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Journal of Swine Health & Production

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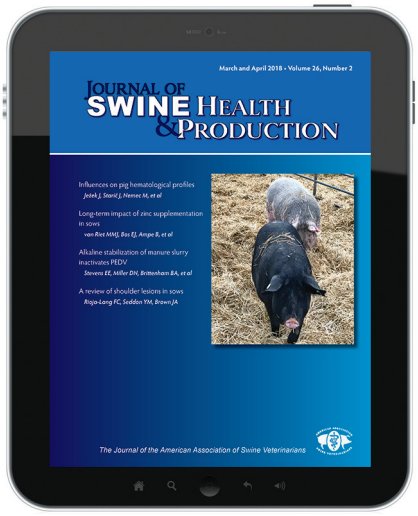

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About the cover…

Iberian-Duroc cross gestating sows in Madrid

Photo courtesy of Dr Antonio Palomo Yagüe

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"Implementation is as fundamental to scientific advancement as the discovery itself."

quoted from the Associate editor's message, page 71

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Thank you!

As my term as AASV president ends, it is a good time to say thank you! We most certainly have an outstanding association and it has been a privilege to serve as its president for the past year.

Often we become involved in activities or leadership roles without fully comprehending their significance until much later. I think all of us at AASV have a high respect and appreciation for all our colleagues who have served in leadership roles in the past – the list is quite long. Their hard work and dedication have helped cement a path that has provided us with the privilege to be recognized as having expertise in helping protect the health and well-being of pigs, as well as the health and well-being of our colleagues. The role of president is not about the office, but rather about the pigs and people we all work with every day.

Swine veterinarians are progressive veterinarians who are always looking forward. Most individuals do not like change, yet progressive individuals are always seeking change, not just for change itself, but rather looking to change to make things better. Our pig farmers have the same attitude and we are proud to work with them every day.

Our association is very lucky to have a highly qualified and dedicated team with Tom, Sue, and Harry. It truly is amazing how easy they make our jobs. They are unselfish and always focused on doing what is best for the association. Just as in any clinic, corporate office, or academic department, the support team is what allows us to be successful. As an organization that is focused on science and having a direct, practical impact in the field, we are quite lucky to have our own journal. The work done by Terri, Karen, Tina, Judi, Sherrie, Laura, Serge, and Zvonimir, along with all the volunteer editorial board, is outstanding. Our webmaster and IT expert, Dave, does amazing work, always behind the scenes. I mention all these names because it is important to remember that without their help and hard work, AASV would not be who we are today. Finally, let us not forget the role you all play in the success of AASV. Our membership is very dedicated and outstanding. All our committees are quite active, and our members are always there to support AASV at any time. It truly is teamwork at all levels that makes our association great!

I have never seen the title of "President" as a title of "power." Instead, it has been a title that has provided me the opportunity to become more involved and serve a great organization. Through this service, one clearly sees that the real power of our association lies in the hands of our membership.

"It truly is teamwork at all levels that makes our association great!"

The mission of AASV is very clear. Our support team and membership continue to do the right things for the right reasons, always focused on the science. Interestingly enough, this mission is the same for all of us involved in the swine industry, regardless of our country of origin or residence. We all have a universal view on one world, one health, and one passion for pigs.

Thank you for the opportunity to serve you!

Alex Ramirez, DVM
AASV President

It is the mission of the American Association of Swine Veterinarians to:

- Increase the knowledge of swine veterinarians
- Protect and promote the health and well-being of pigs
- Advocate science-based approaches to veterinary, industry, and public health issues
- Promote the development and availability of resources that enhance the effectiveness of professional activities
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Swine: Animals intended for human consumption must not be slaughtered within 5 days of receiving a single-injection dose.

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The miracle of life

As swine veterinarians, we are called upon to reduce disease impact and improve the health, well-being, and performance of the pigs in our care. Because the world is not perfect we sometimes have to deal with situations or outcomes that are less than desired. A few times I've felt overwhelmed with the responsibilities we have. It's been helpful for me to draw on my experiences and relationships with family, AASV colleagues, and clients to help work through these difficult situations.

On our home farm, one of my responsibilities in high school was to check the sows in the farrowing house in the evenings. It was a very rewarding experience for me. Night-time at the farm was usually a very quiet time. I would top off feeders in the rooms with sows and older litters. When looking at the sow cards, I was amazed to realize that 90% of the sows had the same previous farrowing date and many had the same farrowing date before that! It was intriguing to me that the sows in this room spent most of their time in close proximity to the same sows. My dad later helped me to understand the impact of maximizing lactation feed intake on wean to service interval and subsequent litter size. He also helped me to understand the consequence of over-feeding when I had to scoop out feeders with wet, spoiled feed after I got carried away. I learned early on that more is not always better.

In the rooms with peri-parturient sows, I would usually leave the lights off so as not to disturb the sows. The amber glow of the suspended heat lamps over the creep areas provided adequate lighting to evaluate each farrowing stall. Most times the sows were fine. It was not unusual to only need to remove afterbirth from farrowing stalls where sows had finished the farrowing process. Occasionally, there would be one or two sows that needed assistance. Sometimes it would just be a breech pig that needed help passing through the birth canal. Other times it could be a combination of a heavier conditioned sow and large birthweight piglets or an older parity sow experiencing uterine inertia and had stopped contracting.

"As swine veterinarians, we have the opportunity to experience this miracle every day."

From my perspective, there are not many things more rewarding than helping a newborn animal into the world. The transition from fetus in the uterus to newborn vigorously nursing colostrum within a few minutes is remarkable. While many sows fare just fine without human assistance, there is no doubt that others benefit from proper oversight and intervention when needed. As swine veterinarians, we have the opportunity to experience this miracle every day. It's easy to take it for granted. Not everyone has this opportunity. Some experience it when their pets give birth, others when they have children. Take some time the next time you are watching a sow farrow to truly reflect on your calling and purpose as a swine veterinarian. I guarantee it will reinvigorate you!

