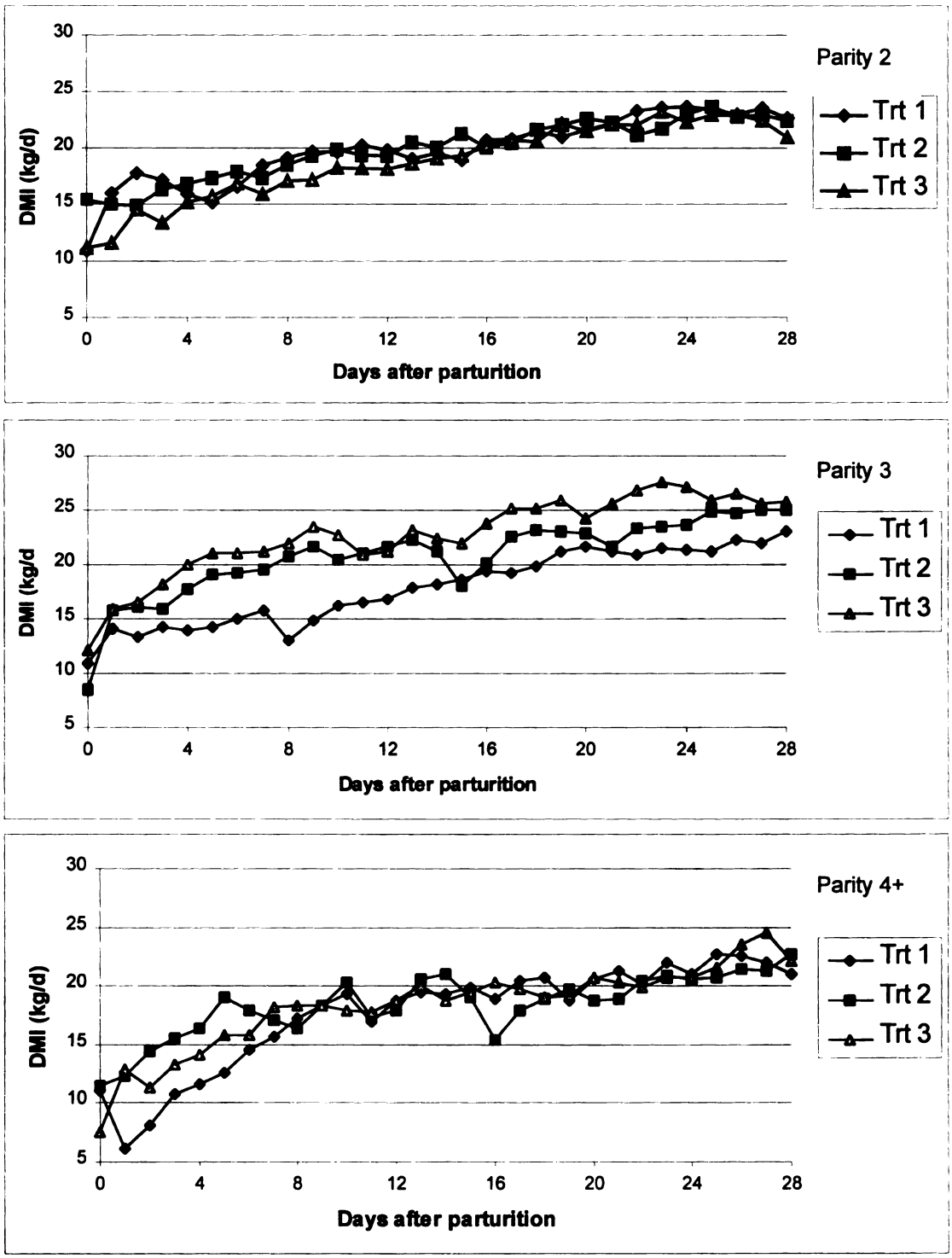


Figure 8. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis), parity, and time on DMI during the 28 d after parturition (SEM = 0.87).

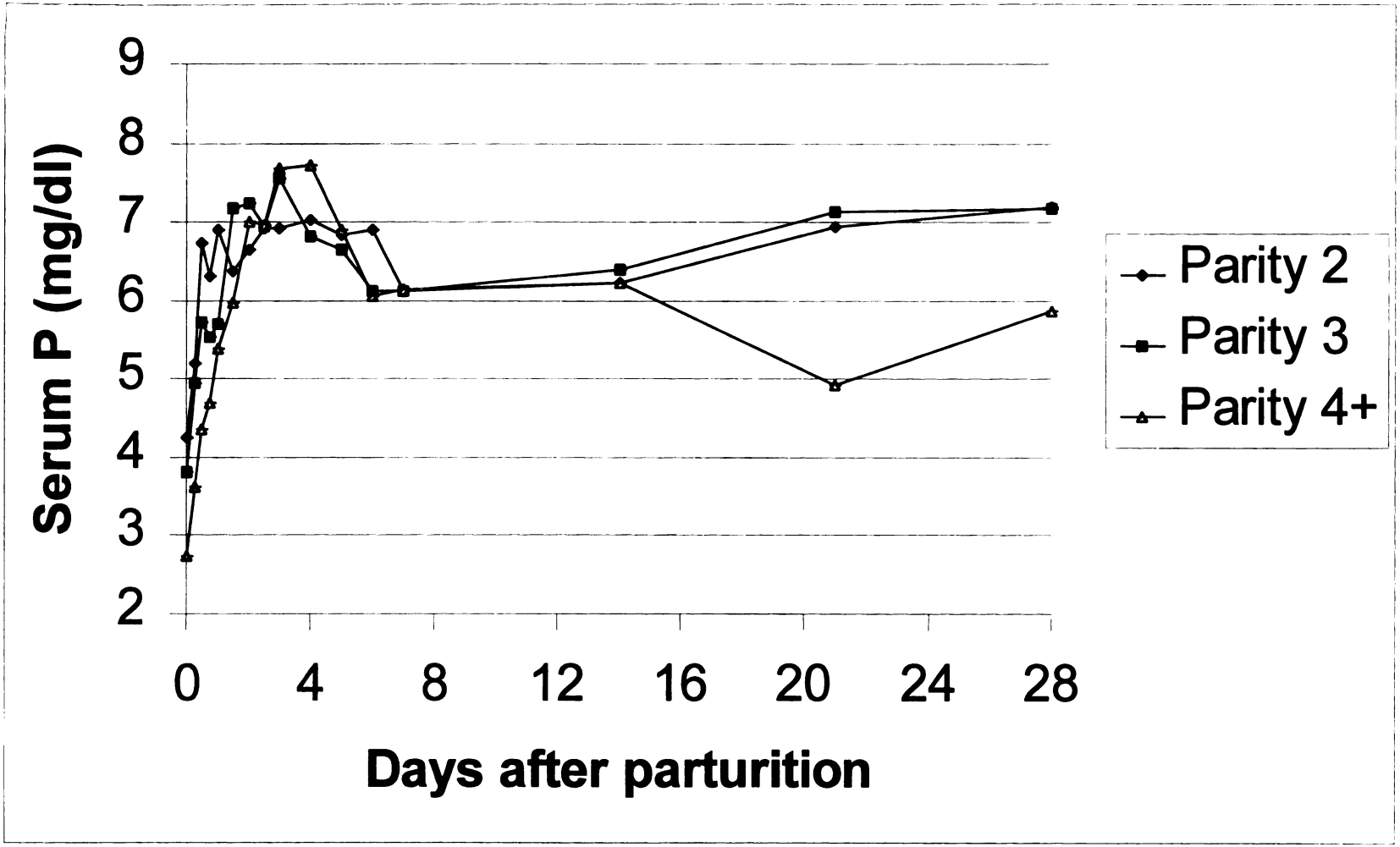
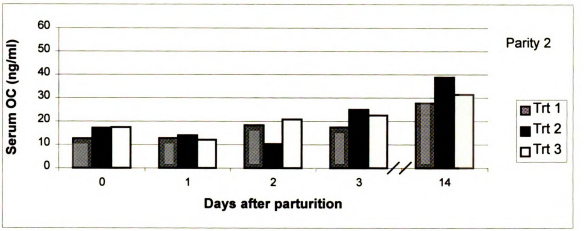
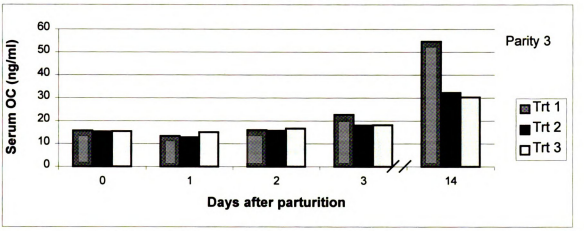


Figure 9. Effects of parity and time on serum P concentrations during the 28 d after parturition (SEM = 0.36).





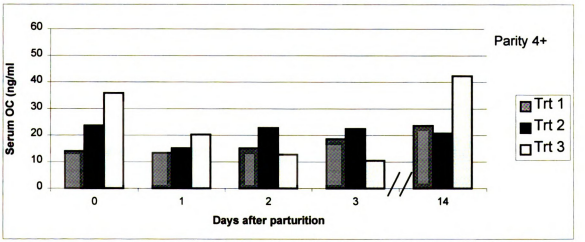


Figure 10. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis), parity, and time on serum osteocalcin concentrations during the 14 d after parturition (SEM = 6.2).

CHAPTER 4

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

In many cases, P is fed above requirements, which may lead to environmental problems through increased P excretion and subsequent application to the soil and runoff into surface waters. Feeding dietary P to match the cow's requirement can result in 25 to 30% less manure P and decrease P supplementation cost by up to $15/cow per yr (Wu et al., 2000).

In our experiment, feeding 0.21% P during the 28 d prepartum resulted in lower prepartum serum P concentrations than feeding 0.31 or 0.44% P. Neither total serum Ca nor plasma ionized Ca was altered by prepartum dietary P concentrations. However, cows fed 0.21% P tended to have lower prepartum OC concentrations than cows fed 0.31 or 0.44% P, suggesting less bone formation. Finally, energy-corrected milk yield was not affected by prepartum dietary P concentration.

Feeding 0.44% P during the 4 wk prepartum was too high and resulted in lower total serum Ca concentrations around parturition compared with 0.21 and 0.31% P. Cows fed 0.21% P consumed slightly above NRC 2001 requirements at 34 g of P/d. These cows fed 0.21% P prepartum had higher total serum Ca and plasma ionized Ca concentrations around parturition compared with 0.31 and 0.44% P. We conclude that consuming 34 g of P/d (0.21% P in this

experiment) was adequate to meet the needs of the periparturient multiparous Holstein cow.

The current experiment provides evidence that feeding at 0.44% dietary P during the 28 d before parturition is unnecessary. However, we do not know if there are any discernable metabolic and production effects of feeding dietary P concentrations between 0.21 and 0.31%. We need to determine the prepartum dietary P requirement of the multiparous Holstein cow not only to meet her needs but also to minimize P excretion in feces. In the practical aspect, decreasing the amount of P excreted in the feces will have environmental benefits because of less potential P run-off. Additionally, feeding at the requirement for prepartum dietary P can decrease the cost of the ration because of the decrease in supplemental P. Not only is determining the optimal P concentration important from 4 wk prepartum until parturition, but it is necessary that the entire lactation cycle (including lactation and the dry period) is evaluated. If producers feed at requirements through an entire lactation cycle, the decrease in fecal P output and cost of the ration could be drastically decreased. However, it is the job of researchers to provide this information to producers.

Another topic that needs further research is bone metabolism in the dairy cow. Assessment of bone metabolism during the periparturient period is necessary to provide a better understanding of P metabolism and how bone contributes to the circulating P and Ca pools. Optimizing the use of bone stores around parturition may decrease the incidence of hypocalcemia and hypophosphatemia around parturition. If we could quantify the grams of P and Ca that bone contributes to the circulating pool around parturition, we could

capitalize on this knowledge. Then, perhaps, we could determine a method to increase bone mobilization prepartum.

Finally, the current NRC (2001) has reported an AC for dietary P of 0.64 for forages, 0.70 for concentrates, and supplemental mineral sources have been assigned individual AC (Table 15-4; NRC, 2001). However, if an AC was also assigned to individual forages and concentrates, then we could determine the AC for the entire ration. With this information, we could balance diets more accurately, providing the cow with adequate P to meet her requirements without overfeeding.

More research is necessary not only on prepartum dietary P requirements, but also on P requirements during the entire lactation cycle. Knowing and manipulating the amount of P and Ca from bone resorption also would be beneficial. Therefore, we should increase our understanding of optimal dietary P utilization by periparturient dairy cows.

APPENDIX A

OSTEOCALCIN ASSAY VALIDATION AND SAMPLE ANALYSIS PROCEDURE

Introduction. The assay used to determine serum OC concentrations is a competitive immunoassay (Novocalcin; Quidel Corporation, San Diego, CA). However, it had not been validated for bovine serum. This kit comes with one 96-well plate with lyophilized OC coated strips, OC standards, high and low OC concentration pools of known concentrations and reagents. To validate an assay, it is necessary to perform recoveries and spikes using unknown and known standards to determine parallelism of the assay. The following is a review of the procedure used to validate the OC assay.

Serum Collection and Storage. Two pools, low and high OC concentrations, were made from serum samples from multiple animals. The low pool was collected from multiparous Holstein cows from 5 to 7 d postpartum. Eight ml of blood was collected from five different cows. The whole blood samples were allowed to clot for 1 h at 10°C. They were then centrifuged at 3000 x *g* for 20 min. Serum was harvested and pooled. After the serum from the 5 cows was mixed, 1-ml aliquots were placed in individually labeled microcentrifuge tubes and immediately frozen at -80°C for later use. The high pool was formed using the previously mentioned method except that serum from five, 1 mo old Holstein heifer calves was used.

