

# Triennial Growth Symposium: Vitamin D—Establishing the Basics to Dispel the Hype

**4 Vitamin D: Bones and beyond.** H. Deluca\* and L. Plum, *Department of Biochemistry, University of Wisconsin-Madison, Madison.*

We now understand that vitamin D is the starting material of an endocrine system primarily devoted to the regulation of serum calcium, serum phosphorus and bone. Vitamin D is normally produced in skin by a photochemical reaction of 7-dehydrocholesterol. It is subsequently converted in the liver to a circulating form (25-hydroxyvitamin D<sub>3</sub>) that is then converted by the kidney to a hormonal form, 1,25-dihydroxyvitamin D<sub>3</sub>. The vitamin D hormone acts through a single nuclear receptor in carrying out these functions. The presence of this receptor in a variety of tissues signals functions beyond bone, calcium and phosphorus. This presentation will provide the basic biology of the endocrine system, the current concepts of the molecular mechanisms by which it functions, and a look at the proven and putative functions of the hormone in the various target tissues.

**Key Words:** vitamin D, 1,25-dihydroxyvitamin D

**5 Basics for establishment of 2011 vitamin D guidelines in humans.** C. M. Weaver\*, *Purdue University, Department of Foods and Nutrition, West Lafayette, IN.*

The 2011 vitamin D recommendations set by the Food and Nutrition Board of the Institute of Medicine was the first time that estimated average requirements (EAR) and recommended dietary intakes (RDA) were given for vitamin D. Previous versions (i.e., 1997 and earlier) used adequate intakes, which implied less certainty of values. Vitamin D is difficult to set requirements for because it behaves more like a hormone than a nutrient and because diet is not the only source of vitamin D. In fact, more vitamin D comes from cutaneous production than from diet for most people. Thus, one cannot use the common factorial approach of estimating daily losses adjusted by absorption and needs during growth to set intake recommendations. The basis for the 2011 vitamin D recommendations was for bone health. Systematic reviews showed vitamin D status levels associated with calcium absorption, bone mineral density, and risk of osteomalacia or rickets. An integrated model was used to set vitamin D status, measured by serum 25(OH)D levels, at 40 nmol/L for the EAR and 50 nmol/L for the RDA. Vitamin D intakes to achieve these levels became the recommended intakes based on a nonlinear model of the relationship of vitamin D intake and achieved serum 25(OH)D levels. The panel assumed all vitamin D intakes should come from diet in their recommendations because many groups are not exposed to UVB light or are elderly or dark skinned which limits cutaneous production. However, intakes are well below recommended levels. Clinical guidelines for patients at risk for vitamin D deficiency were also established in 2011 by the Endocrine Society, which gave physicians more latitude for vitamin D recommendations.

**Key Words:** vitamin D, humans, dietary reference intakes

**6 Novel roles for FGF23 signaling in vitamin D and phosphate homeostasis.** B. Lanske\*, *Harvard School of Dental Medicine, Boston, MA.*

FGF23 is primarily produced in osteoblasts and osteocytes where it is secreted into the blood stream to act hormonally on specific target tissues. In the kidney, it has been shown to inhibit phosphate reabsorption

and vitamin D synthesis by down-regulating the renal sodium phosphate co-transporters NaPi2a and NaPi2c and suppressing the expression of 1 $\alpha$ (OH)ase in the proximal tubules. Mutations in the FGF23 gene itself or in genes regulating its expression and activation have been linked to disease processes including autosomal dominant hypophosphatemic rickets (ADHR), oncogenic osteomalacia (OOM) and X-linked hypophosphatemia (XLH), autosomal recessive hypophosphatemia with hyperphosphaturia (ARHR), confirming the role of FGF23 in regulating phosphate and vitamin D metabolism. A new role for FGF23 was discovered when Klotho, a previously described aging-related gene, was found to be a co-factor for FGF23 signaling. Apart from the kidney Klotho expression has also been detected in the parathyroid gland. More recent experiments have established a role for FGF23 in suppressing PTH transcription and secretion. These findings indicate that FGF23 can indirectly affect calcium homeostasis. Furthermore, the high levels of FGF23 found in patients with chronic kidney failure have been associated with increased risk of mortality and morbidity. FGF-23 has also been found to affect bone mineralization independent of its role in systemic phosphate homeostasis, adding significant new data regarding the physiological properties of FGF-23. Although the underlying mechanisms are still unclear, FGF23 appears to regulate the expression of various matrix mineralization inhibitors such as osteopontin, matrix Gla protein and dentin matrix protein. The novel findings that Klotho might also be expressed in bone cells strengthen the observation that FGF23 has paracrine action on bone. In summary, changes in FGF23 activity and the resulting effects on serum phosphate and calcium homeostasis have severe consequences on bone mineralization and ultimately on survival in mice and men.

