

MINERAL Writes

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In this issue:

- A. Phytase and other nutrients in pigs
- B. Calcium source and particle size effects on laying ducks

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Phytase and other nutrients in pigs

Phosphorus (P) is a limiting nutrient in pig diets. It is not readily available because it is present in almost two-thirds of plant feed-stuffs as phytate. The phytate molecule not only binds phosphorus, making it insoluble in the digestive tract, it also binds to starch and proteins.

Phytase breaks those bindings and improves growth, feed efficiency, nutrient utilization and mineralization. Phytase supplementation is preferred over adding inorganic phosphorus to diets, because the resulting effects are similar, but excretion of nitrogen is reduced. Specifically, phytase improves the digestibility of amino acids, starch, calcium, iron, fat and zinc for improved digestibility. Gross energy of diets is improved by adding phytase. Phytase also can influence the availability of amino acids, peptides, fatty acids, glucose, galactose and fructose as it works to hydrolyze the phytate molecule causing starch, protein and fat to be unbound.

Which nutrients are absorbed, as well as where and how well, throughout the digestive track is influenced by nutrient availability. The effects of phytase increasing nutrient availability influences the activity of the nutrient transporters found on the tip of the membrane of the intestinal absorptive cells. Nutrient fluctuations are sensed by the transporters, which react through gene expression of the required transporters for absorbing the present nutrients.

Vigors, et al. (2014) recently published a paper that looked at how the improvements in growth performance, skeletal bone mineralization and nutrient digestibility on low-phosphorus diets supplemented with phytase are also accompanied by changing gene expression of the nutrient transporters for peptide, mineral, carbohydrate and fatty acid transport.

The Experiment

Researchers used 48 pigs (Meatline boars x [Large White x Landrace] sows), with an

average body weight of 11-76 kg over a 44 day (0-44 days) experimental period to study weaner performance. This was a mixed-sex study.

Three diets were formulated to study the effects on growth performance, measurement of apparent ileal digestibility (CAID), measurement of apparent total tract digestibility (CATTD), bone mineral buildup and intestinal nutrient transporter gene expression in growing pigs (43 kg in body weight). There were two components to the experiment, based on dietary requirements changing as pigs progressed from after weaning (0-23 days, on a nutritionally rich diet) to needing a lower-specification diet (23-44 days).

The three diets during period 1 (0-23 days) were: 1) a high-phosphorus (HP), 2) a low-phosphorus (LP) diet and 3) a phytase supplemented LP diet (PHY). During period 2 (23-44 days) were: 1) a HP Diet, 2) a LP diet, and 3) a phytase supplemented LP diet (PHY). The specific ranges of total P, available P, Ca and Phytase levels are detailed in Table 1. The HP diets were formulated to meet NRC (2012) standards for Ca and P. The LP diets were formulated to contain 16% lower Ca and P levels during period one, and 30% lower during period 2.

Pigs had access to feed and water *ad libitum*. Feed was in the feeders until pigs were weighed weekly, and then feed was weighed to calculate feed conversion ratio (FCR). Fecal samples were collected daily on days 10-15 during period 1 and on days 25-30 during period 2. Feed samples were collected for chemical analysis. Markers were added to the feed to determine the CAID and CATTD. At day 44, the male pigs were slaughtered and the right front foot was cleaned to remove the third and fourth metacarpals. Those bones were used to determine bone ash, P and Ca levels and bone density. The pigs were fasted for 3 hours before slaughter. The entire digestive tract was removed and digesta samples were

Continued on p.4

Calcium source and particle size effects on laying ducks

A great deal of research is devoted to looking at how the dietary particle size and source of calcium influences laying hens. As you know, chickens are not the only animal that lays eggs. Ducks are uniquely different from chickens and it is worth looking at how nutrients may influence production parameters, egg quality and bone development to better understand calcium as a nutrient overall.

One difference between chickens and ducks is that the crop in chickens is a storage organ and in ducks it serves as an expandable gullet as part of the digestive tract. The birds also have different egg laying cycles and times. Given these differences, it is probably worth looking at the species separately instead of trying to make simple predictions that apply to ducks based on findings from experiments with traditional laying hens.