Unknowns. For analysis, whole blood samples were collected from the coccygeal vein in one 10-ml evacuated glass tube. After collection, the tube was placed immediately on ice and allowed to clot at 10°C for 1 h. Tubes were then centrifuged at 3000 x *g* for 20 min. The serum from one tube was divided into equal parts and immediately frozen at –80°C in four 1.5-ml plastic microcentrifuge tubes for later analysis. Four microcentrifuge tubes were collected for OC concentration analysis. Additionally, we recommend that samples only undergo one freeze-thaw cycle because we noted a decrease in OC concentrations after more than one freeze-thaw cycle. Samples were analyzed on microplate reader (SpectraMax 190).

Dilutions. Five different validation plates were run for OC. Each plate contained a standard curve in duplicate (0, 4, 8, 16, 20, 32 ng/ml). The first plate analyzed determined the proper pool dilutions for each the low and high pools. The low pool was diluted with the kit's 1x wash buffer at 1:1, 1:2, 1:3, and 1:4 dilutions. Running the low pool at 1:1 (with no dilution) proved to be a problem perhaps because of pH changes, proteases, etc. The low pool diluted at 1:1 had higher OC concentrations than the highest point on the standard curve and much higher than previously published values. As a result, we do not recommend that bovine serum samples be analyzed without diluting with the 1x wash buffer. The OC concentrations of the 1:4 diluted samples were below the working range of the curve (6.4 to 25.6 ng/ml or the middle 60% of the curve) at 6.06 ng/ml. Because the OC concentrations of the 1:2 and 1:3 dilution samples were within the working range of the curve, averaging 24.24 ng/ml, it is recommended that

serum samples from multiparous cows be diluted at either 1:2 or 1:3 with the kit's 1x wash buffer.

The high pool, from calves at a month of age, also was examined in the first plate. These samples were diluted with the kit's 1x wash buffer 1:8, 1:12, 1:16, 1:20 and 1:24 dilutions. The samples diluted at 1:8 had OC concentrations above the working range of the curve (32.99 ng/ml diluted or 263.92 ng/ml adjusted). This value was not used in the serum OC concentration average. The high pool averaged 240.03 ng/ml and samples can be analyzed at 1:12, 1:16, 1:20 or 1:24 dilutions. These diluted values fell within the working range of the standard curve (6.4 to 25.6 ng/ml). Note that this recommendation is used only if the OC concentration of the high pool runs around 240 ng/ml.

For the five plates (n = 11), the low pool samples averaged 22.89 ng/ml with a standard error of 4.94 ng/ml and a coefficient of variation of 21.59% (inter assay variation). The intra-assay variation for the 5 plates was 8.57%. The inter-assay variation is defined as the variation between plates, which was calculated using a pooled sample that was analyzed in duplicate in every plate. The intra-assay variation is defined as the variation of the pooled sample analyzed in duplicate.

Spiking and Recovery. Spiking of the low pool was analyzed in all five plates. Spiking was done by adding 1 part (50μl) of the low pool to 1 part (50μl) of the spike. Known kit standards were used as spikes. The following table provides the recovery percentages for each spike along with inter- and intra-assay variation for each spike.

Spike (ng/ml)	2	4	8	16	32
Number of Observations	1	3	6	5	3
Average % Recovery	110	105.3	114.2	117.5	131
Inter-Assay Variation (%)	0	60.1	65.1	51.36	19.38
Intra-Assay Variation (%)	17.1	4.57	6.48	11.13	8.27

Spiking is done to determine the linearity of the assay as it relates to the standard curve. It is desirable for the recovery to be constant over all spikes. Therefore the recovery line should be parallel to the standard curve. In this case, however, linearity does not run over the entire standard curve. From the 2 ng/ml spike to the 16 ng/ml spike, the line is linear to the standard curve. As a result, samples were diluted to fall between approximately 10 ng/ml to 20 ng/ml.

APPENDIX B

DEOXYPYRIDINOLINE ASSAY VALIDATION AND SAMPLE ANALYSIS PROCEDURES

Introduction. The assay used to determine serum DPD concentrations is a competitive immunoassay (Total DPD Serum and Serum PYD; Quidel Corporation, San Diego, CA). However, it had not been validated for bovine serum. There are two kits required to analyze serum DPD. The Total DPD Serum kit comes with one 96-well hydrolysis plate and sealer, serum hydrolysis control and reagents. The second kit required is the Serum PYD kit that includes one 96-well plate with lyophilized DPD coated strips, DPD standards, high and low DPD concentration pools of known concentrations and reagents. To validate an assay, it is necessary to perform recoveries and spikes using unknown and known standards to determine parallelism of the assay. The following is a review of the procedure used to validate the DPD assay.

Serum Collection and Storage. Two pools, low and high DPD concentrations, were made from serum samples from multiple animals. The high pool was collected from multiparous Holstein cows from 5 to 7 d postpartum. Eight ml of blood was collected from five different cows. The whole blood samples were allowed to clot for 1 h at 10°C. They were then centrifuged at 3000 x *g* for 20 min. Serum was harvested and pooled. After the serum from the 5 cows was mixed, 1-ml aliquots were placed in individually labeled microcentrifuge tubes and immediately frozen at -80°C for later use. The low

pool was formed using the previously mentioned method except that serum from five, 1 mo old Holstein heifer calves was used.

Unknowns. For analysis, whole blood samples were collected from the coccygeal vein in one 10-ml evacuated glass tube. After collection, the tube was placed immediately on ice and allowed to clot at 10°C for 1 h. At this time, the tubes were centrifuged at 3000 x *g* for 20 min. The serum from one tube was divided into equal parts and immediately frozen at –80°C in four 1.5-ml plastic microcentrifuge tubes for later analysis. Four microcentrifuge tubes were collected for DPD analysis. Additionally, samples are light-sensitive, therefore it is necessary to use caution when exposing them to light which is described in the product insert. Samples were analyzed on microplate reader (SpectraMax 190).

Dilutions. Three different validation plates were run for DPD. Each plate contained a standard curve in duplicate (0, 5, 10, 15, 20, 30 ng/ml). The first plate analyzed determined the proper pool dilutions for each the low and high pools. The high pool was diluted with ultra pure water at 1:1, 1:2, and 1:3 dilutions. Running the high pool at 1:2 and 1:3 resulted in DPD concentrations below the working range of the curve (6 to 24 ng/ml or the middle 60% of the curve). Because the 1:1 dilution samples ran within the working range of the curve, averaging 10.8 ng/ml, it is recommended that serum samples from multiparous cows around parturition not be diluted.

The low pool, from calves at a month of age, also was examined in the first plate. These samples were diluted with ultra pure water at 1:1 and 1:2 dilutions. Again, running the low pool at 1:2 resulted in DPD concentrations

below the working range of the curve. Because the 1:1 dilutions samples ran within the working range of the curve, averaging 7.17 ng/ml, it is recommended that serum samples from 1 mo old Holstein heifer calves not be diluted.

For the three plates (n = 4), the low pool samples averaged 7.17 ng/ml with a standard error of 1.27 ng/ml. The high pool for the three plates (n = 5) averaged 10.8 ng/ml with a standard error of 4.11 ng/ml. The inter-assay variation was 11.0% among the three validation plates. The intra-assay variation for the three plates was 13.7%. The inter-assay variation is defined as the variation between plates, which was calculated using a pooled sample that was analyzed in duplicate in every plate. The intra-assay variation is defined as the variation of the pooled sample analyzed in duplicate in a single plate.

Spiking and Recovery. Spiking of the low pool was performed and analyzed in all three plates. Spiking was done by adding 1 part (50μl) of the low pool to 1 part (50μl) of the spike. Known kit standards were used as spikes. The following table provides the recovery percentages for each spike along with inter- and intra-assay variation for each spike.

Spike (ng/ml)	10	30
Number of Observations	3	3
Average % Recovery	86.67	79.33
Inter-Assay Variation (%)	8.92	14.46
Intra-Assay Variation (%)	5.5	6.47

Spiking is done to determine the linearity of the assay as it relates to the standard curve. It is desirable for the recovery to be constant over all spikes. Therefore the recovery line should be parallel to the standard curve. In this case, the linearity runs from samples averaging 7.17 ng/ml (the average of the 10

ng/ml spike) to 14.48 ng/ml (the average of the 30 ng/ml spike). As a result, it is not necessary to dilute samples if the DPD concentration of samples is between approximately 7 and 15 ng/ml.

APPENDIX C

TREATMENT BY TIME PLOTS
(OR TREATMENT BY PARITY BY TIME PLOTS)
FOR EACH DEPENDENT VARIABLE

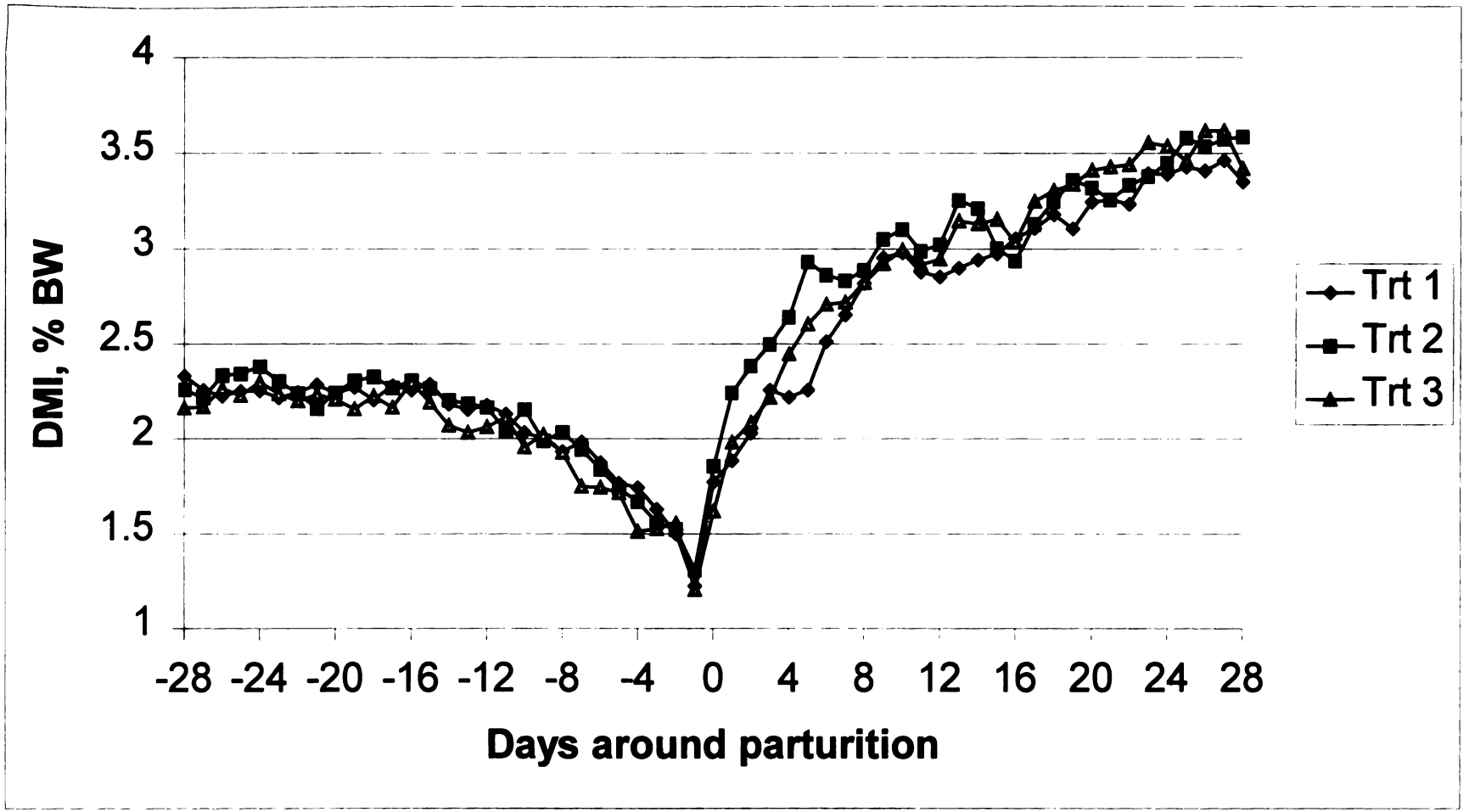


Figure C-1. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on DMI as a percent of BW from 28 d prepartum through 28 d postpartum.

