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Animal production for efficient phosphate utilization: from optimized feed to high efficiency livestock

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Phosphorus (P) is an essential nutrient for livestock but its efficiency of utilization is below 40%, contributing to environmental issues. In this review, we summarize recent approaches to optimize P availability in livestock diets and improve its utilization efficiency. Phase feeding could potentially reduce P excretion by 20%. Addition of phytase enzymes to diets increased P availability from 42 to 95%. Low phytate transgenic plants and transgenic animals increased P availability by 14% and 52–99%, respectively. In practice, a combination of phase feeding and enzymes has the highest potential for P reduction but legislation and ethics implications will prevent using transgenic animals in the short term. Functional and nutritional genomics may provide tools to improve efficiency in the future.

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Introduction

Phosphorus (P) reserves are finite [1]; hence, losses not only cause environmental damage but also waste a depleting resource. Although global estimates are scarce, in the United States, the livestock sector is responsible for about 33% of P load into freshwater resources [2]. This has led to a substantial interest in reducing P content of manure applied to agricultural soils owing to concerns of pollution and regulations from governmental agencies. For example, the US Environmental Protection Agency is mandated to enforce P reductions of at least 0.6 million kg/year in the Chesapeake Bay watershed to maintain water quality [3]. Phosphorus is an essential macromineral for all animals, which should be supplied in sufficient quantities because it plays a major role in bone development, growth and productivity of livestock. The need to use inorganic P in livestock diet arises because feedstuffs may contain organic P, which may not be fully

available for absorption by animals $[4^{\bullet\bullet}]$. Livestock excrete large amount of P [5] because less than 40% of P consumed may be utilized depending on P availability, efficiency of feed conversion and the amount of P consumed in excess of the animal's requirement $[6^{\bullet\bullet}]$.

Opportunities are now available to reduce excess P excretion from livestock. Figure 1 shows the currently available methods of mitigating P excretion. These can be broadly divided in to two categories: improving or optimizing P availability in feed [4^{••},7], and increasing efficiency of livestock through increased P incorporation in product or faster growth [8]. Matching animal P requirement with available P in the diet through accurate predictions [9], ration formulation methods and feeding animals in groups according to their physiological state reduces P excretion drastically. Phosphorus availability can also be substantially improved in monogastric animals by using enzymes such as phytase that break down organic P (Table 1). Livestock can increase their efficiency of P utilization by incorporating genes that express enzyme production that help break down organically bound P and make it available to the animal [8]. Functional and nutritional genomics are new areas that will help us understand how animals interact with their environment and respond to nutritional demands matching P demand with supply in diet more accurately. The objective of this paper is to review methods that will (i) optimize feed offered to animals for better utilization and reduce P excretion to the environment and (ii) improve the efficiency of animals to utilize P in its various forms.

Diet formulation strategies to reduce excess phosphorus

Formulating a complete feed in livestock production systems involves determining a mix of ingredients that meets specific nutrient requirements, such as P, for a targeted level of production. Linear programming is the most widely used optimization tool for formulating diets for livestock. Traditional least-cost formulations seek to minimize the cost of the feed mix without taking into account the environmental consequences of excess P and other nutrients. Hence, a least-cost diet can be nutritionally adequate and economically optimal, but it may still provide significant amounts of indigestible fractions and excess nutrients [10]. Recent developments in mathematical programming have focused on multi-criteria models with the aim of addressing both economic and environmental considerations [11[•]]. Simply increasing P availability in livestock diets does not solve the problem of formulating

Mitigation options to increase P availability in livestock diet			
Species	Mitigation	Increase in available P, %	References
Swine	Phytase ^a	2.0–204.7	[29,44–46]
Poultry	Phytase ^b	3.3–116.9	[47–49]
Ruminants	Phytase ^c	7.6–23.7	[23,26,50]
Swine	Transgenic animal	81.2-90.4	[40]
Swine	Low-phytate plant	38.4-41.3	[37,38]
Swine, poultry	High-phytase plant	18.2–163.2	[39]
Swine	Liquid feeding	18.4–34	[33,34]

^c Phytase doses from 427 to 5000 FTU were used in these studies.

environment friendly diets owing to conflicting relations of P to other nutrients. For example, minimizing P excretion may increase excretion of other pollutants such as nitrogen [11[•]]. Hence, the linear programming concept has been applied to diet formulation at herd level by incorporating nutrient disposal costs into a modified least-cost ration formulation model. This way a joint least-cost decision that minimizes the sum of feed and net nutrient disposal costs can be calculated [12]. It was shown that herd size, land availability and proximity, crop rotation, and initial soil P content are important in determining P disposal costs [12]. In grazing systems, reduction in grazing intensity has frequently been recommended to meet biodiversity and production goals for a more sustainable system by reducing N and P excretions [13].