**Key Words:** osteoblast, Klotho, FGF23

**7 The rise and fall of clinical cases of vitamin D deficiency in commercial swine operations.** D. M. Madson\*, *Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames.*

Hypovitaminosis D is not a new disease; however, in recent years there has been a resurgence of clinical disease within the US swine population. Clinical signs of re-emergence of this disease were first recognized in early 2010 and included lameness, posterior paresis, broken bones, muscle fasciculation/tremors, and sudden death. Morbidity of 50% and mortality in the range of 20% was common. The initial cases of hypovitaminosis D were connected to a particular feed manufacturer supplying a premix devoid of vitamin D which ultimately led to a national recall within five months of the first cases. This recall slowed the number of diagnostic cases and the attention associated with it motivated swine veterinarians and researchers to further investigate this issue. From 2010-present, the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) diagnosed over 100 cases. Case investigations typically involved determination of serum calcium, phosphorus, and vitamin D levels, assessment of bone quality, and microscopic evaluation of bones and joints. In many of these investigations the ISU VDL worked in collaboration with Heartland Labs in Ames, Iowa. These investigations generally led to the realization that large populations of pigs across the U.S. were either clinically or subclinically deficient in vitamin D. Clinical and diagnostic evidence continues to indicate that subclinical hypovitaminosis D as measured by serum vitamin D status is still occur-

ring. Four companies have introduced oral products for supplementation of pigs, particular aimed at the weaned pig. Supplementation along with increased monitoring of bulk feed vitamin D samples, which have been shown to degrade with time, have reduced the incidence within the swine population. Speculations about alternative feed ingredients, calcium-phosphorus ratios, and even phytase usage have been made as a potential contributor to this recent rise in hypovitaminosis D in pigs. However, the primal underlying cause for these continued cases has not been elucidated at this time.

**Key Words:** vitamin D, deficiency, lameness

## 8 Basis for establishment of the 2012 vitamin D requirements in swine. C. Lauridsen\*, Aarhus University, Department of Animal Science, Tjele, Denmark.

In swine nutrition, little is known about vitamin requirements for reproductive processes and bone health, especially vitamin D. Supplemental vitamin D is usually added to animal feed as cholecalciferol (vitamin D<sub>3</sub>), which is transported to the liver and hydroxylated to 25(OH)D<sub>3</sub>, which is released into the circulation. Recently, 25(OH)D<sub>3</sub> has become commercially available (Hy-D, DSM Nutritional Products). A dose-response trial with 4 doses of both vitamin D<sub>3</sub> and 25(OH)D<sub>3</sub> was performed. In Exp. 1, 160 gilts from first estrus until d 28 of gestation were fed diets containing 4 concentrations of 1 of 2 vitamin D sources [200, 800, 1,400, or 2,000 IU·kg<sup>-1</sup> from cholecalciferol or corresponding levels of 5, 20, 35 or 50 µg·kg<sup>-1</sup> from 25(OH)D<sub>3</sub> (Hy-D)]. Concurrently in Exp. 2, the same 8 dietary treatments were fed to 160 multiparous sows from the first day of mating until weaning. In Exp. 1, gilts were killed at d 28 of gestation, the reproductive organs were obtained and number of ovulations was assessed by counting corpora lutea and implanted embryos. In addition, blood samples were obtained at slaughter for plasma separation, and metacarpal bones dissected from the left foot. In Exp. 2, performance data and blood samples were obtained from the sows and the piglets throughout the experiment, and their vitamin D status was assessed by 25(OH)D<sub>3</sub> plasma concentrations. Although no major differences among dietary vitamin D treatments were detected in performance, reproduction and bone status markers of gilts and sows, the observed lower number of still born piglets and the higher vitamin D concentrations may indicate a dietary level around 1,400 IU vitamin D to be recommendable for reproducing swine. The potency of the dietary Hy-D relative to vitamin D<sub>3</sub> depended on the level tested but above 200 IU, Hy-D was more bioavailable than vitamin D<sub>3</sub>, and could as such be considered as an equivalent or even more advantageous source of vitamin D.

