Diagnostic Survey of Biological Measurements Used to Determine Bone Mineralization in Pigs Across the US Swine Industry

Hadley R. Williams
Kansas State University, Manhattan, hadley1@k-state.edu

Mike D. Tokach
Kansas State University, Manhattan, mtokach@k-state.edu

Jason C. Woodworth
Kansas State University, Manhattan, jwoodworth@k-state.edu

See next page for additional authors

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K-STATE
Research and Extension

SWINE DAY 2023

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2023

Diagnostic Survey of Biological Measurements Used to Determine Bone Mineralization in Pigs Across the US Swine Industry[1]

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Summary

Pigs from 64 commercial sites across 14 production systems in the Midwest US were used to evaluate the baseline biological measurements used to determine bone mineralization. Three pigs were selected from each commercial site representing: 1) a clinically normal pig (healthy); 2) a pig with evidence of clinical lameness (lame); and 3) a pig from a hospital pen that is assumed to have recent low feed intake (unhealthy). Pigs ranged in age from nursery to market weight, with the three pigs sampled from each site representing the same age or phase of production. Blood, urine, metacarpal, fibula, 2nd rib, and 10th rib were collected and analyzed. Serum was analyzed for Ca, P, and $25(OH)D_3$, and urine was collected and analyzed for Ca, P, and creatinine. Each bone was measured for density, ash (defatted and non-defatted technique), and breaking strength. A bone × pig type interaction ($P < 0.001$) was observed for defatted and non-defatted bone ash, density, and breaking strength. For defatted bone ash, there were no differences ($P > 0.10$) between pig types for the fibulas, 2nd rib, and 10th rib, but metacarpals from healthy pigs had greater ($P < 0.05$) percentage bone ash compared to unhealthy pigs, with the lame pigs intermediate. For non-defatted bone ash, there were no differences ($P > 0.10$) between pig types for metacarpals and fibulas, but unhealthy pigs had greater ($P < 0.05$) non-defatted percentage bone ash for 2nd and 10th ribs compared to healthy pigs, with lame pigs intermediate. Healthy and lame pigs had greater ($P < 0.05$) bone density than unhealthy pigs for metacarpals and fibulas, with no difference ($P > 0.10$) observed for ribs. Healthy pigs had bones with increased breaking strength compared to lame and unhealthy pigs for metacarpals and 10th ribs ($P < 0.05$) with no differences ($P > 0.05$) between pig types for fibula and 2nd rib. Healthy pigs had greater ($P < 0.05$) serum Ca and $25(OH)D_3$ compared to

unhealthy pigs, with lame pigs intermediate. Healthy pigs had greater ($P > 0.05$) serum P compared to unhealthy and lame pigs, with no differences ($P > 0.05$) between the unhealthy and lame pigs. Unhealthy pigs excreted more ($P < 0.05$) P and creatinine in the urine compared to healthy pigs, with lame pigs intermediate. In summary, there are differences in serum Ca, P, and vitamin D between healthy, lame, and unhealthy pigs. Differences in bone mineralization between the pig types varied depending on the analytical procedure and bone. There was a considerable range in values within pig type across the 14 production systems sampled.

Introduction

Due to improper bone mineralization in pigs, producers in the US swine industry experience economic losses and animal welfare concerns. Diagnosticians rely on bone mineralization as a diagnostic tool to identify cases of lameness in pigs. However, because numerous factors can contribute to lameness, identifying the underlying cause can be difficult. There are different diagnostic assessments to measure bone mineralization, including bone ash, density, and histopathological examination; however, there are limited data that compare these different measurements across pigs with different health status. Recent work evaluating bone mineralization has compared the differences between bone ash procedures available for use. Wensley et al.[5] observed differences in percentage bone ash when comparing bones that had the lipid extracted (defatted) vs. those without the lipid extraction (non-defatted) before ashing of the bone. The authors observed a reduction in standard deviation when the bone is defatted before ashing, and this response was more apparent in finishing pigs compared to nursery pigs due to the increased fat accumulation in bones as pigs grow. Also, Williams et al.[6] observed differences in percentage bone ash and bone density among fibulas, metacarpals, 2nd ribs, and 10th ribs in nursery pigs and differences between analytical methods. Similar to Wensley et al.,[5] defatting bones prior to ashing reduced the amount of variation between the bones compared to not extracting the lipid.

