RESEARCH REPORT
SWINE HEALTH

Title: Quality control assessment of blended source vitamin D supplied to feed manufacturers for use in swine diets - **NPB #11-177**

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Industry Summary

Clinical cases of metabolic bone disease in swine, such as rickets and osteomalacia, have been linked to hypovitaminosis D. Since the primary source of vitamin D for most US swine is feed supplementation, an evaluation of bulk source vitamin D was performed. Five different swine feed suppliers were solicited over a nine month period for bulk source vitamin D samples. These samples were blinded, to keep anonymity, split, and then sent to two separate laboratories to evaluate vitamin D concentration. There was a total of 45 samples evaluated (90 assays), and no individual supplement source was found to be significantly lower than the expected concentration of 500,000 IU/lb. However, variation between sources and testing labs were noted. Results of this study suggest that the rise in clinical swine metabolic bone disease does not appear to be associated with bulk vitamin D that is being supplied to diet manufactures.

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Scientific abstract

In recent years, metabolic bone disease issues have gained interest because of clinical hypovitaminosis D cases in swine. The objective of this study was to assess the quality of vitamin D supplements from multiple commercial suppliers' overtime to determine if variation in feed vitamin D could be part of the increased prevalence within the swine industry. Bulk vitamin D supplement samples, used for inclusion

in swine diets, were solicited once a month of nine months from five different major swine feed suppliers and tested in a blinded fashion at two independent laboratories for vitamin D concentration. Date of manufacturing, company, country of origin, and testing laboratory were subject for evaluation in this study. Of the 45 supplement samples that were evaluated, no individual supplement, from any supplier, was found to be significantly lower than the expected concentration of 500,000 IU/lb. However, there an unexplainable month effect found during testing indicating the potential for difference in bulk vitamin D concentrations received at swine feed suppliers. In addition, a company by laboratory interaction was found indicating test method may give varying results. The results from this project highlight that bulk vitamin D feed supplement concentrations can be variable, but do not appear to be a reason for increased metabolic bone disease seen within the swine industry.

Introduction

Vitamin D is required for the absorption of calcium from the intestine. Sustained hypovitaminosis D can result in metabolic bone disease; a general term used to describe multiple nutritional diseases related to bone growth and/or bone modeling.[6] In growing pigs with hypovitaminosis D, growth plates are still open and become widened due to failure of endochondral ossification; this disease process is known as rickets. In mature animals with hypovitaminosis D, the process is classified as osteomalacia because growth plates have closed and the primary lesion is defective bone remodeling.[6]

Not only does vitamin D have a crucial role in calcium absorption, homeostasis and bone formation, other body systems also utilize vitamin D. Vitamin D3 receptors (VDRs) on the nucleus of activated T-lymphocytes and antigen-presenting cells suggests vitamin D3 aids in preventing uncontrolled immune responses.[7] Vitamin D3 converting enzyme, 25-hydroxyvitamin D3-1-hydroxylase, is also found beyond the kidney suggesting other bodily purposes such as autocrine functions in the immune system and gene expression.[1,7]

Over the past three years, vitamin D deficiency resulting in hypocalcemia has gained interest in the swine industry. In 2010, the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) investigated several cases of sudden death in nursery and finishing pigs that was ultimately attributable to vitamin D deficiency and hypocalcemia. Several cases were associated with feed errors; however, others were not associated with mixing errors or improper ingredients, yet hypovitaminosis D was diagnosed.[3] The objective of the study to further investigate vitamin D related issues assessing the quality of bulk vitamin D feed supplements from multiple commercial suppliers for dietary inclusion to determine if this could be associated with the increase incidence of disease.

Objectives

To investigate the quality of vitamin D sources, from multiple suppliers, being utilized in the manufacturing of swine diets over a period of time and its impact on vitamin D deficiency in pigs.

Materials & Methods

Through collaboration with swine feed suppliers, bulk source vitamin D supplement samples were collected monthly from November 2011 through July 2012. Samples were sent to the ISU-VDL from five independent swine nutrition companies during this time.

Received samples were given a unique identification number, and then all company information was removed. Information recorded for each received sample included: 1) date of collection, 2) date of manufacturing, 3) expected concentration, 4) manufacturer name, 5) manufacturer country of origin, 6) lot number, and 7) supplier name (the swine nutrition company sending the sample).