**Key Words:** requirements, 25(OH)D<sub>3</sub>, reproduction

## 9 Analytical methods to measure vitamin D in blood, feed and tissues: Application to diagnosis of vitamin D deficiency and excess in livestock. R. L. Horst\*, Heartland Assays LLC, Ames, IA.

Contemporary views categorize vitamin D<sub>3</sub> (cholecalciferol) not as a vitamin but as a pro-steroid hormone. The significance of vitamin D<sub>3</sub> as a pro-steroid hormone became clear in 1967 when Deluca's lab isolated a new metabolite of vitamin D<sub>3</sub>, 25-hydroxyvitamin D<sub>3</sub> (25OHD) which acted more rapidly than vitamin D<sub>3</sub> and was established as the precursor to the active form of vitamin D<sub>3</sub> namely 1,25-dihydroxyvitamin D<sub>3</sub> [1,25(OH)<sub>2</sub>D<sub>3</sub>]. A further complication for analysis is the presence of vitamin D<sub>2</sub>. This form and its precursor, ergosterol, are present in plants and is metabolized to vitamin D<sub>3</sub>, thus contributing to the overall vitamin D status in mammals. In addition to skeletal benefits, vitamin

D has significant roles in extraskelatal health. Too little or too much vitamin D can lead to poor performance and death. Access to reliable analytical methods is, therefore, imperative for monitoring vitamin D and/or vitamin D metabolite concentrations in a variety of matrices. 25-Hydroxyvitamin D, the major circulating form is widely accepted as the best in vivo indicator of vitamin D status. Many methods are available but work only with human sera and do not work properly with sera from livestock. For example, methods used widely in human medicine rely on dissociative methods for detaching 25OHD from its binding protein (VDBP). These methods do not work properly with serum/plasma from livestock. Radioimmunoassays involving protein denaturation to release 25OHD from VDBP work quite well and have been used extensively during the past few years to assist clinicians in uncovering widespread deficiencies of vitamin D in swine and other species. Tissue concentrations of vitamin D and to a lesser extent 25OHD can also be of some diagnostic value (particularly liver and kidney) but limited information is available associating tissue and diet concentrations. Feed analysis of vitamin D can be erratic and accuracy is dependent upon proper mixing and sampling techniques. Challenges in applying analytical procedures to measuring vitamin D will be discussed.

**Key Words:** serum 25OHD, radioimmunoassay, vitamin D binding protein

## 10 Novel roles for vitamin D in animal health and immunity. D. R. Barreda\*, University of Alberta, Department of Agricultural, Food and Nutritional Science, and Department of Biological Sciences, Edmonton, Canada.

