IMPACT OF SUPPLEMENTAL PHOSPHORUS SOURCE AND FORM ON UTILIZATION IN LACTATING DAIRY CATTLE

by

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Abstract

Supplemental phosphorus (P) in varying forms and sources: pellet (PELLET), meal (MEAL), liquid (LIQUID) and corn dried distiller's grains with solubles (DDGS) were compared in twelve multiparous Holstein cows producing approximately 43 kg of milk (115 ± 55 DIM) in a 4x4 Latin square with 21d periods. PELLET and MEAL diets contained monocalcium phosphate with a wheat middlings carrier, and the LIQUID contained ammonium polyphosphate in a cane molasses base. The DDGS supplied an organic P source. Cows were blocked by parity, DIM and milk production and randomly assigned to treatments. Data were analyzed using the MIXED model procedure of SAS. Phosphorus intakes of 116, 116, 119 and 118 g/d were similar for PELLET, MEAL, LIQUID and DDGS diets, respectively. Cows consuming the LIQUID diet experienced greater sugar intakes ($P<0.001$). Fat intake was lower ($P<0.001$) for the PELLET, MEAL and LIQUID diets compared to the DDGS diet (1.14, 1.12, 1.07 and 1.36 kg/d, respectively). NE_L intake was similar for all treatments ($P=0.55$). Milk yield differences ($P=0.05$) occurred with the DDGS diet yielding the most milk (34.6, 35.4, 34.1 and 36.5 kg/d). No differences resulted for either milk fat ($P=0.26$) or milk protein ($P=0.33$) percentages or for daily lactose production ($P=0.22$).

Excretion of P in feces tended ($P=0.07$) to differ between treatments (67.4, 66.3, 57.5 and 60.0 g/d) resulting in a trend ($P=0.10$) for greater P retention in diets excreting less P. Secretion of P in milk did not differ ($P=0.51$) between treatments.

Differences ($P=0.04$) occurred in P concentration between diets (0.47, 0.47, 0.49 and 0.47%), but the amount of P fed was not different ($P=0.83$). With similarities for DMI and P concentration in refusals ($P=0.21$) it may be deduced that sorting of the P supplement did not occur.

These data show that supplemental P sources do not affect DMI or P intake, however P source resulted in slight differences in P utilization, but it was not related to sorting of the diet. Utilizing DDGS showed similar responses to inorganic P mineral supplements with favorable production yields making it an adequate substitute for mineral sources of P.

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Dedication

To the One who has given me this life to live. I am grateful for the talents God has given me and I only hope to use them to the best of my ability in the manner He has intended.

To my wife, Melanie, because she is beautiful, amazing, wonderful, delightful, and has a husband who is blessed to be married to her. She has been helpful and supportive through my master's program and is the good woman who helps make a man better.

CHAPTER 1 - Review of Literature

Introduction

Phosphorus (P) is an essential macromineral. While it is present in every cell of the body, 80% of the total body P is found in the teeth and bones. A large portion of P not contained in bone is located mostly in organic combinations including phosphoprotein, nucleoprotein, phospholipids, phosphocreatine and hexose phosphate, and is also a component of many enzyme systems. Phosphorus also makes up 0.15% to 0.2% of the soft tissues. The level of dietary P required by an animal is dependent upon age, production status and species. As the animal advances in age, there is a decrease in the P requirement and also a decreased ability to absorb P. However, an increase in feed intake compensates for decreased P absorption. Increased P intake is due to greater nutrient demands based on animal size and physiological status. As a ruminant animal advances in age and the rumen becomes more highly developed with greater ruminal microbial populations, an increased ability to utilize phytate P occurs. Greater numbers of microbes suggest more enzymatic secretions to act on phytate for P release. Partitioning of minerals for fetal growth in late pregnancy and also increasing milk production levels during peak lactation are when P requirements for cows are greatest. Primiparous cows were found to consume less P, secrete less P in milk, and excrete less total P when compared to multiparous cows (Knowlton et al., 2001). Primiparous cows have less rumen capacity and thus a lower feed intake when compared to multiparous cows and also are not at full potential for milk production. Interactions are present between P and other minerals including iron, aluminum, magnesium and especially calcium, and deem it necessary to balance a ration with these in mind.

Phosphorus Requirements and Importance to Body Function

Deposition of P is an important factor for bone growth in the young animal and in bone maintenance in adult animals. The P requirement for growth is calculated as the sum of the amount of P absorbed and accreted in soft tissues plus the amount deposited in skeletal tissue (National Research Council (NRC), 2001). New bone growth and bone deposition of P are associated with Ca in hydroxy appetite with an estimated accretion ratio of 2.1 g calcium to 1.0 g P (NRC, 2001). The P requirement for bone growth declines as the animal matures.

Depending upon the production level and nutritional status of the dairy cow, diets formulated for P concentrations between 0.32% and 0.42% P are suggested to provide sufficient amounts of absorbable P (NRC, 2001). Calculation of the P requirement was conducted in a factorial approach and is the sum of the P requirements for growth, pregnancy and lactation (NRC, 2001). An adjustment is then made for the availability of P in feedstuffs to calculate the P feeding level. Much like other feedstuffs, the P requirement is based on absorbed values and is dependent upon dry matter intake (DMI). The NRC recommended level was found to be sufficient with few incidences of deficiency in P in a compilation of 8 experiments totaling 162 cows (Weiss and Wyatt, 2004). Utilizing 42 multiparous cows in late gestation, Peterson et al. (2005) fed 3 diets with varying P concentrations (0.21%, 0.31% and 0.44%) to test mineral balance and lactation performance. In concurrence with the NRC, the authors found 34 g (0.21% of diet DM) of P to be sufficient for multiparous cows fed from 28 d prepartum to parturition with no adverse affects on periparturient health, DMI, or lactation performance.

Ruminants suffering from P defficiency show symptoms that include reduced feed intake, reduced rate of gain, low conception rates, reduced milk production, poor appearance, pica and rickets. Long-term deficiencies cause depletion of bone ash resulting in osteomalacia, lameness

and bone fracturing. An experimental comparison of 3 dietary P levels (0.31%, 0.39%, 0.47%) fed for one lactation (most of the animals had been fed a similar diet for one or two lactations previously) indicates that a 0.31% P feeding level for an extended period had no effect on bone strength (Wu et al., 2000). Feeding levels must be in greater deficiency for greater lengths of time to see effects on bone strength, although the authors contend that the 0.31% P feeding level is borderline deficient in cows producing 11,900 kg/308d.

As noted previously, comparing dietary P concentrations that range from sufficient to excess for extended periods of time show little to no positive effects, but dietary P concentrations that range from adequate to deficient may show a negative response. Valk and Sebek (1999) utilized three feeding levels for two lactations at 100%, 80% and 67% of P requirements equaling 3.2 to 3.9, 2.6 to 2.9 and 2.2 to 2.6 g P/kg DM for each feeding level, show the effects of P limitation. Feeding varying amounts of P over multiple lactations demonstrated that cows fed lower levels of P (below requirements) also had lower DMI, which in turn decreased the level of milk production and concluded with an average of 43 kg of body weight loss in the second lactation.

Decreasing P availability to the rumen microbes by diminishing P supply negatively affects digestibility of feedstuffs. Ruminal microbial populations require P for cellulose digestion (Burroughs et al., 1951) with 5 g/kg organic matter digested as the required available P necessary for optimal cell wall degradation (Durand and Komisarczuk, 1987).

Phosphorus is also necessary to maintain reproductive health, although the mechanism to explain the interaction between reproduction and P has not been well defined. Deficiencies in P have been reported to lower conception rate and alter estrous at levels below 0.2% of dietary DM, but P levels above NRC recommended levels have not shown added benefits. Comparing

feeding levels of P (0.31, 0.41 and 0.49%) for the length of a lactation in 26 multiparous cows reveals quadratic effects ($P<0.02$) on days to first estrus (40.6, 77.6 and 43.6) and days to first artificial insemination (69.5, 91.7 and 66.8) (Wu et al., 2000). However, no effects were seen for days open or services per conception. In 54 multiparous cows fed two levels of P (0.35 and 0.47%) beginning at calving, ovarian activity and reproductive performance were measured to analyze the effects of dietary P concentration (Tallam et al., 2005). Cows were synchronized and placed in a breeding program. Results indicated no added benefit of increasing P levels in the diet when measuring ovarian activity and selected reproductive performance measurements.

Although P is necessary for reproductive health, the addition of P above requirements did not show benefits for improving reproductive status. Phosphorus feeding levels suggested by the NRC (2001) are sufficient to maintain reproductive health.

Digestion and Absorption of Phosphorus

Digestion of P occurs in the rumen with the secretion of phytase by rumen microbes. Phytase acts on phytate, or phytic acid, to cleave the inositol ring and release P for absorption. Without phytase, a majority of the P in feed would be unavailable to the ruminant animal because of the bonds binding phosphates to the sugar molecule myoinositol. Approximately 70% of the P in cereal grains is organically bound.