Each sample was homogenized, then split and stored at 4°C until sent to two separate laboratories, DSM Nutritional Products North America (Parsippany, New Jersey) and Heartland Assays, LLC (Ames, Iowa) for vitamin D_3 detection. Detection of vitamin D at both laboratories was accomplished by high-performance liquid chromatography (HLPC) with UV detection. Heartland Assays procedure was done as previous described[4] and DSM Nutritional Products according to their standard operating procedure.

Summary statistics, SAS® (Cary, North Carolina), were used to analyze bulk vitamin D samples. Measured concentrations were evaluated in a mixed effect model with company, laboratory, company of manufacturer, and month as fixed effects, and sample as a random effect. Differences were considered statistically significant at $P < 0.05$ for bulk vitamin D samples.

Results

A total of 45 bulk vitamin D supplement samples were collected, resulting in 90 assays completed for this project. Of the 45 samples received, 23 were manufactured outside the US, and 22 samples originated from US manufactures. Two suppliers solicited in this project provided vitamin D samples sourced strictly from foreign manufacturers during the study period, one supplier provided samples from a US source only, and the remaining two suppliers provided a mix of US and foreign bulk vitamin D for analysis.

Although there was variation of the vitamin D concentration in the supplement samples (Figure 1) that were evaluated, there were no samples found to be statistically lower than the expected concentration of 500,000 IU/lb. Additionally, there was no statistical difference between the country of origin or laboratory utilized for testing. There was a month effect (Figure 2) and company by lab effect with 95% confidence limits (Figure 3).

Figure 1: Comparison of vitamin D supplement concentrations (IU/g) over time by lab from five different swine feed suppliers.

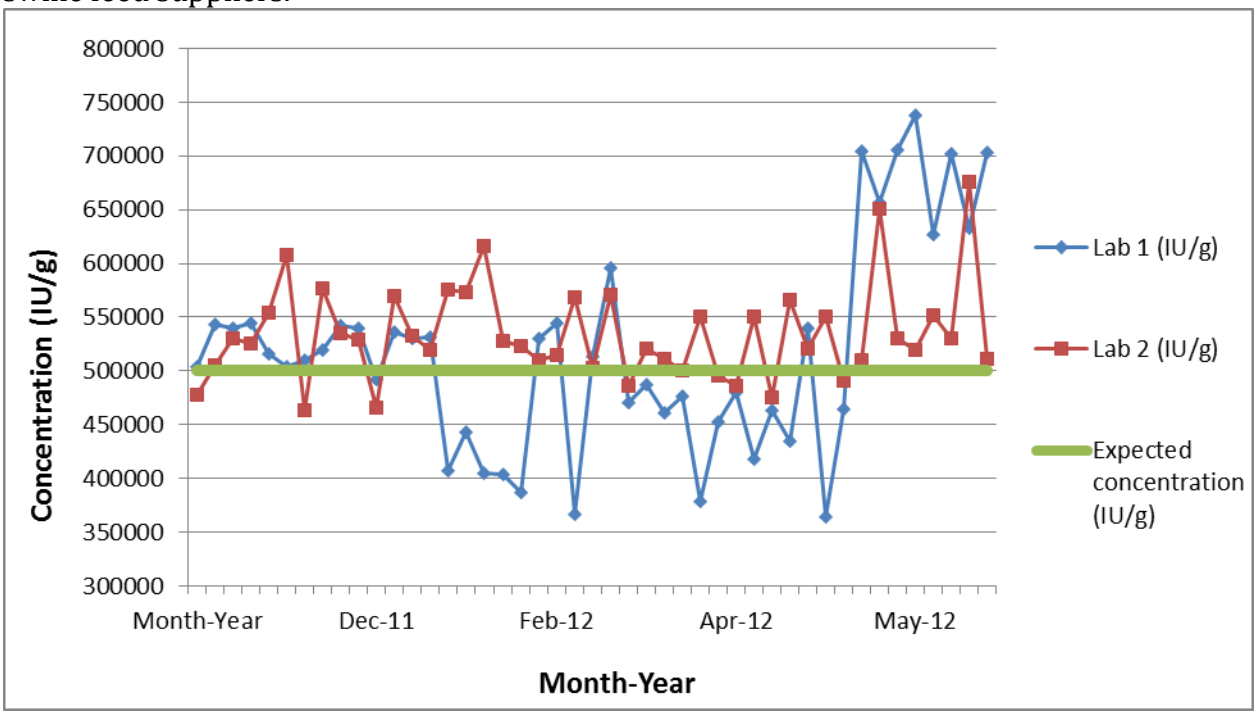


Figure 2: Bulk vitamin D levels over a nine month collection period from five swine supplement suppliers showing a month effect (95% Confidence Limits) of testing.

