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Vitamin D and nursing pigs – clinical investigation, observations and speculation

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In February, while continuing to work on PFTS pigs that would not eat post weaning, we made the observation that ribs appeared to be poorly mineralized. Following up with histopathology on ribs from pigs weaned only a couple of days, we learned that these ribs contained micro-fractures in various stages of healing. Serum was analyzed for calcium, phosphorus and vitamin D. Results demonstrated that pigs had 25-hydroxycholecalciferol (25(OH)D) levels that were from undetectable to low single digit values (ng/ml) instead of the 25-30ng/ml considered to be normal.

Drs. Ron Horst and Jesse Goff, Heartland Assays, Ames Iowa are expert in vitamin D metabolism and analysis and have been guiding the efforts we have undertaken in the field. Formerly with ARS, these researchers developed the assay methodology now used in veterinary medicine. We expanded the serum testing of weaned pigs to additional herds and locations. At this point in the clinical investigation, no pigs have been found with the 25-30ng/ml considered to be the normal target levels. Most levels were only 10%-15% of this level.

Based on the proposal of Ron and Jesse a simple administration of an oral bolus dose of vitamin D at processing, 40,000IU per pig, should create levels that reach the targeted range of normal at weaning. So we initiated a simple 'Proof of Concept' field study. Using vitamin D in peanut oil, prepared by Jesse, we conducted the trial that is reported in the 'Vitamin D Proof of Concept Project' which is attached.

Vitamin D is important for the immune system and immune response, it is critical in calcium-phosphorus metabolism, bone strength and structure and is important to muscle growth. What we want to see is how pigs will perform in many ways if we can confidently assure they have normal vitamin D levels. To get a feel for this it would take a lot of pigs over time and that is what is proposed here. In addition, unlike other drug and vaccine interventions, there is not a downside impact that will result from this targeted supplementation. Thus we have begun oral administration with 40,000IU D at processing in a large population of pigs.

Drs. Horst and Goff, through their research company in Ames, are preparing the vitamin D solution and are assaying product and inputs to assure the dosage is accurate. As a part of our trial treatment they provided an oral solution of vitamin D in peanut oil. This we compared to the commercially available Emcelle D-E combination and the pigs very certainly prefer the peanut oil solution. Reasons for not using the Emcelle include a lower 25-hydroxyvitamin D response, pigs hate it probably due to the cod liver oil carrier and the 2ml dose is a lot to get pigs to swallow. In the one trial we did not achieve expected levels

with a 40,000IU dose of Emcelle. And the cost is greater. To get this right going forward, the following protocol is in place:

- The vitamin D sources used to make up the oral solution vary widely in potency even though they are purchased as 'vitamin D'. This Jesse and Ron have shown repeatedly. So each lot of pure vitamin needs to be analyzed and then the concentration titrated to accurately get the 40,000IU in each dose.

- Vitamin D, like other vitamins, cannot be assumed to have a long shelf life once prepared but it will last for several months. Storage is at room temperature and not in sunlight. So the plan, working with Ron and Jesse, is to come up with a process that assures we are getting and delivering the dose that is predicted. Details are being finalized on how this can be best accomplished, what it will cost and the plan can then be activated. While not final, it is clear the cost will be minimal.

- Pigs like the peanut oil. And it is easier to handle than the cod liver oil base. Again based on Jesse's experience and research, the fatty acid chain length is contributory to the stability and effectiveness as a carrier of vitamin D.

- We really don't know if, over time, the 40,000IU dose is exactly what it should be. This is predicted from other species and appears to achieve expected levels in the limited trials we are doing. So this will be an active and ongoing effort.

Objectively testing the response to vitamin D oral dosing will begin next month, conducted by the K-State Swine Research team, evaluating growth as well as bone structure and vitamin D levels. Conducted with appropriate controls, this will help to answer the questions on growth. The field effort we have undertaken with this treatment of pigs as a part of production has the intent to assess over time and season, in multiple herds, if there is any impact or improvement in several production problems that continue to stump us. The logic behind large numbers in the field is that many of the problems to address, such as PFTS, hump-back pigs, lameness all have relatively low incidence. This makes a prospective, controlled study one of daunting numbers. Thus "treat them all and then evaluate".