C. Scanlon Daniels, DVM
AASV President-elect

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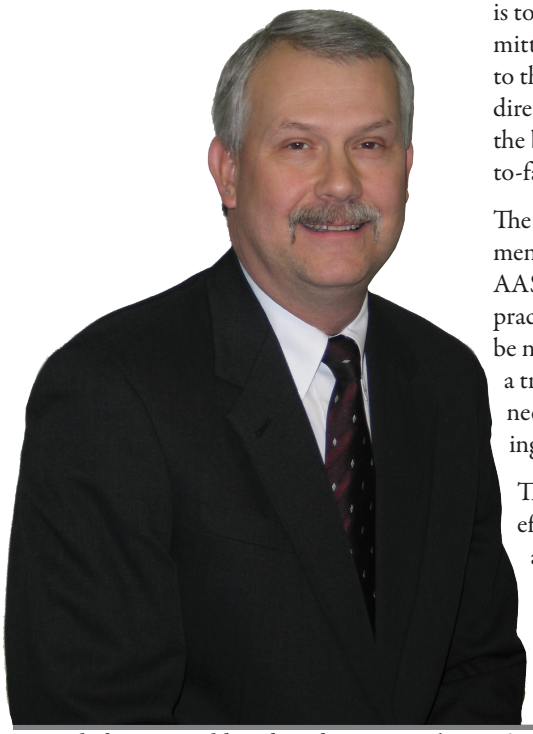
The essentials

The year 2019 will mark 50 years of existence for the AASV as an organization. I have been told by several of our more "experienced" members that they were impressed by how much they benefited from membership in AASP. It was a common comment that they could take knowledge from the Annual Meeting or from the newsletter, later the JSHAP, and put it to use in daily practice. Their conclusion was that this knowledge created value far beyond the cost of membership and became essential to their success as a swine veterinarian.

I would like you to consider what you find essential in your day-to-day practice of swine veterinary medicine. There are probably some common themes surrounding this question. Essentials like diagnostics, disease prevention and treatments, biosecurity, epidemiology, production management, and emerging diseases. Some of what you deem essential may be dependent on the challenges you are currently facing on your clients' farms. Some of these challenges may be quite acute, while others may be more chronic and reoccurring. Other essentials may be related to your own interests. I find that many of our members have inquisitive minds and insatiable curiosities. They value knowledge simply for the sake of knowledge.



As you consider your list of essentials, also think about the role that AASV plays in your daily practice. AASV's mission is to increase the knowledge of swine veterinarians. If we are to remain true to this mission, then we as an organization must strive to have a positive impact on your daily practice. We must have the correct member benefits in place and active. The AASV currently relies on the annual meeting, the digital e-Letter, the *Journal of Swine Health and Production*, the web site, and the AASV office and staff to fulfill the mission.

I would be remiss if I did not ask you to consider how we might strengthen and improve the daily impact of AASV. Member input and participation are essential elements for an organization such as the AASV. One of the greatest fears of an organization is for members to begin to consider the organization to be irrelevant. If that were to happen, then the organization would be short-lived. There are multiple ways for providing input to AASV. You can reach out to any AASV officer, district director, committee chair, or staff member. Another way to provide input is to become a member of an AASV committee. These committees report directly to the board of directors as well as submit direct requests for action and funding from the board. The committee chairs have a face-to-face meeting with the board every fall.

The other area for consideration is the assessment of the future needs and wants of the AASV members. The essentials for swine practice today may not include what will be needed for the future. The time to plant a tree is 15 years before its shade will be needed. The same truth applies to developing future membership benefits today.

The AASV started developing digital benefits in the late 1990's. The AASV web site and e-Letter were started with the idea that their greatest utility was most likely to be better appreciated in the future rather than immediately. This came to be true as the digital world became an essential part of daily activities rather than just a tool for email. The explosion of the use of the Internet, computers, smart phones and tablets gave substance to the predicted need. I give credit to members like Morgan Morrow who had a vision for the future and moved AASV to prepare benefits to meet the needs of swine veterinarians.

> *" With member input, the AASV can continue to be an essential resource for swine veterinarians."*

Going forward, member benefits such as the annual meeting, the *Journal of Swine Health and Production*, and the e-Letter can lead the way to new benefits and technology. Current membership benefits can provide a launching pad for the development of future benefits. However, holding too tightly to a current benefit can also be an anchor holding the AASV in place rather than moving it forward. Balancing between the two can be a challenge. Knowing when and how to move forward is vital to the long-term success of any organization. With member input, the AASV can continue to be an essential resource for swine veterinarians.

Keeping the AASV relevant for swine veterinarians was the challenge for the last 50 years. It remains the challenge for the next 50 years. Providing knowledge that is essential for our members in their daily practice will ensure their success which will in turn ensure the success of the AASV.

Tom Burkgren, DVM
Executive Director

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References: 1. BIAH studies #2013200, 2013232, and 2014001 published on http://productdata.aphis.usda.gov. **2.** Genzow M, Goodell C, Kaiser TJ, Johnson W, Eichmeyer M. Live-attenuated influenza virus vaccine reduces virus shedding of newborn piglets in the presence of maternal antibody. *Influenza Other Respir Viruses*. In review [submission IRV-2017-172]. **3.** Alvarez J, Sarradell J, Kerkaert B, et al. Association of the presence of influenza A virus and porcine reproductive and respiratory syndrome virus in sow farms with post-weaning mortality. *Prev Vet Med*. 2015;121:240–245. **4.** Vincent AL, Ma W, Lager KM, et al. Efficacy of intranasal administration of a truncated NS1 modified live influenza virus vaccine in swine. *Vaccine*. 2007;25:7999–8009. **5.** Janke BH. Influenza A virus infections in swine: pathogenesis and diagnosis. *Vet Pathol*. 2014;51:410–426.

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In pursuit of why

I am very excited to join the *Journal of Swine Health and Production* editorial team. For me, this new career chapter provides an opportunity to continue my passion for promoting scientific research and the pursuit of knowledge.