Currently, reference values used by diagnosticians for bone mineralization and serum vitamin D have been adapted from Field et al.[7] and Arnold et al.[8] Typically, the pigs submitted to diagnostic laboratories are unhealthy or lame and their recent dietary intakes are unknown but likely reduced and highly variable, possibly causing their nutritional status or bone mineralization to be lower than that of healthier pigs in the general population. Thus, interpretation of these results may potentially lead to misdiagnosis relative to the entire population. Recently, Williams et al.[9] evaluated

[5] Wensley, M. R., C. M. Vier, J. T. Gebhardt, M. D. Tokach, J. C. Woodworth, R. D. Goodband, and J. M. DeRouchey. 2020. Technical note: assessment of two methods for estimating bone ash in pigs. J. Anim. Sci. 98:1-8. doi:10.1093/jas/skaa251.

[6] Williams, H. R., T. E. Chin, J. T. Gebhardt, M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, J. R. Bergstrom, M. C. Rahe, C. L. Siepker, P. Sitthicharoenchai, and S. M. Ensley. 2023a. The effect of bone and analytical methods on the assessment of bone mineralization response to dietary phosphorus, phytase, and vitamin D in nursery pigs.

[7] Field, R. A., M. L. Riley, F. C. Mello, M. H. Corbridge, and A. W. Kotula. 1974. Bone composition in cattle, pigs, sheep, and poultry. J. Anim. Sci. 39:3. doi:10.2527/jas1974.393493x.

[8] Arnold, J., D. M. Madson, S. M. Ensley, J. P. Goff, C. Sparks, G. W. Stevenson, T. Crenshaw, C. Wang, and R. L. Horst. 2015. Survey of serum vitamin D status across stages of swine production and evaluation of supplemental bulk vitamin D premixes used in swine diets. J. Swine Health Prod. 23:28-34.

[9] Williams, H. R., J. T. Gebhardt, M. D. Tokach, J. C. Woodworth, R. D. Goodband, J. M. DeRouchey, J. R. Bergstrom, C. W. Hastad, Z. B. Post, M. C. Rahe, C. L. Siepker, P. Sitthiccharoenchai, and S. M.

different bones and various analytical measurements between healthy and unhealthy pigs. The authors observed that healthy pigs have increased serum Ca, P, and vitamin D compared to unhealthy pigs. They also observed no statistical difference between the health statuses for non-defatted bone ash; however, healthy pigs had increased defatted bone ash compared to the unhealthy pigs. Therefore, our objective in the current study was to evaluate the effect of different pig types (healthy, lame, or unhealthy) from pigs in commercial production sites across the Midwestern US on the assessment of bone mineralization.

Procedures

General

The Kansas State University Animal Care and Use Committee approved the protocol used in this study (IACUC no. 4595). The diagnostic survey utilized pigs from commercial production facilities in the Midwest US.

A total of 192 pigs from 64 commercial production sites across 14 different production systems in Minnesota and Iowa were used in the diagnostic survey. All samples were collected from December 2021 to February 2022. At each site, the staff veterinarian selected 3 pigs to be euthanized and used in the diagnostic survey: 1) a clinically normal pig; 2) a pig with locomotive issues and evidence of clinical lameness; and 3) a pig from within a hospital pen with an assumed recent low feed intake (unhealthy pig). Pigs ranged in age from nursery to market weight, with the three pigs sampled from each site representing the same age and phase of production. Information regarding the health status of each pig, building design, and dietary information was obtained. The current report describes analytical results, but further description of correlations of production practices with these measurements will be described in future publications.