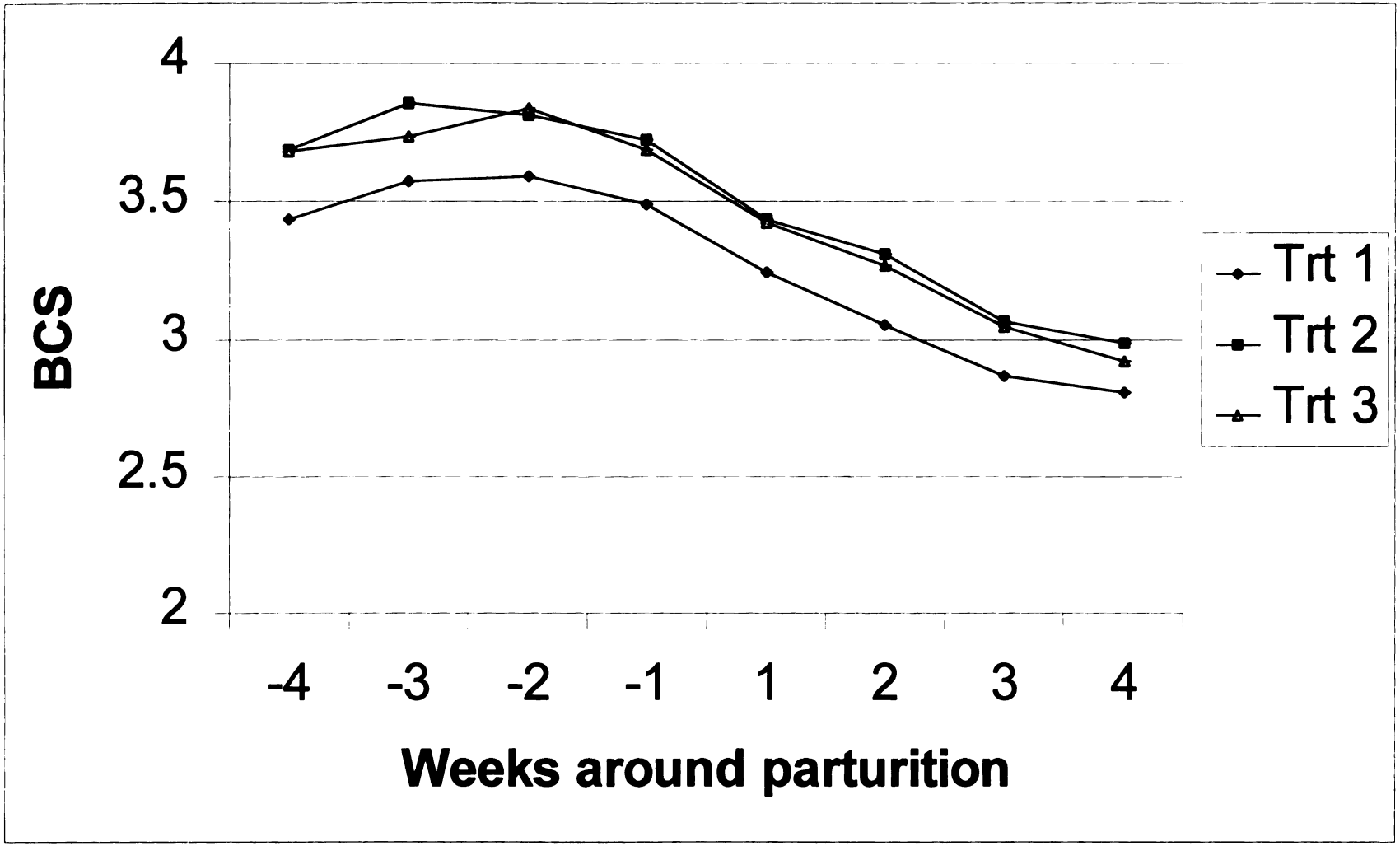


Figure C-2. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on BCS from 28 d prepartum through 28 d postpartum.

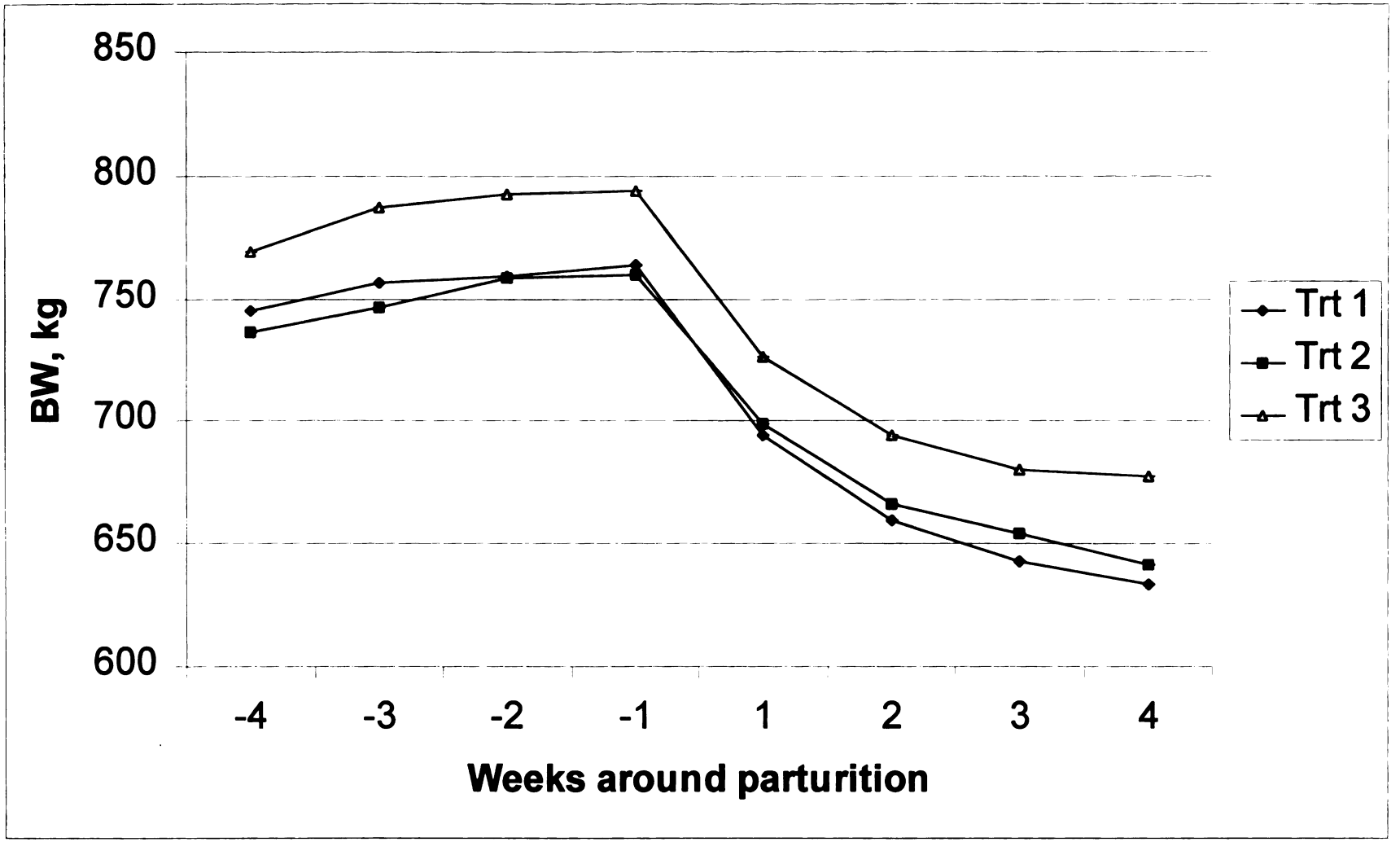


Figure C-3. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on BW from 28 d prepartum through 28 d postpartum.

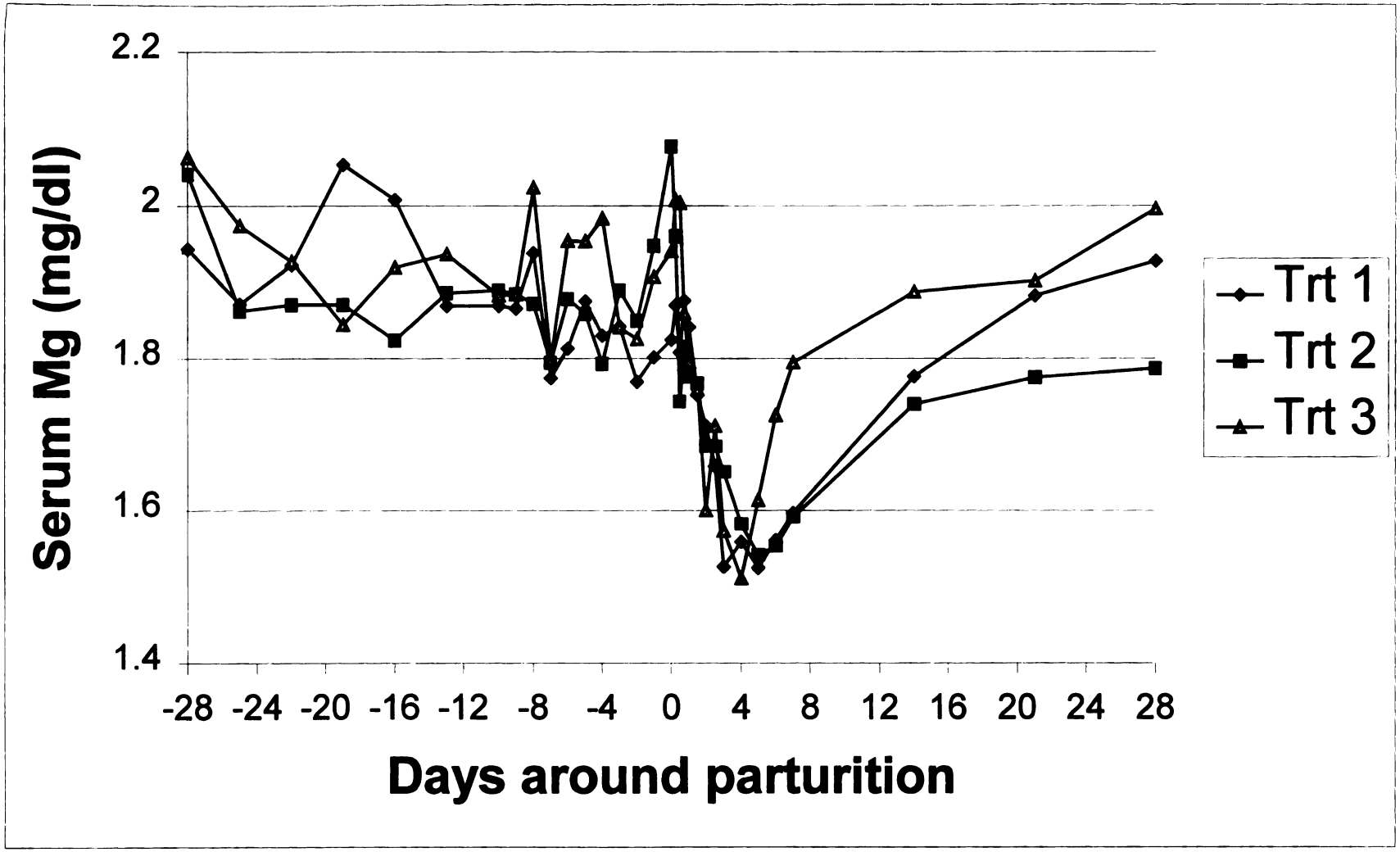


Figure C-4. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on serum Mg concentrations from 28 d prepartum through 28 d postpartum.

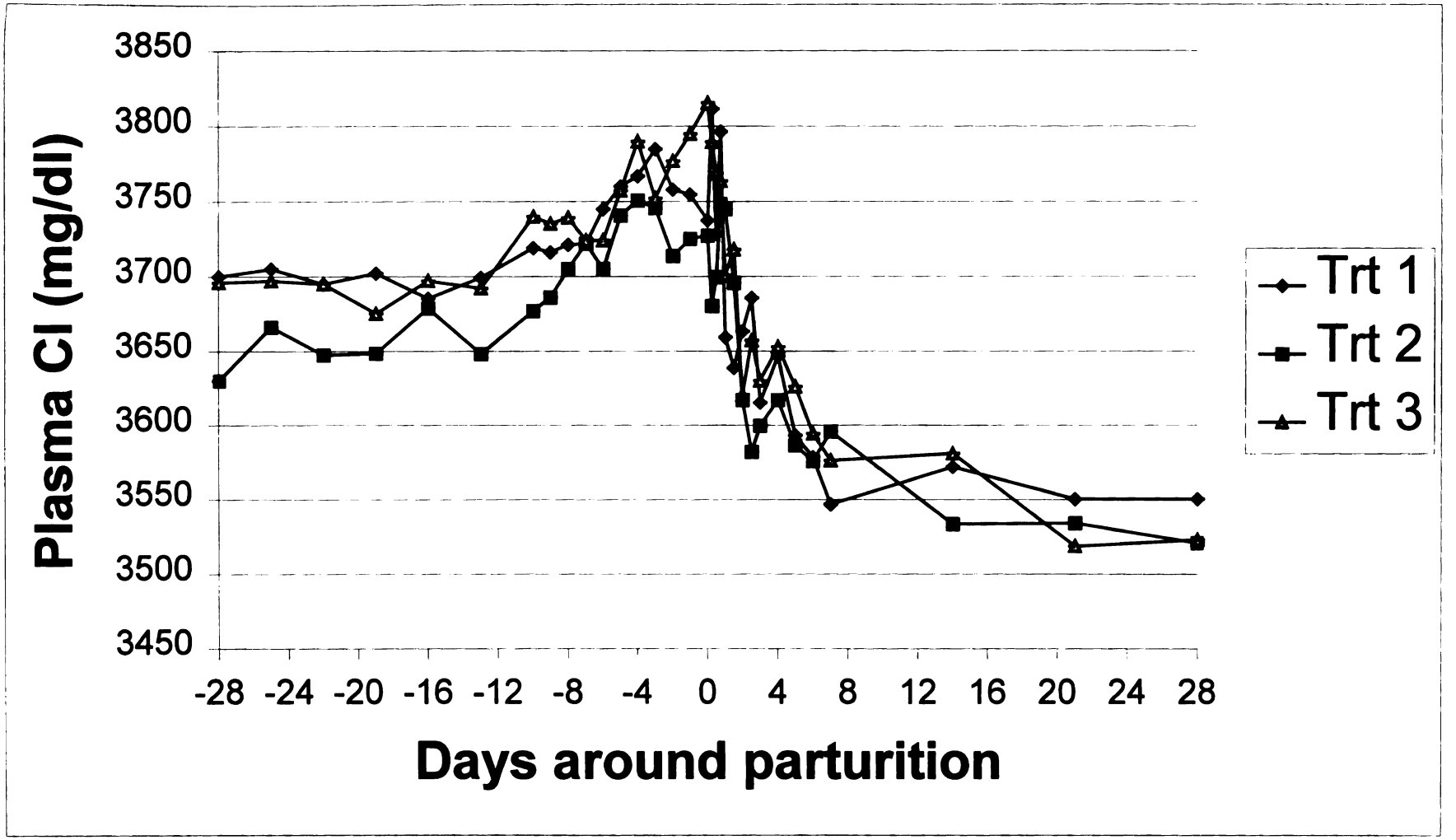


Figure C-5. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on plasma Cl concentrations from 28 d prepartum through 28 d postpartum.

