Phosphorus Balance in Transition Dairy Cows: What We Know and What We Don't

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Abstract

Phosphorus in dairy cow nutrition is a matter of contentious debate. Environmental concerns and increasing scarcity of phosphorus reserves dictate a more restrictive use of this mineral in animal nutrition. Concerns with reducing the dietary phosphorus supply in dairy herds with continuously increasing milk yield do however persist and are difficult to dispel because of an incomplete understanding of the metabolic and clinical effects of inadequate phosphorus supply in cattle. Although our understanding of the regulation of the phosphorus balance in ruminants made considerable progress in recent years, important knowledge gaps remain. An important advancement is that in dry cow nutrition overfeeding rather restricted feeding of phosphorus has been identified as a risk factor for transition failure in dairy cows. The period of greatest risk for phosphorus balance disorders becoming clinically apparent is in the first weeks of lactation. While the role of phosphorus in the etiology of some of the clinical presentations commonly associated with phosphorus deficiency, such as the downer cow syndrome, remains doubtful; other clinical signs such as feed intake depression or postparturient hemoglobinuria that predominantly occur in the first weeks of lactation could be induced experimentally by restricting the dietary P supply after calving. This paper reviews the current understanding of the regulation of the P balance in cattle and identifies knowledge gaps requiring urgent work-up in order to be able to give evidence-based advice on phosphorus requirements of the transition dairy cow.

Introduction

Concerns with environmental pollution with phosphorus **(P)** of animal origin and shrinking P reserves in the world are drawing increased attention onto P in ruminant nutrition. Phosphorus contained in animal waste and applied to agricultural land tends to leach into lakes and ponds and thereby contributes to eutrophication of surface waters. Another incentive for a more sustainable, i.e., restrictive use, of this mineral is the shrinking availability of phosphate rock that is the primary and non-renewable source of P (Schroder et al., 2010). On the other hand, providing high producing dairy cows with adequate amounts of dietary P is critical to sustain health and productivity. Producers, nutritionists, and veterinarians voice concerns with restricting the dietary P supply in dairy herds with continuously increasing productivity. Many of these concerns are nurtured by our incomplete understanding of the metabolic and clinical impact P balance disturbances may have in cattle and in dairy cows in particular.

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Background

With increasing intensification of agriculture, the use of P as fertilizer increased through most of the last century. Forage with high P content harvested from these fertilized soils and the widely used practice to copiously supplement ruminant rations with this mineral resulted in continuously increasing amounts of P of animal origin entering the environment (Bomans et al., 2005). To counteract this development, governmental authorities across the world adopted a variety of strategies. Approaches aiming at containing environmental pollution with P originating from animal waste include limiting the amount of manure applicable to agricultural land, reducing livestock density, or implementing taxes penalizing the excessive use of P in animal feed (Bomans et al., 2005). The objective of all these measures is to provide incentives to lower the dietary P supply to cattle to the lowest possible level that does not yet compromise health and productivity. Increasing costs for P as feed supplement as reserves of this mineral dwindle provides another incentive to consider a more restrictive use of P in animal nutrition. Scarcity of P has the potential to hamper the future capacity of the agriculture worldwide to produce sufficient food for a rapidly growing population (Schroder et al., 2010).

In this context, estimates of daily P requirements of cattle also underwent a thorough reappraisal over the last decades. Studies conducted over the last 40 years indicated that true absorption of dietary P in cattle was considerably underestimated for most of the last century, which led to an unnecessary, and to some extent even harmful, oversupply of this mineral (AFRC, 1991; NRC, 2001). Official recommendations for dietary P requirements that are reviewed by governmental authorities in regular intervals were adjusted accordingly in many countries. Current recommendations handled in different parts of the world do vary within a reasonable range, which is primarily due to differences in the estimated P absorption from feed on which these recommendations are based (vary between 64 and 75%). A considerable body of evidence confirming that current recommendations are adequate and do not jeopardize health and productivity has been generated over the years. These new lower estimates of dietary P requirements for cattle were nonetheless received with skepticism by the dairy industry. Phosphorus deficiency is considered to cause or at least to contribute to impaired productivity, poor fertility, and clinical disease in cattle, and common wisdom states that P supply must not restricted in particular during the transition period (Morrow, 1969; Call et al., 1987). Before legal incentives aiming at mitigating environmental pollution with P were implemented, motivation to adhere to these revised recommendations was limited, and feeding P in excess of requirements was commonplace in particular in the dairy industry (NRC, 2001; Plaizier et al., 2004).