Recent years have seen an increased interest in the extra-skeletal roles of vitamin D<sub>3</sub>. Pleiotropic effects on the immune system include those on monocyte, macrophage, neutrophil, B and T lymphocyte, and dendritic cell functions. The impacts to cellular immune mechanisms are relevant for host antimicrobial responses, inflammation, hematopoietic cell turnover and others. This presentation will discuss a recent study that examined the modulation of weanling pig cellular immunity in response to diet supplementation with 25-hydroxyvitamin D<sub>3</sub> [25(OH)D<sub>3</sub>]. Analysis of functional traits showed that leukocyte cell numbers ( $P < 0.05$ ) as well as proportions of individual granulocyte ( $P < 0.01$ ) and lymphocyte subpopulations ( $P < 0.05$ ) were significantly and differentially affected. There was also positive modulation of leukocyte survival across blood ( $P < 0.01$ ) and bronchoalveolar ( $P < 0.05$ ) compartments, highlighting the potential contributions to systemic and mucosal antimicrobial responses. This was consistent with a parallel upregulation in phagocytic functional responses in Hy-D supplemented pigs ( $P < 0.05$ ). Importantly, challenges remain as we consider the mechanistic basis of these cellular changes. Recent approaches in cellular analysis provide novel alternatives for analysis, particularly in non-classical animal model systems where reagents such as leukocyte antibody-based markers are not as readily available.

**Key Words:** vitamin D, cellular immunity, animal health

## 11 The role of vitamin D in skeletal muscle development and growth. J. D. Starkey\*, Texas Tech University, Lubbock.

The extra-skeletal functions of vitamin D have gained greater attention in recent years. Emerging evidence that vitamin D and its metabolites are indeed involved in the proper development and growth of skeletal muscle in both mammalian and avian species will be the primary focus of this presentation. Recent work conducted in swine suggests that oral administration of 25-hydroxycholecalciferol (25OHD<sub>3</sub>) in place

of a majority of dietary vitamin D<sub>3</sub> improves both maternal and fetal vitamin D status as measured by circulating concentrations of 25OHD<sub>3</sub>. These improvements in vitamin D status resulted in enhancement of fetal skeletal muscle fiber number and alteration of fetal skeletal muscle satellite cell proliferation kinetics. These changes in fetal skeletal muscle developmental characteristics may result in improvements in the postnatal growth potential and ultimate red meat yield of these offspring. The cellular and molecular mechanisms by maternal supplementation and vitamin D status improvement augmented fetal development will require further effort. There is a growing body of commercial field research in which up to a 2% increase in breast meat yield has been observed when 25OHD<sub>3</sub> is included in broiler chicken diets. As is common in the broiler industry, replacement of the greater part of dietary vitamin D<sub>3</sub> with 25OHD<sub>3</sub> in caged-raised broilers resulted in a classic satellite cell-mediated muscle hypertrophy response in fast-twitch (pectoralis major) muscle. This conclusion is based on observation of increases in satellite cell density on d 14 and 21, nuclear density on d 28, and muscle fiber cross-sectional area at the conclusion of the study on d 49 in 25OHD<sub>3</sub>-fed birds. A mystery remains as to why a similar response was not observed in slow-twitch (biceps femoris) muscles also examined in the study. These findings shed light on the cellular mechanism behind the intriguing results obtained in field studies. Further investigation into the mechanisms by which vitamin D and its metabolites affect both pre- and postnatal development and growth of skeletal muscle is certainly warranted given the large number of questions that remain unanswered at this time.

**Key Words:** vitamin D, skeletal muscle, satellite cells

## 12 Practical applications of vitamin D supplements in swine diets. G. Weber\*, *DSM Nutritional Products, Basel, Switzerland.*