Sample collection and analysis

A blood sample was taken from the jugular vein of each pig prior to euthanasia. Blood samples were analyzed for Ca and P (Iowa State University Veterinary Diagnostic Lab) and $25(OH)D_3$ (Heartland Assays, Ames, IA). A 10 mL urine sample was collected shortly after euthanasia and later analyzed for Ca, P, and creatinine (Iowa State University Veterinary Diagnostic Lab, Ames, IA).

The metacarpal, 2nd rib, 10th rib, and fibulas were analyzed for bone density, bone-breaking strength, bone ash (de-fatted and non-defatted), and bone Ca and P. The left-over extraneous soft tissues and cartilage were removed from the bones prior to assessment. The Archimedes principle was used for bone density. Bone breaking strength was determined with an Instron (Instron 5569, NV Lab, Norwood, MA). For bone ash, both the defatted and the non-defatted methods were used (Williams et al., 2023c). These methods were used to determine the total bone ash weight and percentage ash relative to dried bone weight. Bone Ca (AOAC 985.01, 2006) and P (AOAC 985.01, 2006) quantity were measured by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Concentrations of Ca and P were calculated as a percentage of the total bone ash.

Ensley. 2023b. The effect of bone and analytical method on the assessment of bone mineralization in response to dietary phosphorus, phytase, and vitamin D in finishing pigs.

Statistical analysis

For the statistical analysis, bone data were analyzed by fitting a linear mixed model using the lmer function from the lme4 package in R (version 3.5.1 (2018-07-02), R Foundation for Statistical Computing, Vienna, Austria). The pig type, bone, and the associated interaction were included as fixed effects. System and pig type within system were included as random effects to account for systems having multiple sites sampled and multiple bones being collected from each pig type for each site. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

Diagnostic survey

For the bone analysis, a significant bone × pig type interaction ($P < 0.001$; Table 1) was observed for percentage bone ash (defatted and non-defatted), defatted bone ash weight, bone density, non-defatted bone ash weight, and bone breaking strength. For each analysis, the response to pig type varied depending on the bone analyzed. For defatted percentage bone ash, there was no difference ($P > 0.10$) between the pig types for the fibulas, 2nd ribs, or 10th ribs. For the metacarpals, the bones from healthy pigs had greater ($P < 0.05$) defatted percentage bone ash than unhealthy pigs, with the lame pigs intermediate. For the 10th rib and metacarpal defatted bone ash weight, healthy and lame pigs had greater bone ash weight compared to unhealthy pigs ($P < 0.05$) with no difference ($P > 0.05$) between healthy and lame pigs. For the fibulas and 2nd ribs, healthy pigs had greater bone ash weight compared to unhealthy pigs ($P < 0.05$), with lame pigs intermediate. For non-defatted percentage bone ash, there was no difference ($P > 0.05$) between pig types for metacarpals and fibulas. For 2nd and 10th ribs, unhealthy pigs had greater non-defatted percentage bone ash compared to healthy pigs, with lame pigs intermediate ($P < 0.05$). For non-defatted bone ash weight, metacarpals and 10th ribs from healthy and lame pigs were greater than unhealthy pigs ($P > 0.05$) but weights had no difference ($P > 0.10$) between healthy and lame pigs. For fibulas and 2nd ribs, healthy pigs had greater non-defatted bone ash weight compared to unhealthy pigs, with lame pigs intermediate ($P < 0.05$). Metacarpals and 10th ribs from healthy and lame pigs had greater ($P < 0.05$) non-defatted bone ash weight compared to unhealthy pigs, with no difference ($P > 0.05$) between healthy and lame pigs. Healthy pigs had greater ($P < 0.05$) fibula non-defatted bone ash weight compared to unhealthy pigs, with lame pigs intermediate. For bone density, there was no difference ($P > 0.10$) between pig types when 2nd and 10th ribs were analyzed. Healthy pigs had greater bone ($P < 0.05$) density for metacarpals and fibulas compared to unhealthy pigs, with no difference between healthy and lame pigs ($P > 0.05$). There was no difference ($P > 0.10$) between pig types when fibulas and 2nd ribs were analyzed for bone breaking strength. For the 10th ribs, healthy and lame pigs had increased ($P < 0.05$) bone-breaking strength compared to unhealthy pigs, with no difference ($P > 0.10$) between healthy and lame pigs. For metacarpals, healthy pigs had greater ($P < 0.05$) bone breaking strength compared to lame pigs, and lame pigs had greater ($P < 0.05$) bone breaking strength compared to unhealthy pigs.