Optimization of phosphorus utilization through phase feeding

Phase feeding is a concept that is based on the premise that the population requirement for P (as well as other essential nutrients) changes during the stage of growth, lactation and gestation. This can be exploited by feeding multiple diets where each of the diets provides optimal nutrient densities at the midpoint of each subinterval (phase). A recent evaluation of the phase feeding concept in growing pigs, covering the growth period from 20 to 120 kg body weight, has shown a 20% reduction in P consumption without hampering growth performance [14[•]]. Phase feeding is a mature technology that has been adopted by the livestock industry worldwide owing to the reduction in feed costs [14[•]].

Precision feeding with individualized requirements

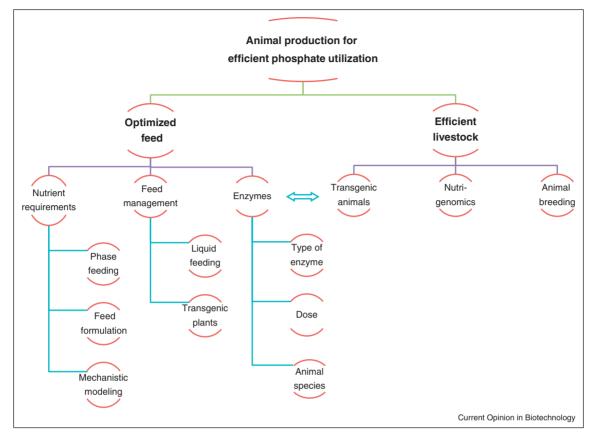
Although phase feeding provides significant improvements in nutrient and P utilization, diets are still formulated to meet the requirements of a group of animals. Nutritional requirements can vary greatly between animals in a given population and each animal follows individual patterns during the course of growth. To maximize the desired population response, such as body weight gain or milk production, requirements are formulated to meet the demands of the lower third of the population, resulting in excess nutrient received by the majority of the animals and thus reducing their efficiency of nutrient utilization [15]. Daily feeding programs tailored to individual animals based on their individualized requirements may yield significant reductions in nutrient and P excretion. For example, André et al. [16] developed adaptive models for online estimation of individual cow's requirement based on milk yield. A recent simulation study has also shown that individualized nutrition can potentially reduce P excretion from growing pigs by 38% [17[•]]. However, it must be noted that the technology is still premature and hardware and software needs further development before industry wide implementation. For example, such a system [9] requires continuous monitoring of growth and feed intake and algorithms are needed to process this information and predict future individualized P requirements.

To optimize P utilization, prediction of the amount of P absorbed by the animal should be improved by taking into account the main factors of variation. Mechanistic models describe degradation and absorption of P processes in the digestive tract and excretion of P to the environment. Létourneau-Montminy [17[•]] developed a model that represented the main metabolic processes occurring along the gastro-intestinal tract of growing pigs, such as P digestion, phytate-P hydrolysis, and absorption. Model parameters governing these flows were derived from *in vitro* and *in vivo* literature data. A combined approach where *in vitro* systems are used to measure and estimate degradation parameters [18,19] and then convert it to an *in vivo* P digestibility equivalent was implemented. Such a model may eventually be used to find economically optimal diets.

Use of enzymes in animal feed

Plants used as animal feed mostly store their P as phytate (myo-inositol hexakisphosphate, Figure 2), which is poorly available to monogastric animals. However, phytate P can be converted to lower inositol phytate or inorganic P, which is easily digestible by animals using an enzyme called phytase. Phytase belongs to a class of phosphatases that enables dephosphorylation of phytate in the digestive tract of the animal or in the feed before ingestion [4^{••},20-22]. Phytase naturally originates from plants, gut microbes and intestinal mucosa, but the activity of the latter two is minimal in monogastric animals [22]. Ruminants on the contrary are more effective in degrading phytate because microbes in the rumen produce phytase [23]. Microbial phytase can be added to diets to increase availability of phytate bound P. The most common microbial phytase used in swine and poultry diets are from fungi (Aspergillus niger and Peniophora lycii) and bacteria (Escherichia coli) [22]. The activity of phytase depends mainly on pH, temperature, dose, and diet composition [24-27]. Phytase supplementation has mostly been studied in monogastric animals, and improvement of the P digestibility has been observed in