Before looking at the research on calcium effects on duck egg laying, production performance and bone development markers, one should understand some well established findings on particle size and calcium source in laying hens. Calcium size and source influences the solubility of the nutrient in the digestibility tract. Larger, less soluble particles generate more available calcium in poultry stomachs over time for adequate absorption from the intestine into the bloodstream and then to the shell gland.

Recently, Wang, et al. (2014) conducted an experiment to determine specific effects of two sources of calcium (oyster shell or limestone) and two particle sizes on laying performance, egg quality and tibial developmental markers in laying ducks. The authors' objective was to determine specific parameters for ducks instead of continually making predictions based on findings in traditional laying hen re-

search experiments.

The Experiment

The researchers divided 288 Longyan laying ducks into four groups, with six replications, of 12 birds. The birds were approximately the same body weight at 24 weeks and fed one of four treatment diets. The study ran for a 12 week period.

Diets were formulated for laying ducks, with four different calcium components. (Tables 1 and 2). Each diet provided 3.6% calcium which exceeds NRC recommendations (NRC, 1994). The researchers compared limestone to oyster shell and ground the calcium source coarsely or finely. Large particles (LP) were ground between 1.18 and 2 mm and small particles (SP) were less than 0.1 mm.

Birds had seven days to adjust to the treatment diets. Researchers recorded daily feed intake and the total laid, broken and shell-less number of eggs. Each individual egg was weighed and graded. Researchers calculated egg production, weight and yield (average egg weight per day per duck), average daily feed intake (ADFI), and feed conversion ratio (FCR) daily.

Findings

Particle size did not significantly impact egg production among the birds. By increasing particle size, (small to large), egg yield increased and FCR decreased. Calcium source did not affect productive performance markers significantly over the entire 12 week duration of the experiment.

Large particle sizes had significant impact on several egg quality markers. Breaking strength of the shell,

Table 1. Composition and nutrient levels of the diets (g/kg, as fed basis)

Item	Small Particles (SP)		Large Particles (LP)	
	Limestone	Oyster Shell	Limestone	Oyster Shell
Ingredient				
Corn	552.9	553.1	554	555
Soybean Meal	242	244	245	244.5
Wheat bran	87	82.7	79	80.3
Calcium hydrogen phosphate	12.2	12.2	12.3	12.2
Limestone (LP 34.23%) ¹	--	--	94	--
Oyster shell (LP 34.85%) ¹	--	--	--	92.3
Limestone (SP 35.63%) ¹	90.2	--	--	--
Oyster shell (SP 34.85%) ¹	--	92.3	--	--
DL-Methionie	1.65	1.65	1.65	1.65
L-Lysine-HCL	0.55	0.55	0.55	0.55
Sodium chloride	3.5	3.5	3.5	3.5
Premix	10	10	10	10
Total	1,000	1,000	1,000	1,000
Calculated analysis				
AME (kcal/kg)	2.5	2.5	2.5	2.5
CP (%)	17.25	17.83	17.46	17.17
Total Lys (%)	0.87	0.87	0.87	0.87
Total Met (%)	0.43	0.43	0.43	0.43
Ca (%)	3.45	3.37	3.49	3.28
Total P (%)	0.57	0.54	0.54	0.52
Available P (%)	0.35	0.35	0.35	0.35

¹ Measured values of calcium content.

Table 2. Particle size distribution of limestone and oyster shell

Size (mm)	Small Particles (%)		Size (mm)	Large Particles (%)	
	Limestone	Oyster Shell		Limestone	Oyster Shell
>0.18	0.36	0.82	>2	1.1	0.38
0.15-0.18	0.2	0.34	1.18-2	95.32	84.96
0.1-0.15	1.06	1.02	0.85-1.18	3.56	13.52
<0.1	98.28	97.7	<0.85	0.11	1.14

and egg appearance based on the increased albumen height and Haugh units were increased. Eggshells also contained higher phosphorus and lower magnesium levels from birds on the LP diets. Calcium content in the shells was not affected by particle size. Calcium source influenced albumen height, shell calcium and phosphorus content. Egg quality results were higher on the limestone diets.