Healthy pigs had greater serum $25(OH)D_3$ and serum Ca levels compared to unhealthy pigs, with lame pigs intermediate, but significantly different than the healthy and unhealthy pigs ($P < 0.05$; Table 2). Healthy pigs had greater serum P compared to unhealthy and lame pigs ($P < 0.05$) with no difference between unhealthy and lame pigs ($P > 0.10$). There was no significant difference for urine Ca between the different pig

types ($P > 0.10$). Unhealthy pigs were excreting greater concentrations ($P < 0.05$) of P and creatinine in urine compared to healthy pigs, with lame pigs intermediate. There was no difference observed between the pig types for Ca:creatinine and P:creatinine ratios ($P > 0.10$).

For the main effect of pig type, there was no difference between pig types for defatted bone ash percentage ($P = 0.122$; Table 3). Healthy and lame pigs had increased defatted bone ash weight compared to unhealthy pigs ($P < 0.05$). There was a significant difference between pig types for percentage non-defatted bone ash, non-defatted bone ash weight, bone density, and breaking strength ($P < 0.05$). For non-defatted bone ash, unhealthy pigs had greater percentage bone ash compared to healthy pigs, with lame pigs intermediate; however, healthy and lame pigs had greater bone ash weight compared to unhealthy pigs. Healthy and lame pigs had greater bone density compared to unhealthy pigs. Healthy and lame pigs required greater force to break bones compared to unhealthy pigs ($P < 0.05$). Healthy and lame pigs had increased grams of Ca and P in defatted bone ash compared to unhealthy pigs ($P < 0.05$).

For the main effect of bone, there were significant differences for defatted and non-defatted bone ash weight, defatted and non-defatted percentage bone ash, bone density, and bone breaking strength between bones ($P < 0.05$; Table 4). For defatted bone ash weight, metacarpals and 10th ribs had greater bone ash weight compared to the fibula and 2nd rib ($P < 0.05$). Fibulas had the greater defatted percentage bone ash compared to the 2nd and 10th ribs, with the metacarpals having the lowest defatted percentage bone ash ($P < 0.05$). Metacarpals and 10th ribs had greater non-defatted bone ash weight compared to fibulas and 2nd ribs ($P < 0.05$), with the 2nd ribs being the lowest. For non-defatted percentage bone ash, 10th ribs had the greatest percentage bone ash, metacarpals the lowest, with 2nd ribs and fibulas intermediate ($P < 0.05$). Fibulas had the greatest bone density, metacarpals had the lowest bone density, 2nd and 10th ribs were intermediate, with 10th ribs having greater bone density than 2nd ribs ($P < 0.05$). Metacarpals had the greatest bone breaking strength ($P < 0.05$), the 2nd ribs had the lowest, and fibulas and 10th ribs were intermediate, and 10th ribs had greater bone breaking strength than fibulas. There were no differences between defatted bone ash of metacarpals and 10th ribs for grams and percent Ca and P ($P > 0.10$).

When looking at the main effect of production system, there was considerable variation in serum $25(OH)D_3$ between production systems (Figure 1).

