The primary roles for the involvement of vitamin D in Ca homeostasis have been established over the past 4 decades. More recent discoveries of extra-skeletal roles of vitamin D metabolites in cellular pathways, beyond those involved in Ca homeostasis, have rekindled interest in research to better understand the vitamin D needs of humans and animals. The objective of this Triennial Growth Symposium was to establish a foundation for the principles involved in the roles of vitamin D in animals. Ten invited speakers were selected to provide a breadth of topics concerning vitamin D. Authors from 2 abstracts selected from the general program were invited by the organizing committee for oral presentations at the symposium. All speakers were encouraged to distinguish between roles for vitamin D that can be supported by scientific principles vs. speculative roles that are not fully supported. This approach will hopefully help dispel some unrealistic hype generated by speculative inferences.

The foundation for the discovery and delineation of the vitamin D endocrine system can be mostly attributed to the meticulous research by Hector F. DeLuca (University of Wisconsin, Madison) and his coworkers over the past 6 decades. The proximal convoluted tubule cells of the kidney are the primary location of the highly regulated conversion of 25-hydroxyvitamin D3 (25-OH-D3) to the active metabolite, 1α,25-dihydroxyvitamin D3 [1,25-(OH)2D3] by the enzyme CYP27B1. However, CYP27B1 has more recently been detected in extra-renal cells. Specific molecular signals regulated by 1,25-(OH)2D3 have been identified in skeletal, intestinal, and renal tissues. Verification of a direct role for 1,25-(OH)2D3 in the induction of these signals required identification of the target receptor, vitamin D receptor (VDR), in these tissues. The VDR was initially identified in the intestinal enterocyte, the osteoblast, and renal cells. Additional cells now known to contain VDR include parathyroid cells, keratinocytes, activated lymphocytes, islet cells of the pancreas, pituitary cells, ovarian cells, and aortic endothelial cells. Evidence from in vivo experiments to support nongenomic mechanisms for vitamin D actions that are independent of VDR-mediated responses is not convincing. DeLuca (2014) also summarized his current work focused on extra-skeletal roles of vitamin D metabolites and the use of active, synthetic vitamin D analogs to successfully treat diseases such as psoriasis, postmenopausal osteoporosis, and type I diabetes. No direct evidence to support effective roles for vitamin D in treatment of multiple sclerosis, colorectal cancer, or breast cancer has been reported, despite retrospective epidemiological studies that support the concept for therapeutic or preventive roles. Interestingly, DeLuca (2014) identified many areas of research still needed...
to understand the roles of vitamin D in both skeletal and extra-skeletal tissues.

Fueled by prospects for reduced risks from a variety of diseases, vitamin D recommendations are as vigorously debated and contested for humans, as they are for animals. As a member of the Food and Nutrition Board of the Institute of Medicine, Connie M. Weaver (Purdue University, West Lafayette, IN) provided an overview of the basis for establishment of the 2011 vitamin D guidelines in humans (Weaver, 2014). The intent of the Triennial Growth Symposium committee for this presentation was to help bridge an understanding of approaches used to set human and animal nutrient requirements. The 2011 recommendations were the first to provide an estimated average requirement (EAR) and recommended dietary allowance (RDA) for vitamin D in humans. Weaver described the challenges to establish recommendations for a nutrient such as vitamin D, which functions more as a hormone and can be synthesized within the body following ultraviolet light (UVB) exposure. The 2011 EAR and RDA were established using an integrated, nonlinear model based on measures of bone health as a function of serum 25-OH-D concentrations. Evidence for other health indicators, besides bone, was not sufficient to establish the requirements. Justification for use of serum 25-OH-D concentrations as an indicator of vitamin D status was based on the assumptions that the serum concentrations reflect both diet and cutaneous synthesis, that 25-OH-D is not as readily degraded as the active vitamin D metabolite, and that 25-OH-D is not a homeostatic regulator which responds rapidly to serum Ca.