What are the health questions that vitamin D deficiency might pose? It is possible that vitamin D deficiency in this critical stage of life may be having some profound effects on pigs especially as they move from milk to solid food at weaning. For many pigs, their body mass will increase five-fold over this critical period. The list below attempts to capture these thoughts and why this is worth a try on a herd level basis. Oral dosing is a simple way to change the pig's status and then maybe we can just evaluate what the pigs are telling us week by week.

- We know – poor bone mineralization with micro-fractures in ribs occurs in pigs that have very low to undetectable levels of serum D. This was found in samples from failure to start on feed pigs (PFTS) in recent months. These lesions may have been there for a long time but now we are finally looking for them. Also could this have ramifications in long term lameness- i.e. lesions and defects derived in early life make for a weak bone later in life.

- We know – Vitamin D levels have been uniformly quite low in all 21d old pigs we have tested at weaning. See table for assay results.

- We know – that pigs receive their vitamin D supply from colostrum. This colostral D must suffice until pigs begin eating solid food, or spend adequate time in the sun. Pigs have the ability to synthesize vitamin D from ultraviolet sunlight radiation, in fact have more of the cholesterol precursor concentration in the skin than most other species. So the source of D has to be diet or sunlight which leads to additional thoughts when applied to the modern pig.

- For the pig in the wild or outdoors, vitamin D is not an issue since sunlight allows synthesis even for very young pigs.

- UV light does not come through glass and is not produced by our building lighting or heat lamps. So under the modern methods of production, UV light is really not hitting the skin and the natural synthesis does not occur. Diet is the sole avenue.

- Perhaps – the sow really never needed to pass high levels of D on to her pigs in colostrum? From an evolutionary standpoint there was little need as sunlight and early consumption of materials containing D before weaning resulted in deficiency not being a problem. Not a proven fact, just a thought to consider from Ron that I find rather logical. With no evolutionary benefit, there is little reason for sows to burn resources to provide it for their babies.

- Colostrum – provides the D quantity until solid food, diet supplemented with vitamin, is being consumed by the baby pig. It is reported that a baby pig assimilates about 10-15% of the level of the sow's serum level of D from colostrum. Reported normal levels for sows are 50-60ng/ml so a pig would get 5-9ng/ml as expected blood levels. In our small study, levels in colostrum-suckled piglets averaged about 3.5ng/ml, below expectations. Many possible reasons to be tested as to why but it is speculated:

    - Litters today are much larger than when the 10-15% general relationship was established from Jesse's PhD research.

    - We don't know the sow actual levels for the litters tested in our study. As we do survey testing in sow herds, so far the 25(OH)D levels in sows are also below expected values.

    - We do know, based on research of Dr. Goff, that it is unlikely that sow levels can be elevated to any degree by feeding levels; it took injection to elevate sow serum levels substantially in a research setting. And, as a side note, the injection of A-D combinations for sows is probably counter-productive due to an interaction (Dr. Horst).

- The 'solid-food' consideration as source of dietary vitamin D also brings in great avenues for speculation. Today's pig and production systems are very different than the '90s when research on vitamin D relationships was done. And these facets may have great impact on the pig. Some of these differences in and for the pig include:

    - Creep feed is much less commonly used. And even when it is, as K-State research and others have shown, a significant percentage of suckling pigs never do eat creep feed even if provided. My recollection is 30% and many of these are the more robust pigs who find milk to be all they need.

    - Weaning ages have increased and thus the exposure to diets supplemented with vitamins occurs later and for an older pig. This means extending the time over which colostral vitamin D must support the pig. Given the tremendous growth of pigs between 10 and 21 days of age, there is substantial requirement for D just to support growth.