My graduate career started at the University of Illinois where, like most animal science majors, I was planning to enter the veterinary profession. However, I quickly changed course when I caught the research "bug." What began as an undergraduate project in a stress physiology lab gradually morphed into a Master's of Science degree. My area of focus was exploring how animals perceive various factors in their environment and the impact those perceptions have on their physiology and performance, behavior, and affective states. It was a bonus that I worked primarily with pigs.

I have spent the last 12 years working with US pig farmers to identify on-farm issues that impact them and the well-being of their pigs and building relationships among the academic, veterinary, and farmer communities to work towards solutions. This included using Checkoff dollars to fund research and utilizing outcomes to develop science-based educational materials promoting best practices for pig care and well-being.

On a personal note, my toddler is almost 2 and I often say he is the coolest science experiment I have had the pleasure to be a part of. It is fascinating to watch his physical and mental development; he is the ultimate scientist. "What happens if I grab the cat's tail? What will dad do if I throw food on the floor?" While he's not quite to the "why" stage, I'm really looking forward to it. I see it as an opportunity to look at everyday objects and routines through a new lens and have longstanding ideals tested. It's also an opportunity to find new ways to deliver complex information to a unique audience. He is only 2 after all.

I firmly believe that knowledge is power. Knowledge helps us make better decisions, be better people and ultimately become a better society. As scientists and veterinarians, we endeavor to answer the why's and how's that continue to challenge pig health and production. We continue to challenge the status quo in search of improvement.

"I have spent the last 12 years working with US pig farmers to identify on-farm issues that impact them and the well-being of their pigs and building relationships among the academic, veterinary, and farmer communities to work towards solutions."

As scientists, we must remember that the scientific method does not end with data collection and analysis. We must interpret and determine the implications of the data generated. Submitting our data, interpretations and theories to peer review with the intent to publish the work helps to strengthen, enhance, and expand the scientific body of knowledge. Science is a social process, in that peer-review, publication, and replication must occur before information tends to be accepted and implemented by the greater scientific community. Implementation is as fundamental to scientific advancement as the discovery itself.

I look forward to seeing the latest and greatest scientific submissions to the journal and working with authors to refine their work for publication. I also look forward to continuing in the footsteps of my predecessor, Dr Judi Bell. For the past 17 years, she has worked to make JSHAP a high-quality publication allowing readers to glean important information, implement findings on-farm, and generate the next new hypothesis.

Sherrie Webb, MSc
Associate Editor

Original research

Peer reviewed

The influence of age, farm, and physiological status on pig hematological profiles

Jožica Ježek, DVM, PhD; Jože Starič, DVM, PhD; Marija Nemec, DVM, MS; Jan Plut, DVM; Irena Golinar Oven, DVM, PhD; Martina Klinkon, DVM, PhD; Marina Štukelj, DVM, PhD

Summary

Objectives: To evaluate influence of age, farm, and physiological status on pig hematological profiles.

Materials and methods: This study was carried out on five 1-site, farrow-to-finish pig farms in Slovenia, where a total of 382 clinically normal pigs were sampled. All farms were free of Aujeszky's disease (pseudorabies), classical swine fever, and porcine reproductive and respiratory syndrome. Blood samples were taken from the anterior vena cava. Hematological analyses were performed with an automated hematological analyser. The following hematological variables were measured: red blood cell count (RBC), white blood cell count (WBC), hematocrit (Hct), hemoglobin concentration (Hb), erythrocyte indices, and platelet count (PLT). Differential WBC counts were determined manually using stained smears.

Results: The farms themselves influenced all of the investigated variables except RBC and WBC differential (ie, lymphocytes, monocytes, and band neutrophils). A trend of lower values of RBC, Hb, and Hct, higher WBC numbers, and a higher percentage of segmented granulocytes were observed in lactating sows when compared to pregnant sows. Age significantly influenced hematological values and differential WBC counts except basophils, monocytes, and band neutrophils. Values of mean corpuscular volume (MCV) increased with age, the highest values being found in sows. Numbers of WBC and PLT decreased with age, the lowest number being observed in sows.

Implications: Hematological examination may be an important diagnostic tool in the assessment of pig health status, but to interpret the results properly, it is important to consider pig age, health history, and clinical data.

Keywords: swine, pig, hematology, pig health status

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Accepted: October 20, 2017

Resumen – La influencia de la edad, granja, y estado fisiológico en los perfiles hematológicos del cerdo

Objetivos: Evaluar la influencia de la edad, granja, y estado fisiológico en el perfil hematológico del cerdo.

Materiales y métodos: Este estudio fue realizado en cinco granjas de un sitio, nacimiento a venta, en Eslovenia, donde se tomaron 382 muestras totales de cerdos clínicamente normales. Todas las granjas estaban libres de la enfermedad de Auyesky (pseudorabia), fiebre porcina clásica, y síndrome reproductivo y respiratorio porcino. Las muestras de sangre se tomaron de la vena cava anterior. Se realizaron análisis hematológicos con un analizador hematológico automatizado. Se midieron las siguientes variables hematológicas: conteo de glóbulos rojos (RBC por sus siglas en inglés), conteo de glóbulos blancos (WBC por sus siglas en inglés), hematocrito (Hct por sus siglas en inglés), concentración de hemoglobina (Hb por sus siglas en inglés) índices de eritrocitos, y conteo de plaquetas (PLT por sus siglas en inglés). Los conteos diferenciales de WBC se determinaron manualmente utilizando laminillas teñidas.

Resultados: Las granjas mismas influenciaron todas las variables investigadas excepto el diferencial de RBC y WBC (ie, linfocitos, monocitos, y neutrófilos en banda). Se observó una tendencia de valores inferiores de RBC, Hb, Hct, con números más altos de WBC, y un porcentaje más alto de granulocitos segmentados en hembras lactantes al compararlos con los de hembras gestantes. La edad influyó significativamente los valores hematológicos y los conteos diferenciales de WBC excepto basófilos, monocitos y neutrófilos en banda. Los valores de volumen corpuscular medio (MCV) aumentaron con la edad, encontrándose los valores más altos en hembras. Los números de WBC y PLT disminuyeron con la edad, observándose el número más bajo en hembras.