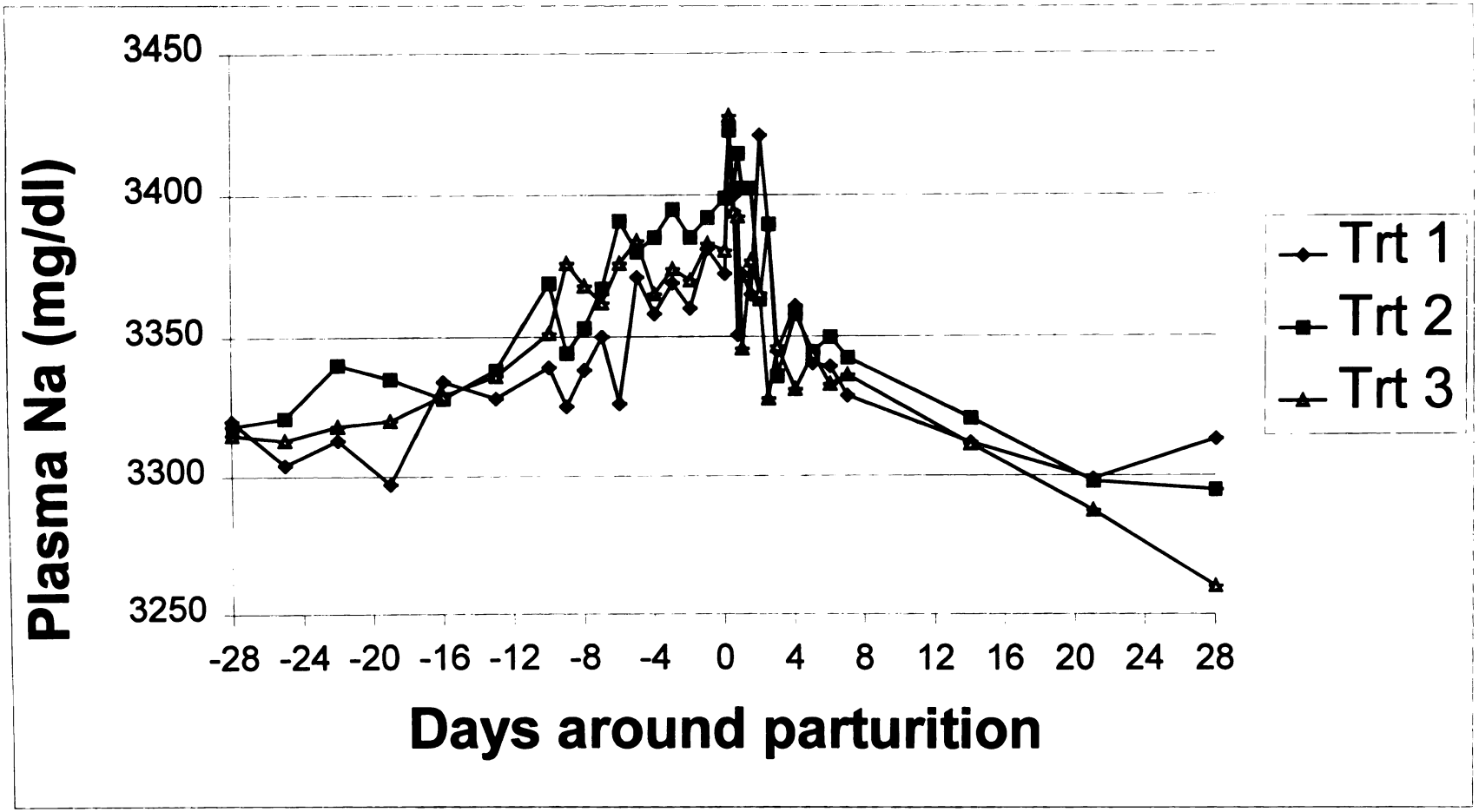


Figure C-6. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on plasma Na concentrations from 28 d prepartum through 28 d postpartum.

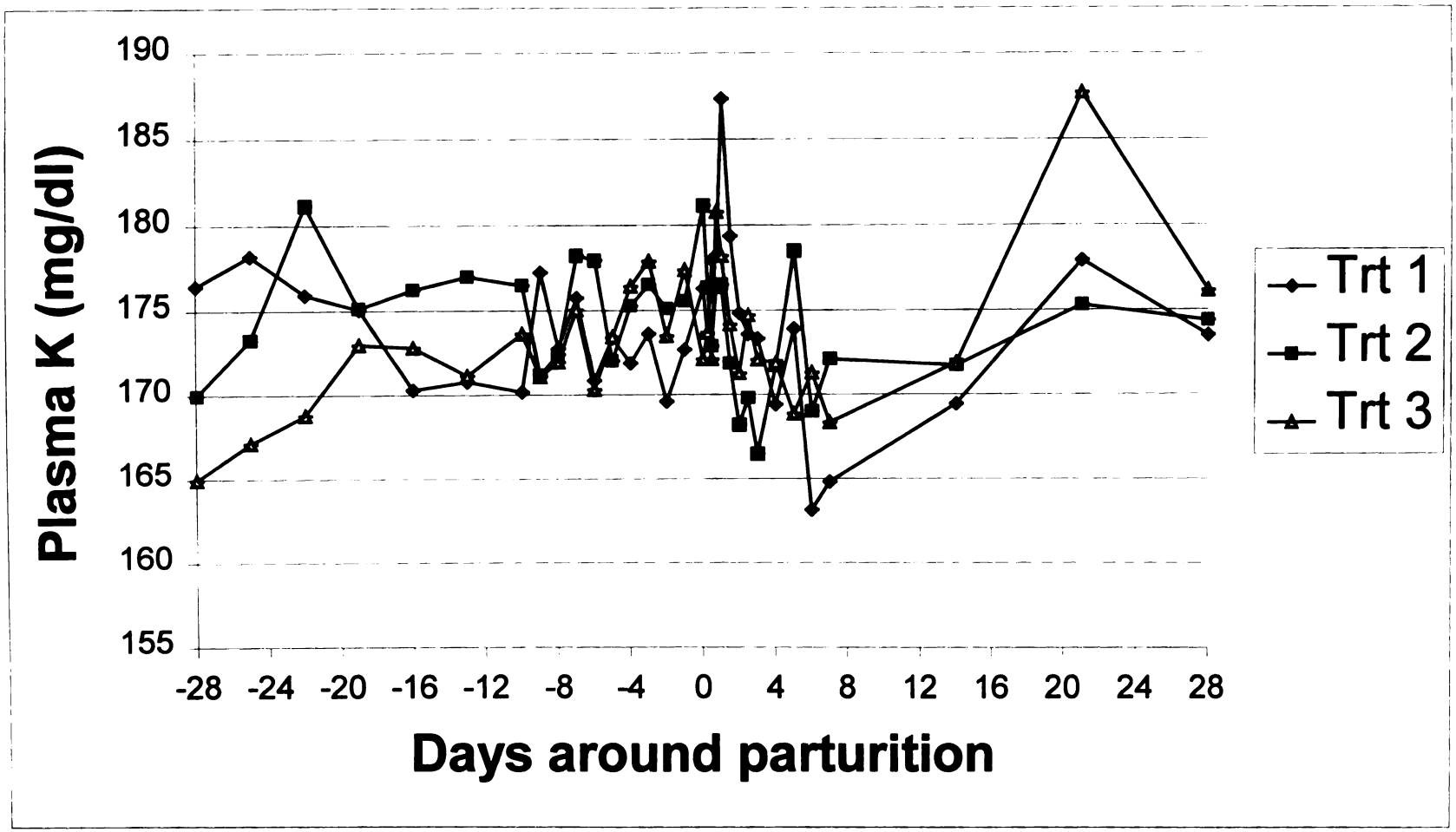


Figure C-7. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on plasma K concentrations from 28 d prepartum through 28 d postpartum.

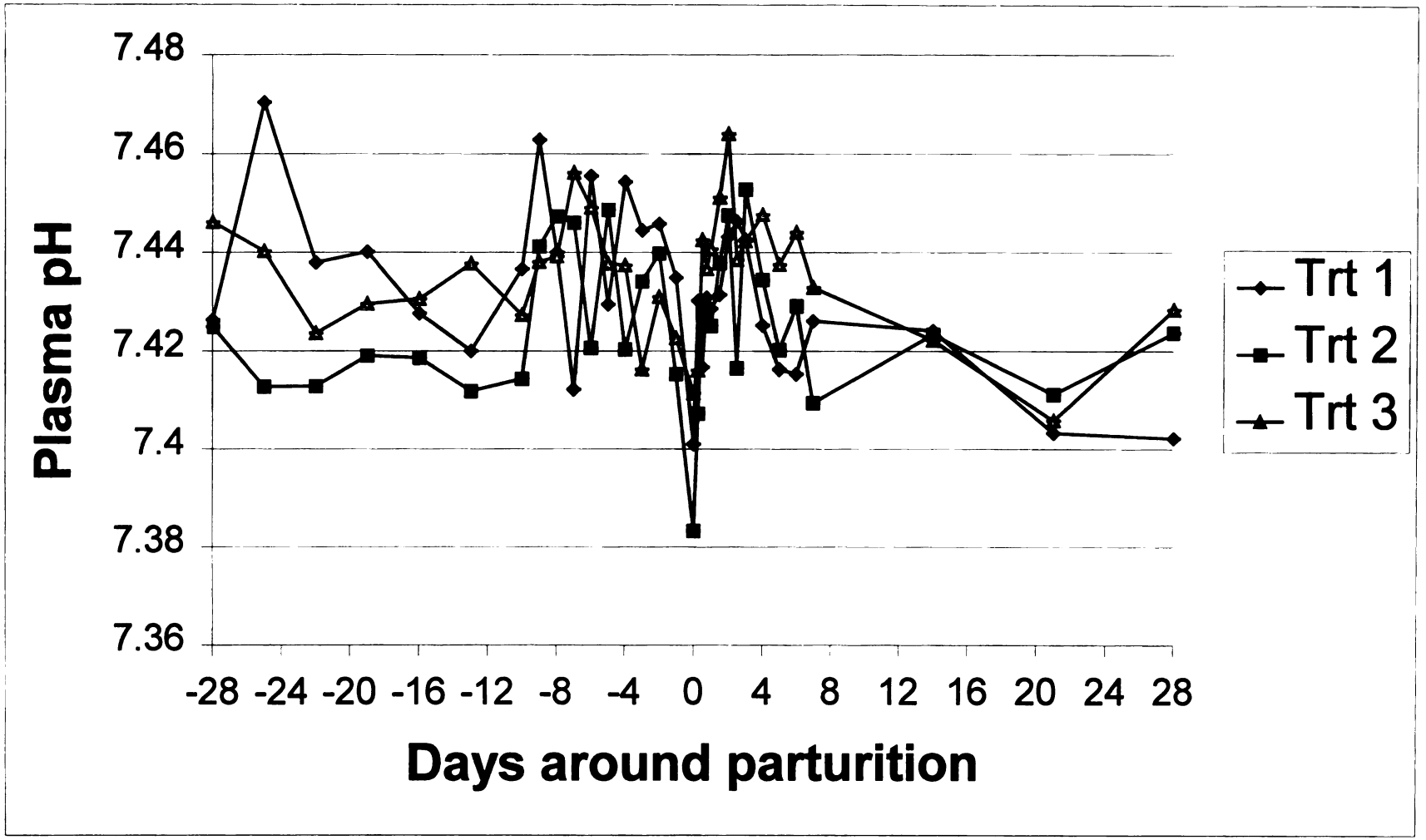


Figure C-8. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on plasma pH from 28 d prepartum through 28 d postpartum.