The absorption of P occurs in small amounts in the rumen, omasum and abomasum, but the majority is taken up in the small intestine via active and passive processes. The active process is stimulated via the vitamin D pathway and is readily saturated to allow for passive absorption to occur at higher luminal concentrations (Horst, 1986). Higher P levels in the diet lead to increased absorption but decreased efficiency of absorption. Absorption or regulation of P status and also Ca status are modulated by parathyroid hormone and 1, 25-dihydroxyvitamin

D, a metabolite of vitamin D. When a P deficiency occurs, such that the amounts of P consumed and that absorbed does not meet requirements, decreased plasma P concentrations will occur. The lower plasma P levels influence the pituitary mediated response from the kidneys, a release of 1 α-hydroxylase. This enzyme increases the blood concentration of 1,25 dihydroxycholecalciferol, leading to improvements in P absorption efficiency in the intestine (Horst, 1986). Aluminum contaminants reduce the biological value of phosphate (Church, 1988). Absorption of P is affected when there is a large intake of iron, aluminum or magnesium due to the formation of insoluble phosphate salts. Beryllium also decreases P absorption. Ruminants are able to withstand large calcium (Ca) to P ratios if vitamin D is adequate. A 2 to 1 Ca to P ratio is sufficient, but ratios of 1.1 to 1 or less lead to metabolic or other problems. A great excess or deficiency of Ca disrupts the absorption of P, with P having the same effects on Ca in similar situations. Absorption of P occurs in the orthophosphate form, with the more water-soluble sources generally having higher biological values (Church, 1988). The organic form of P, phyate, must first be converted to an inorganic form, orthophosphate, in the rumen to ensure utilization by ruminal microbes and then absorbed in the small intestine. Phytate may bind protein and Ca leading to insufficient available dietary levels in nonruminant animals which lack the necessary phytase to extract these nutrients.

Evidence of the conversion from organic to inorganic P was demonstrated through fecal analysis (Toor et al., 2005). Inorganic orthophosphate increased from a concentration of 55% in the diet to 62% in the feces while the proportion of phytic acid decreased from 32% in the diet to 18% in the feces.

Altering ruminal pH affects P disappearance. Mineral in situ disappearance was measured by Emanuele and Staples (1994) using treatments including a control, an acid

treatment, an energy supplement and a buffered energy supplement. Lowering of rumen pH improved the extent of P release (87.8 vs. 90.2%). Increased daily outflow of P from the rumen was also reported, especially in the liquid flows when animals experience lower ruminal pH.

The addition of dietary fat may also play a role in P absorption. Calcium salts of palm fatty acid distillate (CS) and animal-vegetable fat (AV) were added to the diets of five ruminally and duodenally cannulated cows at levels of 0%, 2.5% and 5% (Rahnema et al., 1993). Supplementing dietary fat increased P intake and resulted in increased apparent total tract P absorption and total tract P absorption. The authors speculate that the increased absorption may be the result of increased P supplied to the tract from residual phospholipids in the AV, thus resulting in increased absorption and not necessarily due to the addition of fat to the diet. Wu et al., (2008) found that supplementing soy oil tended to decrease fecal P. This may be the result of increased apparent P absorption.

Phosphorus absorption occurs mainly in the small intestine. The amount of P available for absorption is dependent upon the presence of adequate phytase to release phytate bound P and also ensuring that P is not bound in an insoluble phosphate with other minerals. Increasing the dietary P level results in increased absorption but decreases the efficiency of absorption. Calcium and P play important roles in the absorption of each mineral. Ruminal pH and the inclusion of dietary fat may also affect the absorption of P.

Phosphorus Availability in Feedstuffs

Phosphorus availability varies between feedstuffs. Phytate P as the main source of P in concentrates, cereal grains and grain byproducts and is made available to ruminants by the enzyme, phytase. This enzyme is supplied by the rumen microbial population. Microbial populations including *Selenomonas ruminantium* and *Megasphaera elsdenii* are main suppliers

of phytase with *S. ruminantium* as the main supplier in concentrate diets (Yanke, 1998). The absence or low concentration of this enzyme in nonruminants greatly reduces P availability. Utilizing rumen fluid, in vitro phytate P disappearance was measured by Morse et al. (1992) in 6 concentrates including cottonseed meal, dried distillers grains, ground corn, hominy, peanut meal, rice bran, soybean meal and wheat middlings. Phytate P disappearance was measured at over 99% after 24 h. These results were reinforced through an in vivo trial displaying similar results of minimal phytate P occurring in the feces. These results led the authors to conclude that phytate P from concentrates used in dairy cow diets is available for absorption.

Ruminal solubility of minerals was analyzed in alfalfa hay, fescue hay, bromegrass hay and corn silage individually (Ledoux and Martz, 1991). Results show 48 h ruminal in situ P disappearance being greatest for fescue (87%), followed by corn silage (79.5%), the average of two alfalfa samples (68%) and bromegrass (41%).

Martz et al. (1990) utilized 4 lactating cows in a trial comparing an alfalfa-based diet to an alfalfa-corn silage based diet without supplemental P to determine the true absorption of P. It was determined that an alfalfa hay-corn silage based diet had similar true absorption of P compared to an alfalfa hay based diet (74.6% vs. 64.4%). True absorption was calculated on a percentage basis as the difference of intake and the sum of daily fecal mineral excretion and daily endogenous fecal loss of mineral divided by intake of mineral. This calculation is dependent upon the determination of endogenous fecal loss, conducted through a single intravenous pulse dose of isotopes and collection of plasma and feces over time. An equation based on the specific activity in the feces and plasma is then utilized to calculate endogenous loss. A second way to determine endogenous loss is by feeding diets devoid of P. Apparent absorbed P was different ($P<0.01$) with the alfalfa-corn based ration having greater absorption

than the alfalfa based ration (26.23g vs. 14.82g). Apparent absorption is the difference of mineral intake and mineral fecal content and may be used to estimate the amount of mineral retained in the body. Substituting soy hulls, a more digestible non-forage fiber source of P, for alfalfa hay reduced fecal output (Wu, 2005). Forages are extensively used in dairy cattle diets, and the P availability from diets with higher forage contents may affect P utilization. A comparison of varying forage levels (48% vs. 58%) and P levels (0.33% vs. 0.42%) were conducted to determine forage level effect on P output (Wu et al., 2003). Forty-four multiparous cows and 4 dietary treatments were utilized: low P, low forage; low P, high forage; high P, low forage; and high P, high forage. Results indicate that daily P absorption is not affected by forage level (28.6, 22.3, 32.1 and 29.7 g). However, the supplemental P resulted in increased P intake, fecal P content and fecal excretion. The authors concluded that the overall impact of the amount of forage in the diet had little effect on estimated P output, and more to do with P intake than forage proportion.

Soybean meal is a typical protein source in dairy cow diets. Substituting blood meal for soybean meal in 36 Holstein cows decreased P retention and P absorption (Knowlton et al., 2001). Comparing corn to barley as the principle grain source indicated that cows consuming corn grain in a total mixed ration (TMR) had the greatest P intakes but the lowest fecal excretions of P (Kincaid et al., 2005). Guyton et al. (2003) compared steam flaked corn and dry ground corn and the effects on P digestibility. Cows consuming diets with steam flaked corn had similar P digestibility and apparent P absorption as compared to diets containing dry ground corn.

Phosphorus disappearance in corn and soybean by products has little been studied or measured. Mjoun et al. (2008) conducted 2 in situ studies to measure P disappearance from corn

or soybean-based feedstuffs, including 3 sources of distiller's grains with solubles, corn, corn germ, solvent extracted soybean meal, expeller soybean meal, extruded soybeans and soyhulls. Results of the first study indicate that ruminal P disappearance was similar for all feedstuffs except soyhulls. Soyhulls had the lowest P disappearance, from hour 12 to the conclusion of the experiment at 48 h. Corn by products and corn germ contained the highest amounts of the rapidly soluble P fraction while the soy-based feedstuffs contained the highest amounts of potentially releasable P fraction. Soybean meal had greater extent of P digestion than corn by products in the first 12 h.

The second experiment by Mjoun et al. (2008) compared P disappearance from 3 dried distillers grains with solubles samples (DG1, DG2 and DG3) and one sample of wet distillers grains with solubles (WDG). Differences were seen between dry distillers grains samples and also the wet distillers grains sample in the rapidly soluble P fraction up to 3 h, but at the completion of 36 h, no difference was measured in P disappearance. Overall, WDG was similar to DG2 in effective disappearance of P (88.1 and 89.8%), but both previously mentioned samples were different from DG1 and DG3 with effective disappearance values of 92.1 and 92.8%. DG1 and DG3 did not differ from each other.

Although phytate P is made available by the enzyme phytase, it may not be fully hydrolyzed in the high producing cow with high ruminal dilution rates. This results in phytate P from undigested grains escaping the rumen (Clark et al. 1986).

Phytate P is found to be highly available in the rumen due to the presence of phytase produced by the rumen microbial population. Different forages and combinations of forages may affect phytate P availability, but starch source does not affect P digestibility. Ethanol by products were found to have similar availabilities as various soybean products. Although phytate

P may be highly available in most feedstuffs, some may remain undigested due to the high ruminal dilution rates in high producing cows.