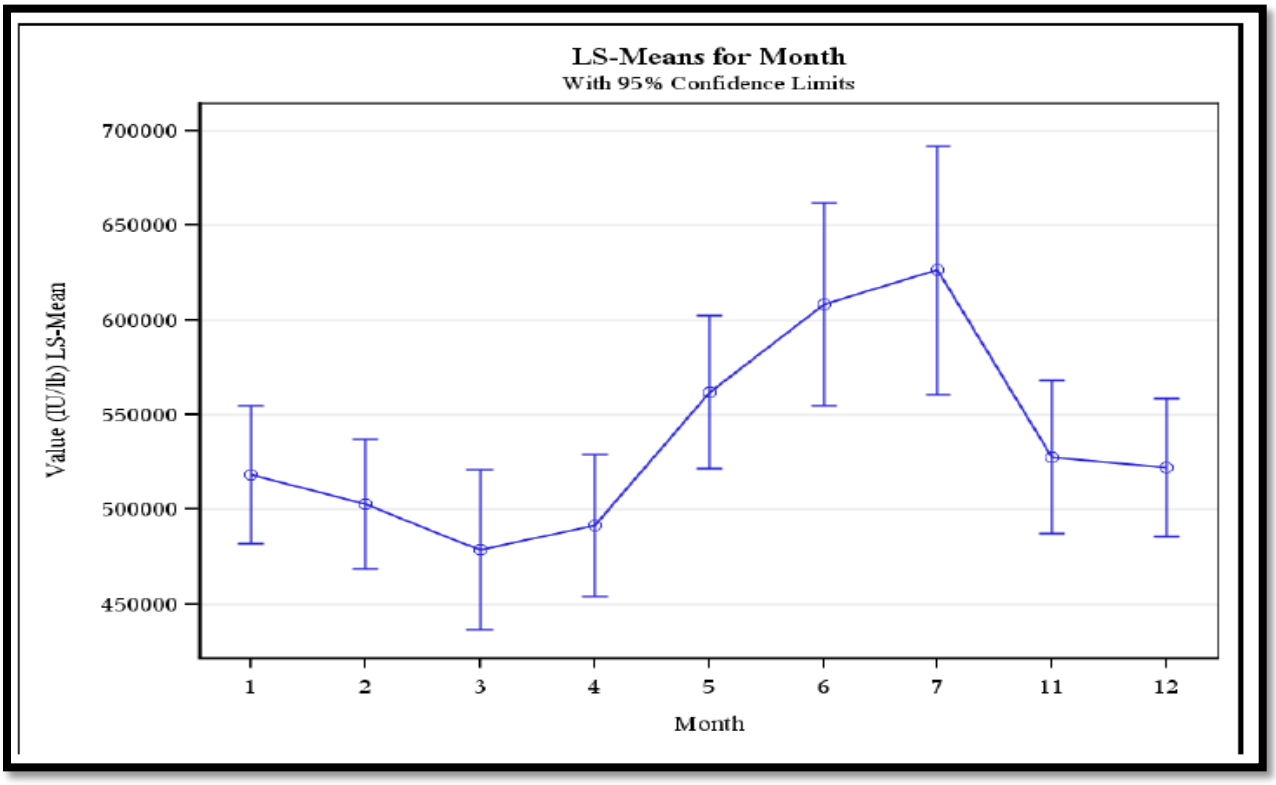
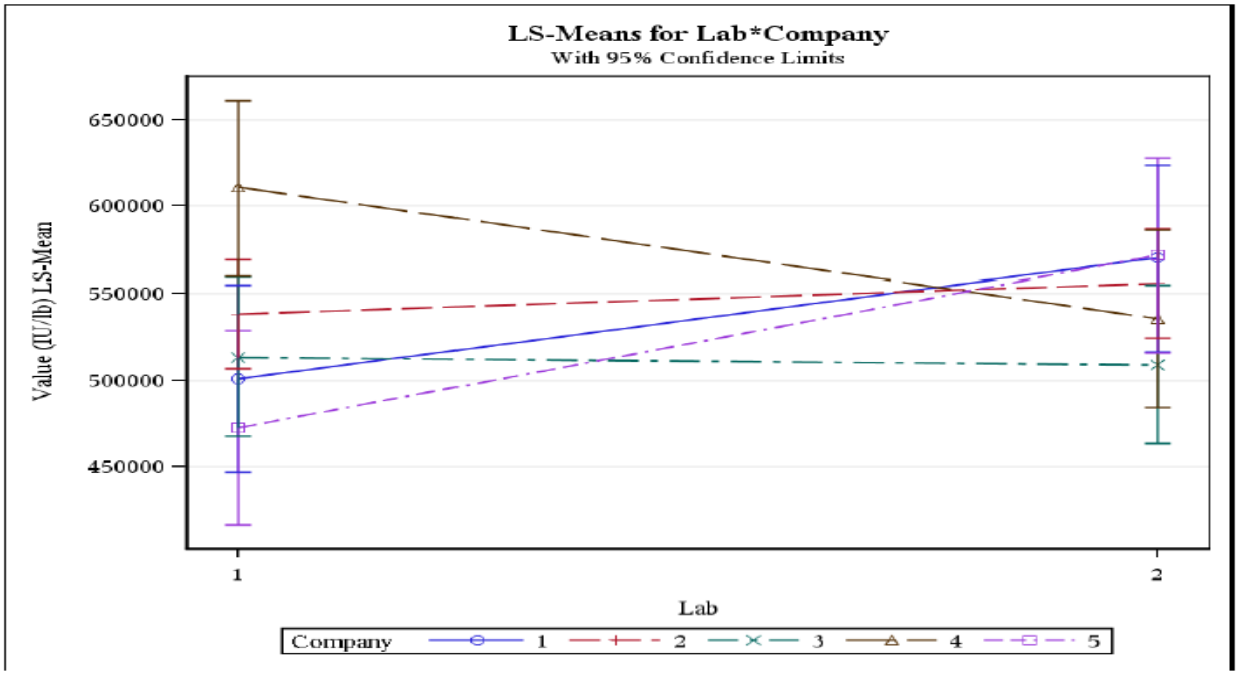