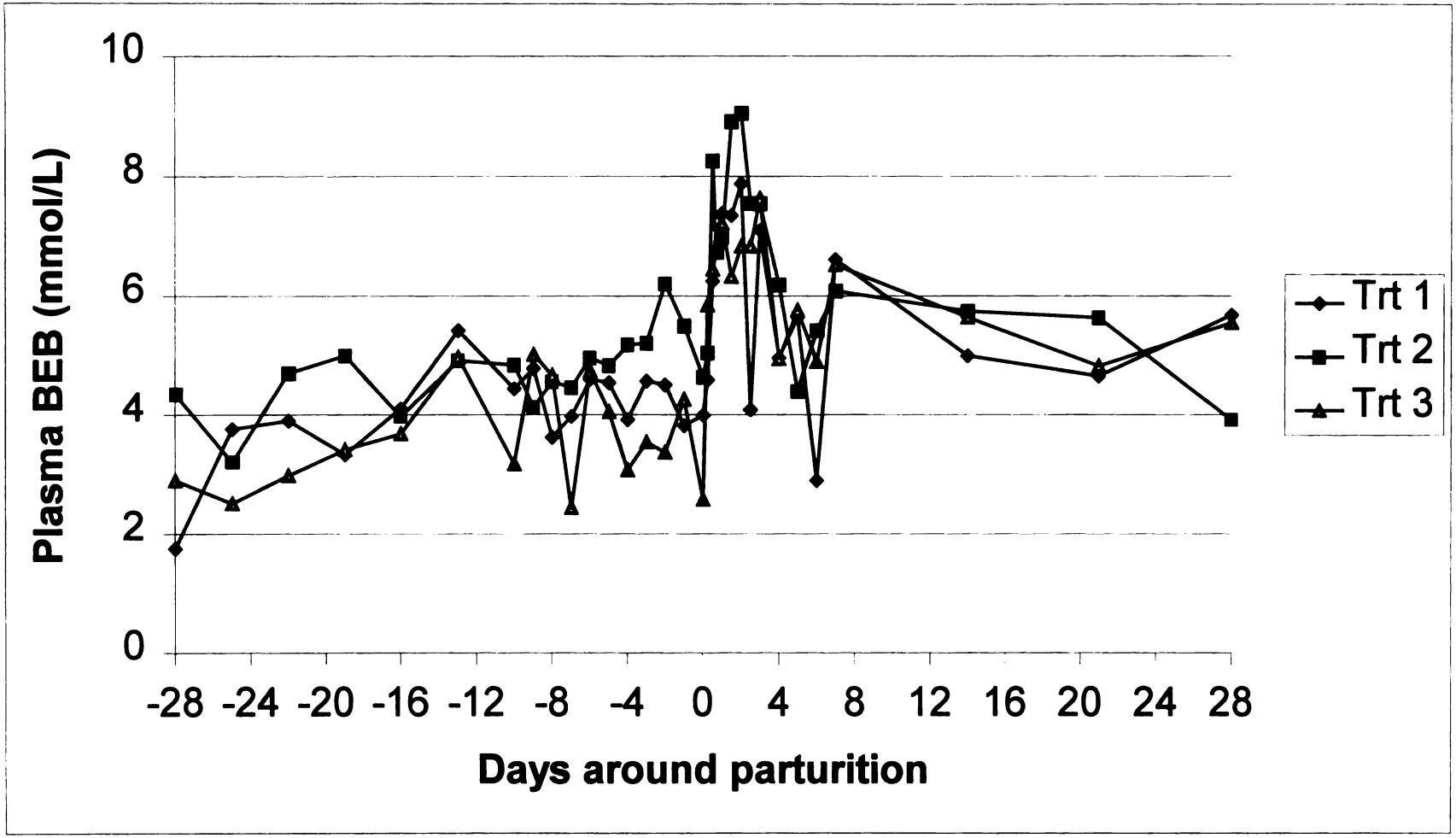


Figure C-9. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on plasma BEB concentrations from 28 d prepartum through 28 d postpartum.

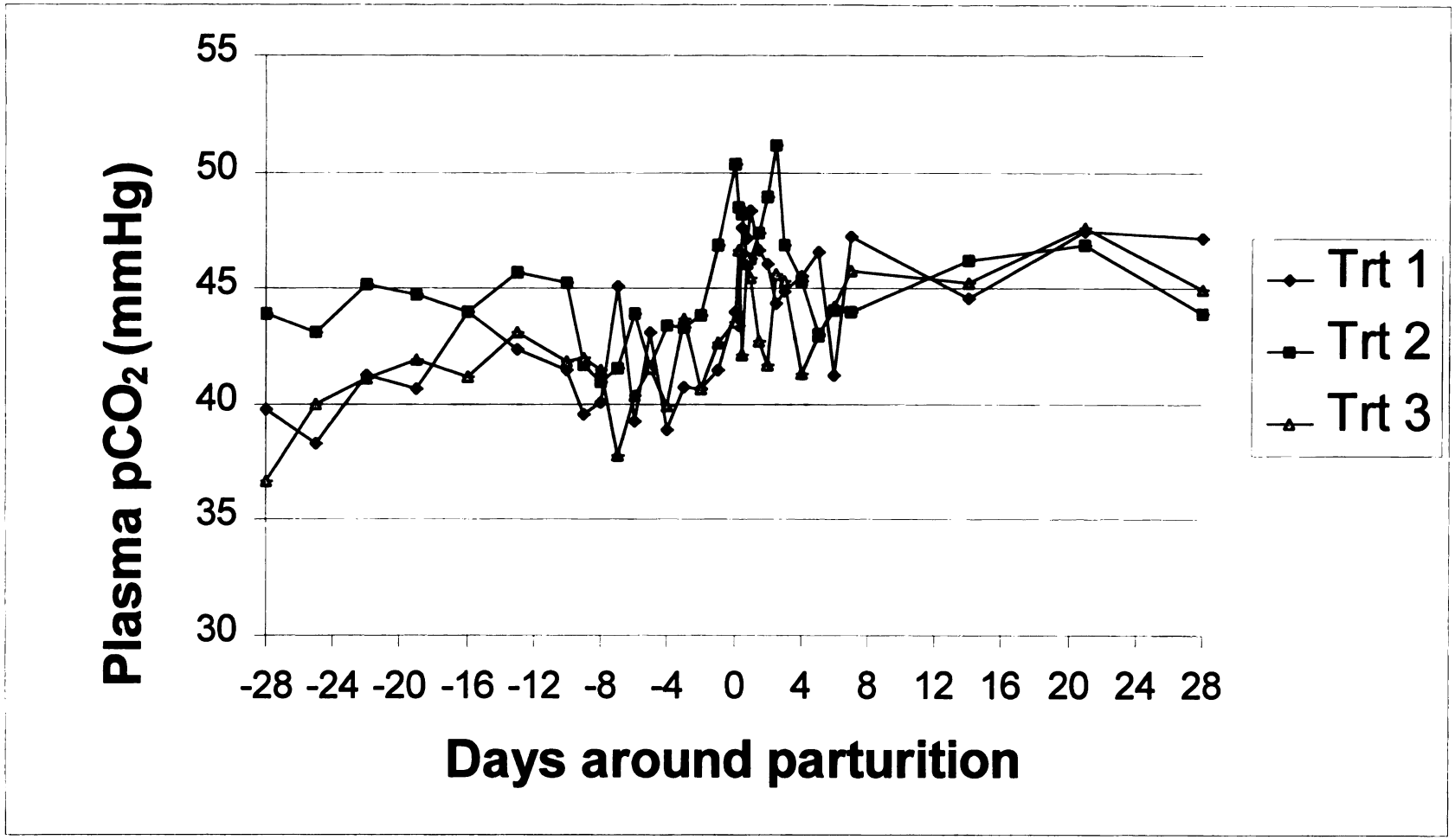


Figure C-10. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on plasma pCO_2 concentrations from 28 d prepartum through 28 d postpartum.

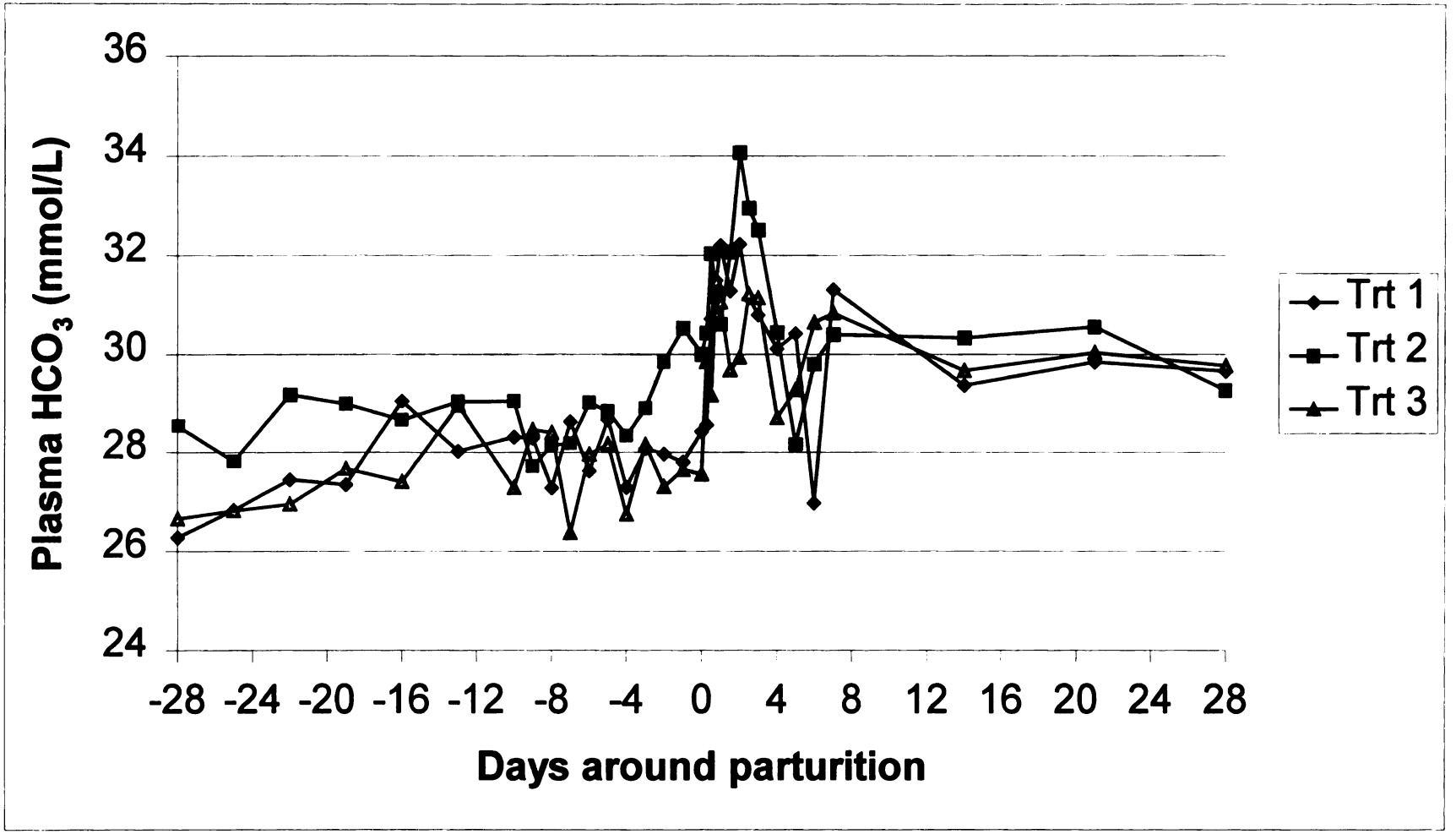


Figure C-11. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on plasma blood HCO_3 concentrations from 28 d prepartum though 28 d postpartum.

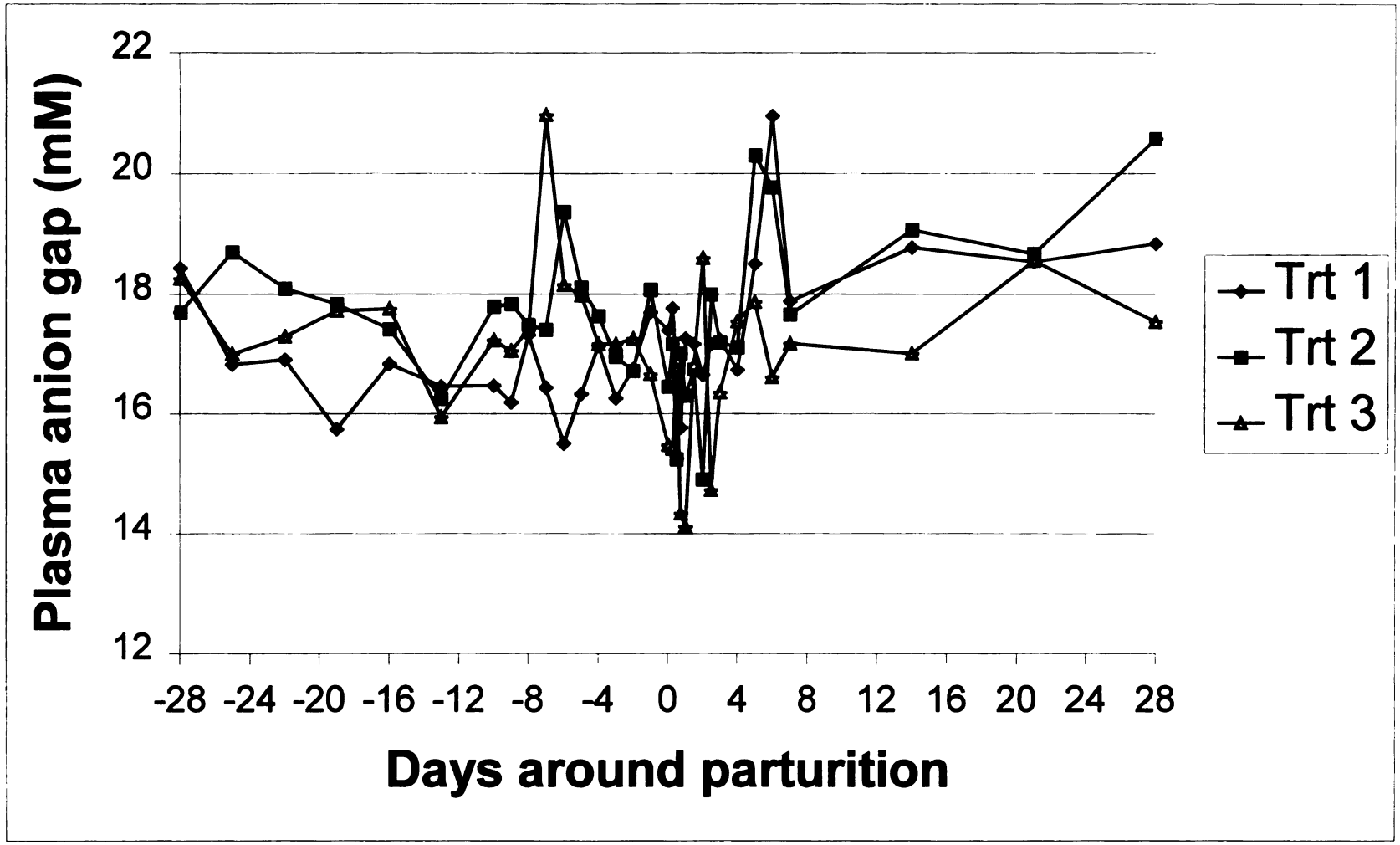


Figure C-12. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on plasma anion gap from 28 d prepartum though 28 d postpartum.