Phytase and Enzymes

As mentioned previously, the enzyme phytase is naturally produced in the rumen of the ruminant animal, but supplementing additional phytase to increase P release may show positive results. Kincaid et al. (2005), supplemented phytase as a treatment with a barley grain diet and a corn grain diet. Results indicate decreased levels of phytate P in feces and increased hydrolysis of phytate P. Tendencies for decreased fecal P and improved P digestibility were also discovered when exogenous phytase was added. Similar results were found with decreased fecal P and also decreased total fecal output when exogenous enzymes were added to TMR diets in a phytase-cellulase enzyme combination (Knowlton et al., 2007). Utilizing the enzyme combinations of phytase and cellulase, dietary comparisons were conducted between high P (0.49%), low P (0.32%) and low P with the added enzyme (Knowlton et al., 2005). Similar to the previous study, no treatment effects were seen on DMI, milk yield or milk components. However, there were no effects on the amount fecal DM or DM digestibility. Fecal P excretion was decreased with the addition of enzyme in the former and latter experiments.

The addition of phytase to non-ruminant diets is essential. Phosphorus digestibility in barrows was improved by 24 percentage points with the addition of 800 phytase units/kg when compared to diets not containing phytase (Mroz et al., 1994). There were also improvements in digestibilities of dry matter, organic matter, crude protein and Ca. Adding phytase along with organic acids also demonstrates the importance of phytase. Omogbenigun et al. (2003) reported significant improvements in P digestibility and an improved ratio of P excretion to feed consumed, resulting in a 19.4% decrease in P excretion for starter pigs. In swine, phytate P is

more available in dried distillers grains than in corn, but a decreased fecal phytate P concentration was still observed with the inclusion of phytase in lactating sows (Hill et al., 2007).

Phytase continues to play a role in non-ruminant diets for greater digestibility of P as well as protein and selected minerals bound to phytate in feedstuffs. Ruminant diets see small improvements in P digestibility due to added phytase. Exogenous phytase may be used to assist in phytate digestion in high producing cows with high ruminal flow rates, an issue that may decrease the ability of the microbial phytase to fully hydrolyze phytate P.

Although phytase is naturally present in the rumen of the ruminant animal, the addition of exogenous phytase may improve P availability in cow with high ruminal flow rates. The addition of exogenous phytase is essential for non-ruminant animals due to minimal secretions of this enzyme. Adding phytase to the non-ruminant diet allows for greater digestibility of not only P but also Ca and protein.

Mineral and Non-mineral Phosphorus Sources

Mineral Phosphorus Sources

Ruminal availabilities of varying mineral P sources including sodium phosphate, mono-dicalcium phosphate (21% P), mono-dicalcium phosphate (18.5% P) and defluorinated rock phosphate were measured by Witt and Owens (1983). In vitro, sodium phosphate proved to be the most ruminally available, followed by the 21% P and 18.5% P mono-dicalcium phosphate supplements, and lastly the defluorinated rock phosphate (100, 87.6, 61.5, 39.7%, respectively). Values were based on a linear standard curve for sodium phosphate fed to steers at increasing levels. Solubility was also measured in vitro using abomasal fluid with similar results as the

availability trial. Sodium phosphate was most soluble followed by the 21% and 18.5% mono-dicalcium phosphate supplements and then the defluorinated rock phosphate (100, 76.6, 43.9, 35.5, respectively).

Non-mineral Phosphorus Sources

Supplementating phytic acid, a non-mineral P form, resulted in a tendency for greater apparent absorption but no difference in apparent digestibility when compared to non-supplemented diets (Guyton et al., 2003). Supplemental phytic acid increased P intake by 29 g from the unsupplemented treatment. This resulted in greater total P excretion, but did not affect DMI, milk yield or milk components.

Wheat bran was utilized as a supplemental P source in comparison to a mineral source in 36 Holstein cows. The experiment was comprised of 2 periods including early lactation and midlactation cows to measure P partitioning (Knowlton et al., 2001). Apparent P digestibility and milk P concentrations were not affected by treatment, but P intake was greater for the mineral treatment. Increased average P intake (14 g/d) by the mineral P source over the wheat bran resulted in more total P excreted (46.8 vs. 40.3 g/d), more P retained (13.4 vs. 7.6 g/d) and more P excreted in feces (46.1 vs. 39.7 g/d). Milk yield and milk components were unaffected by treatment.

Utilizing a non-mineral or organic source of P may allow for a reduction in use of mineral sources and feed cost due to similar production results because the ruminant animal has the capability to utilize phytate P from feedstuffs.

Route of Phosphorus Output

Routes for P excretion or secretion include feces, milk, urine and saliva. Fecal matter is the primary route while urine contains minimal amounts of P when feeding levels are within the suggested range. Excretion of P is limited in urine (~1 g/cow per day) because the kidney is not a major route of P excretion in the ruminant animal (Horst, 1986), but levels ranging from 1.3 to 4.9 g P/d have been shown when P levels exceed 0.40% of the diet (Morse et al., 1992; Knowlton and Herbein, 2002).

Internally, salivary P secretion supplies approximately 40% of the total P to the rumen. Saliva contributes about 80% of the total endogenous P, with 30 to 90 g P/d typically occurring (Reinhardt et al., 1988). Saliva P levels are reported to be 4 to 5 times that of plasma (NRC, 2001). Also, decreasing plasma P concentration reduces saliva P concentration. Saliva P levels were measured for 2 lactations and 2 dry periods to analyze the effects of P concentration (Valk et al., 2002). Salivary P levels varied from 4.9 to 8.6 mmol/L with the 67% P feeding level usually having the lowest concentration when compared to diets with P concentrations at 80 and 100% of P requirements. Feeding high concentrate diets decrease saliva flow and salivary P, and also increase urinary P excretion (Scott and Buchan, 1985). Decreased saliva production in high concentrate diets is a result of decreased rumination time from smaller particles not requiring further breakdown by mastication.

Other routes for P include shedding of the hair coat and skin cells. The P concentration of the hair coat was measured in seven Hereford cows (O'Mary et al., 1969) using samples of white hair and red hair collected from the face and rump, respectively, and analyzed for P. Results indicated P concentrations as 164 ppm for red hair and 144 ppm for white hair. P analysis of hair samples collected from the forelock on 359 Black Pied Lowland cattle showed P

concentration of 232 ppm (Neseni and Koriath, 1967). While P concentration appears to be variable in cattle hair, a value close to 200 ppm is accurate and would be approximately double the concentration of sloughed skin cells. These outlets for P are ongoing processes as hair regrows and replaces hair that has fallen out and skin cells are being regenerated.

Milk Secretion

Milk secretion of P is broken down into 20% esterified in casein, 40% as colloidal inorganic calcium phosphate, 30% as phosphate ions in solution and 10% associated with the lipid fraction of milk (Jenness and Patton, 1959). Milk P secretion is dependent upon milk production, retained P (Morse et al., 1992) as well as breed (Cerbulis and Farrell, Jr., 1975) and accounts for approximately 1 g P per kg of milk. Reductions in milk yield in later lactation reduce milk P content (Forar et al., 1982). Modeling of P digestion and metabolism by Hill et al. (2008) in lactating dairy cows fitted to 2 studies from the literature found that 30% of absorbed inorganic P was used for milk synthesis. Feeding differing levels of P at 0.31, 0.39 or 0.47%; 0.37 or 0.57%; 0.34, 0.51 or 0.67% of the diet DM showed no effects on milk yield or milk components with varying P concentrations (Wu et al., 2001; Lopez et al., 2004; Knowlton and Herbein, 2002). In the study by Wu et al., milk production tended to be greater for cows consuming diets including P at 0.31% of diet DM, as opposed to 0.39%. Cows fed for P intakes at 67% of requirement for two lactations had lower milk production in the first twenty-one weeks of the second lactation when compared to cows fed at 100% or 80% of requirement (Valk and Sebek, 1999). While milk production suffered, no difference was detected between treatments on milk components.

Fecal Excretion

Fecal P excretion is the major route of excretion, accounting for 60-70% of total P intake. Spiekers et al. (1993) noted that fecal P can be categorized into 3 components. The first portion is labeled unavailable dietary P, referring to dietary P that cannot be absorbed under any conditions. The second portion is labeled inevitable P loss, the portion consisting of microbial residue P and metabolic P from used microbial cells or sloughed cells from the digestive tract. The third portion is labeled regulated P, a component that varies according to P intake relative to cow requirement. This portion is regulated through excretion of excess dietary or salivary P dependent upon the physiological status of the cow.

Quantifying each portion of fecal P may be of value. Inevitable P loss in feces amounted to 1.2 g/kg DMI (Spiekers et al., 1993) in 2 sets of dairy cows fed diets with low P concentrations assuming P at 100% availability. However, Wu et al. (2000) suggest 85% as the maximum amount of P available with this level only being attained in animals very deficient in P. Utilizing the suggested level of 85%, the authors find inevitable P loss to be 0.9 g/kg of DM consumed. Endogenous fecal P loss was estimated using isotopes to label P and measure true absorption in lactating dairy cows (Martz et al., 1990) with results of 0.9-1.5 g/100 kg of body weight being found. Regulated P would then be the difference of P contents of intake, milk, urine, inevitable P and unavailable P.