Figure 3: Company by laboratory interaction of bulk vitamin D samples



Discussion

The two forms of vitamin D that are available to swine are either through (1) sunlight and conversion within the skin or, (2) dietary sources. Since the majority of US swine production is indoors, the main source of vitamin D is obtained by dietary supplementation. The objective of the current study was to evaluate the quality of bulk vitamin D3 used for inclusion in swine diets. Currently, there are several types of dietary supplements available. There are powder supplements that can be mixed into total feed rations (evaluated in this project), injectable products, oral drenches that can be given to piglets at processing or weaning, and liquid products that can be supplied through water sources.

Some forms of vitamin D added to feed may contain large quantities of inactive metabolites and the quantity of pure vitamin D3 can be overestimated.[8] Therefore, accurate analyte testing is essential to determine the true active amount present. In this study, we found that specific laboratories may give different results, of the same sample, based on the analytical method used to measure the quantity of vitamin D. Testing for vitamin D is not easy, and method used will give different results.[2] HPLC with UV detection is considered the gold standard for quantifying vitamin D3,[2] yet extraction or internal controls are important and may result in different quantities detected between similar analytic methods.[4] However, our results show that there is no individual supplement or supplier (US or foreign) was found to be significantly lower than the expected concentration based testing of both laboratories.

Vitamin D_3 is susceptible to degradation by heat, moisture and contact with minerals, such as ferrous sulfate and manganese oxide.[9] Time of bulk vitamin D storage within a facility could have an effect. Anecdotally, this was an issue in a couple of instances of metabolic bone disease cases in Iowa.[5] However, we did not see this trend if the same lot number of vitamin D was received in consecutive months in this study (data not shown). We can therefore not explain why there is a month effect overtime with the samples. Our only speculation would be that vitamin D suppliers were actually adding more available vitamin D within the bulk sample because of the hypersensitivity of the issue within the swine industry.

The results from this analysis of vitamin D supplements indicate there can be variation between various supplement suppliers and the specific detection from different laboratories. However, no individual supplement or supplier (US or foreign) was found to be significantly lower than the expected

concentration of 500,000 IU/lb. Results of this study suggest that the rise in clinical swine metabolic bone disease does not appear to be associated with bulk vitamin D that is being supplied to diet manufactures.

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