Particle size and calcium source did not significantly affect plasma concentrations of calcium or phosphorus or alkaline phosphatase activity. Large particle size with limestone produced the best markers for tibial characteristics (breaking strength, dry bone weight and calcium content) overall.

What this means for the industry

Previous research on ducks specifically is limited on this subject and nothing was found to support or refute these findings by the researchers. Therefore, this experiment was original research conducted on ducks. Similar findings from experiments on hens were observed (Skřivan et al., 2010, Fleming et al., 1998 and Richter et al., 1999). The current experiment found a positive effect on most of the productive traits such as egg yields and FCR with an intake of larger particles regardless of calcium source. Particle size of the calcium source also influenced bone quality in ducks, as demon-

strated by tibia breaking strength, dry defatted weight and calcium content. This did not influence the calcium content in eggshells though.

Generally, the calcium source (limestone or oyster shell) did not affect productive performance, egg quality or tibial markers in ducks. Limestone diets had some effect with higher albumen height, calcium and phosphorus content in the shells and breaking strength of the tibia. Oyster shell decreased albumen height and marginally reduced Haugh units. The researchers theorize that this may compromise the freshness of the eggs. The researchers go further to hypothesize that the compromised freshness was due to microbial contamination from meat residue contained in the oyster shells. Oyster shell is also less soluble than limestone, if source particles of the same size experience similar retention time in the digestive track, but calcium and phosphorus net absorption in ducks was reduced and resulted in decreased shell contents of those nutrients and lower breaking strength of the tibia than in ducks fed limestone.

Ducks fed limestone with large particle size had increased productive performance and improved egg and bone quality. Both calcium sources with large particle size positively influenced productive performance, egg appearance, egg quality and tibia qualities in ducks experiencing heavy produc-

tion. It should be expected that producers may experience a decrease in egg quality and tibial characteristics to some degree if substituting oyster shell for limestone. Feeding limestone of large particle size to ducks provides a better calcium source.

Information for this article from: Fleming, R., H. McCormack and C. Whitehead. 1998. Bone structure and strength at different ages in laying hens and effects of dietary particulate limestone, vitamin K and ascorbic acid. *Br. Poult. Sci.* 39:434-440.

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Phytase and other nutrients in pigs

Table 1. Composition and chemical analysis of the experimental diets (as-fed basis; g/kg unless stated)

Dietary Treatments	Period 1 (0-23 days)			Period 2 (23-45 days)		
	HP	LP	PHY	HP	LP	PHY
Analysis						
Ca (g/kg)	7.0	5.9	5.9	6.7	4.7	4.7
P (g/kg)	5.9	4.9	4.9	5.9	4.1	4.1
Available P (g/kg)*	3.4	1.9	1.9	3.0	1.7	1.7
Phytase activity (FTU/kg)	0	0	1020	0	0	1030

* = Calculated for the tabulated nutritional composition.

recovered from the ileum to measure CAID of nitrogen, dry matter and gross energy.

Samples from the jejunum and ileum were collected and prepared for RNA extraction and identification of nutrient transporter genes. Specifically, the researchers were identifying the following nutrient transporters: 1) peptide transporter 1; 2) sodium-glucose-linked transporter 1; 3) fatty acid binding protein 2; 4) iron-regulated transporter; 5) a cluster of differentiation 36/fatty acid translocase; 6) membrane calcium channel; 7) calcium-binding protein; 8) plasma membrane calcium ion ATPase; and 9) vitamin-D receptor.