The currently available methods of mitigating P excretion.

poultry [21,28**] and swine [22,29]. However, there is a wide variation in the effect of phytase added at the same phytase dose and type of diet [30], contributing to its unreliability in diet formulation. Addition of phytase to dairy cow and goat diets has also been shown to improve P availability [23,25-26]. Owing to differences in pH of the digestive tract of ruminants and monogastric animals, different types of phytases are required for ruminants [31[•]]. It is expected that the continued development of phytase through improved understanding of its ability to breakdown organic P will produce more effective classes of phytases. Commercially, phytase in swine and poultry diets is used at a dose of 500-750 FTU/kg (FTU = the amount of enzyme that liberates 1 μ mol inorganic PO₄ per minute from 1.5 mmol/L of sodium phytate at pH 5.5 and 37 °C) [4**]. Superdosing with up to 12 000 FTU/kg has been tested [4**,27,32] and in one study with broilers [32], an increase of phytase from 93 to 12 000 FTU/kg increased the phytate-P digestibility from 42 to 95%.

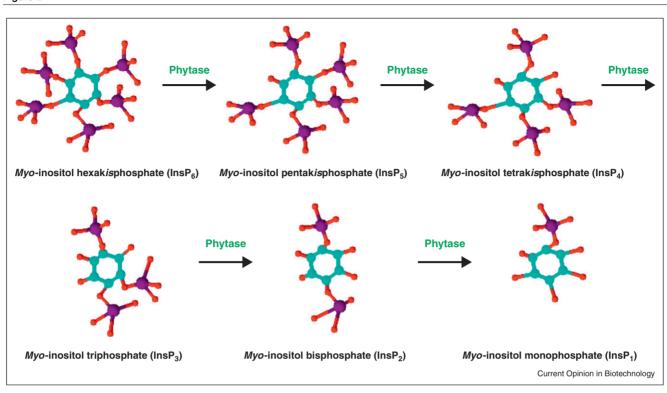
Management and genetic manipulation of feed

Provision of feed in liquid form is practiced predominantly in northern Europe and it can be a potential tool for optimizing P utilization. The use of fermented liquid feeding or soaking of dry feed for swine is a method to pre-digest phytate before feeding. When the feed is mixed with water, phytate will be subjected to degradation by microbial or plant phytases [33–36]. The degree of phytate degradation depends on phytase concentration in the plant, addition and type of microbial phytase, soaking or fermentation time and whether or not the cereal was heat-treated before mixing into the ration [34].

Transgenics has been used to develop low-phytate plants such as soybean meal [37], high available P corn [38] and canola seeds expressing a phytase gene [39] for inclusion in swine and poultry diets. Hill *et al.* [37] fed growing pigs a diet containing low-phytate corn and soybean meal, and the total P digestibility increased from 34 to 48% compared to a conventional corn–soybean meal diet. Addition of microbial phytase to the diet increased the total P digestibility further from 48 to 60%.

Transgenic animals, functional and nutritional genomics

In 2001, the EnviropigTM, a transgenic pig producing salivary phytase (*E. coli* phytase), was developed in



Example of enzymatic degradation of phytate (*myo*-inositol hexakisphosphate ($InsP_6$)) to lower inositol phosphates ($InsP_5$, $InsP_4$, $InsP_3$, $InsP_2$ and $InsP_1$) by the enzyme phytase. Different types of phytases start to cleave phosphate off at different positions.

Canada [8,40] to increase utilization and minimize excretion of P. Transgenic weanling and growing-finishing pigs obtained a true P digestibility of 88 and 99%, respectively, when fed a corn and soybean meal based diet, whereas the non-transgenic weanling and growing-finishing pigs had a true P digestibility of 49 and 52%, respectively. The transgenic and non-transgenic pigs had the same growth rate from weaning to slaughter [40] and an analysis of the health of the EnviropigTM did not show any problems [41].