Most common clinical signs and symptoms associated to P balance disturbances in cattle are the so called downer cow syndrome, postparturient hemoglobinuria, and anorexia, as well as impaired productivity and fertility (Grunberg, 2014). The association between these presentations and low P supply or hypophosphatemia is in most cases solely based on empirical observation and without good understanding of underlying mechanisms which results in ongoing controversy (Grünberg, 2014).

Regulation of the Phosphorus Homeostasis in Cattle

Differences between ruminant and monogastric species

The P balance of ruminants differs fundamentally from monogastric species. While feed is the only P source of the body for all species, the excretion of this mineral differs fundamentally between ruminants and non-ruminants (Horst, 1986). Monogastric animals excrete P primarily with urine. Ruminants in contrast depend on maintaining a continuous and adequate P supply to the rumen to assure proper fermentation activity of the microbiome and a stable acid-base equilibrium (Breves and Schröder, 1991). Considerable amounts of P entering the rumen are of endogenous origin contained in saliva, much of which is reabsorbed from the small intestine only to be available for resecretion into the rumen. This P recycling in the upper digestive tract of ruminants is apparently very dynamic, but the regulatory circuits are however poorly understood.

Lactating cows furthermore excrete large amounts of P through the mammary gland. Phosphorus loss through the udder presents a challenge, in particular at the onset of lactation. The combination of the sudden onset of milk production and the low dry matter intake around calving, as well as compartmental shifts of P between intra- and extracellular space that are presumably driven by hormonal changes occurring at calving all present metabolic challenges that have the potential to overwhelm counter regulatory mechanisms.

It is evident form this that the regulation of the P balance in cattle must differ from other species and that the transition period presents the greatest risk for P balance disturbances, in particular for high yielding dairy cows.

Sense and nonsense of a combined regulation of the calcium and phosphorus homeostasis

For decades, common wisdom stated that the P homeostasis of cattle and other species is regulated directly but rather indirectly through mechanisms maintaining the calcium **(Ca)** balance in equilibrium (Goff, 2000). Accordingly, the key hormones thought to maintain the P-balance were parathyroid hormone **(PTH)**, a peptide hormone synthetized and excreted from the parathyroid glands and calcitriol ($1,25(OH)_2$-D_3), that is the bioactive form of vitamin D_3 (Horst, 1986). The concept of a combined regulation of Ca and P may be biologically meaningful in growing animals, in particular when not (yet) ruminating, as both Ca and P are accrued in bone at a precise Ca: P ratio (Chen et al., 1998). Supplying Ca and P through feed in at a certain ratio may also appear meaningful to some extent in lactating dairy cows, which excrete both minerals through the mammary gland at a relatively constant ratio as well (Cerbulis and Farrell Jr, 1976). Beyond bone mineralization and milk production, however, Ca and P have only limited common ground metabolically that would support the concept of common regulatory circuits.

A major difference between Ca and P is that the former is a predominantly extracellular mineral, while P prevails in the intracellular space (Grünberg, 2014). Ca in the extracellular space is tightly regulated in cattle and other species, and imbalances with concentrations 25% or more above or below the reference range are associated to well defined clinical signs (Horst, 1986). In contrast, imbalances of the P concentration in serum or plasma (equivalent to the extracellular space) are not nearly regulated as tightly as Ca. Furthermore, clinical symptoms associated with subnormal blood P concentrations are ill defined and not consistently reproducible (Grunberg, 2014).

A series of studies investigating the effects of restricted P supply in cattle at different stages of lactation consistently reported that the P concentration in serum or plasma is easy to disequilibrate, and that this disequilibrium is in most cases without unambiguous and consistent symptomatology. The intracellular P balance characterized by the P content of tissues and cells like muscle, liver or erythrocytes could not be disrupted in these studies (Grunberg et al., 2015a; Grunberg et al., 2015b; Grünberg et al., 2019). It was thus proposed that the focus of the body's P regulation may be on maintaining the intracellular rather than extracellular P balance.