    - The combination of older wean age, greater milk production capability and genetic capacity to grow resulted in a substantially heavier pig at weaning than was the case just a decade ago. Pigs today are almost a third heavier by age at 21 days than they were not that long ago. So whatever vitamin D supplies are provided in colostrum must support a much greater deposition of muscle and bone than in the recent past.

Is this just vitamin D? – One of the concerns, since the mechanism of transfer of all fat soluble vitamins is strongly tied to colostrum, was *'is this D only or are A and E also deficient in these pigs?'* Again from our small trial it appears that vitamins A and E were at normal levels in weaned pigs. Supplementation with the Emcelle D-E which contains 500IU/ml of vitamin E as well as D did not impact levels. From our albeit small 10 litter trial, it appears the game is vitamin D.

What might this have to do with health of weaned pigs? Speculation – how might all of these pieces involving vitamin D deficiency, as they are parsed above, result in or associate with what seem to be a diverse and unconnected set of 'conditions' we struggle with the weaned pig? This is where thinking outside the box comes into play. And the questions are now testable as it relates to vitamin D if we have a means to create pigs that are in the reported normal levels at weaning. We can find out if this is just a veterinarian's imagination or something with substance fairly easily. Following are the puzzles and commentary on common, but intractable, problems in weaned pigs that might have roots in vitamin deficient pigs. The only way to learn is to correct the problem and observe pigs.

- PFTS – the failure to thrive, starve out pig post-weaning just won't go away. Good to best pigs at weaning, big frames and good condition enter the nursery and consume very little if any feed. The spiral down is now pretty well described with cachexia, fatty liver disease, atrophic enteritis, behavioral change and oral mastication/chomping behavior until final euthanasia or death. Clinical observations include:
    - Not a condition we recognized when weaning younger and lighter pigs, transitioning to solid diets at younger ages and lighter weights.
    - There is a seasonal variability with PFTS in which the incidence in all affected herds begins to increase in November and wane in May in Kansas. Of course this leads to the speculation that, indeed, there is perhaps a reduction in 25(OH)D levels when UV light levels are low and are naturally increased by light in spring and summer.
    - Affected pigs seem to be among the best at weaning – body condition and frame, robust pigs – and not the smaller, challenged pigs at weaning. It is a condition of the 'physically buff and affluent' at weaning.
    - Effort continues to define pathology and infectious agents in these pigs, so far without any direct indicators. The syndrome occurs in high health (Mpp, PRRS free) herds even as it does in conventional herds. Many terminal cachectic pigs have been necropsied and demonstrate normal respiratory tracts, but fatty liver and atrophic enteritis, likely of starvation.
    - Cardinal presentation of PFTS in a group is the stark contrast between the affected population, usually 3-7%, and the others in the cohort. The rest of the pigs are clinically normal, perform well or even very well, without any indication of an illness that can be related to their affected brethren.
- Structural abnormalities, specifically 'hump-back' pigs, have long been a frustration and a problem the genesis of which has been largely unexplained. Association with vitamin D deficiency has been noted by Drs. Goff and Horst. In their studies on D deficiency and pigs the appearance of hump-back pigs was considered a cardinal sign of D deficiency. (Interestingly, Dr. Duane Ullrey, the reknown comparative nutritionist emeritis, Michigan State, made that observation to Steve in 1988 when they served together at FDA. This is not "news" but the how and why of development are now more clear.) Clinically the incidence increases dramatically following any of a number of insults (PRRS, respiratory diseases, some enteric diseases) that occur at and around weaning. A period of little or no feed intake, depressed appetite and other illness precedes the recognition of these stunted and misshapen pigs. The speculation is that vitamin D deficiency, even over a short time but at a critical developmental phase, may at least in part explain the structural changes and the growth suppression.