Findings

Overall, the pigs fed the LP diet had lower average daily gain, final body weight and increased feed conversion ratio compared to the pigs fed the HP and PHY diets. Pigs fed the PHY diet had higher CAID of gross energy and nitrogen compared to the digestibility of those nutrients in pigs fed the HP. Compared to the LP diet, the CAID of P, ash and gross energy was higher in pigs fed the PHY diet. The measurement of apparent total tract digestibility of Ca, P and ash when fed the PHY diet was higher than the pigs fed either the HP and LP diets during both periods. Pigs fed the LP diet had a lower CATTD of P compared to the pigs fed the HP diet. Bone ash content, bone P content and bone density were decreased in pigs fed the LP diet compared to the pigs fed the HP and PHY diets.

Gene expression was influenced by the PHY and HP diets for some nutrients in the jejunum. The Ca and P transporters demonstrated a numerical increase in pigs fed the PHY diets over the pigs fed the HP diet. The pigs fed the HP diets demonstrated an increased gene expression of amino acid transporter on the PHY diets. Amino acid transporters were also increased in pigs on the HP diets compared to the LP diet.

The expression of gene transporters was

also influenced in the ileal tract. Gene expression was lower for peptide, fatty acid, sodium-glucose linked transporters when fed the LP diet compared to the PHY and HP diets. Pigs fed the LP diet showed an increase in the gene expression of the membrane calcium, calcium bindings and ATPase transporters over pigs fed HP and PHY diets.

What this means for the industry

The study by Vigors et al. (2014), confirmed previous studies that diets supplemented with phytase improve the digestibility of nutrients, growth performance and bone mineralization. Phytase supplemented diets release nutrients bound by the phytate molecule. Specific to this experiment, there was no difference in the measurements of the apparent total tract digestibility of nitrogen and gross energy, which suggests that the phytase activity is influenced in the large intestine. Other studies (Woyengo, et al., 2008) found that hindgut fermentation masks PHY effects. Nutrients are absorbed more efficiently in the small intestine compared to in the hindgut. Nutrients (such as amino acids) that do not get absorbed in the small intestine do not have nutritional value to pigs.

The second portion of this study sought to understand the fluctuations of nutrients in the intestine by changing gene expression of intestinal nutrient transporters. Gene expression fluctuates in the presence of varying nutrients. Further, Heim et al. (2014), found that as nutrient digestibility improves, the intestinal nutrient transporter gene expression improves specifically for intestinal glucose. This study (Vigors et al., 2014) goes further with their aim to determine if the same effects can be found for multiple nutrients with the inclusion of phytase. What they found was that phytase increased nitrogen availability by influencing the gene expression of peptide transporters.

The authors suggest that the improved gene expression of the sodium-glucose linked transporters when fed the HP diet is because the higher levels of Ca and P available may

inhibit the phytate molecule to bind to free glucose in the digestive tract. Mineral phytate complexes also prohibit fat digestibility. Phytase supplementation improves fat digestibility by reducing soap forming in the digestive tract (Ravindran et al., 2001).

Phytase is known to free calcium for digestibility and absorption. When animals are calcium deficient, their digestive systems increase the gene expression for calcium transporters to maximize the utilization of what calcium is available in their ileum. If pigs are fed HP and phytase supplemented diets, most of the calcium absorption takes place in the central region of the small intestine, since there is an increased amount available from the inorganic source and degradation of the phytate molecule. Phosphorus digestibility occurs in the same region in both HP and PHY diets, because of the increased availability of the nutrient triggering the gene expression of the transporters.

Phytase supplementation improved growth performance and bone mineralization even more than when fed a HP diet with inorganic P supplementation. The gene expression of transporters improves nutrient uptake of not only P and Ca, but also of gross energy, peptides (amino acids), and fatty acids throughout the digestive tract, particularly the ileum. It is logical to assume that the digestive tract is going to modify itself (through gene expression for example) to adjust to the available nutrients. Understanding that this happens is essential to managing feeding practices and production performance for the maximum beneficial results to livestock operations.

Information for this article taken from:

Heim, G., A.M. Walsh, and T. Sweeney. Effect of seaweed-derived laminarin and fucoidan and zinc oxide on gut morphology, nutrient transporters, nutrient digestibility, growth performance and selected microbial populations in weaned pigs. *British Journal of Nutrition*. 111:1577-1585.

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