A main driver for the concept of a joint Ca and P regulation were that the hormones regulating the Ca balance (PTH and 1,25$(OH)_2$-D) undisputedly also alter the P homeostasis. Also to this day, no mechanism through which the body could sense a deregulation of the P balance either in the intra- or the extracellular space has been identified. A series of recent studies conducted in cattle and small ruminants demonstrated unequivocally, however, that the ruminant organism responds efficiently to P balance disturbances, even in states of unaltered Ca balance, which refutes the concept of a Ca-dependent P regulation (Grunberg et al., 2015b; Cohrs et al., 2018, Köhler et al., 2021).

What we know about regulatory mechanisms of the P balance

Breakdown of bone matrix through osteoclast activation in states of P deprivation appears to be a primary counter regulatory pathway leading to release of Ca and P from bone. Upregulated osteoclast activity was reported in P deprived cattle at different stages of lactation. Dietary P deprivation results in increased concentrations of bone biomarkers in urine or plasma within a matter of days (Grunberg et al., 2015b; Cohrs and Grunberg, 2018). The mechanisms of osteoclast activation in P deficient ruminants remains enigmatic, but differs from the activation pathway in Ca deficient cattle, where elevated PTH excretion in response to low blood Ca levels is the trigger (Horst, 1986). Such a peak in PTH secretion was not observed in P deprived cattle with unaltered Ca balance (Cohrs and Grunberg, 2018; Wächter et al., 2022b; Keanthao, 2022).

The jury is still undecided on the role of vitamin D_3 as a regulatory hormone of the P homeostasis. Negative associations of calcitriol ($1,25(OH)_2D_3$), the active form of vitamin D_3 with blood [Pi] were reported in P deprived sheep, but not in sheep on adequate dietary P supply (Köhler et al., 2021). A regulatory circuit activated in states of P deficiency that will be discussed below (downregulation of FGF23) was shown to upregulate the conversion of vitamin D_3 from its inactive form calcidiol ($25(OH)_2D_3$) to calcitriol. Clinical trials did, however, not consistently determine elevated calcitriol levels in P deprived cattle, which led to assume that vitamin D_3 is probably not a primary regulatory hormone of the P homeostasis in ruminants (Schröder et al., 1995; Wächter et al., 2022b; Keanthao, 2022).

A novel compound identified early in this millennium that is thought to be a key player in the regulation of the P balance, at least in monogastric species, is fibroblast growth factor 23 **(FGF23)** (Razzaque, 2009). This hormone is synthetized by osteocytes apparently in response to hyperphosphatemia. Main functions of FGF23 reported in monogastric species are upregulation of renal P excretion and downregulation of the conversion of calcidiol to calcitriol (Kuro-o, 2010). This mechanism of action has not yet been confirmed in ruminants due to difficulties with the measurement of this compound in ruminants. Determination of the mRNA transcription rate of FGF23 in sheep after 6 weeks of either adequate

or restricted P supply gave markedly lower FGF23 transcription rates in P deprived than in sheep adequately supplied with P (Köhler et al., 2021). FGF23 may thus not only play a role in the counter regulation of hyperphosphatemia but could rather be counter regulating imbalances of the P homeostasis in both directions. Lower levels of FGF23 in states of P deprivation should at least in theory result in enhanced production of the enzyme 1α-hydroxylase in the kidney that is responsible for the conversion of calcidiol to calcitriol (Köhler et al., 2021). This suggests that vitamin indeed pertains to the regulatory circuits maintaining the P balance.

In summary considerable advances were made in the understanding of the regulation of the P homeostasis in cattle. This progress most of all led to the awareness that earlier concepts of the P regulation cannot be sustained anymore. The overall understanding of how the body maintains an adequate P balance, however, remains patchy.