Implicaciones: El examen hematológico puede ser una herramienta de diagnóstico importante en la valoración del estado de salud del cerdo, pero para interpretar los resultados correctamente, es importante considerar la edad del cerdo, la historia de salud y los datos clínicos.

Résumé – Influence de l'âge, de la ferme d'élevage, et du statut physiologique sur les profils hématologiques du porc

Objectifs: Évaluer l'influence de l'âge, de la ferme d'élevage, et du statut physiologique sur les profils hématologiques du porc.

Matériels et méthodes: Cette étude a été menée sur cinq sites uniques de fermes porcines de type naisseur-finisseur en Slovénie, où un total de 382 porcs cliniquement normaux ont été échantillonnés. Toutes les fermes étaient exemptes de pseudorage, de peste porcine classique, et du syndrome reproducteur et respiratoire porcin. Des échantillons sanguins ont été pris dans la veine cave antérieure. Les analyses hématologiques ont été réalisées à l'aide d'un analyseur hématologique automatisé. Les

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variables hématologiques suivantes ont été mesurées: comptage des globules rouges (GR), comptage des globules blancs (GB), hématocrite (Hct), concentration en hémoglobine (Hb), indices érythrocytaires, et comptage des plaquettes (PLT). Des dénombrements différentiels des GB ont été réalisés manuellement sur des frottis colorés.

Résultats: Les fermes elles-mêmes influençaient toutes les variables étudiées sauf les GR et les différentiels des GB (ie, lymphocytes, monocytes et neutrophiles immatures). Une tendance à des valeurs inférieures pour les GR, Hb, et Hct, et un nombre plus élevé de GB, et un pourcentage plus grand de granulocytes segmentés ont été observés chez les truies en lactation comparativement aux truies gestantes. L'âge influençait de manière significative les valeurs hématologiques et les compte différentiels de GB sauf pour les basophiles, les monocytes et neutrophiles immatures. Les valeurs du volume corpusculaire moyen (VCM) augmentaient avec l'âge, les valeurs les plus élevées étant retrouvées chez les truies. Le nombre de GB et de PLT diminuait avec l'âge, les valeurs les plus basses étant observées chez les truies.

Implications: L'examen hématologique peut être un outil diagnostique important dans l'évaluation de l'état de santé des porcs, mais afin d'interpréter les résultats de manière adéquate, il est important de considérer l'âge des porcs, l'historique de santé, et les données cliniques.

Despite the common availability of hematological tests in veterinary medicine, they are still rarely performed in the routine evaluation of pig health status. Low individual animal intrinsic value, blood sample collection difficulty, different husbandry techniques, large variations between hematologic values in a given population, and the reality that reference value ranges are relatively wide are the main reasons for the low utility of swine hematology.[1] Measuring animal hematological parameters can provide important information on animal health and is a practical tool for assessing pathological conditions in individual animals and for monitoring the health status of groups of animals. Additionally, hematological values reflect the response of the animal to its environment and may reveal adverse conditions, even though animals may not be displaying clinical signs of disease.[2]

A variety of factors should to be taken into account when evaluating individual animal results, collating data, and drawing whole herd or population conclusions. The interpretation of hematological data is often limited pursuant to the broad animal-to-animal variations occurring in normal populations. Friendship et al[3] placed emphasis on the division of animals in different production groups, as some parameters vary greatly, even on a daily basis. Furthermore, gender, breed, and stage of gestation are factors influencing variability of blood values. However, very few recent studies have investigated the influence of age,[4,5] reproductive status (gestating versus lactating sows),[6-8] or individual farm effects[9] in relation to pig hematological parameters.

Diet and disease are important external influencers of certain hematological values. An increase in tannic acid concentration (125 mg to 1000 mg per kg) in the diet of weanling pigs linearly reduced total red blood cell count (RBC), hemoglobin (Hb), and hematocrit (Hct) on days 21 and 28 of treatment.[10] The long-term dietary use of clinoptiolite-rich tuff at the inclusion rate of 2% was not associated with adverse effects on growing and finishing pigs' overall condition and health status or significant changes of their hematological profiles.[11] Infectious agent health challenge often results in changes in white blood cell count (WBC), as well as differences in RBC and plasma parameters depending on temporal occurrence, severity of clinical signs, and magnitude of immune response. Increased sedimentation rate, as well as decreased Hb and Hct values, are most commonly present[1,12] with disease challenges. Anemia is a cardinal clinical sign of *Mycoplasma suis* infection in pigs.[13] *Mycoplasma suis* blood loads had significant negative correlation with RBC, Hct, and Hb.[14] Hematological alterations appear in bacterial septic response and viral diseases. Leukocytosis, a reflection of the initial inflammatory response primarily mediated by neutrophils, was evident 12 hours after induction of septic injury of the abdomen in female pigs (27 to 37 kg), and was followed by a steady decrease.[15] The number and percentage of neutrophils were both twofold higher in septic pigs 24 hours after *Escherichia coli* injection than their own basal values on day 0.[16] Porcine reproductive and respiratory syndrome virus (PRRSV)-positive young pigs (age 28 to 160 days) had significantly lower WBC than PRRSV-negative pigs.[17] Unthrifty nursery pigs had higher Hb concentrations and Hct values when compared to those of healthy pigs, indicating dehydration and (or) malnourishment in unthrifty pigs.[18] In pigs aged 22 to 26 days fasted for 72 hours, Hct values started to increase at the onset of the fasting period and continued to increase (33.5% to 40.1%) throughout the fasting period.[19] In diseased pigs, appetite and water intake are often decreased, and with diarrhea they lose more fluid, which may contribute to hemoconcentration.

The aim of this study was to analyze the blood samples of pigs from five small one-site farms to evaluate influence of age, farm, and physiological status (pregnant versus lactating sows) on the hematological profiles to acquire orientational reference values for different categories of pigs to then serve as an additional diagnostic tool in the assessment of pig health status.