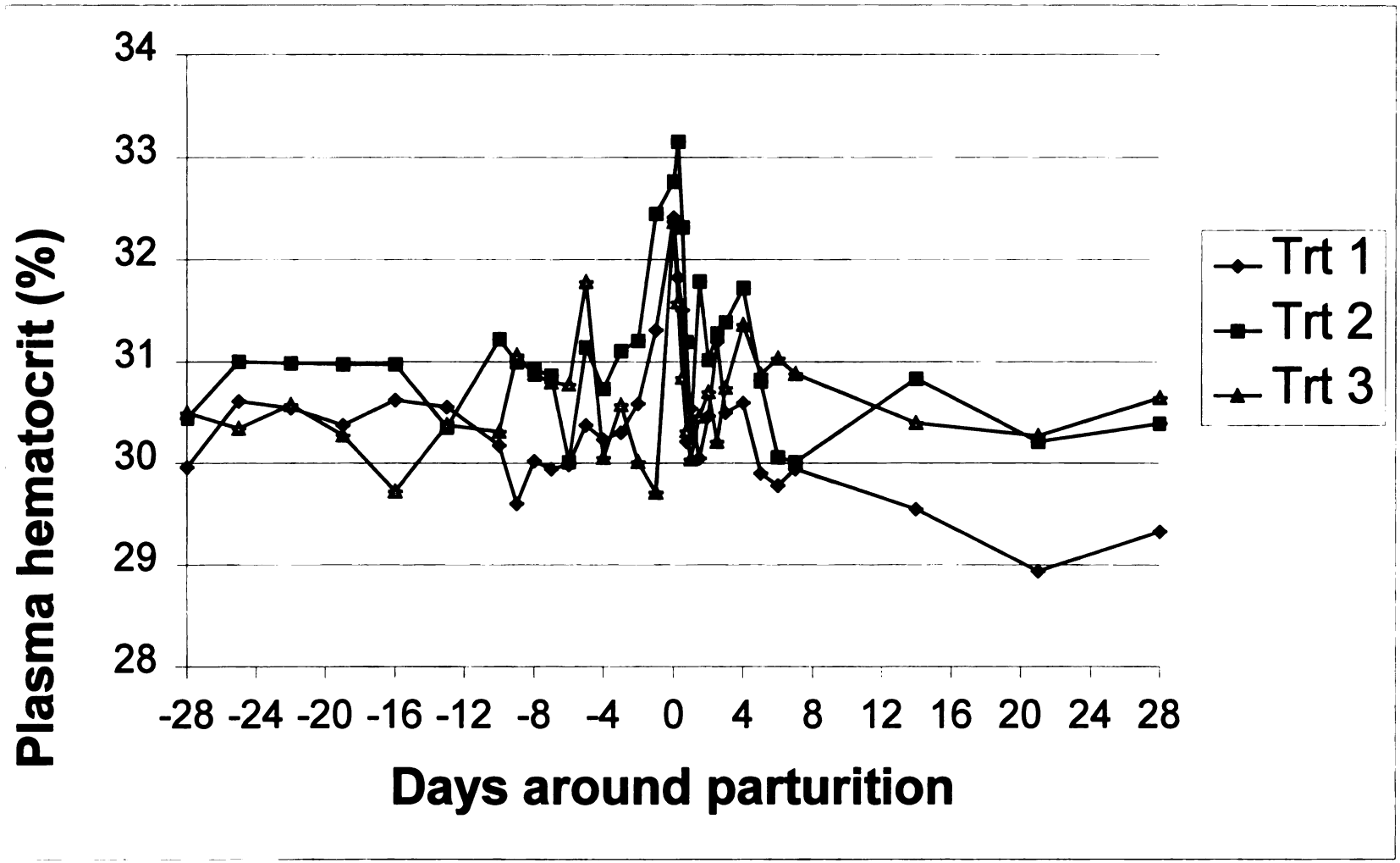


Figure C-13. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis) and time on plasma hematocrit from 28 d prepartum though 28 d postpartum.

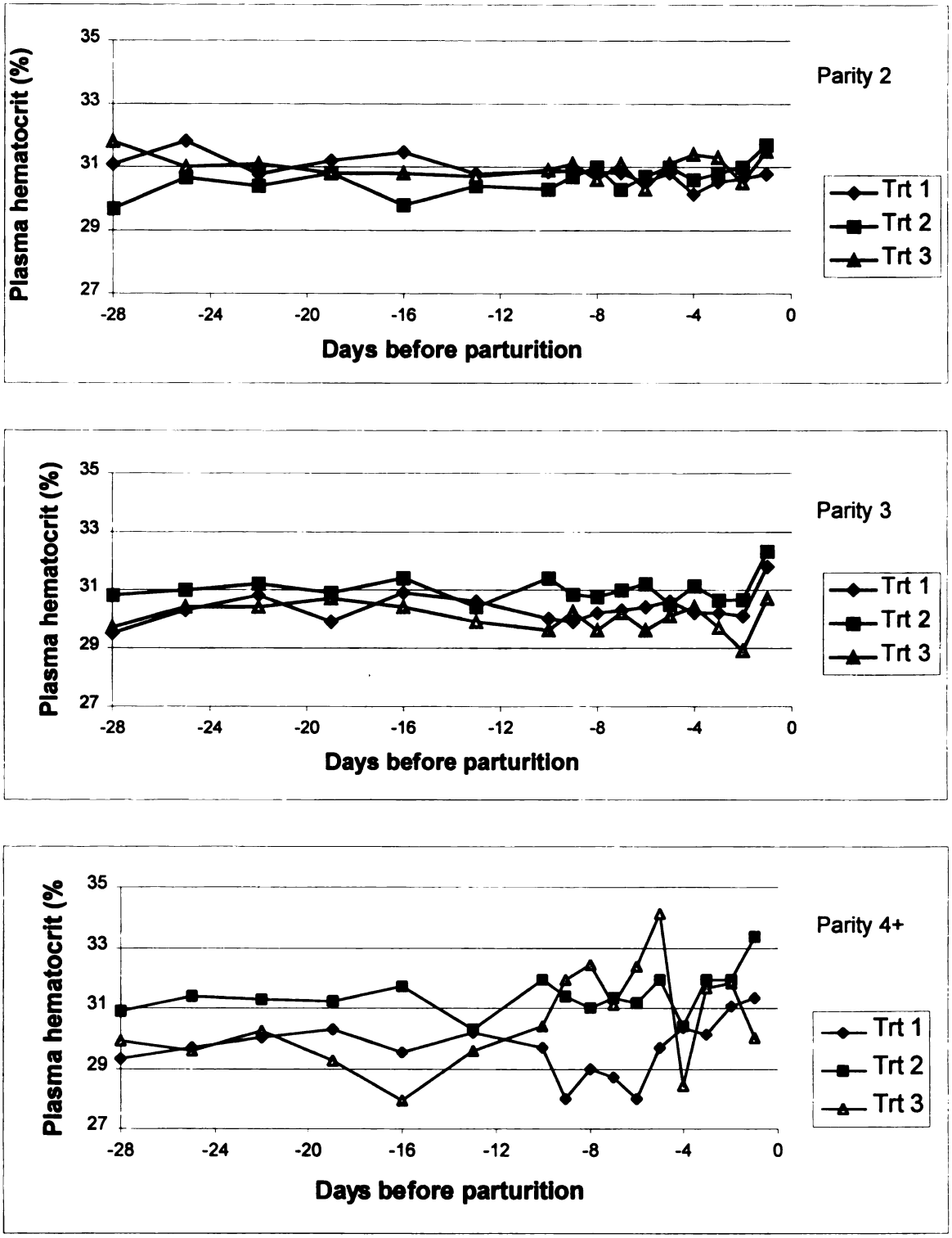


Figure C-14. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt 3 = 0.44% P, dry basis), parity and time on plasma hematocrit during the 28 d before parturition.

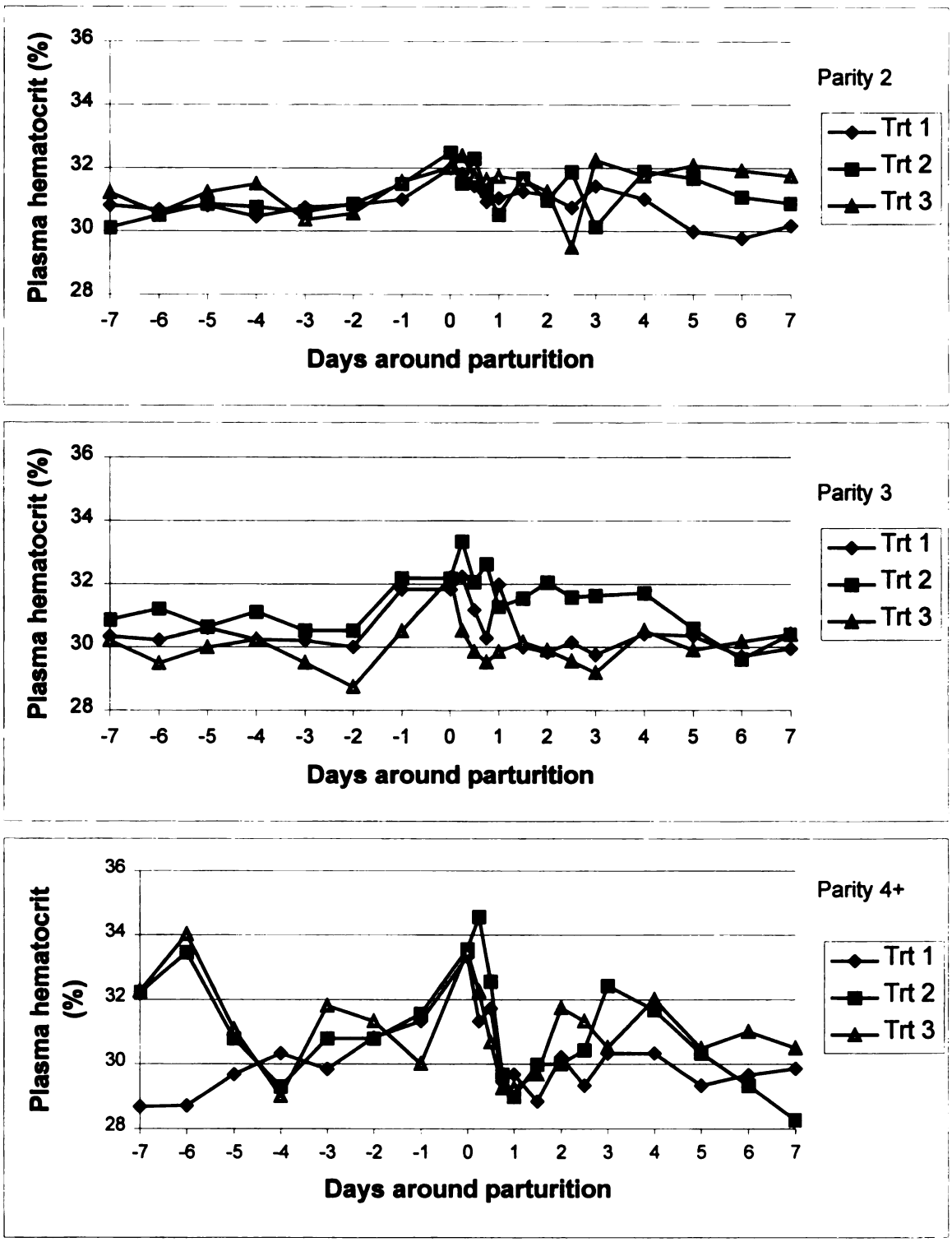


Figure C-15. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt = 0.44% P, dry basis), parity and time on plasma hematocrit from 7 d prepartum through 7 d postpartum.