Vitamin D<sub>3</sub> is an essential micronutrient, which regulates the absorption of calcium and phosphorus. This homeostatic function of vitamin D<sub>3</sub> is important to provide sufficient minerals for incorporation into the bone matrix and to avoid disorders of various origins. To become active, vitamin D<sub>3</sub> has to be metabolized through two subsequent hydroxylation steps, first to the 25-hydroxyvitamin D<sub>3</sub> (25-OH-D<sub>3</sub>) in the liver and second to 1,25-dihydroxyvitamin D<sub>3</sub> (1,25-(OH)<sub>2</sub>-D<sub>3</sub>) in the kidney. The major form of vitamin D<sub>3</sub> in blood plasma of animals is 25-OH-D<sub>3</sub>. If absorption of vitamin D<sub>3</sub> is hampered or the metabolic function of the liver is impaired by diseases or stress, the released 25-OH-D<sub>3</sub> may not suffice to secure adequate mineral supply. Feeding high-yielding farm animals with 25-OH-D<sub>3</sub> (Hy-D) could therefore optimize mineral homeostasis and improve productivity. Partial or full replacement of vitamin D<sub>3</sub> by Hy-D in swine feeds resulted in higher concentrations of 25-OH-D<sub>3</sub> in blood and in several tissues. In sows 25-OH-D<sub>3</sub> plasma levels are cyclic, being highest in early gestation and lowest at weaning. In a five-parity study, Hy-D supplementation secured that 25-OH-D<sub>3</sub> plasma concentrations never dropped below the threshold to vitamin D insufficiency. The elevated 25-OH-D<sub>3</sub> levels during lactation resulted also in an increased plasma concentration of

the active form 1,25-(OH)<sub>2</sub>-D<sub>3</sub>. Higher 25-OH-D<sub>3</sub> levels in milk were measured as well, which improved the vitamin D<sub>3</sub> status of the suckling piglets. In a growth trial 50 mcg 25-OH-D<sub>3</sub>/kg improved performance of growing pigs in comparison to a control group with 2000 IU vitamin D<sub>3</sub>/kg. Bone strength and bone mineralization were better with Hy-D both in the post-weaning and in the growing-finishing period. Optimum leg confirmation is of particular importance for the selection of gilts for breeding. When supplementing Hy-D during the full growth cycle, the selection rate of gilts was markedly improved under various production systems. Furthermore, the productivity of sows was ameliorated, related to piglets, born alive and litter weight at weaning. In conclusion, Hy-D is a versatile nutritional supplement, improving swine production at different levels.

**Key Words:** vitamin D, swine, bone strength

## 13 Practical application for use of dietary vitamin D to promote structural soundness in swine. M. A. Pérez-Alvarado, D. Braña, and J. A. Cuarón\*, *Centro Nacional de investigación Disciplinaria en Fisiología Animal, INIFAP, México.*

Even with safety margins of vitamin D (VD), Ca and P, over 8% of growing pigs may either be culled, or experience reduced growth due to structural unsoundness. Structural unsoundness is also a major cause for removal of breeding sows. Recently, the incidence of osteochondrosis (OC) lesions in sows was not linked to bone mineral concentrations. However, diets that provide excess Ca and P may increase mineral density of subchondral bone, possibly creating a rigidity stress on the cartilage preventing chondroblasts differentiation. A deficiency of VD may induce osteomalacia in adults, but bone mineralization in the offspring may also be affected. These responses may be mediated by P through FGF23. Thus, development of dietary inputs to regulate Ca and P homeostasis are necessary to sustain animal wellbeing. Traditional skeletal and extra-skeletal effects of VD are well accepted but an understanding of the non-classical responses within bone, such as effects on cartilage via cell differentiation may expand the practical applications for a role of VD in structural soundness. Epidemiological studies and empirical trials are needed to titrate VD requirements relative to structural soundness, but these trials are hampered by methods used to assess responses beyond those of bone mineralization. Assuming a greater absorption of 25OHVD than VD, our approach has used an assessment of a soundness scores to evaluate apparent OC in concert with measurements of Ca and P balance, particularly in lactating first litter gilts fed diets with varied VD, Ca and P concentrations. Growth performance and fibula ash validated the method of visual appraisal for structural soundness and gilts fed diets with 25OHVD had a greater number of femur osteoblast cells ( $P < 0.02$ ). During lactation, at lower intakes, Ca and P retention were similar or improved ( $P < 0.01$ ) by 25OHVD. Thus provision of greater dietary levels of VD from 25OHVD is a resource to sustain structural soundness of gilts and to protect efficiency of Ca and P use.

**Key Words:** structural soundness, 25-OH-D<sub>3</sub>, osteochondrosis