As P feeding levels increase above requirement, an increasing amount is partitioned toward excretion in the feces. Increasing P intake from 82 g/d to 112 g/d (0.41% to 0.56% of diet DM) resulted in a 48.6% increase in fecal P excretion, while a decrease in P intake from 82 g/d to 60 g/d (0.41% to 0.30%) resulted in a 22.7% decrease in fecal P excretion in multiparous lactating cows fed 20 kg DM/d (Morse et al., 1992). At feeding levels of 0.31, 0.39 and 0.47%

of diet dry matter, Wu et al. (2001) found for every 0.08% increase in P in the diet, fecal P excretion increased by approximately 20 grams. Utilizing a model, Weiss and Wyatt (2004) created an equation that concurs with Wu et al. (2001) that fecal excretion of P increases linearly as intake of P increased. Also discovered by the authors was that fecal excretion of P was negatively related to apparent DM digestibility.

Results by Toor et al., (2005) demonstrate the conversion of organic P to inorganic P in the digestive tract as phytic acid concentration is decreased by over 40% from the diet to the feces (32% vs. 18%). Water-soluble P is a form more available to the environment. At feeding levels of 3.4, 3.1 and 3.7 g/kg DM as the base diet P levels in three trials, water soluble P accounted for 56, 58 and 64% of the total P in the feces (Dou et al., 2002). The authors also found that much of the water soluble P was in an inorganic form (80, 84 and 92%, respectively).

Fecal samples from over 30 farms in Northeastern and Mid-Atlantic regions of the United States contained an average of 50% water soluble P, with 83% of the water-soluble fraction as inorganic P (Chapuis-Lardy et al., 2004). Utilizing 13 manure samples to analyze P content and P fractions showed results for inorganic P varying between 1000 and 6800 mg P/kg DM and organic P ranging between 130 and 1700 mg P/kg of total P in manure (He et al., 2004). Correlation analysis results for the water-soluble fraction reveal a higher correlation between inorganic P and total manure ($r^2 = 0.62$) than the correlation between organic P and total manure ($r^2 = 0.24$). Differences are present when analyzing feces as opposed to manure since manure is the inclusion of feces, urine, bedding, and dirt. Manure from dairy farms has been shown to contain numerically greater percentage of inorganic P (73 vs. 62%) and also significantly lower concentrations of organic P in the phytic acid form (18 vs. 9%) than feces collected from the same farm (Toor et al., 2005). The authors speculate that alterations in the concentration of P

form may be attributable to phytase present in the feces or in the manure storage area which further converted phytic acid to inorganic orthophosphate. Further analysis showed that along with decreased phytic acid, phospholipids and DNA in manure were also decreased compared to feces, thus contributing to the increased inorganic P level present in stored manure.

Overfeeding Effects

Overfeeding of P in lactating cow diets is a cause of concern due to the high probability of excess P entering surface water by washing out of manure applied to cropland. The ideal situation would be to know the precise amount of P to be fed to cattle to optimize health and performance. There are many obstacles that arise and many factors that play into understanding how much P to feed how much P can be digested and absorbed by the animal so that there is a minimal percentage that is passed through the body.

Increasing the amount of P above NRC recommendations was not shown to have effects on milk yield or major effects on milk fat, milk protein or the incidence of health problems in cows fed from parturition to 165 days in milk (DIM) (Lopez et al., 2003). Results of Wu et al. (2000) agreed that feeding excess P to early lactation cows is not necessary since the cow has the ability to mobilize a minimum of 500-600 g of P from bones in early lactation. Mineralization of bone occurs over the length of a lactation to replenish minerals drawn from bones in early and peak lactation. As milk production decreases and the demand for minerals for milk production decreases, more can be partitioned to replacing bone minerals.

Surveys have shown that 34% of farms in the northeast United States are feeding above the P guidelines set by the NRC, with the majority of these farms following the recommendations of their consulting nutritionist (Dou et al., 2003). A survey of 54 farms in Wisconsin revealed an average feeding level of 4.1 g P/kg, a level above the NRC recommended

level of 3.8 g P/kg (Powell et al., 2006). Supplementing P above requirements for early lactation cattle leads to increased fecal and urinary excretion (Knowlton, 2002).

The majority of excess P in diets was excreted in feces with almost the entirety of the excess in the water-soluble form (Dou, 2002). The environmental concern about P, especially the water-soluble form, is the ease at which this P form can leach out of manure into surface water. Water-soluble P is inorganic and originates from saliva. Salivary P, which has been recirculated (Valk, 2000), contributes a large portion of P to the rumen (Wu et al., 2000). As feeding level of P increased (3.4, 5.1, and 6.7 g P/kg feed intake) nearly all the P consumed above the base diet level was excreted in the feces and mostly in the water-soluble form (Dou et al., 2002). The level of inorganic, water-soluble P is affected by fecal pH and exists mostly as Ca-P complexes (Chapuis-Lardy et al., 2004). Further analysis by Chapuis-Lardy revealed fecal Ca having a major impact on the proportion of water-soluble inorganic P in total phosphorus excretion.

Application of manure to cropland is a typical method of manure management on dairy farms. Difficulties are encountered with land closely adjoining the dairy, especially if the dairy has been located in the same area for some time, and surface application of manure has been practiced for a similar length of time. Land closest to the dairy is utilized most often for manure application due to its close proximity to the dairy and shorter hauling distances. Overfeeding of P increases fecal P excretion, resulting in greater amounts of P applied to cropland. Over time, P builds in soil and leaching of P in runoff becomes an increasing risk to surface water. When applying manure at similar rates, but differing concentration of P from diets with high and low concentrations of P, runoff was 4 times greaster for soils receiving the high P manure (Ebeling et

al., 2002). Simulating rainfall on manure packed columns showed a linear relationship between P loss in leaching and concentration of water-soluble P in manure (Sharpley and Moyer, 2000).

Utilizing whole farm nutrient balancing to monitor P importing and exporting on the farm is useful. This method shows the benefits of manipulating diet composition to ensure the balance between nutrients entering or leaving the farm. Exporting of P from a farm occurs in 3 main ways including milk, manure and meat. A model utilized by Kebreab et al. (2008), assuming 0.39% P in the diet and over 340,000 cows, indicated that reducing the dietary P concentration by 0.02% would save 620 metric tons of P per year. Cerolsaletti et al. (2004) implemented nutrient balancing on 4 farms. Adjusting the dietary P level contributed by concentrates reduced the amount of P remaining on the farm by 49% in adjusted balances. The amount of P remaining on the farm was less than 45%. Season of the year for application, type of crop utilized and soil type are also factors considered in utilizing nutrient balancing.

Conclusion

The results of this review are much the same as what previous research and reviews have shown. Prediction of P requirement is possible but accuracy will be affected by many factors. The level of excretion is highly related to the intake level of P, with the majority of the excess excreted in the feces. There is variability between farms as well as within farms in the amount of P excreted (Chapuis-Lardy, 2004) and may also be a result of the fecal sampling due to the time of day the sample was taken (Wu, 2000) or the type of analysis, wet versus dried-ground, that was performed (Chapuis-Lardy, 2004). Variability is less common within research facilities due to the ability to control factors including dry matter intake and total collection of urine and feces. However, variability still exists and once again is related to factors stated previously. As the level of P consumed increases, the amount of excretion and secretion increase depending upon

the level of milk production and physical state of the animal. Urine excretion of P is relatively stable with minimal increases in amount as P intake increases. Utilizing organic sources of P may reduce the need for inorganic supplementation. Decreasing the amount of P excreted is essential to decreasing dietary costs of supplementation and also decreasing the environmental impact. This suggests that further research is necessary to further develop options to improve P utilization.

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CHAPTER 2 - Impact of supplemental phosphorus source and form on utilization in lactating dairy cattle

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Abstract

Varying forms and sources of supplemental phosphorus (P) were compared in this experiment to test the effects on P intake, utilization, and excretion. Twelve multiparous Holstein cows producing approximately 43 kg of milk (115 ± 55 DIM) were utilized in a 4x4 Latin square design with 21d periods. Four total mixed rations (TMR) were formulated for similar P concentrations. Supplemental P was provided in a pellet (PELLET), meal (MEAL) and liquid (LIQUID) form. Wheat middlings were used as a carrier for monocalcium phosphate in the pellet and meal forms while a cane molasses based liquid was the delivery form for ammonium polyphosphate in the third diet. Corn dried distillers grains with solubles (DDGS) were used in the fourth diet for comparison between organic and inorganic P sources. Cows were blocked by parity, lactation number and milk production, then randomly assigned to treatments. Data were analyzed using the MIXED model of SAS. Dry matter intake (DMI) was not affected by diet and P intake was similar for the PELLET, MEAL, LIQUID, and DDGS diets (116, 116, 119 and 118 g/d, respectively). Cows consuming the LIQUID supplementation diet experienced increased sugar intakes ($P<0.001$) and also the lowest daily milk production. Daily fat intake was significantly greater ($P<0.001$) for the DDGS than the PELLET, MEAL, and LIQUID supplemented diets (1.36, 1.14, 1.12 and 1.07 kg/d, respectively). The distillers grains replaced a portion of the corn grain in the DDGS diet which decreased ($P<0.03$) starch intakes of cows consuming that diet, but intake of NE_L was similar ($P=0.55$) for all diets. Differences ($P=0.05$) in milk production were noticed with the DDGS supplemented diet resulting in the greatest milk production (34.6, 35.4, 34.1 and 36.5 kg/d). Milk fat percentage was greatest for the MEAL diet but no difference was seen between diets for milk protein percentage ($P=0.33$) or lactose production ($P=0.22$).