Within the past decade, novel functional roles for a hormone, fibroblast growth factor 23 (FGF-23) have been identified. Beate Lanske (Harvard School of Dental Medicine, Boston, MA) has provided major research efforts focused on FGF-23 signaling. Lanske reviewed current evidence for direct feedback relationships between vitamin D and FGF-23 and the role of FGF-23 in regulation of P homeostasis (Lanske, 2013). Evidence of severe osteomalacia, downregulated renal Na-P transporter expression, decreased serum P and 1,25-(OH)2D3 concentrations with increased parathyroid hormone (PTH) concentrations in transgenic mice overexpressing FGF-23 was presented. Studies designed to assess loss of function via FGF-23 knockout models were also summarized. The FGF-23 knockout mice displayed abnormal mineral metabolism. Mice had increased serum Ca and P in the presence of an up-regulated renal Na-P transporter. Knockouts also had increased serum 1,25-(OH)2D3 that was exacerbated by an overexpression of 1,α-hydroxylase, despite decreased PTH concentrations. The knockout mice displayed skeletal abnormalities with numerous callouses and evidence of soft tissue calcification due to abnormal Ca and P homeostasis. These models led to the concept of a PTH, vitamin D, and FGF-23 axis that involves FGF-23 downregulation of renal 1,α-hydroxylase and PTH activity.

Darin M. Madson, (Veterinary Diagnostic Laboratory, Iowa State University, Ames) was involved in swine lameness and mortality cases that were diagnosed as hypovitaminosis D. A dramatic increase and then decrease in the number of cases diagnosed as hypovitaminosis D were recorded between 2010 and 2013. Madson reported results from a follow-up survey that involved collections of 8 blood samples from 15 sites for 5 age groups of pigs at each site (Madson, 2013). Serum 25-OH-D concentrations were consistently below reference ranges, especially for pigs in the younger age groups. Additionally, analysis of feed samples collected from 5 manufacturers during a 12-mo period revealed that none of the samples were significantly below target levels. Dietary vitamin D concentrations did vary with season, which raised concerns for vitamin D stability. The abrupt decrease in clinical cases was attributed to an increase in dietary vitamin D supplementation and an increased awareness by veterinarians and nutritionists to potential problems in quality control issues that affect vitamin D.

The time over which a dramatic increase in hypovitaminosis D diagnosis occurred in the Midwest region of the United States coincided with efforts to revise the nutrient requirements for swine. In contrast to the publications focused on vitamin D supplements for humans, data to estimate vitamin D requirements of swine, especially breeding-age animals, are scarce. Charolette Lauridsen (Aarhus University, Tjele, Denmark) presented results from recent research that contributed to the basis for the current vitamin D requirements of sows (Lauridsen, 2014). Results from 2 experiments with gilts and reproducing sows were used to assess responses to varied concentrations of 2 sources of vitamin D, vitamin D3 vs. a commercial source of 25-OH-D (Hy-D, DSM Nutritional Products, Basel, Switzerland). Lauridsen concluded that diets for gilts and gestating sows with 800 IU D3/kg diet provided beneficial bone trait and reproductive responses compared with responses of animals fed diets that supplied only basal concentrations (200 IU D3/kg). Sows fed diets supplemented with Hy-D above the basal concentration had improved reproductive traits, but not bone traits compared with sows fed similar amounts of vitamin D3. Lauridsen further discussed the use of different response criteria such as bone traits, reproductive response traits, and serum 25-OH-D concentrations in the establishment of vitamin D requirements.

From the first invited abstract (Rortvedt-Amundson and Crenshaw, 2013), Laura A. Rortvedt-Amundson (University of Wisconsin, Madison) presented results from continued efforts to assess the role of vitamin D signals in maternal-fetal development of skeletal tissues.
A vitamin D-induced kyphosis model was used to evaluate carryover effects of maternal vitamin D3 intake on pig bone traits. Sows fed diets with no supplemental vitamin D induced negative bone traits in pigs at 8 wk of age even though influences of maternal diets were not detected in pigs at birth and weaning.