Phosphorus Balance Disturbances and Their Potential Effect on Health and Productivity of the Dairy Cow

A variety of clinical presentations has been associated with hypophosphatemia or P deficiency in cattle. Associations for example of anorexia, the downer cow syndrome, or postparturient hemoglobinuria with hypophosphatemia or P deprivation are based on empirical observation and lack plausible mechanistic explanations (Grunberg, 2014). Complicating factors are that these presentations do not consistently occur in all animals showing hypophosphatemia or P deprivation and that they are in many cases not experimentally reproducible.

The main challenge in determining deleterious effects of P balance disturbances is the lack of suitable diagnostic parameter for this purpose. The concentration of P in serum or plasma is the established standard to diagnose P balance disturbances. This is however primarily for reasons of convenience and practicality. Phosphorus in serum or plasma is a decent reflection of the short-term P supply and the extracellular P pool that represents less than 1% of the total P pool, but a poor indicator of the total P balance of the body (Grunberg, 2014). This parameter is furthermore affected by a number of factors that are not directly related to the P balance of the body. These include diurnal effects, feed intake, hydration status, acid-base balance, or carbohydrate metabolism just to name a few. Other parameters such as P in rumen fluid, saliva, or red blood cells have been explored for their suitability as diagnostic alternatives with sobering results (Breves et al., 1987; Sharifi et al., 2007; Grunberg, 2014).

Hypophosphatemia is mainly diagnosed in cows in early lactation, which is also the period with the highest disease incidence. In many instances, it remains uncertain if hypophosphatemia is causing or at least contributing to the observed clinical presentation, if it is consequence rather than cause of the disease, or if periparturient hypophosphatemia is just a sign of adaptation to lactation of the fresh cow and is entirely unrelated to the diagnosed disease.

Postparturient hypophosphatemia

Postparturient hypophosphatemia is defined as the decline of the P concentration in serum or plasma that is observed in the majority of clinically healthy dairy cows during the first hours, days, and even weeks of lactation. This presentation should be differentiated from hypophosphatemia occurring in sick fresh cows because the etiology of hypophosphatemia in these cases is likely to be different. A large scale

metabolic profiling study (including over 7000 dairy cows) reported hypophosphatemia ([Pi] < 3.9 mg/ dL) occurring in 71, 21, and 10% of healthy fresh cows on the 1st, 3rd, and 5th day of lactation, respectively (Hansen, 2018). The relevance of this drop of the serum P level below the reference range for health and productivity of the dairy cow remains obscure (Grunberg, 2014). Specifically, it remains unresolved whether transient postparturient hypophosphatemia that is devoid of any clinical symptoms is just part of the physiologic adaption process to lactation, or if it should rather be seen as a harbinger for more severe metabolic disturbances to come.

Downer cow syndrome

Although the mechanism through which disturbances of the P balance could result in involuntary recumbency remains obscure, P deficiency or hypophosphatemia have been incriminated for decades as a cause of this clinical presentation (Menard and Thompson, 2007). Affected cows are typically in early lactation, mentally alert with posture suggesting hind limb paresis, and do not respond to intravenous treatment with Ca salts. A consistent finding in affected animals is again hypophosphatemia that may or may not be associated with hypocalcemia (Hofmann and el-Amrousi, 1971; Stolla et al., 2000; Menard and Thompson, 2007). While P depletion and hypophosphatemia can be experimentally induced without much effort, there is to this date not one single documented case of experimentally induced hypophosphatemic recumbency in cattle. Treatment responses to administration of P salts either orally or intravenously are anecdotal and inconsistent at best.

Postparturient hemoglobinuria ***(PPH)***

Postparturient hemoglobinuria is characterized by intravascular hemolysis predominantly occurring in multiparous dairy cows in their first weeks of lactation. The disease occurs as sporadic but potentially lethal disease affecting only a minority of cows of herd even when the majority of fresh cows display hypophosphatemia (Macwilliams et al., 1982). It was only recently that PPH could be induced experimentally by P depriving animals during the transition period in a reproducible manner in a controlled study (van den Brink et al., 2023). This study furthermore reported the existence of a subclinical form of the disease characterized by pronounced intravascular hemolysis occurring in a subset of P deprived cows that was, however, not severe enough to result in hemoglobinuria (van den Brink et al., 2023).