- Micro-fractures and weak, soft bone result from abnormal, imbalanced or deficiencies of calcium, phosphorus and vitamin D. These interact and are especially critical in growing pigs as they have a lot of bone to build to support muscle and weight. Recently, with bone samples from weaned pigs, we have found soft bone with fractures. These are called micro-fractures and are more like multiple cracks that are in various stages of healing and repair. Testing for calcium, phosphorus and vitamin D has focused on D as the abnormal and deficient nutrient. We have not found weaned pigs, at this point, that have serum vitamin D levels considered to be in the normal range. The speculation is that the bone condition that is soft and weak results in pain which may have consequences we just aren't able to appreciate in pigs. As we have more and more pigs that have been treated at processing with oral D the clinical observation suggests pain may just be a large part of the reason behind what we think of as "failure to thrive". The pain of rachitic pigs is well known and recognized. But the pain of micro-fractures, soft bone and likely muscle insertion pain are below our recognition level as veterinarians. We just cannot objectively assess low level chronic pain. But watching these pigs that have been treated, it is subjectively apparent that they have different activity level than was observed in untreated pigs.

- Vitamin D is an important component of the immune response as well. What these low levels may be doing regarding general immunity to a host of viral and bacterial pathogens is unknown. It is expected that pigs with normal vitamin D levels would better respond to, for example, challenges from H parasuis, PRRS, and influenza. Clearly, there are methods and mechanisms to objectively assess immune response on down the road should that be of interest.

- Finally, adequate vitamin D is necessary for maximum muscle growth. It is expected that additional weight gain over time, ADG, would increase if vitamin D deficiency was prevented.

Hopefully this background helps to explain and support the logic of the planned addition of oral vitamin D at processing. We are still very early in the process – and don't know where it will lead. But the first goal is to prepare pigs with 'normal' ranges of vitamin D and then observe the results. Material will be coming for use beginning week 20. Then we'll watch and let the pigs tell us, along with the K-State controlled research into growth.

The conclusion, to be validated with research and clinical observation, is that just as is the case with iron, baby pigs must be supplemented with vitamin D soon after birth unless they are reared outdoors.

Experimental product, testing protocol and procedures

The product we are using is Wean-D, produced by Jesse Goff. It is experimental at this point. We are the facilitators for distribution to selected persons. Abilene Animal Hospital is serving as the distribution and accounting facilitator for GlycoMyr and Heartland, the private research companies owned and operated by Drs. Horst and Goff. The product is being made into quarts (960 pig doses at 1ml/pig at processing) and in 4 ½ gal (17,280 pig dose) quantities.

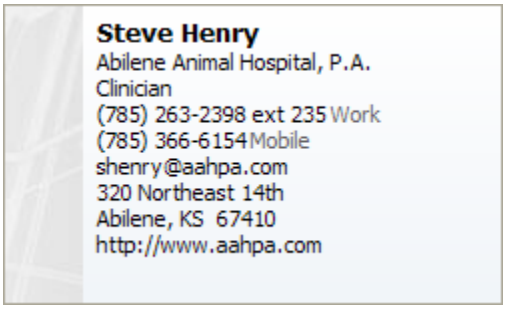
Running baseline values in a herd prior to initiating supplementation with Wean-D is important. Following are details on the sampling protocol:

1) Don't have to sample many to get a profile plan of a herd. Can do 1 pig per litter from 10 litters for example.
2) We have been picking the bigger pigs as they are most likely to be low.
3) Most interested in the levels in pigs near weaning age. The goal of supplementation at processing is to create pigs with normal levels at the time of weaning, 25-30ng/ml of 25(OH)D.
4) Must have clear, non-hemolyzed serum to have valid assay. (So we do about 15 and toss any that aren't pristine and clear, then hold under refrigeration)
5) Send to the address below – cost is $35/sample so good that it doesn't take many to set the profile.