Materials and methods

Farms and animals

The blood samples were taken from pigs on five small, one-site farms included in a serological study of selected pathogens. All procedures complied with the relevant Slovenian legislation (Animal Protection Act, Official Gazette of the republic of Slovenia, No 43/2007).

The study involved five small one-site farrow-to-finish pig farms in Slovenia between January 2014 and September 2014. All farms were free of pseudorabies, classical swine fever, and porcine reproductive and respiratory syndrome (PRRS). Age groups were established (Table 1) and animals from each farm were sampled. A total of 382 blood samples were taken. All pigs included in the study were clinically healthy at the time of blood sampling.

Previous treatment

The pigs from all five farms were vaccinated against *Mycoplasma hyopneumoniae.* Additionally, sows from Farm 1 were vaccinated against atrophic rhinitis, weaners from Farm 2 were vaccinated against porcine circovirus 2 (PCV2), sows from Farm 4 were vaccinated against *E coli*, and sows and weaners from Farm 5 were vaccinated against *E coli* and PCV2, respectively. Treatments for endo- and ectoparasites were performed on all sows on the 100th day of pregnancy, as well as on all growing pigs at approximately 25 to 30 kg of body weight. Group samples of feces were collected on each farm and examined for internal parasites using flotation and sedimentation

methods; low, clinically insignificant numbers of the protozoan *Balantidium coli* were found in all five group samples of feces.

Nutrition

Each of the five farm owners provided information about the feeding regimes on their farm, including feeding intervals, composition of category-dependent diets, and feed consumption. Breeding animals were fed twice daily, between 6 and 7 AM and 4 and 7 PM in the afternoon-evening, and growers and finishers were fed ad libitum. Diets were composed of corn, barley, wheat, and soybeans, supplemented with complementary feed and mineral and vitamin mixtures according to NRC[20] category recommendations. No other additives (eg, therapeutics or nostrums) were added to the feed on any of the five farms.

Blood sample collection

All blood samples were collected from the anterior vena cava; animals were restrained using wire-noose snares. Blood samples for hematological analysis were collected in evacuated blood collection tubes containing the anticoagulant K_3EDTA; the tubes were gently vibrated by hand for 30 seconds to assure the proper contact and mixing of the blood and anticoagulant. Blood samples were collected at 9 AM on all farms, approximately 2 hours after the morning feeding. One blood sample was obtained from every animal in this study.

Hematological analysis

All hematological analyses were performed on the day of sampling, utilising the Scil Vet abc Plus (Horiba, Japan) automated hematological analyzer. The following hematological variables were measured: RBC, WBC, Hct, Hb, and erythrocyte indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC]), and platelet count (PLT). Differential WBC was determined according to the standard procedure: smears stained with Hemacolor (Merck, Darmstadt, Germany) and manually counted via microscopic examination. The laboratory where the analyses were performed participates in the Randox International Quality Assessment Scheme (RIQAS) hematology program.

Table 1: Number of blood samples taken per age group from each of five 1-site, farrow-to-finish Slovenia farms

Age group/farm	Sows	Boars	Growers (age 7-14 weeks)	Finishers	Total/farm
1	42	1	10	7	**60**
2	39	1	15	5	**60**
3	45	3	10	9	**67**
4	61	1	20	5	**87**
5	92	1	10	5	**108**
Total/age group	**279**	**7**	**65**	**31**	**382**

Statistical analysis

Statistical analysis of hematological data was performed using the SPSS software package (SPSS 22.0 for Windows, Chicago, Illinois). For hematologic variables, descriptive statistics were calculated with regard to farm, physiological status, and age. Data were checked for normality, and reference intervals were calculated in accordance with Farver.[21] Reference values are presented as mean (median) values, and the range between the 2.5th and 97.5th percentiles. Ninety-five percent confidence intervals were not used because individual pig data were not normally distributed. Physiological state and farm effects were assessed using the GLM variance analysis procedure, with physiological state and farm being fixed factors, in accordance with the following model:

$$Y_{ij} = \mu + F_i + S_j + e_{ij}$$

where F means effect of herd (herd 1/.../herd 5) and S means physiological state (pregnant or lactating).

The effect of age was assessed using GLM variance analysis procedure in a separate model, which only includes the influence of age on hematological variable values,

$$Y_i = \mu + V_i + e_i$$

where V means effect of age (breeding sows or finishers or growers [11 to 14 weeks] or weaners [7 to 10 weeks]).

The level of significance was set at $P < .05$.

Results

Table 2 presents sow hematological values from all farms and a comparison with reference values from the existing literature.

Figure 1 presents hematological values for pregnant (n = 224) and lactating (n = 48) sows. Lower RBC, Hb, and Hct values were observed in lactating sows when compared to pregnant sows. A higher number of WBC and a higher percentage of segmented granulocytes were found in lactating sows.

The results of hematological variables for sows at the herd level are presented in Table 3. Herd significantly influenced Hb, Hct, MCV, MCH, MCHC, PLT, and differential WBC values except lymphocytes, monocytes, and band neutrophils.

The hematological values and reference intervals of growers and finishers are presented in Table 4. Age significantly influenced values of RBC ($P < .001$), WBC ($P < .001$), Hb ($P < .001$), Hct ($P < .001$), MCV ($P < .001$), MCH ($P < .001$), MCHC ($P < .001$), PLT ($P < .001$) and WBC differential, except basophils, monocytes, and band neutrophils.

Significantly lower RBC numbers were observed in sows than in young pigs. The highest values of Hct were found in finishers compared to other age groups, and the highest values of MCV were found in sows. Numbers of WBCs and PLTs decreased with age, the lowest numbers being observed in sows. A significantly higher percentage of eosinophils was found in sows than in younger pigs.

Discussion

Hematological reference values for sows were established in this study. When comparing with the reference values from the literature,[1,22,23] clinically relevant differences were found for the hematological parameters such as RBC, Hb, and Hct. These values may have differed because published reference values in the literature are not specified by age, or perhaps in part because laboratory methods differed.