Modern biotechnology has led to new fields such as functional genomics (i.e. the study of the genome of an animal and how it regulates homeostasis and responds to stimuli), which is providing a clearer understanding of how animals interact with their environments and respond to nutrients. The shortcoming of classical nutrient requirement specification is that it is based on past information, which is extrapolated to the current production setting where the genotypes are unknown. Hence, current nutritional guidelines for P [42] do not consider the differences in the individual animal response to P, as current dietary recommendations are appropriate for the average animal only. Nutritional genomics can provide valuable information about the individual genotypes by means of high throughput single nucleotide polymorphism genotyping technology, which can be obtained at birth of the animal. The genome-tailored nutritional regimen (i.e. personalized nutrition) is an emerging field in human nutrition, but efforts in animal nutrition have so far been limited $[43^{\bullet\bullet}]$.

Conclusions

Optimizing P availability in feed and improving efficiency of utilizing P in livestock are expected to reduce P excretion to the environment and help preserve a finite resource. Diet formulation needs to take into account the individual animal, or at least a group of animals' requirement according to their stage of growth and level of production. Phase feeding has been shown to reduce P consumption by 20% without compromising production, which has positive economic and environmental implications. Enzymes such as phytases are commercially available, which increase availability of P and reduce the need of supplementation with inorganic P to livestock diets. Over 50% improvement of P digestibility can be expected with the right amount of phytase enzyme inclusion in monogastric diets. Advances in transgenics, such as development of transgenic plants (low phytate corn and soybeans) have shown a 14% increase in P availability. Transgenic animals also offer a way of increasing livestock efficiency in using P, however, owing to ethics considerations we do not expect their use in livestock production systems in the near future. Nutritional genomics, on the contrary, will play a role in establishing better nutrient requirement estimates, which will lead to higher efficiency and lower P excretion to the environment. In the short term, a combination of reduced P, phase feeding, precision feeding and enzymes offer a realistic way of improving sustainability in the use of P in livestock production systems.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- . of outstanding interest
- Gilbert N: The disappearing nutrient. Nature 2009, 461:716-718. 1.
- U.N. Food and Agriculture Organization: Livestock's long 2. shadow - environmental issues and options. FAO; 2006.
- 3. U.S. Environmental Protection Agency: The next generation of tools and actions to restore water quality in the Chesapeake Bay. A revised report fulfilling section 202a of executive order 13508. EPA; 2009.
- 4. Adeola O, Cowieson AJ: Opportunities and challenges in using exogenous enzymes to improve nonruminant animal ...
- production. J Anim Sci 2011, 89:3189-3218.

A well-written review describing the use and mode of action of exogenous enzymes in improving monogastric animals' ability to use phytate phosphorus.

Van Vuuren DP, Bouwman AF, Beusen AHW: Phosphorus 5. demand for the 1970-2100 period: a scenario analysis of resource depletion. Global Environ Change 2010, 20:428-439.

Vitti DMSS, Kebreab E: Phosphorus and Calcium Utilization and 6. Requirements in Farm Animals. CAB International; 2008. Most recent book that covers bioavailability, nutrition, metabolism, and requirements of phosphorus and calcium in ruminants and monogastric animals.

- Leytem AB, Thacker PA: Phosphorus utilization and 7. characterization of excreta from swine fed diets containing a variety of cereal grains balanced for total phosphorus. J Anim Sci 2010, 88:1860-1867.
- Forsberg CW, Phillips JP, Golovan SP, Fan MZ, Meidinger RG, Ajakaiye A, Hilborn D, Hacker RR: **The Enviropig physiology,** 8 performance, and contribution to nutrient management advances in a regulated environment: the leading edge of change in the pork industry. J Anim Sci 2003, 81:E68-E77
- Pomar C, Hauschild L, Zhang G, Pomar J, Lovatto P: Precision 9. feeding can significantly reduce feeding cost and nutrient excretion in growing animals. In Modelling Nutrient Digestion and Utilisation in Farm Animals. Edited by Sauvant D, Van Milgen J, Faverdin P, Friggens N. Wageningen Academic Publishers; 2011: 327-334.
- 10. Pomar C, Dubeau F, Létourneau-Montminy MP, Boucher C, Julien PO: Reducing phosphorus concentration in pig diets by adding an environmental objective to the traditional feed formulation algorithm. Livest Sci 2007, 111:16-27.
- 11. Dubeau F, Julien PO, Pomar C: Formulating diets for growing pigs: economic and environmental considerations. Ann Oper Res 2011, 190:239-269.

An article describing how multi-criteria models can be used to optimize economic and environmental considerations during diet formulation.

Hadrich JC, Wolf CA, Black JR, Harsch SB: Incorporating 12. environmentally compliant manure nutrient disposal costs into least-cost livestock ration formulation. J Agric Appl Econ 2008, 40:287-300.