In summary, this diagnostic survey demonstrates the differences between healthy, lame, and unhealthy pigs for serum Ca, P, and vitamin D, as well as the differences across the various bones and analytical measurements to assess bone mineralization. By using the defatted bone ash method, the amount of variation across bones and different pig types was reduced, but no differences were observed between healthy, lame, and unhealthy pigs. For non-defatted bone ash, there was more variation, with unhealthy pigs having greater percentage bone ash compared to healthy pigs because of lipid mobilization from the bones of unhealthy pigs. When comparing all swine production systems included in this diagnostic survey, we observed considerable variation across different measurements, with serum vitamin D having a wide range between the different pig types. Although serum vitamin D had the highest mean serum concentration for healthy pigs, there were healthy pigs below the reference range threshold for clinically

normal serum vitamin D analysis. These data can be used to help guide future diagnostic investigations with baseline levels of different methodologies to determine bone mineralization in pigs.

Table 1. Interactive effect of bone and pig type[1]

	Pig type						
	Healthy		Lame		Unhealthy		
Item	Mean	Range[2]	Mean	Range	Mean	Range	SEM
Defatted bone ash, %[3]							
Metacarpal	60.9[a]	51.0-69.3	59.8[b]	49.3-69.1	58.7[c]	47.3-63.7	0.37
Fibula	64.4	56.0-67.9	64.6	59.8-67.8	63.7	50.7-69.8	
2nd rib	62.2	56.5-65.3	62.4	59.2-65.0	62.4	56.8-66.4	
10th rib	62.3	55.5-65.0	62.7	59.5-67.0	62.3	51.4-66.3	
Defatted bone ash, g							
Metacarpal	5.19[a]	0.74-10.45	4.53[a]	0.58-10.34	3.46[b]	0.48-8.56	0.352
Fibula	2.94[a]	0.42-7.47	2.52[ab]	0.33-7.72	1.96[b]	0.19-6.06	
2nd rib	2.91[a]	0.37-6.92	2.53[ab]	0.20-6.25	1.99[b]	0.14-6.33	
10th rib	5.03[a]	0.79-11.54	4.62[a]	0.62-14.12	3.39[b]	0.34-10.86	
Defatted bone ash content							
Ca, g							
Metacarpal	2.53[a]	0.30-6.09	2.19[ab]	0.21-5.92	1.68[b]	0.20-4.62	0.220
10th rib	2.41[a]	0.36-6.62	2.25[a]	0.26-7.41	1.55[b]	0.18-4.50	
P, g							
Metacarpal	1.21[a]	0.14-2.96	1.01[ab]	0.10-2.83	0.79[b]	0.09-2.16	0.104
10th rib	1.16[a]	0.18-3.18	1.09[a]	0.12-3.47	0.74[b]	0.08-2.17	
Ca, %							
Metacarpal	48.50	35.04-66.84	47.24	33.54-64.17	46.56	31.33-62.07	0.916
10th rib	47.64	29.92-63.30	47.45	33.12-65.33	45.24	34.37-60.42	
P, %							
Metacarpal	23.25	16.75-33.62	22.51	15.62-30.44	22.22	15.23-30.32	0.476
10th rib	22.83	14.31-29.42	22.81	15.82-31.18	21.66	16.41-29.59	
Non-defatted bone ash, %[4]							
Metacarpal	43.2	35.1-54.0	43.3	34.8-56.4	44.4	36.2-58.2	0.57
Fibula	54.0	44.6-62.3	53.9	39.1-61.1	55.0	48.1-65.1	
2nd rib	54.3[b]	46.1-60.3	55.4[ab]	44.1-62.8	56.0[a]	46.9-62.1	
10th rib	55.4[b]	45.9-61.2	56.6[ab]	47.6-62.4	57.2[a]	48.8-62.3	
Non-defatted bone ash, g							
Metacarpal	5.14[a]	0.83-10.44	4.53[a]	0.64-10.32	3.51[b]	0.48-8.67	0.369
Fibula	3.43[a]	0.63-7.41	2.99[ab]	0.21-8.34	2.41[b]	0.25-7.60	
2nd rib	3.01	0.31-7.52	2.66	0.30-7.63	2.17	0.16-7.17	
10th rib	5.29[a]	0.38-13.16	4.71[a]	0.47-13.59	3.58[b]	0.19-12.18	