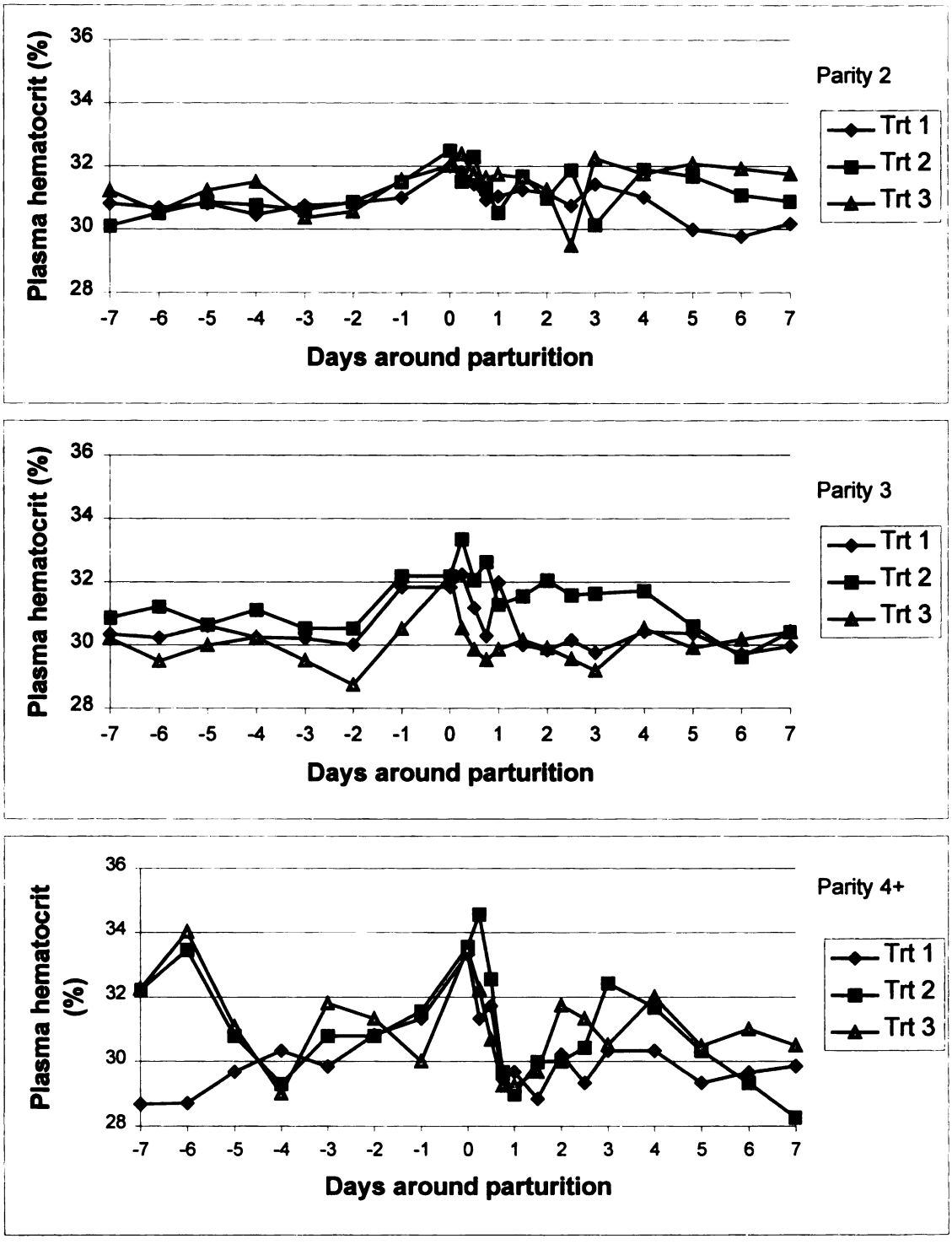


Figure C-15. Effects of dietary treatments (Trt 1 = 0.21; Trt 2 = 0.31; Trt = 0.44% P, dry basis), parity and time on plasma hematocrit from 7 d prepartum through 7 d postpartum.

APPENDIX D

PROBABILITY TABLES FOR EACH DEPENDENT VARIABLE

Table D-1. Probability values from least-squares analysis of variance for dependent variables during the pre-experiment period (during 35 and 42 d before expected calving date; standardization period).

	DMI[1] kg/d	DMI % BW	BCS[2]	BW[3] kg
Treatment (Trt)	0.36	0.65	0.05	0.29
Orthogonal contrasts				
Trt 1 vs. 2,3	0.06	0.22	0.01	0.65
Trt 2 vs. 3	0.28	0.42	0.01	0.72
Time[4]	0.01	0.14	0.01	0.01
Parity	0.44	0.67	0.35	0.07
Trt*Time	0.12	0.93	0.27	0.56
Parity*Trt	0.84	0.64	0.57	0.92
Parity*Time	0.72	0.75	0.73	0.34
Parity*Trt*Time	0.36	0.36	0.19	0.57

[1]DMI = Dry matter intake.

[2]BCS = Body condition score.

[3]BW = Body weight.

[4]Time = 35 and 42 d before expected calving date.

Table D-2. Probability values from least-squares analysis of variance for dependent variables in serum[1] and plasma during the pre-experiment period (during 35 and 42 d before expected calving date; standardization period).

	P mg/dl	Ca mg/dl	Mg mg/dl	iCa[2] mg/dl	Cl mg/dl	Na mg/dl	K mg/dl	pH	BEB[3] mmol/L	pCO_2 mmHg	HCO_3 mmol/L	Anion Gap mM	Hematocrit %
Treatment (Trt)	0.21	0.79	0.99	0.68	0.93	0.13	0.22	0.07	0.18	0.44	0.72	0.58	0.89
Orthogonal contrasts													
Trt 1 vs. 2,3	0.68	0.36	0.71	0.71	0.40	0.83	0.44	0.13	0.44	0.33	0.71	0.48	0.72
Trt 2 vs. 3	0.68	0.39	0.52	0.50	0.41	0.43	0.16	0.37	0.13	0.73	0.88	0.32	0.47
Time[4]	0.82	0.99	0.28	0.95	0.73	0.25	0.15	0.10	0.24	0.65	0.89	0.83	0.68
Parity	0.84	0.40	0.87	0.68	0.58	0.93	0.61	0.85	0.16	0.70	0.51	0.52	0.69
Trt*Time	0.22	0.97	0.15	0.42	0.88	0.19	0.23	0.07	0.83	0.40	0.57	0.93	0.46
Parity*Trt	0.63	0.49	0.85	0.99	0.75	0.68	0.72	0.55	0.64	0.91	0.74	0.28	0.61
Parity*Time	0.84	0.49	0.83	0.73	0.37	0.28	0.27	0.31	0.48	0.95	0.67	0.37	0.77
Parity*Trt*Time	0.49	0.76	0.13	0.78	0.45	0.57	0.38	0.17	0.22	0.47	0.72	0.17	0.63

[1]P, Ca, and Mg were analyzed in serum; remaining variables were analyzed in plasma.

[2]iCa = Ionized Ca.

[3]BEB = Base excess.

[4]Time = 35 and 42 d before expected calving date.

Table D-3. Probability values from least-squares analysis of variance for dependent variables from 28 d prepartum until

Table D-3. Probability values from least-squares analysis of variance for dependent variables from 28 d prepartum until parturition.

	DMI[1] kg/d	DMI % BW	P Intake g/d	BCS[2]	BCS change	BW[3] kg	BW change
Treatment (Trt)	0.65	0.73	0.01	0.21	0.74	0.17	0.30
Orthogonal Contrasts							
Trt 1 vs. 2,3	0.36	0.62	0.01	0.08	0.28	0.64	0.65
Trt 2 vs. 3	0.50	0.43	0.01	0.11	0.42	0.60	0.72
Time[4]	0.01	0.50	0.01	0.01		0.01	
Trt*Time	0.91	0.95	0.02	0.89		0.28	
Orthogonal Contrasts[5]							
Trt 1 vs. 2,3 * L time	0.76	0.73	0.01	0.74		0.16	
Trt 1 vs. 2,3 * Q time	0.91	0.46	0.07	0.98		0.98	
Trt 1 vs. 2,3 * C time	0.38	0.88	0.92	0.80		0.39	
Trt 2 vs. 3 * L time	0.43	0.37	0.02	0.83		0.59	
Trt 2 vs. 3 * Q time	0.80	0.26	0.26	0.73		0.36	
Trt 2 vs. 3 * C time	0.79	0.87	0.83	0.15		0.12	
Parity	0.95	0.14	0.96	0.33	0.18	0.09	0.13
Parity*Trt	0.86	0.90	0.70	0.33	0.15	0.96	0.91
Parity*Time	0.09	0.62	0.13	0.43		0.04	
Parity*Trt*Time	0.83	0.83	0.35	0.26		0.01	

[1]DMI = Dry matter intake.

[2]BCS = Body condition score.

[3]BW = Body weight.

[4]Time = Daily for DMI and P intake and weekly for other variables.

[5]L = linear, Q = quadratic, C = cubic effects of time.

Table D-4. Probability values from least-squares analysis of variance for dependent variables in serum[1] and plasma from 28 d prepartum until parturition.

	P mg/dl	Ca mg/dl	Mg mg/dl	iCa[2] mg/dl	Cl mg/dl	Na mg/dl	K mg/dl	pH	BEB[3] mmol/L	pCO_2 mmHg	HCO_3 mmol/L	Anion Gap mM	Hematocrit %
Treatment (Trt)	0.01	0.80	0.69	0.20	0.19	0.07	0.57	0.22	0.29	0.11	0.18	0.04	0.25
Orthogonal contrasts													
Trt 1 vs. 2,3	0.01	0.97	0.67	0.11	0.42	0.03	0.87	0.16	0.84	0.29	0.55	0.02	0.5
Trt 2 vs. 3	0.01	0.71	0.99	0.30	0.14	0.03	0.51	0.09	0.33	0.07	0.17	0.01	0.18
Time[4]	0.01	0.10	0.13	0.01	0.01	0.01	0.48	0.43	0.10	0.38	0.38	0.62	0.76
Trt*Time	0.87	0.80	0.91	0.01	0.75	0.46	0.08	0.28	0.92	0.68	0.76	0.81	0.01
Orthogonal contrasts[5]													
Trt 1 vs. 2,3 * L time	0.62	0.50	0.40	0.01	0.20	0.27	0.01	0.56	0.81	0.92	0.69	0.97	0.74
Trt 1 vs. 2,3 * Q time	0.43	0.76	0.10	0.80	0.70	0.25	0.20	0.68	0.35	0.43	0.20	0.22	0.10
Trt 1 vs. 2,3 * C time	0.97	0.05	0.20	0.68	0.97	0.59	0.10	0.05	0.78	0.22	0.60	0.84	0.01
Trt 2 vs. 3 * L time	0.38	0.85	0.87	0.04	0.71	0.97	0.03	0.14	0.76	0.21	0.90	0.74	0.02
Trt 2 vs. 3 * Q time	0.65	0.30	0.58	0.23	0.28	0.34	0.55	0.88	0.26	0.24	0.06	0.94	0.01
Trt 2 vs. 3 * C time	0.69	0.74	0.56	0.26	0.78	0.19	0.74	0.84	0.79	0.94	0.22	0.54	0.01
Parity	0.07	0.01	0.81	0.62	0.27	0.01	0.09	0.39	0.25	0.41	0.20	0.39	0.56
Parity*Trt	0.76	0.06	0.90	0.93	0.44	0.92	0.31	0.92	0.76	0.94	0.86	0.18	0.49
Parity*Time	0.91	0.80	0.70	0.43	0.44	0.24	0.11	0.12	0.14	0.02	0.02	0.81	0.01
Parity*Trt*Time	0.91	0.27	0.99	0.06	0.61	0.75	0.19	0.29	0.47	0.35	0.76	0.18	0.01

[1]P, Ca, and Mg were analyzed in serum; remaining variables were analyzed in plasma.

[2]iCa = Ionized Ca.

[3]BEB = Base excess.

[4]Time = 28, 25, 22, 19, 16, 13, 10, 9, 8, 7, 6, 5, 4, 3, 2, and 1 d prepartum.

[5]L = linear, Q = quadratic, C = cubic effects of time.

Table D-5. Probability values from least-squares analysis of variance for dependent variables in serum from 16 d prepartum until parturition.

	Osteocalcin ng/ml	Deoxypyridinoline ng/ml
Treatment (Trt)	0.15	0.80
Orthogonal contrasts		
Trt 1 vs. 2,3	0.06	0.52
Trt 2 vs. 3	0.07	0.67
Time[1]	0.01	0.30
Trt*Time	0.13	0.66
Orthogonal contrasts[2]		
Trt 1 vs. 2,3 * L time	0.13	0.56
Trt 1 vs. 2,3 * Q time	0.58	0.34
Trt 1 vs. 2,3 * C time	0.21	0.23
Trt 2 vs. 3 * L time	0.13	0.41
Trt 2 vs. 3 * Q time	0.02	0.72
Trt 2 vs. 3 * C time	0.49	0.48
Parity	0.93	0.33
Parity*Trt	0.34	0.46
Parity*Time	0.01	0.58
Parity*Trt*Time	0.17	0.54

[1]Time = 16, 3, 2 and 1 d prepartum for all variables.

[2]L = linear, Q = quadratic, C = cubic effects of time.

Table D-6. Probability values from least-squares analysis of variance for dry matter intake and blood serum[1] and plasma during the peripartum period (7 d prepartum through 7 d postpartum).