- 13. Orr RJ, Griffith BA, Cook JE, Champion RA: Ingestion and excretion of nitrogen and phosphorus by beef cattle under contrasting grazing intensities. Grass Forage Sci 2012, 67:111-118
- 14. Kebreab E, Strathe AB, Yitbarek A, Nyachoti CM, Dijkstra J,
- Lopez S, France J: Modeling the efficiency of phosphorus utilization in growing pigs. J Anim Sci 2011, 89:2774-2781.

A good article quantifying the the amount of phosphorus that can be saved by phase feeding according to the requirements and addition of phytase in the diet.

- 15. Hauschild L, Pomar C, Lovatto P: Systematic comparison of the empirical and factorial methods used to estimate the nutrient requirements of growing pigs. Animal 2010, 4:714-723
- 16. André G, Engel B, Berentsen PBM, Van duinkerken G, Oude lansink AGJM: Adaptive models for online estimation of individual milk yield response to concentrate intake and milking interval length of dairy cows. J Agric Sci 2011, 149.769-781
- Létourneau-Montminy MP, Narcy A, Lescoat P, Magnin M,
 Bernier JF, Sauvant D, Jondreville C, Pomar C: Modeling the fate of dietary phosphorus in the digestive tract of growing pigs. J Anim Sci 2011, 89:3596-3611.

This article is one the few studies in the literature that takes a mechanistic approach to model digestion and absorption of phosphorus in nonruminants.

- 18. Liu J. Ledoux DR. Veum TL: In vitro prediction of phosphorus availability in feed ingredients for swine. J Agric Food Chem 1998, **46**:2678-2681.
- 19. Bollinger DW, Tsunoda A, Ledoux DR, Ellersieck MR, Veum TL: A simple in vitro test tube method for estimating the bioavailability of phosphorus in feed ingredients for swine. J Agric Food Chem 2004, 52:1804-1809.
- 20. Haefner S, Knietsch A, Scholten E, Braun J, Lohscheidt M, Zelder O: Biotechnological production and applications of phytases, Appl Microb Biotechnol 2005, 68:588-597
- 21. Woyengo TA, Nyachoti CM: Review: supplementation of phytase and carbohydrases to diets for poultry. Can J Anim Sci 2011, 91:177-192.
- 22. Selle PH, Ravindran V: Phytate-degrading enzymes in pig nutrition. Livest Sci 2008, 113:99-122.
- 23. Knowlton KF, Taylor MS, Hill SR, Cobb C, Wilson KF: Manure nutrient excretion by lactating cows fed exogenous phytase and cellulase. *J Dairy Sci* 2007, **90**:4356-4360.
- 24. Poulsen HD, Blaabjerg K, Feuerstein D: Comparison of different levels and sources of microbial phytases. Livest Sci 2007, 109:255-257
- 25. Bravo D, Meschy F, Bogaert C, Sauvant D: Effects of fungal phytase addition, formaldehyde treatment and dietary concentrate content on ruminal phosphorus availability. Anim Feed Sci Technol 2002. 99:73-95
- 26. Kincaid RL, Garikipati DK, Nennich TD, Harrison JH: Effect of grain source and exogenous phytase on phosphorus digestibility in dairy cows. J Dairy Sci 2005, 88:2893-2902
- 27. Cowieson AJ, Wilcock P, Bedford MR: Super-dosing effects of phytase in poultry and other monogastrics. World's Poult Sci J 2011. 67:225-236
- 28. Slominski BA: Recent advances in research on enzymes for poultry diets. Poult Sci 2011, 90:2013-2023.

The review discusses efficacy of enzyme use and for the expansion of the use of enzymes to accommodate a wide array of dietary constituents used in poultry feeding programs.

- 29. Kerr BJ, Weber TE, Miller PS, Southern LL: Effect of phytase on apparent total tract digestibility of phosphorus in cornsoybean meal diets fed to finishing pigs. J Anim Sci 2010, 88:238-247
- 30. Johansen K, Poulsen HD: Substitution of inorganic phosphorus in pig diets by microbial phytase supplementation - a review. Pig News Inf 2003, 24:77-82.

- 31. Brask-Pedersen DN, Glitsø LV, Skov LK, Lund P, Sehested J:
- Effect of exogenous phytase on feed inositol phosphate hydrolysis in an in vitro rumen fluid buffer system. J Dairy Sci 2011, 94:951-959.

The paper describes an *in vitro* evaluation of newly developed phytases for use in ruminants.