Fecal P excretion tended (P=0.07) to differ between treatments (67.4, 66.3, 57.5 and 60.0 g/d) resulting in a trend (P=0.10) for greater P retention in treatments with lower fecal P excretion. Apparent P absorption (P=0.19) and secretion of P in milk did not differ (P=0.51) between treatments.

While analysis of dietary treatments displayed differences (P=0.04) in P concentration between diets (0.47, 0.47, 0.49 and 0.47%), the actual amount of P fed was not different (P=0.83). After noticing similarities in DMI and P concentration in refusals (P=0.21), it may be deduced that sorting of the P supplement did not take place.

These data show that supplemental P sources in this experiment did not affect DMI or P intake, however P source resulted in slight differences in utilization between treatments, but P source or form were not related to sorting of the diet. Utilizing the organic P source in DDGS shows responses similar to inorganic P mineral supplements with favorable production yields making it an adequate substitute for mineral sources of supplemental P.

Introduction

Developing feeding strategies to reduce P excretion is crucial to alleviate the potential environmental hazards of P entering surface water and the resulting algal growth and eutrophication. The NRC (2001) recommended P feeding level is 0.32 to 0.42%, depending upon the physiological status of the animal, has well been shown to be sufficient and negates the need to provide P at levels that exceed this range (Wu et al., 2000; Knowlton and Herbein, 2002; Lopez et al., 2004; Weiss and Wyatt, 2004). Overfeeding of P has been practiced to ensure adequate absorption P to meet the needs of the animal for milk production and reproduction. Reproduction efficiency may be decreased at dietary levels below 0.2%, but P feeding levels greater than or equal to the NRC (2001) suggested amounts result in no negative effects on

reproductive performance as measured by number of days open and services per conception (Wu et al., 2000; Tallam et al., 2005). Dietary manipulation of the P supplementation level has been shown to be an effective method for decreasing P output and also decreasing cost. A model utilized by Kebreab et al. (2008) showed that reducing feeding levels from 0.41% of dietary DM to 0.35% decreased P output into the environment by 1.3 kilotons/yr and also saved producers $20 Canadian per cow/yr. Utilization of organic P sources, such as phytic acid or other feedstuffs to meet the animal's P requirement, may be of value to reduce the use of inorganic P supplementation. The objective of this experiment was to analyze the effects of varying sources of supplemental P presented in three forms on milk production and composition, P partitioning in the cow and diet sorting in lactating dairy cows.

Materials and Methods

Cows, Diets and Sampling

All procedures were conducted under the approval of the Kansas State University Institutional Animal Care and Use Committee. Twelve multiparous lactating dairy cows (115 ± 55 DIM) (44.45 ± 6.35 kg milk) were fed one of four diets with similar P levels but differing in form of P supplementation and source in a 4 x 4 Latin square design with three replications. Treatment periods were 21 d in length with the first 14 d serving as adaptation and the last 7 d for data collection. Cows were randomly assigned to treatments after treatment groups were balanced by DIM, milk yield and parity. Phosphorus supplementation included a wheat middlings based meal (MEAL) and pellet (PELLET) containing monocalcium phosphate and a molasses based liquid (LIQUID) containing ammonium polyphosphate. The fourth diet used corn dried distillers grains with solubles (DDGS) as an organic source of supplemental phosphorus for comparison to inorganic sources and was presented in a meal form.

Cows were housed in a tie-stall facility at the Kansas State University Dairy Teaching and Research Center. The animals were individually fed a total mixed ration (TMR) twice daily at 500 and 1700 h with feed and orts weighed daily to offer feed at 5 to 10% in excess of intake from the previous day. TMR and orts were sampled daily during the collection week, frozen and later composited by treatment and period. Feed ingredients were sampled weekly, frozen and later composited by period. Body weights were collected at the beginning of the trial and on d 20 and 21 of each period following the p.m. milking. Body condition scoring (Wildman et al., 1992) was performed following collection of body weights on the same d.

Cows were milked twice daily at 500 and 1600 h. Milk weights were recorded at every milking and 2 milk samples were collected for 6 consecutive milkings beginning d 1 of the collection week each period. One sample was collected in a vial with preservative, the second was collected in a vial without preservative and frozen for later analysis. Fecal grab samples were collected every 8 h for 4 d beginning on d 1 of the collection week with sampling time advanced 2 h each day to account for diurnal variation (Knowlton et al., 2007). Fecal samples were frozen after collection was completed for all cows and later composited by cow and period based upon sample weight. Blood samples were collected on d 4 and 5 of the collection week at approximately 90 minutes post feeding (Kincaid et al., 2005) via coccygeal vein utilizing a 20-gauge needle, and 10 mL non-heparinized vaccutainer blood tubes. Samples were allowed to clot, centrifuged at 2400 rpm for 20 min and serum was transferred by pipette to labeled microcentrifuge tubes and frozen for later analysis.

Laboratory Analysis

Weekly silage samples were collected for DM analysis to allow for dietary adjustments. Minimal variation occurred, not requiring dietary adjustment for silage DM. Orts and feed

ingredients were analyzed by Cumberland Valley Analytical Services (Hagerstown, MD) utilizing wet chemistry for dry matter, crude protein, acid detergent fiber (ADF) (adapted from AOAC, 2000), neutral detergent fiber (NDF) (adapted from Van Soest et al., 1991), lignin (modified procedure of USDA research service, handbook number 379), ash (adapted from AOAC, 2000). Calcium, P, magnesium, potassium, sodium, iron, manganese, zinc and copper were analyzed per adaptation from AOAC (2000) (Perkin Elmer 3300 XL and 5300 DV ICP, Perkin Elmer, Shelton, CT). Analysis for chloride (extraction with 1% Nitric acid and determination of chloride ions with a Corning 925 Chloride Analyzer) and sulfur (Leco Organic Application Note, form 203-821-169, 06/03-REV1; Leco S-144DR Sulfur Combustion Analyzer, Leco Corporation, St. Joseph, MO) and also concentration of fat in feedstuffs and diets were conducted (AOAC, 2006; Tecator Soxtec System HT 1043 Extraction unit, Eden Prairie, MN). Analysis of pH on corn silage was also conducted (Mettler DL12 Titrator, Mettler-Toledo, Inc., Columbus, OH). Also included in analysis from the external laboratory were starch (modification of Holm et al., 1986) and sugar (Dubuis et al., 1956.)

The following analyses were performed in the ruminant nutrition laboratory at Kansas State University. Fecal samples were thawed and composited on a wet weight basis. Representative subsamples were dried in a forced air oven at 55°C and ground through a 1 mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). Duplicate 2 g samples were dried at 105°C for laboratory DM. Samples were then combusted at 450°C for 8h in 50 mL beakers that were washed in phosphate free detergent to prevent sample contamination by non-fecal P. Fecal ash samples were then solubilized in 10 mL 6 N HCl, and analyzed for P by colorimetry (AOAC, 1990). An acid molybdate reagent consisting of sulfuric acid and ammonium molybdate was added to the solubilized fecal sample followed by the addition of a

reducing agent consisting of sodium bisulfate and p-methylaminophenol. Water was then added and the color was allowed to develop for 20 minutes prior to reading color intensity (Perkin Elmer Lambda 3B UV/VIS Spectrophotometer, Oak Brook, IL).

Serum was thawed at room temperature and deproteinized using 10% sulfosalicylic acid (1 mL SSA/1 mL serum), then placed on ice for 15 minutes. Samples were then centrifuged at 20,000 x g, and supernatant was retained for P analysis by colorimetry similarly to fecal samples (AOAC, 1990) (Lambda 3B, Perkin Elmer, Oak Brook, IL). A dilution of 4 to 1 was required for the serum samples and a dilution of 8 to 1 was required for the fecal samples to remain within the linear range for the P analysis.

Samples of milk containing preservative were analyzed for fat, protein, lactose, somatic cell count (SCC) and milk urea nitrogen (MUN) content by Heart of America DHIA Laboratory (Manhattan, KS). Fat, protein and lactose content in milk were determined using near infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments Inc.). A flow cytometer laser (Somacount 500, Bentley Instruments Inc.) was used to determine SCC and chemical methodology from a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments Inc.) to measure MUN. Urea in milk was measured by an enzymatic reaction that splits urea to ammonia that is quantified colorimetrically.

Milk samples collected without preservative were thawed at room temperature, then warmed in a water bath at 38.5°C with occasional agitation. Samples were then composited by cow and period on a volume basis and mixed thoroughly. Duplicate 2.5 g samples were obtained while mixing and transferred by pipette into tared 50 mL beakers prepared similarly to beakers utilized during fecal preparation. After transfer, samples were placed in an oven at 105°C for 24 h, removed to a dessicator and reweighed before being placed in a muffle furnace at 500°C for at

least 4 h. Ash samples were then solubilized in 6 N HCl and analyzed for P similarly to fecal and serum samples (milk preparation for P analysis adapted from Morse et al. 1992).