Physiological status influenced the values of hematological parameters in our study. The lower values of Hb and Hct established in lactating sows may be related to physiological appearance.[1] A higher number of WBCs and a higher percentage of neutrophils were observed in lactating sows than in pregnant sows, consistent with Elbers et al,[7] who reported a higher leukocyte count, mostly accounted for by a higher segmented neutrophil count, in the blood samples of sows obtained at weaning when compared with those obtained between 4 and 5 weeks of gestation. During lactation, a physiologic leukocytosis caused by lymphocytosis with or without neutrophilia may occur.[24] Increased WBC can be associated with inflammatory response to uterine involution or responses to infections, such as uterine infection. Interpretation of results of lactating sows should take into account that the values of RBC, Hb, and Hct may be slightly lower and WBC values slightly higher than in the reference intervals for sows.

Farm was a significant source of variation in our statistical model for most of the investigated hematological parameters. The influence of farm on biochemical parameter values was evident in a study performed on eight farms in the Netherlands and Belgium.[25] No publication about influence of farm on hematological profiles in sows were found. Farm also significantly influenced the blood profiles of pigs from 40 finishing herds in the Netherlands.[9] The authors suspect that differences in health status between farms could account for some of the variance in the values of blood parameters, and that the magnitude of between-herd variation in some of the blood parameters can be a useful parameter for herd-health control, as it may reflect herd-health status.[9] For example, PRRSV-positive pigs had significantly lower values of WBC in comparison to PRRSV-negative pigs.[17] Nutrition may also contribute to some differences in hematological profile.[8] Alterations in hematological profile found in a single herd may indicate subclinical disease or other disturbances. Monitoring the levels in individual animals compared to other herd data and clinical examination of suspicious animals with deviating values may facilitate the early detection of disease or conditions that may be subclinical.

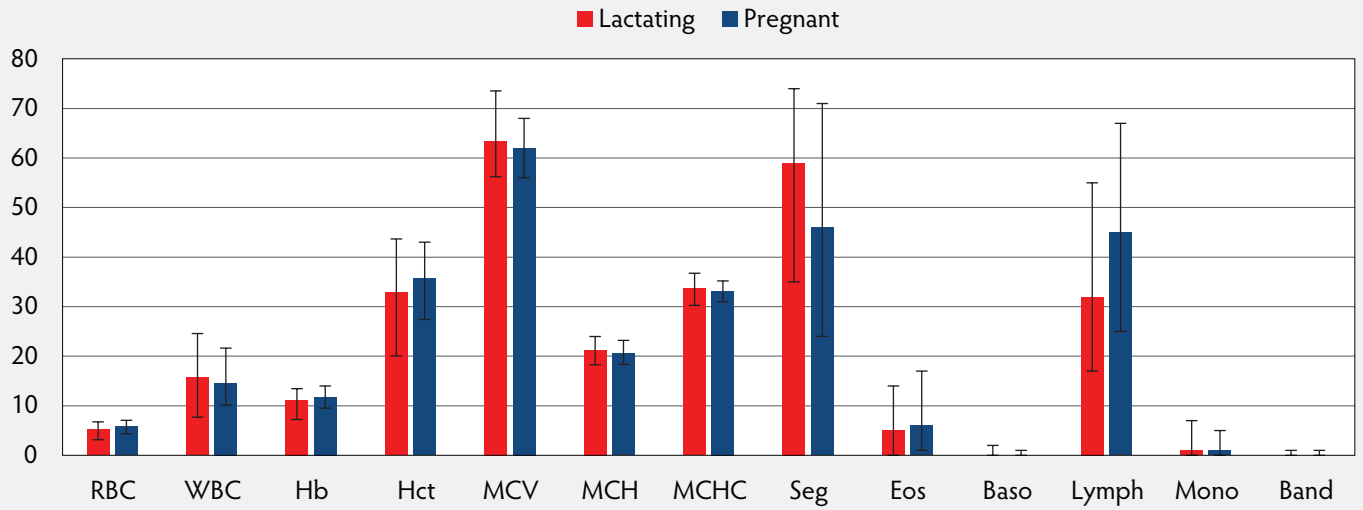
Age significantly influenced most hematology variables in our study, which is in accordance with the findings of other studies[4,26]

Table 2: Hematological values for sows (n = 272) from five Slovenian farrow-to-finish pig farms and a comparison with reference values from the existing literature

Parameter (unit)	Mean (median)	Range*	Reference values
RBC (10^{12}/L)	5.62 (5.63)	4.3-7.0	5.8-8.1[22] 5.0-8.0[1,23]
WBC (10^9/L)	15.21 (14.90)	9.78-22.67	10.0-20.0[23] 10-22[22] 11.0-22.0[1]
Hb (g/dL)	11.6 (11.5)	9.3-13.8	10.0-15.5[23] 10.8-14.8[22] 10.0-16.0[1]
Hct (%)	34.9 (34.6)	27.1-42.9	32-47[23] 33-45[22] 32-50[1]
MCV (fL)	62.2 (62.0)	56.0-68.0	50-68[1,23] 50-65[22]
MCH (pg)	20.6 (20.6)	18.4-23.2	17-21[1,22,23]
MCHC (g/dL)	33.2 (33.2)	31.0-35.4	30-34[1,23] 30-35[22]
PLT (10^9/L)	274 (273)	134-429	250-600[23] 220-620[22] 325-715[1]
Seg (%)	48 (48)	25-72	28-52[23] 10-39[22] 28-47[1]
Eos (%)	7 (6)	1-17	0.5-11[1] 1-8[23] 0-6[22]
Baso (%)	0 (0)	0-2	0-2[1,22,23]
Lymph (%)	43 (43)	23-66	40-64[23] 49-85[22] 39-62[1]
Mono (%)	1 (1)	0-5	2-8[23] 0-5[22] 2-10[1]
Band (%)	0 (0)	0-1	0-4[1,23] 0-7[22]