Following a career in vitamin D-related research, Ronald L. Horst (Heartland Assays, Ames, IA) co-founded 2 companies. One company is involved in developing vitamin D products for use in treatment or prevention of human and animal diseases. The second company provides analytical services to evaluate vitamin D in foods, feeds, and tissues. With the recent upsurge of interest in the role of vitamin D deficiency in the onset of many diseases in humans and animals, Horst and coworkers have established expertise in assay procedures to measure vitamin D and related metabolites. Based on current practices, Horst concluded (Horst, 2013) that serum concentrations of 25-OH-D offer the best indicator of vitamin D status, as tissue and feed concentrations are problematic. Cautions were presented for use of commercially available assays for 25-OH-D, because the assays may be species specific and may be affected by numerous and variable concentrations of other vitamin D metabolites. The advantages and disadvantages of various assay methods, including competitive protein binding assays, RIA, HPLC, and liquid chromatography/mass spectrophotometry (LC/MS/MS) procedures were discussed. Quantitation by HPLC, the “long time gold standard”, has now been replaced by LC/MS/MS procedures for analysis of all vitamin D metabolites. Cautions were discussed in the use of automated instrumentation methods developed for human serum 25-OH-D assays, as these assays are not accurate for swine samples. Preferred assays for swine serum 25-OH-D analysis include RIA, or LC/MS/MS methods. Preferred assays for tissue concentrations, including liver samples, are HPLC or LC/MS/MS procedures. Assays of feed samples are a challenge, in part, due to difficulty in obtaining a uniform sample. The preferred methods for feed samples are HPLC for premixes and LC/MS/MS for complete feeds.

From the second invited abstract (Flohr et al., 2013), Gilbert M. Weber (DSM Nutritional Products Ltd., Basel, Switzerland) presented results of additional research designed to assess the practical applications of vitamin D3 and Hy-D dietary supplements to sows during gestation and lactation (Weber et al., 2014). Across multiple parities and stages of lactation, sows fed diets with Hy-D, consistently had greater plasma concentrations of 25-OH-D than sows fed vitamin D3, but plasma 1,25-(OH)2D3 concentrations only differed between diet treatments of 25-OH-D than sows fed vitamin D3, but plasma 1,25-(OH)2D3 concentrations only differed between diet supplements on d 5 of lactation. Differences between vitamin D sources were detected incolostrum and milk 25-OH-D concentrations, consistent with responses observed in plasma. Only minor differences were detected in sow reproductive responses or pig survival and growth.

Jessica D. Starkey (Texas Tech University, Lubbock) presented results in the use of imaging flow cytometry and multivariate analysis of immune cell function were presented using results from experiments designed to evaluate the contributions of vitamin D supplements on the capacity of weanling pigs to mount effective immune responses to pathogens. Pigs fed diets with Hy-D vs. vitamin D3 produced leukocytes with an upregulated phagocytic capacity. Specific mechanisms that explain the differences observed between the 2 vitamin D sources will require additional research to identify the best overall impact on animal health.

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José A. Cuarón (CNID-Fisiología, INIFAP, Querétaro, México) presented results from efforts to identify practical applications for vitamin D sources. Diets formulated with either vitamin D$_3$ or Hy-D were fed to growing gilts to assess effects on structural soundness (Pérez-Alvarado et al., 2013). Approximately 30% more gilts fed Hy-D vs. vitamin D$_3$ were visibly appraised to be structurally fit for breeding herd replacements. A visual assessment of structural unsoundness is the primary method used in practical settings for removal of developing gilts as prospective breeding herd replacements. The decisions based on visual assessments were validated by growth performance and fibula ash measurements. Thus, feeding Hy-D in diets for developing gilts improved structural soundness and improved the efficiency of producing replacement gilts. Additional beneficial effects of Hy-D included improvements in Ca balance of lactating sows fed Hy-D vs. vitamin D$_3$ in diets formulated with phytase.

In conclusion, the presenters were constructively critical of their research results and strived to present results supported by scientific principles. Presentations included conflicting results that challenged each of our professional biases. The presenters confirmed the needs for additional, carefully designed experiments, which will withstand constructive challenges from our colleagues and help to unravel the multifaceted roles of vitamin D. Additional research efforts were especially evident in areas associated with swine nutrition. A critical evaluation of traits used to define vitamin D status would contribute clarity for future research. Efforts to establish the biological functions, requirements, and safe feeding guidelines for swine, will help serve as a model to establish vitamin D guidelines for humans.

**LITERATURE CITED**