- Shirley R, Edwards H: Graded levels of phytase past industry standards improves broiler performance. *Poult Sci* 2003, 82:671-680.
- 33. Lyberg K, Lundh T, Pedersen C, Lindberg JE: Influence of soaking, fermentation and phytase supplementation on nutrient digestibility in pigs offered a grower diet based on wheat and barley. *Anim Sci* 2006, **82**:853-858.
- Blaabjerg K, Jørgensen H, Tauson A-H, Poulsen HD: Heattreatment, phytase and fermented liquid feeding affect the presence of inositol phosphates in ileal digesta and phosphorus digestibility in pigs fed a wheat and barley diet. *Animal* 2010, 4:876-885.
- 35. Blaabjerg K, Poulsen HD: Microbial phytase and liquid feeding increase phytate degradation in the gastrointestinal tract of growing pigs. *Livest Sci* 2010, **134**:88-90.
- Columbus D, Niven SJ, Zhu CL, de Lange CFM: Phosphorus utilization in starter pigs fed high-moisture corn-based liquid diets steeped with phytase. J Anim Sci 2010, 88:3964-3976.
- Hill BE, Sutton AL, Richert BT: Effects of low-phytic acid corn, low-phytic acid soybean meal, and phytase on nutrient digestibility and excretion in growing pigs. J Anim Sci 2009, 87:1518-1527.
- Sands JS, Ragland D, Baxter C, Joern BC, Sauber TE, Adeola O: Phosphorus bioavailability, growth performance, and nutrient balance in pigs fed high available phosphorus corn and phytase. J Anim Sci 2001, 79:2134-2142.
- Zhang ZB, Kornegay ET, Radcliffe JS, Wilson JH, Veit HP: Comparison of phytase from genetically engineered Aspergillus and canola in weanling pig diets. J Anim Sci 2000, 78:2868-2878.
- Golovan SP, Meidinger RG, Ajakaiye A, Cottrill M, Wiederkehr MZ, Barney DJ, Plante C, Pollard JW, Fan MZ, Hayes MA et al.: Pigs expressing salivary phytase produce low-phosphorus manure. Nat Biotechnol 2001, 19:741-745.

- Golovan SP, Hakimov HA, Verschoor CP, Walters S, Gadish M, Elsik C, Schenkel F, Chiu DKY, Forsberg CW: Analysis of Sus scrofa liver proteome and identification of proteins differentially expressed between genders, and conventional and genetically enhanced lines. *Comp Biochem Phys D* 2008, 3:234-242.
- 42. Nutrition NRCSoS: *Nutrient Requirements of Swine*. National Academies Press; 1998.
- 43. Neibergs H, Johnson K: Nutrition and the genome. J Anim Sci
 2012, 90:2308-2316.

A good article describing how nutritional genomics will offer new opportunities to investigate the complex interactions of the genome and an animal's diet.

- 44. Goebel KP, Stein HH: Phosphorus digestibility and energy concentration of enzyme-treated and conventional soybean meal fed to weanling pigs. *J Anim Sci* 2011, **89**:764-772.
- Rojas OJ, Stein HH: Digestibility of phosphorus by growing pigs of fermented and conventional soybean meal without and with microbial phytase. J Anim Sci 2011, 90:1506-1512.
- Poulsen HD, Blaabjerg K, Strathe A, Ader P, Feuerstein D: Evaluation of different microbial phytases on phosphorus digestibility in pigs fed a wheat and barley based diet. *Livest Sci* 2010, 134:97-99.
- Powell S, Bidner TD, Southern LL: Phytase supplementation improved growth performance and bone characteristics in broilers fed varying levels of dietary calcium. *Poult Sci* 2011, 90:604-608.
- Woyengo TA, Slominski BA, Jones RO: Growth performance and nutrient utilization of broiler chickens fed diets supplemented with phytase alone or in combination with citric acid and multicarbohydrase. *Poult Sci* 2010, 89:2221-2229.
- Lan GQ, Abdullah N, Jalaludin S, Ho YW: In vitro and in vivo enzymatic dephosphorylation of phytate in maize-soya bean meal diets for broiler chickens by phytase of Mitsuokella jalaludinii. Anim Feed Sci Technol 2010, 158:155-164.
- Knowlton KF, Parsons CM, Cobb CW, Wilson KF: Exogenous phytase plus cellulase and phosphorus excretion in lactating dairy cows. Prof Anim Sci 2005, 21:212-216.