Acid detergent insoluble ash (ADIA) analysis was conducted on fecal, feed ingredient and ort samples. Orts and feed ingredients containing greater than 5% fat were twice, shaken ten times and soaked for 10 minutes in fresh acetone to remove fat that may affect ADIA analysis. Following ADF analysis (ANKOM Technology Corp., Fairport, NY), samples were soaked in acetone for 3 minutes, removed and allowed to air dry before drying at 105°C for at least 2h. Once dried and cooled to room temperature in a dessicator, bags were weighed and combusted at 450°C for 8 h to measure ash content. ADIA was calculated as (ash content – bag contribution)/original sample at laboratory DM weight x 100 (Cochran et al., 1986).

Statistical Analysis

Prior to analysis all data were averaged by period within cow. Body weights and body condition score were averaged, then subtracted from the previous period to determine the period difference. Cow somatic cell counts were log (base 10) transformed to normalize the data prior to analysis. Data were then analyzed using the MIXED procedure of SAS version 9.1 (SAS Institute Inc., Cary, NC). The model statement included the effects of diet, replication and the interaction between diet and replication as fixed effects. Random effects were set as cow and period. Treatment means were determined by using LSMEANS option and orthogonal contrasts were performed. Significance was determined at $P \leq 0.05$.

Results and Discussion

Feed Intake, Milk Yield, and Milk Components

Ingredients included in the diets are listed in Table 1. The diets were formulated to be similar for all nutrients and adjustments were made for the inclusion of distillers grains in the

DDGS diet. Corn grain and Soybest™ (Grain States Soya, West Point, NE) were reduced to account for the protein and energy supplied by the distillers grains. Since the PELLET, MEAL and LIQUID diets only differed in P supplementation form, a base TMR was mixed daily and sufficient amounts were obtained to feed the cows on the selected treatment. The supplemental P was added to the base TMR and mixed in a stand alone drum tumble mixer (Data Ranger, American Calan, Northwood, New Hampshire). The DDGS diet was mixed separately and the distillers grains were added as a component of a grain mixture.

Dietary nutrient composition analysis (Table 2) shows the dietary P concentrations as 0.46, 0.47, 0.49 and 0.47% for the PELLET, MEAL, LIQUID and DDGS diets. The LIQUID diet contained approximately 19% more sugar than the PELLET and MEAL diets and approximately 27% more sugar than the DDGS diet. Fat content of the DDGS diet was over 17% greater and starch was 11% less than the other diets. There appeared to be small differences in mineral composition, however all other nutrients appeared to be similar.

Dry matter intake was similar across treatments ($P=0.97$), giving explanation for similarities across treatments for intake of selected nutrients (Table 3). Fat intake was significantly greater ($P<0.0001$) for the DDGS diet than other treatments. The solubles portion of the distillers grains are added back to the distillers grains after starch extraction from the corn endosperm for ethanol production. The solubles contain oil from the corn germ and increase the fat content of the DDGS. Decreasing corn grain in the DDGS diet decreased ($P=0.004$) the starch intake compared to PELLET and MEAL diets and showed a tendency ($P=0.08$) for lower starch intake compared to the LIQUID diet. The additional molasses supplied by the P supplement in the LIQUID diet significantly increased ($P<0.0001$) sugar intake above all other treatments. Values for NE_L intake were not different between treatments ($P=0.55$). Similarly,

intakes of NSC were not different (P=0.34) despite differences in starch and sugar intakes. Ash intake was not different (P=0.89) across treatments but instances of differing mineral intakes exist due to differences in mineral content of the diets, but all minerals were within sufficient ranges that would not impact experimental treatments.

Differences in milk production and other performance measurements (Table 4) occurred. Contrasts were performed to compare the inorganic P source supplied in the PELLET and MEAL diet with the organic P source in the DDGS diet, the inorganic P source to the LIQUID diet, and the LIQUID diet to the organic P source. Phosphorus source affected milk production (P=0.05); the DDGS supported 1.5 kg/d more milk production than the inorganic (P=0.05) sources and 2.4 kg/d more milk production than the LIQUID (P=0.01). The increase in milk production is not necessarily due to P intake. Studies have shown similar production responses from the addition of distillers grains compared to a control diet (Anderson et al., 2006; Kleinschmit et al., 2006; Janicek et al., 2008), but the production response is not due to increased energy consumption since intakes of NE_L did not differ between treatments. Milk fat (P=0.26) and milk protein (P=0.33) percentages were unaffected by treatment, however the LIQUID diet produced significantly less fat than the inorganic (P=0.05) and organic (P=0.01) sources. Small decreases in milk fat can occur when supplementing molasses (Broderick and Radloff, 2004). Sugar from molasses is readily soluble in the rumen by the ruminal microbes which increases propionate production. This leads to a decrease in the amount of acetate available for milk fat synthesis in the mammary gland. Daily milk protein production favored the DDGS diet with greater protein production than both the inorganic (P=0.03) and LIQUID (P=0.01) supplemented diets, while no difference existed between inorganic and LIQUID sources. Increases in daily protein and fat production follow an increase in milk yield. A tendency (P=0.08) for greater

daily lactose production was present for DDGS over inorganic, but the DDGS diet was significantly greater than the LIQUID (P=0.01), while no difference occurred in contrast analysis between inorganic and the LIQUID P supplementation.

Udder health quantified as the number of somatic cells times 1,000 cells/ml, found no differences between treatments (P=0.46). The negative log transformation of somatic cell counts also did not differ (P=0.29) between treatments.

Energy corrected milk (ECM), fat corrected milk (FCM) and solids corrected milk (SCM) are measures utilized to equally compare production between cows based on equal energy in milk, 4% fat and also adjusted solids in milk. The LIQUID was significantly lower in each of the measured categories in contrast to the organic P supplement (P=0.006, 0.009, 0.006, respectively). LIQUID was also significantly lower for ECM and FCM in contrast to the inorganic source (P=0.06, 0.05) and tended to be lower for SCM (P=0.07) compared to the inorganic P source. The LIQUID was affected by lower milk production and milk components.

Phosphorus supplementation form and source were shown to not affect average body condition score (BCS) or body weight and also did not effect any change in BCS or body weight (Table 4). Differences may have been difficult to detect due to the somewhat short duration of the trial and especially the short length of time between observations of BCS and body weight.

Measurements for efficiency of production (Table 5) were not different (P=0.27) for milk, ECM (P=0.25), FCM (P=0.30) or SCM (P=0.23) between treatments. However, trends for greater efficiency exist when comparing the organic source to the LIQUID for each mentioned category. Improving milk production and subsequently daily milk component production, while maintaining similar feed intake improves efficiency.

Phosphorus Utilization

As formulated, and along with similarities in DMI, P intakes were similar across diets, while utilization was different in some instances (Table 6). Dietary levels of P in this experiment would be sufficient for lactating cows producing greater than 40 kg/d based on NRC (2001) values. Mean milk production over the length of the trial was below 40 kg/d, resulting in cows being over supplemented with P near the end of the trial when milk production was decreasing. Fecal P excretion tended to be greater (P=0.07) for the inorganic diets than the organic diet, but was significantly greater (P=0.02) for the inorganic diet than the LIQUID diet, while no difference existed between the LIQUID and the organic dietary P supplements. Fecal P can be broken down into three portions: an inevitable portion, the portion that will be secreted in any instance due to the inability of the P to be utilized; a second portion consisting of endogenous losses from microbial cells and sloughed cells from the digestive tract; and lastly, a regulated portion that is determined by P intake levels. Similar P intake and differences in fecal P would indicate greater partitioning of P to other outlets. Average fecal P output (62.8 g/d) is similar to cows consuming 97.5 g P/d (Wu et al., 2001), 95.7 g P/d (Wu et al., 2003), and 117g P/d (Kincaid et al., 2005).

Phosphorus digestibility tended (P=0.09) to differ between treatments. Contrasts revealed that the LIQUID was significantly (P=0.02) more digestible than the inorganic supplemented diets. However, no difference occurred between contrasts of inorganic and organic (P=0.18) and LIQUID and organic (P=0.29) P supplements. Increased digestibility would typically affect apparent absorption, however no dietary effects were seen (P=0.19) for absorption, except in contrasts where greater absorption (P=0.04) followed greater digestibility for the LIQUID versus the inorganic P supplement. Molasses supplementation has been shown to increase diet digestibility (Broderick and Radloff, 2004). Supplying readily soluble

carbohydrates to the rumen, such as the sugar in molasses, increases microbial growth. More microbes would be available to produce phytase, thus releasing more of the P bound to phytate and increasing the P supply available for absorption in the small intestine. Apparent absorption of P averaged 54 g/d across treatments, a level greater than previous research with similar P intakes (Guyton et al., 2003; Kincaid et al., 2005). Limited data exists for comparison of a liquid supplemental P source, but utilizing mineral or non-mineral sources show apparent P digestibility within a range of five percentage points (Knowlton et al., 2001) of P digestibilities in this experiment.