Feed intake depression

Anorexia or feed intake depression is the most consistent symptom associated with P deficiency across species (Fuller et al., 1976; Knochel, 1977; Call et al., 1987; Ternouth and Sevilla, 1990). The mechanism through which P deprivation supply hampers voluntary feed intake has again not been elucidated. In ruminants, impaired rumen fermentation activity in states of P deprivation was proposed as possible cause for anorexia, a mechanism that does however not explain similar symptoms observed in a variety of non-ruminant species when P deprived (Breves and Schröder, 1991).

Long episodes of restricted dietary P supply of months or even years are well documented to result in reduced dry matter intake, and in severe cases even in pica (Call et al., 1987, Valk and Sebek, 1999). Feed intake depression was also observed after shorter periods of P deprivation in transition dairy cows. Specifically, dairy cows that were on restricted P supply from 4 weeks before to 4 weeks after calving (diets with 0.16% P in DM before, and 0.20% P in DM after calving) did not increase

their dry matter intake after the first week of lactation (Puggaard et al., 2014; Grünberg et al., 2019). In contrast, cows where restricted P supply was discontinued at the time of calving did not show a reduced dry matter intake (Wächter et al., 2022a). These results suggest that restricted dietary P supply, particularly when occurring in the fresh cow period, has the potential to result in feed intake depression.

Impaired fertility

Impaired growth and poor fertility were historically among the first signs attributed to inadequate P supply in ruminants (Gartner et al., 1982; Milton and Ternouth, 1985). Current wisdom states that negative effects on fertility or weight gain are not directly caused by deficient P supply, but rather by inadequate energy and nutrient intake resulting from feed intake depression (Grünberg, 2014). Feeding P in excess of requirements has no beneficial effect on growth or fertility, nonetheless poor fertility remains a common justification to feed P in excess of current recommendations (Wu et al., 2000; Bach et al., 2020).

Dietary Phosphorus Requirements in Transition Cows

As mentioned above, current estimates of daily P requirements for cattle are generally considered to be safe for the use in high producing dairy cows and are even thought to provide a certain safety margin with being based on rather conservative estimates of P absorption rates. Notwithstanding, the adequate dietary P content in rations fed to transition cows remains an issue of controversy. Historically, it was common practice to feed transition cows well in excess of requirements for a variety of reasons. In some instances, the main rationale for oversupplying dry cows with P was to mitigate postparturient hypophosphatemia, while in other instances, high P in dry cow rations was advocated to contain the risk of milk fever or fertility problems in early lactation (Boda and Cole, 1954; Morrow, 1969; NRC, 2001). In the meantime, feeding P in excess of current recommendations in dry cows has been unequivocally identified as being counterproductive and rather more harm than help a smooth transition process (Lean et al., 2006; Keanthao et al., 2021).

Phosphorus in dry cow nutrition

Estimating P requirements of dry cows presents a variety of challenges. The main difficulty arises from the lack of a suitable parameter to identify dry cows in negative P balance. Further to this, the limited duration of the dry period, the impossibility to include productive or reproductive parameters, and the lack of unambiguous clinical signs attributable to P deprivation all contribute to the difficulty of determining at what dietary P level dry cows enter into negative P balance.

Current recommendations for dietary P supply to dry cows range between 0.20 to 0.25% in DM in different countries (CVB, 2005; Landwirtschaft, 2021; National Academies of Sciences and Medicine, 2021). Dry cow rations with 0.30 to 0.40% P and even higher are widely used in the field and can be considered as oversupply. Formulating rations with a P content of 0.25% or below using standard feed ingredients can however be a challenge. This underscores the unlikelihood of P deficiency occurring in dry cows when using standard diet ingredients, as long as these are not produced on heavily P deficient soils. It furthermore underscores that there is in general no justification for including P in a mineral mix for dry cow diets.