Samples should be submitted to Dr. Ron Horst. Contact information for Ron and Jesse, as well as our group is included below:

Submit clear, chilled serum samples to:

Attn: Dr. Ron Horst
Heartland Assays
2325 N Loop Drive, Suite 6300
Building 6
Ames, IA 50010



Published normal ranges	
Age of animal	25-OH D3 ng/ml
Neonate	5-15
10 days	8-23
3 to 4 weeks	25-30
Finishing pigs	30-35
Mature	35-70
Parturition	35-100

Farm 1	5.1	Pre-weaning 20±3d
Farm 1	7.7	Pre-weaning 20±3d
Farm 1	6.4	Pre-weaning 20±3d
Farm 2	4.8	Pre-weaning 20±3d
Farm 2	8.4	Pre-weaning 20±3d
Farm 2	3.7	Pre-weaning 20±3d
Farm 2	7.9	Pre-weaning 20±3d
Farm 2	5.1	Pre-weaning 20±3d
Farm 3	6.2	Pre-weaning 20±3d
Farm 3	5.8	Pre-weaning 20±3d
Farm 3	4.9	Pre-weaning 20±3d
Farm 3	5.1	Pre-weaning 20±3d
Farm 3	4.8	Pre-weaning 20±3d
Farm 4	7.0	Pre-weaning 20±3d
Farm 4	12.1	Pre-weaning 20±3d
Farm 4	8.6	Pre-weaning 20±3d
Farm 4	10.2	Pre-weaning 20±3d
Farm 4	14.3	Pre-weaning 20±3d
Farm 5	6.2	Pre-weaning 20±3d
Farm 5	5.5	Pre-weaning 20±3d
Farm 5	7.5	Pre-weaning 20±3d
Farm 5	5.9	Pre-weaning 20±3d
Farm 5	8.4	Pre-weaning 20±3d
Farm 5	8.2	Pre-weaning 20±3d
Farm 5	7.3	Pre-weaning 20±3d
Farm 5	4.8	Pre-weaning 20±3d
Farm 5	5.9	Pre-weaning 20±3d
Farm 5	9.6	Pre-weaning 20±3d
Farm 5	5.6	Pre-weaning 20±3d
Farm 5	9.4	Pre-weaning 20±3d
Farm 5	6.9	Pre-weaning 20±3d
Farm 5	6.4	Pre-weaning 20±3d

Farm 5	6.2	Pre-weaning 20±3d
Farm 5	6.5	Pre-weaning 20±3d
Farm 5	7.7	Pre-weaning 20±3d
Farm 5	7.4	Pre-weaning 20±3d
Farm 5	8.8	Pre-weaning 20±3d
Farm 6	4.3	Microfracture pigs, 28doa
Farm 6	6.1	Microfracture pigs, 28doa
Farm 6	5.6	Microfracture pigs, 28doa
Farm 6	5.1	Microfracture pigs, 28doa
Farm 6	3.5	Microfracture pigs, 28doa
Farm 7	7.2	Pre-weaning 20±3d
Farm 7	7.1	Pre-weaning 20±3d
Farm 7	7.2	Pre-weaning 20±3d
Farm 7	5.6	Pre-weaning 20±3d
Farm 7	6.8	Pre-weaning 20±3d
Farm 7	8.7	Pre-weaning 20±3d
Farm 7	5.6	Pre-weaning 20±3d
Farm 7	6.1	Pre-weaning 20±3d
Farm 8	8.4	Pre-weaning 20±3d
Farm 8	3.7	Pre-weaning 20±3d
Farm 8	7.9	Pre-weaning 20±3d
Farm 8	5.1	Pre-weaning 20±3d
Farm 10	5.1	Pre-weaning 20±3d
Farm 10	7.7	Pre-weaning 20±3d
Farm 10	6.4	Pre-weaning 20±3d
Farm 11	4.8	Pre-weaning 20±3d
Farm 11	43.0	Sows 1wk prepartum
Farm 11	36.9	Sows 1wk prepartum
Farm 11	38.8	Sows 1wk prepartum
Farm 11	36.3	Sows 1wk prepartum
Farm 11	36.7	Sows 1wk prepartum
Farm 11	33.9	Sows 1wk prepartum
Farm 11	38.9	Sows 1wk prepartum
Farm 12	40.7	6mo crippled boars
Farm 12	38.3	6mo crippled boars
Farm 12	27.3	6mo crippled boars
Farm 12	38.4	6mo crippled boars