Calculating P balance as P intake minus fecal and milk P resulted in a tendency (P=0.10) for organic to retain approximately 9 g/d more P than the inorganic supplement. The LIQUID also tended (P=0.02) to retain over 13.5 g/d more P more than the inorganic supplemented diets. No differences existed (P=0.43) between the LIQUID and the organic P supplements. Increased retention of P coincides with lower fecal P output. Higher values (>20 g/d) for retained P have been demonstrated in a previous study (Morse et al., 1992) in cows consuming 112 g P/d. Results of Knowlton and Herbein (2002) displayed two incidences of retained P similar to this experiment: in wk 9 of lactation for cows consuming 144.5 g/d and in week 11 of lactation for cows consuming 179 g/d of P. Retained P may have increased due to increased P availability as noted by increased digestibility. Bone mineralization is one route for retained P and was found to be deposited in bone at a rate of 4 g/d in a model by Hill et al. (2008). Replenishing bone P occurs after a period of P removal, such as early lactation, but once replenished will no longer occur at this rate. Spilling of P into urine may also inflate the retained P values obtained in this experiment because urine was not collected. Excretion of urinary P is assumed to be 1 g/d, while

studies have shown amounts ranging from 1 to 5 g/d at similar feeding levels (Morse et al., 1992; Knowlton and Herbein, 2002).

Serum samples analyzed for inorganic P displayed no statistical significance (P = 0.26) between treatments although a trend (P=0.06) for greater serum P concentration exists for the LIQUID compared to the organic diet. Serum P levels are not highly regulated and thus may be affected by P intake, but in this trial a trend may have resulted due to the increased digestibility of P in the LIQUID diet. Results are not atypical as values are within the suggested range (1.3 to 2.6 mmol/l) (Forar et al, 1982) and also coincide with another experiment at similar P feeding levels (Knowlton and Herbein, 2002).

Phosphorus concentration in the refusals utilized for conclusions about sorting of the diet can be seen in Table 7. The analysis of dietary treatments displayed no differences (P=0.13) in P concentration between diets (0.47, 0.47, 0.49 and 0.47%), and the actual amount of P fed also was not different (P=0.83). After noticing similarities in DMI, P intake and P concentration in refusals (P=0.21), it may be deduced that sorting of the P supplement did not take place.

Conclusion

Offering supplemental P in varying forms and from differing sources in this experiment did not affect DMI or P intake, however P source resulted in slight differences in utilization between treatments. Sorting of the P source was not evident, demonstrating that P form does not play a role in P intake. Utilizing the organic source of P in DDGS shows responses similar to inorganic P mineral supplements with favorable production yields, making it an adequate substitute for mineral sources of supplemental P.

Further Research

In concluding this thesis there appear to be unanswered questions that may be resolved with further research to follow up and attempt to answer these questions. The balance of P, or P that was retained in the body, tended to be greater for cows consuming the LIQUID and the DDGS P supplemented diets. One route of P excretion, urine, was not collected in this study. Were greater amounts of P portioned toward excretion in urine?

An in situ and a metabolism study may offer answers for digestibility of the diet and diet components to analyze P release. Would the P source affect rumen fermentation since greater intakes of fat and sugar occurred for the LIQUID and DDGS P supplements?

A study including heifers at breeding age and primiparous cows may give insight into the effects of these P sources in growing dairy animals. Phosphorus requirements would be greater for these groups since mature size has not been attained. Would responses in milk production occur in primiparous cows similarly to multiparous cows? Sorting of the diet did not occur in mature cows, would younger animals be more apt to sort out smaller particles?

Continued research is necessary in an attempt to reduce overfeeding of P and excessive excretion of P into the environment. Altering the P source may be one way to alleviate this problem with the help of ongoing research.

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Table 1. Ingredient composition of total mixed rations

Ingredient	Treatments[1]			
	Pellet	Meal	Liquid	DDGS
	% of DM			
Corn Silage	33.28	33.28	33.56	32.38
Alfalfa Hay	25.66	25.66	25.97	24.98
Whole cottonseed	5.14	5.14	5.14	5.00
Corn grain, ground	18.40	18.40	18.68	14.65
Soybest™ (Grain States Soya, West Point, NE)	10.15	10.15	10.15	2.88
Sodium Bicarbonate	0.76	0.76	0.76	0.74
Magnesium oxide	0.13	0.13	0.13	0.13
MFP™ (Novus International, Inc., St. Charles, MO)[2]	0.09	0.09	0.09	0.09
Zinpro 4-Plex™ (Zinpro Corp., Eden Prairie, MN)[3]	0.05	0.05	0.05	0.05
Sodium Selenite, 0.06%	0.04	0.04	0.04	0.04
Vitamin A premix, 30,000/g	0.02	0.02	0.02	0.02
Vitamin E premix, 20,000/g	0.19	0.19	0.19	0.18
XP Yeast™ (Diamond V Mills, Inc., Cedar Rapids, IA)	0.21	0.21	0.21	0.21
Rumensin™ (Elanco, Greenfield, IN)	0.01	0.01	0.01	0.01
Cane molasses	0.90	0.90	2.88	0.68
DDGS[4]	-	-	-	16.43
Wheat middlings	3.55	3.55	-	-
Salt	0.28	0.28	0.32	0.28
Calcium carbonate	0.76	0.76	1.00	1.10
Monocalcium Phosphate	0.38	0.38	-	0.15
Ammonium Polyphosphate	-	-	0.80	-

[1] Supplemental P sources: wheat middling base containing monocalcium phosphate in pelleted (Pellet) and meal (Meal) forms; (Liquid) cane molasses base containing ammonium polyphosphate; (DDGS) corn dried distillers grains with solubles.
[2] Dry source of 84% active methionine.
[3] Nutrient premix containing 2.58% zinc, 1.43% manganese, 0.90% copper, 0.18 % cobalt, 8.21% methionine, 3.80% lysine, 11.5% protein, 1.5% fat, 22.0% fiber and 26.5 %ash.
[4] Corn dried distillers grains with solubles.

Table 2. Nutrient composition of total mixed rations containing varying phosphorus supplements

	Treatments[1]			
Nutrient	Pellet	Meal	Liquid	DDGS
	% of DM			
CP, %	16.6	16.6	16.8	17.2
Soluble protein, % CP	4.8	4.8	4.7	5.3
ADF, %	18.6	18.5	18.5	19.0
NDF, %	29.9	29.9	29.2	31.3
Lignin, %	3.7	3.7	3.7	3.9
Fat, %	4.7	4.6	4.6	5.6
Starch, %	26.1	26.1	25.4	23.3
Sugar, %	5.2	5.2	6.4	4.7
NSC[2], %	43.4	43.3	42.4	41.2
NE_L, Mcal/kg	1.70	1.69	1.66	1.73
Ash, %	7.7	8.0	8.0	7.8
Ca, %	1.0	1.1	1.1	1.2
P, %	0.46	0.47	0.49	0.47
Mg, %	0.29	0.29	0.29	0.28
K, %	1.6	1.6	1.7	1.5
Na, %	0.34	0.36	0.37	0.38
Cl, %	0.36	0.39	0.46	0.4
S, %	0.22	0.22	0.22	0.27
Fe, mg/kg	195.0	205.0	227.0	179.4
Mn, mg/kg	42.3	42.0	40.0	36.5
Zn, mg/kg	59.8	54.2	51.6	55.6
Cu, mg/kg	15.5	13.8	13.7	13.5

[1]Supplemental P sources: wheat middling base containing monocalcium phosphate in pelleted (Pellet) and meal (Meal) forms; (Liquid) cane molasses base containing ammonium polyphosphate; (DDGS) corn dried distillers grains with solubles.
[2]Non-structural carbohydrates.

Table 3. Effect of supplemental phosphorus source and form on intake of selected dietary nutrients in total mixed rations

	Treatment[1]								
	Pellet	Meal	Liquid	DDGS			Contrast		
Item	----------Intake, kg----------				SEM	P=	I vs O[4]	I vs L[5]	L vs O[6]
DMI[2]	24.7	24.5	24.2	24.5	0.80	0.97	0.90	0.67	0.78
CP	4.1	4.1	4.1	4.2	0.14	0.59	0.24	0.85	0.24
ADF	4.4	4.4	4.4	4.5	0.15	0.77	0.46	0.75	0.36
NDF	7.2	7.2	7.0	7.5	0.23	0.25	0.19	0.31	0.05
Fat	1.1[a]	1.1[a]	1.1[a]	1.4[b]	0.05	<0.0001	<0.0001	0.49	<0.0001
NSC[3]	10.8	10.7	10.3	10.2	0.35	0.34	0.11	0.19	0.80
Starch	6.4[b]	6.4[b]	6.2[ab]	5.7[a]	0.21	0.03	0.004	0.24	0.08
Sugar	1.3[b]	1.2[ab]	1.5[c]	1.1[a]	0.05	<0.0001	0.02	<0.0001	<0.0001
NE_L, Mcal/d	42.3	41.9	40.6	42.6	1.4	0.55	0.68	0.26	0.18
Ash	1.9	2.0	1.9	1.9	0.09	0.89	0.84	0.84	0.73
Ca, g/d	247.8[a]	277.1[ab]	255.3[a]	290.1[b]	13.36	0.05	0.05	0.60	0.04
Mg, g/d	72.1	71.2	72.0	68.5	2.37	0.44	0.16	0.89	0.18
K, g/d	394.3	385.4	409.4	364.4	14.00	0.06	0.08	0.16	0.01
Na, g/d	82.5	87.0	89.1	94.7	4.86	0.21	0.05	0.38	0.33
Cl, g/d	89.1[a]	95.1[a]	110.2[b]	99.7[ab]	5.97	0.05	0.23	0.009	0.16
S, g/d	55.0[a]	54.9[a]	53.4[a]	66.8[b]	1.99	<0.0001	<0.0001	0.32	<0.0001
Fe, g/d	46.8[ab]	48.7[b]	53.1[c]	43.7[a]	2.00	0.002	0.03	0.007	0.0002
Mn, g/d	10.4[b]	10.2[b]	9.6[ab]	8.9[a]	0.37	0.008	0.001	0.06	0.11
Zn, g/d	14.8[c]	13.4[ab]	12.6[a]	13.8[b]	0.49	0.004	0.43	0.003	0.03
Cu, g/d	3.8[b]	3.4[a]	3.3[a]	3.3[a]	0.16	0.01	0.05	0.08	0.86

[a,b,c] Means within a row with differing superscripts are different ($P < 0.05$).