Feeding dry cow diets with a P content of 0.36% in DM or above was associated with lower blood Ca concentrations and higher

incidences of hypocalcemia around calving when compared to cows fed diets with 0.22% P in DM (Lean et al., 2006; Keanthao et al., 2021). Feeding cows well short of 0.20% P in DM for the last 4 weeks of the dry period was associated with significantly increased blood Ca concentrations around calving and significantly decreased incidences of clinical and subclinical hypocalcemia compared to dry cows on adequate dietary P supply (Wächter et al., 2022b). The presumed mechanism through which P deficient dry cow diets improve the Ca balance around calving is activation of bone mobilization during the last days of gestation, as described above (Cohrs et al., 2018). In contrast, the precise mechanisms through which dietary P well in excess of requirements obviously hampers bone mobilization is not entirely understood.

Recently restricted P supply during the dry period was discussed as a possible strategy to mitigate periparturient hypocalcemia in dairy cows (Cohrs et al., 2018; Wächter et al., 2022b). Concerns that dietary P deprivation during late gestation could exacerbate the occurrence of postparturient hypophosphatemia and other presentations commonly associated with P deprivation could not be confirmed in small scale studies and as long as restricted P supply was discontinued at the time of calving (Wächter et al., 2022a). These results however certainly deserve to be confirmed in field trials on a larger scale. A major problem with the use of dry cow diets with low P content would be the difficulty to formulate rations with a P content sufficiently low to trigger counter regulation (0.16% P in DM or below) that are otherwise balanced. Feed additives capable of binding dietary P as it is the case for zeolites may facilitate the induction of a negative P balance when included in the dry cow ration. Inducing P deficiency with the objective of triggering bone mobilization may be achieved more easily by using P binding compounds as feed additives than solely by restricting the dietary P content. Phosphorus binders not only make dietary P unavailable for reabsorption, they may furthermore have the potential to withdraw P from the body by also making endogenous P unavailable for recycling.

Taken together the current evidence indicates that feeding P in excess of current recommendations during the dry period is to be avoided whenever possible. Restricting the P supply during the dry period is an interesting approach, potentially supporting health and productivity of the transition cow, which deserves to be explored on a larger scale in future studies.

Phosphorus in fresh cow nutrition

The fresh cow period is the phase of the lactation cycle with the highest risk for P deprivation becoming clinically apparent in dairy cows. Studies investigating the effect of P deprivation in cattle consistently reported the most obvious clinical signs in the first weeks of lactation (Valk and Sebek, 1999; Puggaard et al., 2014; Grünberg et al., 2019). Studies investigating effects of P deprivation in transition cows report considerable negative impact on health and productivity when P deprivation was extended into early lactation. Most prominent observations were marked feed intake depression, decreased productivity, and increased disease incidences. In contrast, when P deprivation was limited to the dry period and adequate amounts of P were provided from the moment of calving, only the beneficial effect on the Ca balance, without any of the side effects mentioned above was observed (Wächter et al., 2022a). From the available data, it is not clear if deleterious effects of P deprivation in early lactation are the result of prolonged P deprivation during late gestation and early lactation, or if P depriving cows only during early lactation would have been sufficient to induce feed intake

depression and increased disease incidence. As mentioned above, the precise mechanisms through which P deprivation in early lactation causes havoc remains obscure.

Based on current knowledge, recommendation for dietary P supply to lactating cows are adequate for fresh cows, and P deprivation limited to the dry period facilitates rather than complicates adaptation of the P homeostasis to lactation. Combining dietary P deprivation with the sudden losses of P through the mammary gland at the onset of lactation, and the low dry matter intake around calving has the potential to overwhelm the cows' counter regulating capacity. Depending on the presence of other metabolic disturbances commonly encountered in fresh dairy cows, the effect of P deprivation or hypophosphatemia and the ensuing clinical presentation may vary from animal to animal. Phosphorus deprivation in early lactation is generally a result of high milk yield in combination of either diets with inadequately low P content, insufficient access to feed, or feed intake depression as it may occur in sick animals. Other factors such as an increased acidotic burden for example when feeding acidogenic compounds like anionic salts (e.g., $CaCl_2$), leading to increased renal absorption, as well as large or repeated oral doses of compounds capable to bind rumen P can exacerbate P depletion (Grunberg, 2014)

Until better understanding of the P homeostasis in fresh cows is available, every effort should be made to avoid P deprivation in this critical period of lactation.

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