continued

Table 1. Interactive effect of bone and pig type[1]

| | Pig type | | | | | | |
| | Healthy | | Lame | | Unhealthy | | |
Item	Mean	Range[2]	Mean	Range	Mean	Range	SEM
Bone density, g/mL[5]							
Metacarpal	1.30[a]	1.17-1.41	1.28[a]	1.12-1.38	1.26[b]	1.13-1.36	0.008
Fibula	1.38[a]	1.25-1.61	1.37[a]	1.26-1.54	1.34[b]	1.16-1.50	
2nd rib	1.32	1.24-1.74	1.32	1.24-1.47	1.30	1.20-1.44	
10th rib	1.34	1.30-1.55	1.34	1.28-1.50	1.33	1.22-1.51	
Breaking strength, kg							
Metacarpal	115.2[a]	3.12-328.3	99.6[b]	18.8-280.1	73.1[c]	7.7-196.8	6.00
Fibula	47.7	9.9-100.1	43.1	6.6-131.9	32.4	4.3-91.8	
2nd rib	39.3	7.0-97.9	35.0	4.5-93.8	26.8	0.9-103.3	
10th rib	74.8[a]	11.3-168.0	67.6[a]	11.0-206.7	47.5[b]	5.7-170.2	

[abc]Means within a row with different superscripts differ ($P < 0.05$).
[1]Bone × pig type interaction ($P < 0.001$) for defatted bone ash percentage, defatted bone ash grams, non-defatted bone ash, bone density, and bone breaking strength. SEM for the interaction is reported.
[2]The range is the minimum and maximum value for each bone within each pig type.
[3]All bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 d to remove water and fat. Bones were then dried at 221°F (105°C) for 7 d, and then ashed in a muffle furnace at 1,112°F (600°C) for 24 h.
[4]All bones were cleaned of tissue and then dried at 221°F (105°C) for 7 days and then ashed in a muffle furnace at 1,112°F (600°C) for 24 h.
[5] Bone density was measured on each bone based on Archimedes principle.

Table 2. The effect of pig type on serum and urine analysis

| | Pig type | | | | | | | |
| | Healthy | | Lame | | Unhealthy | | | |
Item	Mean	Range	Mean	Range	Mean	Range	SEM	P =
Serum analysis[1]								
25(OH)D$_3$, ng/mL	25.2[a]	5.6-78.9	21.0[b]	4.6-64.0	15.5[c]	2.8-71.8	1.88	< 0.001
Ca, mg/dL	10.4[a]	6.9-14.4	9.7[b]	6.7-13.4	9.2[c]	6.6-14.5	0.16	< 0.001
P, mg/dL	10.2[a]	6.4-18.0	9.4[b]	5.5-15.8	8.8[b]	4.5-14.7	0.26	< 0.001
Urine analysis[2]								
Ca, mg/dL	10.9	1.0-35.6	11.1	1.0-41.0	9.5	1.0-35.6	1.88	0.740
P, mg/dL	50.9[b]	5.5-142.9	75.2[ab]	5.5-903.0	96.9[a]	5.5-286.0	11.76	0.003
Creatinine, mg/dL	103.1[b]	9.1-255.9	135.1[ab]	3.2-378.3	151.3[a]	16.4-381.1	14.08	0.022
Calcium:creatinine	0.13	---	0.19	---	0.13	---	0.048	0.456
Phosphorus:creatinine	0.63	---	0.71	---	0.94	---	0.151	0.303

[abc]Means within a row with different superscripts differ ($P < 0.05$).
[1]Serum Ca and P were measured at Iowa State Veterinary Diagnostic Lab (Ames, IA). The vitamin D serum analysis was conducted at Heartland Assays (Ames, IA).
[2]Urine was collected from the bladder of each pig and analyzed for Ca, P, and creatinine at Iowa State Veterinary Diagnostic Lab (Ames, IA).