* 2.5^{th} to 97.5^{th} inter-percentile range.
RBC = red blood cell count; WBC = white blood cell count; Hb = hemoglobin; Hct = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelets; Seg = segmented neutrophils; Eos = eosinophils; Baso = basophils; Lymph = lymphocytes; Mono = monocytes; Band = band neutrophils

Figure 1: Hematological values of sows from five 1-site, farrow-to-finish pig farms in Slovenia are presented (median, range) in terms of physiological status. RBC = red blood cell count; WBC = white blood cell count; Hb = hemoglobin; Hct = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelets; Seg = segmented neutrophils; Eos = eosinophils; Baso = basophils; Lymph = lymphocytes; Mono = monocytes; Band = band neutrophils.



and is related to physiological changes.[1,24] Reference intervals for growers and finishers were calculated because differences in values of some parameters (RBC, Hb, Hct, MCV, PLT) between these age groups seem to be clinically relevant. Reference intervals for growers and finishers differ from reference intervals for sows, and this should be considered when interpreting the results.

Implications

The hematological data in this study originates from clinically healthy pigs on small commercial farms. The data suggest the following:

- Farm and physiologic state significantly influence the majority of hematological variables;
- Age-related changes in hematological values occur;
- Sources of normal variation must be considered to allow for an appropriate interpretation of the values;
- Reference values serve as a guideline for interpreting the results from individual pigs and can also be used to assess health status in herds when the values of groups of pigs are considered;
- Herd anamnestic and clinical data should be considered when making interpretations.

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Conflict of interest

None reported.

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Table 3: Sow hematological values in terms of herd*

Parameter	Farm 1 N = 39 Mean (median) Range	Farm 2 N = 39 Mean (median) Range	Farm 3 N = 45 Mean (median) Range	Farm 4 N = 61 Mean (median) Range	Farm 5 N = 88 Mean (median) Range	*p*†
RBC (10^{12}/L)	5.76 (5.78) 4.49-7.15	5.93 (5.92) 4.39-6.99	5.40 (5.37) 3.20-6.62	5.56 (5.60) 3.52-7.36	5.60 (5.65) 3.74-7.03	.102
WBC (10^9/L)	16.29 (16.24) 8.90-24.50	16.16 (15.80) 11.20-24.86	14.77 (14.10) 8.23-26.77	14.82 (14.20) 8.66-23.34	14.80 (14.75) 9.72-19.63	.436
Hb (g/dL)	12.2 (12.5) 9.5-14.6	11.9 (12.0) 9.7-14.2	11.0 (10.8) 6.9-13.7	11.5 (11.4) 7.8-13.6	11.5 (11.6) 7.7-14.0	.003
Hct (%)	36.6 (36.3) 27.8-44.1	37.9 (37.8) 30.0-44.8	33.2 (32.9) 20.0-42.3	34.0 (33.8) 22.5-41.4	34.4 (34.3) 21.9-42.3	< .001
MCV (fL)	63.6 (63.0) 58.0-71.9	64.0 (63.0) 59.0-73.8	61.6 (62.0) 55.1-67.8	61.5 (62.0) 54.5-66.4	61.7 (61.0) 56.0-68.8	< .001
MCH (pg)	21.2 (21.1) 19.2-23.9	20.2 (20.0) 18.4-23.0	20.4 (20.4) 18.1-23.2	20.8 (20.8) 18.2-23.2	20.7 (20.7) 18.3-23.3	.012
MCHC (g/dL)	33.3 (33.2) 32.0-34.9	31.5 (31.5) 30.1-32.8	33.1 (33.0) 32.0-34.7	33.8 (33.6) 32.3-36.7	33.7 (33.5) 32.3-36.1	< .001
PLT (10^9/L)	208 (210) 80-343	284 (294) 149-383	310 (316) 103-459	281 (282) 160-424	276 (268) 157-466	< .001
Seg (%)	58 (59) 31-81	45 (46) 29-72	45 (44) 19-68	48 (49) 21-71	47 (46) 23-71	.014
Eos (%)	5 (5) 0-10	7 (6) 1-18	9 (9) 3-22	7 (6) 0-17	6 (6) 1-14	.005
Baso (%)	0 (0) 0-1	0 (0) 0-1	0 (0) 0-1	0 (0) 0-3	0 (0) 0-1	.020
Lymph (%)	35 (35) 16-66	45 (48) 25-62	44 (44) 26-64	43 (44) 21-63	45 (45) 22-70	.062
Mono (%)	1 (1) 0-7	2 (2) 0-6	2 (1) 0-6	1 (1) 0-4	1 (1) 0-5	.065
Band (%)	0 (0) 0-1	0 (0) 0-1	0 (0) 0-1	0 (0) 0	0 (0) 0-1	.370

* 2.5^{th} to 97.5^{th} inter-percentile range.
† Data were analyzed by general linear model (GLM) analysis of variance.
RBC = red blood cell count; WBC = white blood cell count; Hb = hemoglobin; Hct = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelets; Seg = segmented neutrophils; Eos = eosinophils; Baso = basophils; Lymph = lymphocytes; Mono = monocytes; Band = band neutrophils.

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Table 4: Hematological values of growers (7-14 weeks) (n = 54), and finishers (n = 31) from five Slovenian farrow-to-finish pig farms