	DMI[2] kg/d	P mg/dl	Ca mg/dl	iCa[3] mg/dl	Mg mg/dl	Cl mg/dl	Na mg/dl	K mg/dl	pH	BEB[4] mmol/L	pCO_2 mmHg	HCO_3 mmol/L	Anion Gap mM	Hematocrit %
Treatment (Trt)	0.78	0.41	0.55	0.73	0.67	0.51	0.38	0.98	0.45	0.44	0.23	0.25	0.71	0.43
Orthogonal contrasts														
Trt 1 vs. 2,3	0.56	0.22	0.95	0.65	0.46	0.93	0.51	0.83	0.90	0.48	0.71	0.63	0.99	0.37
Trt 2 vs. 3	0.48	0.40	0.62	0.48	0.71	0.45	0.24	0.85	0.59	0.25	0.24	0.22	0.65	0.21
Time[5]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Trt*Time	0.51	0.01	0.82	0.01	0.93	0.64	0.57	0.39	0.15	0.83	0.04	0.21	0.06	0.01
Orthogonal contrasts[6]														
Trt 1 vs. 2,3 * L time	0.43	0.01	0.58	0.01	0.83	0.55	0.24	0.56	0.23	0.57	0.28	0.86	0.01	0.87
Trt 1 vs. 2,3 * Q time	0.82	0.08	0.02	0.03	0.76	0.61	0.64	0.03	0.05	0.91	0.11	0.92	0.04	0.01
Trt 1 vs. 2,3 * C time	0.54	0.01	0.63	0.99	0.36	0.76	0.18	0.04	0.01	0.97	0.06	0.48	0.38	0.02
Trt 2 vs. 3 * L time	0.29	0.55	0.47	0.92	0.97	0.90	0.93	0.98	0.42	0.26	0.80	0.49	0.07	0.45
Trt 2 vs. 3 * Q time	0.51	0.26	0.21	0.43	0.16	0.15	0.54	0.29	0.66	0.10	0.02	0.02	0.87	0.01
Trt 2 vs. 3 * C time	0.21	0.78	0.03	0.65	0.17	0.94	0.94	0.89	0.27	0.73	0.17	0.65	0.53	0.95
Parity	0.49	0.16	0.02	0.30	0.44	0.49	0.07	0.28	0.93	0.33	0.60	0.29	0.47	0.44
Parity*Trt	0.50	0.34	0.11	0.67	0.37	0.88	0.49	0.62	0.54	0.32	0.45	0.37	0.90	0.70
Parity*Time	0.03	0.05	0.82	0.01	0.81	0.11	0.34	0.11	0.09	0.68	0.02	0.07	0.65	0.01
Parity*Trt*Time	0.20	0.82	0.52	0.04	0.29	0.96	0.26	0.65	0.37	0.87	0.10	0.68	0.53	0.01

[1]P, Ca, and Mg were analyzed in serum; remaining variables were analyzed in plasma.

[2]DMI = Dry matter intake.

[3]iCa = Ionized Ca.

[4]BEB = Base excess.

[5]Time = 7, 6, 5, 4, 3, 2, and 1 d prepartum; 0, 6, 12, 18, 24, 36, 48, 60, 72 h; 4, 5, 6, and 7 d postpartum for all variables except DMI. Time = 7, 6, 5, 4, 3, 2, and 1 d prepartum; 0, 1, 2, 3, 4, 5, 6, and 7 d postpartum for DMI.

[6]L = linear, Q = quadratic, C = cubic effects of time.

Table D-7. Incidence rates[1] of hypophosphatemia[2] and hypocalcemia[3] at parturition (0 h) and 6 h after parturition.

Variable	Time	Treatments[4]			Treatment	
		1	2	3	1 vs. 2, 3	2 vs. 3
					P <	
Hypophosphatemia						
	0 h	64.3	38.5	25.0	0.04	0.14
	6 h	50.0	15.4	14.3	0.01	0.09
Hypocalcemia (total Ca)						
	0 h	50.0	69.2	75.0	0.10	0.20
	6 h	64.3	61.5	64.3	0.98	0.93
Hypocalcemia (ionized Ca)						
	0 h	14.3	38.5	25.0	0.16	0.12
	6 h	35.7	38.5	23.1	0.72	0.87

[1]Incidence rates are expressed as a percentages.

[2]Hyphosphatemia was defined as serum P concentrations less than 3.5 mg/dl (T. H. Herdt, personal communication).

[3]Hypocalcemia was defined as serum Ca concentrations less than 8 mg/dl or plasma ionized Ca concentrations less than 4 mg/dl (T. H. Herdt, personal communication).

[4]Dietary treatments: 1 = 0.21; 2 = 0.31; 3 = 0.44% P, respectively.

Table D-8. Probability values from least-squares analysis of variance for dependent variables parturition through 28 d of lactation.

	DMI[1] kg/d	DMI % BW	BCS[2]	BCS change	BW[3] kg	BW change
Treatment (Trt)	0.46	0.65	0.19	0.96	0.05	0.27
Orthogonal contrasts						
Trt 1 vs. 2,3	0.22	0.37	0.08	0.96	0.11	0.37
Trt 2 vs. 3	0.34	0.51	0.09	0.94	0.12	0.83
Time[4]	0.01	0.01	0.01		0.01	
Trt*Time	0.80	0.43	0.92		0.96	
Orthogonal contrasts[5]						
Trt 1 vs. 2,3 * L time	0.20	0.62	0.63		0.64	
Trt 1 vs. 2,3 * Q time	0.95	0.73	0.36		0.60	
Trt 1 vs. 2,3 * C time	0.07	0.32	0.60		0.50	
Trt 2 vs. 3 * L time	0.11	0.98	0.65		0.53	
Trt 2 vs. 3 * Q time	0.51	0.23	0.89		0.56	
Trt 2 vs. 3 * C time	0.43	0.54	0.59		0.69	
Parity	0.26	0.11	0.59	0.11	0.01	0.19
Parity*Trt	0.31	0.44	0.26	0.71	0.41	0.63
Parity*Time	0.21	0.06	0.33		0.99	
Parity*Trt*Time	0.04	0.37	0.88		0.99	

[1]DMI = Dry matter intake.

[2]BCS = Body condition score.

[3]BW = Body weight.

[4]Time = Daily for DMI and weekly for remaining variables.

[5]L = linear, Q = quadratic, C = cubic effects of time.

Table D-9. Probability values from least-squares analysis of variance for dependent variables in serum[1] and plasma from parturition through 28 d of lactation.

	P	Ca	Mg	iCa[2]	Cl	Na	K	pH	BEB[3]	pCO_2	HCO_3	Anion Gap	Hematocrit
	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl		mmol/L	mmHg	mmol/L	mM	%
Treatment (Trt)	0.78	0.76	0.68	0.87	0.50	0.33	0.94	0.20	0.72	0.31	0.41	0.08	0.25
Orthogonal contrasts													
Trt 1 vs. 2,3	0.48	0.70	0.63	0.69	0.83	0.90	0.84	0.38	0.56	0.88	0.77	0.20	0.13
Trt 2 vs. 3	0.51	0.52	0.97	0.86	0.76	0.51	0.75	0.96	0.78	0.52	0.46	0.90	0.10
Time[4]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.37	0.01	0.01	0.01
Trt*Time	0.03	0.98	0.75	0.01	0.46	0.38	0.76	0.88	0.94	0.08	0.32	0.86	0.76
Orthogonal contrasts[5]													
Trt 1 vs. 2,3 * L time	0.01	0.05	0.39	0.05	0.52	0.14	0.14	0.26	0.70	0.33	0.91	0.46	0.03
Trt 1 vs. 2,3 * Q time	0.01	0.04	0.82	0.01	0.65	0.66	0.20	0.74	0.39	0.79	0.62	0.97	0.39
Trt 1 vs. 2,3 * C time	0.01	0.67	0.99	0.02	0.72	0.25	0.75	0.25	0.79	0.22	0.76	0.76	0.81
Trt 2 vs. 3 * L time	0.98	0.79	0.07	0.25	0.24	0.89	0.26	0.23	0.24	0.01	0.11	0.37	0.08
Trt 2 vs. 3 * Q time	0.44	0.18	0.95	0.77	0.61	0.45	0.91	0.79	0.79	0.22	0.41	0.96	0.14
Trt 2 vs. 3 * C time	0.91	0.38	0.86	0.90	0.64	0.75	0.39	0.56	0.28	0.51	0.32	0.46	0.13
Parity	0.17	0.03	0.29	0.21	0.36	0.14	0.41	0.88	0.58	0.33	0.35	0.10	0.38
Parity*Trt	0.25	0.42	0.49	0.86	0.85	0.32	0.95	0.69	0.75	0.75	0.67	0.80	0.70
Parity*Time	0.01	0.18	0.92	0.01	0.03	0.56	0.71	0.08	0.88	0.33	0.51	0.74	0.53
Parity*Trt*Time	0.62	0.07	0.13	0.01	0.89	0.07	0.62	0.26	0.91	0.06	0.58	0.94	0.84

[1]P, Ca, and Mg were analyzed in serum; remaining variables were analyzed in plasma.

[2]iCa = Ionized Ca.

[3]BEB = Base excess.

[4]Time = 0 (parturition), 6, 12, 18, 24, 36, 48, 60, and 72 h; 4, 5, 6, 7, 14, 21, and 28 d postpartum.

[5]L = linear, Q = quadratic, C = cubic effects of time.

Table D-10. Probability values from least-squares analysis of variance for dependent variables in serum from parturition through 14 d of lactation.

	Osteocalcin ng/ml	Deoxypyridinoline ng/ml
Treatment (Trt)	0.92	0.29
Orthogonal contrasts		
Trt 1 vs. 2,3	0.70	0.37
Trt 2 vs. 3	0.71	0.92
Time[1]	0.01	0.30
Trt*Time	0.28	0.63
Orthogonal contrasts[2]		
Trt 1 vs. 2,3 * L time	0.19	0.73
Trt 1 vs. 2,3 * Q time	0.23	0.76
Trt 1 vs. 2,3 * C time	0.49	0.15
Trt 2 vs. 3 * L time	0.61	0.26
Trt 2 vs. 3 * Q time	0.04	0.18
Trt 2 vs. 3 * C time	0.86	0.40
Parity	1.00	0.09
Parity*Trt	0.72	0.89
Parity*Time	0.09	0.52
Parity*Trt*Time	0.04	0.41

[1]Time = 0 (parturition), 1, 2, 3, and 14 d postpartum for all variables.

[2]L = linear, Q = quadratic, C = cubic effects of time.

Table D-11. Probability values from least-squares analysis of variance of dependent variables for milk yield and milk components from parturition through 28 d of lactation.

	ECM[1] Yield kg/d	Adjusted ECM Yield, kg/d	P mg/dl	Fat[3] %	Fat kg/d	Protein[3] %	Protein kg/d	Lactose[3] %	Lactose kg/d	SNF[3,4] %	SNF kg/d	SCC[3]
Treatment (Trt)	0.84	0.54	0.01	0.48	0.98	0.51	0.54	0.23	0.41	0.42	0.39	0.03
Orthogonal contrasts												
Trt 1 vs. 2,3	0.72	0.37	0.01	0.38	0.88	0.92	0.27	0.15	0.20	0.43	0.19	0.01
Trt 2 vs. 3	0.95	0.62	0.06	0.73	0.85	0.63	0.38	0.09	0.35	0.23	0.35	0.04
Parity	0.79	0.94	0.01	0.69	0.98	0.06	0.07	0.23	0.05	0.06	0.09	0.02
Parity*Trt	0.10	0.15	0.11	0.34	0.45	0.52	0.81	0.47	0.72	0.70	0.74	0.31

[1]ECM = Energy Corrected Milk Yield [ECM(kg) = 0.3246*Milk yield (lb) + 12.86*Fat (lb) + 7.04*Protein (lb)/2.204].

[2]Adjusted ECM = ECM yield using SCC as a covariate in the statistical model.

[3]Analysed at Dairy Herd Improvement Association (DHIA) East Lansing, Michigan.

[4]SNF = Solids not fat.

Table D-12. Least-squares means of treatment, parity and treatment by parity for milk yield and milk components from parturition through 28 d of lactation.

	ECM[1] Yield kg/d	Adjusted ECM Yield, kg/d	P mg/dl	Fat[3] %	Fat kg/d	Protein[3] %	Protein kg/d	Lactose[3] %	Lactose kg/d	SNF[3,4] %	SNF kg/d	SCC[3]
Treatment (Trt)												
Trt 1	53.39	54.36	76.88	5.43	2.25	3.06	1.23	4.65	1.89	8.61	3.49	1447
Trt 2	53.24	53.23	70.55	5.32	2.29	3.11	1.29	4.76	2.00	8.77	3.67	592
Trt 3	52.23	51.69	65.50	5.04	2.26	3.00	1.30	4.70	2.04	8.63	3.75	354
Parity												
Parity 2	53.75	53.06	76.59	5.12	2.34	2.98	1.32	4.81	2.12	8.67	3.82	209
Parity 3	52.69	52.72	72.68	5.35	2.30	3.18	1.33	4.74	1.99	8.84	3.71	790
Parity 4+	52.43	53.50	63.66	5.34	2.16	3.02	1.18	4.57	1.83	8.51	3.38	1394
Trt * Parity												
Trt 1 * Parity 2	53.99	53.47	78.26	5.25	2.36	2.97	1.32	4.81	2.15	8.66	3.87	268
Trt 1 * Parity 3	54.13	55.03	75.61	5.64	2.40	3.12	1.27	4.70	1.88	8.74	3.52	1485
Trt 1 * Parity 4+	52.03	54.57	76.77	5.41	1.99	3.10	1.11	4.43	1.64	8.44	3.07	2189
Trt 2 * Parity 2	54.84	54.00	80.59	5.22	2.43	3.11	1.33	4.82	2.07	8.83	3.80	130
Trt 2 * Parity 3	55.91	55.59	71.33	5.76	2.43	3.19	1.34	4.74	2.01	8.85	3.75	463
Trt 2 * Parity 4+	48.98	50.10	59.74	4.98	2.01	3.04	1.20	4.73	1.92	8.64	3.47	1183
Trt 3 * Parity 2	52.40	51.72	70.93	4.88	2.25	2.84	1.29	4.79	2.12	8.51	3.79	228
Trt 3 * Parity 3	48.03	47.53	71.11	4.64	2.07	3.24	1.39	4.77	2.08	8.92	3.87	422
Trt 3 * Parity 4	56.27	55.82	54.47	5.61	2.48	2.91	1.23	4.55	1.92	8.46	3.59	411

[1]ECM = Energy Corrected Milk Yield [ECM(kg) = 0.3246*Milk yield (lb) + 12.86*Fat (lb) + 7.04*Protein (lb)/2.204].

[2]Adjusted ECM = ECM yield using SCC as a covariate in the statistical model.

[3]Analysed at Dairy Herd Improvement Association (DHIA) East Lansing, Michigan.

[4]SNF = Solids not fat.

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