Table 3. The effect of pig type on bone analysis[1]

	Healthy		Lame		Unhealthy			
	Pig type							
Item	Mean	Range	Mean	Range	Mean	Range	SEM	*P* =
Defatted bone ash[2]								
Bone ash, g	4.02[a]	0.37-11.54	3.55[a]	0.20-14.12	2.71[b]	0.14-10.86	0.337	0.001
Bone ash, %	62.4	51.0-67.9	62.3	49.3-69.1	61.8	47.3-69.8	0.29	0.122
Non-defatted bone ash[3]								
Bone ash, g	4.23[a]	0.31-13.16	3.73[a]	0.21-13.59	2.92[b]	0.16-12.18	0.354	0.001
Bone ash, %	51.7[b]	38.1-62.3	52.3[ab]	34.8-65.8	53.1[a]	36.2-65.1	0.53	0.037
Bone density, g/mL[4]	1.33[a]	1.16-1.56	1.33[a]	1.16-1.52	1.31[b]	1.14-1.44	0.007	0.001
Breaking strength, kg	69.1[a]	3.1-328.3	61.1[a]	4.5-280.1	44.9[b]	0.9-196.8	5.61	0.001
Defatted bone ash content[5]								
Ca, g	2.47[a]	0.30-6.62	2.22[a]	0.21-7.41	1.62[b]	0.18-4.62	0.214	0.001
P, g	1.18[a]	0.14-3.18	1.05[a]	0.10-3.47	0.76[b]	0.08-2.17	0.100	0.001
Ca, %	48.0	29.9-66.8	47.4	33.1-65.3	45.9	31.3-62.1	0.648	0.064
P, %	23.0[a]	14.3-33.6	22.7[ab]	15.6-31.2	21.9[b]	15.2-30.3	0.354	0.050

[abc]Means within a row with different superscripts differ (*P* < 0.05).

[1]The means of all 4 bones measured are combined for each pig type.

[2]All bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 d to remove water and fat. Bones were then dried at 221°F (105°C) for 7 d, and then ashed in a muffle furnace at 1,112°F (600°C) for 24 h.

[3]All bones were cleaned of tissue and then dried at 221°F (105°C) for 7 d and then ashed in a muffle furnace at 1,112°F (600°C) for 24 h.

[4]Bone density was measured on each bone based on the Archimedes principle.

[5]After bone ash processing was completed, the ash samples from the metacarpal and 10th ribs were digested and analyzed for Ca and P using ICP-OES by the K-State Research and Extension Soil Testing Laboratory, Manhattan, KS.

Table 4. Effect of different bones on bone analysis of pigs

	Metacarpal	Fibula	2nd rib	10th rib	SEM	P =
Defatted bone ash[1]						
Bone ash, g	4.39[a]	2.47[b]	2.48[b]	4.35[a]	0.287	0.001
Bone ash, %	59.7[c]	64.2[a]	62.3[b]	62.5[b]	0.25	0.001
Non-defatted bone ash[2]						
Bone ash, g	4.39[a]	2.94[b]	2.61[c]	4.53[a]	0.301	0.001
Bone ash, %	43.6[d]	54.3[c]	55.2[b]	56.4[a]	0.44	0.001
Bone density, g/mL[3]	1.28[d]	1.36[a]	1.31[c]	1.33[b]	0.006	0.001
Bone breaking strength, kg	96.7[a]	41.0[c]	33.6[d]	63.2[b]	4.63	0.001
Defatted bone ash content[4]						
Ca, g	2.14	---	---	2.07	0.177	0.281
P, g	1.00	---	---	0.99	0.084	0.799
Ca, %	47.4	---	---	46.8	0.532	0.381
P, %	22.7	---	---	22.4	0.304	0.537