[1]Supplemental P: wheat middling base containing monocalcium phosphate in pelleted (Pellet) and meal (Meal) forms; (Liquid) cane molasses base containing ammonium polyphosphate; (DDGS) corn dried distillers grains with solubles.

[2]Dry matter intake.

[3]Non-structural carbohydrates.

[4]Inorganic P source vs Organic P source = Pellet and Meal vs DDGS.

[5]Inorganic P source vs Liquid P source = Pellet and Meal vs Liquid.

[6]Liquid P source vs Organic P source = Liquid vs DDGS.

Table 4. Effect of supplemental phosphorus source and form in total mixed rations on lactating dairy cattle performance

	Treatment[1]						Contrast		
Item	Pellet	Meal	Liquid	DDGS	SEM	P=	I vs O[6]	I vs L[7]	L vs O[8]
Milk, kg/d	34.6[a]	35.4[ab]	34.1[a]	36.5[b]	1.27	0.05	0.05	0.25	0.01
Milk fat, %	3.68	3.83	3.58	3.68	0.16	0.26	0.47	0.11	0.43
Milk protein, %	3.05	3.09	3.08	3.12	0.06	0.33	0.13	0.67	0.33
Milk fat, kg/d	1.27[ab]	1.35[b]	1.22[a]	1.34[b]	0.08	0.05	0.50	0.05	0.03
Milk protein, kg/d	1.05[a]	1.09[ab]	1.05[a]	1.13[b]	0.04	0.04	0.03	0.34	0.01
Milk lactose, %	4.73	4.79	4.70	4.76	0.07	0.22	0.93	0.15	0.19
Milk lactose, kg/d	1.6	1.7	1.6	1.7	0.07	0.06	0.08	0.21	0.01
SNF[2], kg/d	3.0[a]	3.1[ab]	3.0[a]	3.2[b]	0.13	0.05	0.06	0.24	0.01
SCC, x 1,000 cell/ml	429	171	318	423	163	0.46	0.44	0.91	0.57
Log SCC	2.09	1.90	2.03	2.21	0.18	0.29	0.14	0.82	0.27
Milk urea N	14.9	14.7	15.4	14.3	0.73	0.12	0.19	0.14	0.02
ECM[3], kg/d	35.3[ab]	36.9[bc]	34.5[a]	37.4[c]	1.60	0.02	0.12	0.06	0.006
FCM[4], kg/d	32.8[ab]	34.4[b]	32.0[a]	34.6[b]	1.55	0.03	0.21	0.05	0.009
SCM[5], kg/d	37.9[ab]	39.6[bc]	37.0[a]	40.2[c]	1.76	0.02	0.12	0.07	0.006
Avg. BCS	3.40	3.41	3.42	3.39	0.10	0.86	0.64	0.64	0.42
Avg. weight, kg	697.2	691.3	699.1	697.0	13.29	0.48	0.54	0.29	0.69
BCS change	0.06	0.15	0.14	0.11	0.04	0.46	0.83	0.52	0.71
Weight change, kg	5.5	-3.0	3.1	8.0	3.76	0.17	0.13	0.66	0.33

[a,b,c]Means within a row with differing superscripts are different ($P < 0.05$).

[1]Supplemental P source: wheat middling base containing monocalcium phosphate in a pelleted (Pellet) and meal (Meal) form; (Liquid) cane molasses base containing ammonium polyphosphate; (DDGS) corn dried distillers grains with solubles.

[2]Solids Non-Fat.

[3]Energy Corrected Milk = (0.327 x kg of milk) + (12.95 x kg of milk fat) + (7.2 x kg of milk protein).

[4]Fat Corrected Milk = (0.4 x kg of milk) + (15 x kg of milk fat).

[5]Solids Corrected Milk = (0.0752 x kg of milk) + (12.3 x kg of milk fat) + (6.56 x kg of SNF).

[6]Inorganic P source vs Organic P source = Pellet and Meal vs DDGS.

[7]Inorganic P source vs Liquid P source = Pellet and Meal vs Liquid.

8Liquid P source vs Organic P source = Liquid vs DDGS.

Table 5. Effect of supplemental phosphorus source and form on efficiencies of production in lactating dairy cattle

	Treatment[1]						Contrast		
Item	Pellet	Meal	Liquid	DDGS	SEM	P=	I vs O[6]	I vs L[7]	L vs O[8]
Milk efficiency[2]	1.43	1.45	1.43	1.50	0.07	0.27	0.10	0.69	0.08
ECM efficiency[3]	1.46	1.51	1.44	1.54	0.08	0.25	0.28	0.29	0.07
FCM efficiency[4]	1.36	1.41	1.33	1.42	0.08	0.30	0.40	0.26	0.10
SCM efficiency[5]	1.57	1.63	1.55	1.65	0.09	0.23	0.28	0.29	0.07

[1]Supplemental P source: wheat middling base containing monosodium phosphate in a pelleted (Pellet) and meal (Meal) form; (Liquid) cane molasses base containing ammonium polyphosphate; (DDGS) corn dried distillers grains with solubles.
[2]Milk efficiency = Milk yield kg / DMI kg.
[3]Energy corrected milk efficiency = ECM kg / DMI kg.
[4]Fat corrected milk efficiency = FCM kg / DMI kg.
[5]Solids corrected milk efficiency = SCM kg / DMI kg.
[6]Inorganic P source vs Organic P source = Pellet and Meal vs DDGS.
[7]Inorganic P source vs Liquid P source = Pellet and Meal vs Liquid.
[8] Liquid P source vs Organic P source = Liquid vs DDGS.

Table 6. Effect of supplemental phosphorus source and form on phosphorus partitioning in lactating dairy cattle

	Treatments[1]						Contrast		
Item	Pellet	Meal	Liquid	DDGS	SEM	P=	I vs O[2]	I vs L[3]	L vs O[4]
P intake, g/d	116	116	119	118	5.05	0.93	0.75	0.53	0.79
Fecal P excretion, g/d	67.4	66.3	57.5	60.0	3.54	0.07	0.07	0.02	0.54
Apparent P absorption, g/d	48.3	50.1	61.4	57.5	5.77	0.19	0.16	0.04	0.56
Apparent P digestibility, %	41.3	42.8	51.3	46.9	3.52	0.09	0.18	0.02	0.29
Milk P concentration, g/d	36.6	39.0	36.4	37.2	1.84	0.51	0.72	0.39	0.66
P balance, g/d	11.7	11.1	25.1	20.3	5.23	0.08	0.10	0.02	0.43
Milk P, % of P intake	32.0	33.8	31.1	31.8	1.59	0.54	0.50	0.29	0.72
Serum P concentration, mmol/L	1.46	1.50	1.55	1.41	0.05	0.26	0.30	0.23	0.06

[1]Supplemental P source: wheat middling base containing monocalcium phosphate in a pelleted (Pellet) and meal (Meal form; (Liquid) cane molasses base containing ammonium polyphosphate; (DDGS) corn dried distillers grains with solubles.
[2]Inorganic P source vs Organic P source = Pellet and Meal vs DDGS.
[3]Inorganic P source vs Liquid P source = Pellet and Meal vs Liquid.
[4]Liquid P source vs Organic P source = Liquid vs DDGS.

Table 7. Effect of supplemental phosphorus source and form on sorting of total mixed ration

	Treatment[1]						Contrast		
Item	Pellet	Meal	Liquid	DDGS	SEM	P=	I vs O[2]	I vs L[3]	L vs O[4]
Diet P, %	0.47	0.47	0.49	0.47	0.008	0.13	0.83	0.03	0.08
Ort P, %	0.45	0.45	0.51	0.43	0.005	<0.0001	0.005	<0.0001	<0.0001
P fed, g/d	126	130	131	129	5.54	0.83	0.88	0.54	0.68
P refuse, g/d	10.7	14.0	12.6	11.6	1.32	0.21	0.58	0.86	0.52

[1]Supplemental P source: wheat middling base containing monocalcium phosphate in a pelleted (Pellet) and meal (Meal form; (Liquid) cane molasses base containing ammonium polyphosphate; (DDGS) corn dried distillers grains with solubles.
[2]Inorganic P source vs Organic P source = Pellet and Meal vs DDGS.
[3]Inorganic P source vs Liquid P source = Pellet and Meal vs Liquid.
[4]Liquid P source vs Organic P source = Liquid vs DDGS.