[abc]Means within a row with different superscripts differ ($P < 0.05$).
[1]All bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 d as a means of removing water and fat. Bones were then dried at 221°F (105°C) for 7 d, and then ashed in a muffle furnace at 1,112°F (600°C) for 24 h.
[2]All bones were cleaned of tissue and then dried at 221°F (105°C) for 7 d and then ashed in a muffle furnace at 1,112°F (600°C) for 24 h.
[3]Bone density was measured on each bone based on Archimedes principle.
[4]After bone ash processing was completed, the ash samples from the metacarpal and 10th ribs were digested and analyzed for Ca and P using ICP-OES by the K-State Research and Extension Soil Testing Laboratory, Manhattan, KS.
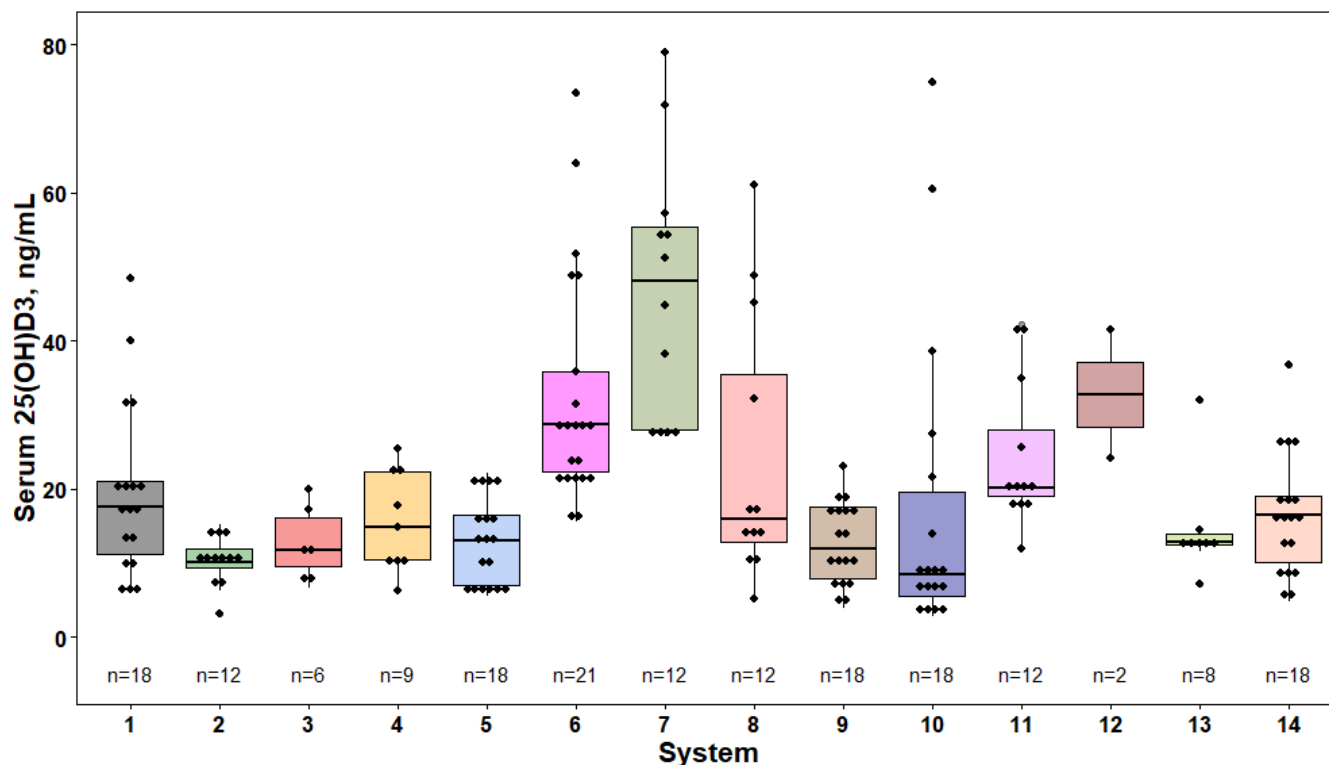

Figure 1. Serum vitamin D levels comparing all of the samples across the systems.