Parameter	Age	Mean (median)	Range*
RBC (10^{12}/L)	Growers	6.43 (6.46)	5.40-7.28
	Finishers	6.92 (6.89)	5.74-8.63
WBC (10^9/L)	Growers	22.44 (22.60)	13.70-34.12
	Finishers	20.97 (20.30)	14.10-32.10
Hb (g/dL)	Growers	10.9 (11.0)	9.2-12.5
	Finishers	12.6 (12.6)	11.1-14.4
Hct (%)	Growers	35.3 (35.2)	28.0-41.7
	Finishers	39.9 (39.3)	34.1-48.7
MCV (fL)	Growers	54.9 (55.0)	47.7-63.0
	Finishers	57.8 (58.0)	50.0-64.8
MCH (pg)	Growers	17.1 (17.3)	14.0-18.5
	Finishers	18.4 (18.5)	16.1-20.9
MCHC (g/dL)	Growers	31.1 (31.1)	28.8-33.5
	Finishers	31.7 (31.8)	29.2-33.7
PLT (10^9/L)	Growers	483 (486)	273-730
	Finishers	336 (329)	134-584
Seg (%)	Growers	48 (48)	30-71
	Finishers	41 (41)	15-67
Eos (%)	Growers	2 (1)	0-9
	Finishers	5 (4)	0-22
Baso (%)	Growers	0 (0)	0-2
	Finishers	0 (0)	0-1
Lymph (%)	Growers	49 (47)	27-69
	Finishers	52 (55)	27-77
Mono (%)	Growers	1 (1)	0-7
	Finishers	1 (0)	0-4
Band (%)	Growers	0 (0)	0-1
	Finishers	0 (0)	0-0

* 2.5th to 97.5th inter-percentile range.

RBC = red blood cell count; WBC = white blood cell count; Hb = hemoglobin; Hct = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelets; Seg = segmented neutrophils; Eos = eosinophils; Baso = basophils; Lymph = lymphocytes; Mono = monocytes; Band = band neutrophils.

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Original research

Long-term impact of zinc supplementation in sows: Impact on zinc status biomarkers and performance

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Summary

Objectives: To evaluate the long-term impact of zinc (Zn) supplementation on performance and Zn status biomarkers in sows and on whether this possible impact depends on housing conditions.

Materials and methods: Six groups of sows were allotted to group housing on two different floor types during gestation. Within each group, sows were randomly allocated to one of three diets varying in the amount of Zn supplemented (0, 50, or 100 mg added Zn per kg diet; 50% ZnO:50% organic Zn) to a basal diet containing 46.6 and 128.9 mg Zn per kg during gestation and lactation, respectively. Blood was collected at days 0, 50, 108, and 143 of every cycle and analyzed for plasma Zn and copper and serum metallothionein (MT) concentrations. After slaughter, mineral concentrations of metacarpals, liver, and abaxial horn wall were determined.

Results: Dietary Zn supplementation beyond basal dietary Zn concentrations did not influence serum MT concentrations ($P = .77$) and Zn concentrations in blood plasma ($P = .13$), liver ($P = .54$), bone ($P = .26$), and horn wall ($P = .39$). The 100-mg Zn per kg supplemented sows had lower bodyweight, body condition score, and backfat thickness ($P < .001$). The lack of impact of Zn supplementation may have been (partly) attributed to the unexpected high supply of Zn through premix in the lactation diet.

Implications: Under these study conditions, commercially grown sows might not need Zn supplementation during gestation when their basal diet contains Zn with phytase.

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Resumen – Impacto a largo plazo del suplemento de zinc en hembras: Impacto sobre los biomarcadores del nivel de zinc, y en el desempeño

Objetivos: Evaluar el impacto a largo plazo del suplemento de zinc (Zn) en el desempeño y en los biomarcadores de nivel de zinc en hembras, y si este posible impacto depende de las condiciones del alojamiento.

Materiales y métodos: Durante la gestación, se asignaron seis grupos de hembras en alojamiento grupal en dos diferentes tipos de piso. Dentro de cada grupo, las hembras fueron asignadas aleatoriamente a una de tres dietas variando en la cantidad de Zn suplementado (0, 50, ó 100 mg de Zn adicionado por kg de dieta; 50% OZn:50% Zn orgánico) a una dieta básica con un contenido de 46.6 y 128.9 mg de Zn por kg, respectivamente durante la gestación y lactancia. Se tomaron muestras de sangre en el día 0, 50, 108, y 143 de cada ciclo y se analizaron en busca de concentraciones de Zn, cobre, y metalotioneína (MT por sus siglas en inglés) en suero. Después del sacrificio, se determinaron las concentraciones de minerales de los metacarpos, hígado, y pared abacial de la pezuña.

Resultados: El suplemento dietético de Zn extra a las concentraciones dietéticas básicas no afectó las concentraciones de suero de MT ($P = .77$), ni las concentraciones de Zn en el plasma de la sangre ($P = .13$), hígado ($P = .54$), hueso ($P = .26$), y pared de la pezuña ($P = .39$). Las hembras suplementadas con100-mg de Zn por kg tuvieron un peso corporal, puntaje de condición corporal, y grosor de grasa de lomo ($P < .001$) menor.

La falta del impacto de la suplementación de Zn, puede atribuirse (parcialmente), a la inesperada a alta inclusión de Zn en la premezcla de la dieta de lactancia.

Implicaciones: Bajo las condiciones de este estudio, las hembras criadas comercialmente pueden no necesitar un suplemento de Zn durante la gestación cuando su dieta básica contenga Zn con fitasa.

Résumé – Impact à long terme d'une supplémentation en zinc chez les truies: Impact sur les biomarqueurs du statut du zinc et les performances

Objectifs: Évaluer l'impact à long terme d'une supplémentation en zinc (Zn) sur les performances et les biomarqueurs du statut du Zn chez les truies et si cet impact possible dépend des conditions d'hébergement.

Matériels et méthodes: Six groupes de truies ont été répartis en hébergement en groupe sur deux types différents de plancher durant la gestation. À l'intérieur de chaque groupe, les truies ont été aléatoirement assignées à une des trois diètes qui variaient quant à la quantité de Zn ajoutée (0, 50, ou 100 mg de Zn ajoutés par kg de nourriture; 50% ZnO:50% Zn organique) à l'aliment de base contenant 46,6 et 128,9 mg de Zn durant la gestation et la lactation, respectivement. Du sang a été prélevé aux jours 0, 50, 108, et 143 de chaque cycle et analysé pour les concentrations de Zn et de cuivre plasmatiques et pour les concentrations de métallothionéine (MT) sériques. Suite à l'abattage, les concentrations minérales ont été déterminées dans les métacarpes, le foie, et la paroi abaxiale de l'onglon.

Résultats: Un supplément de Zn alimentaire au-delà des concentrations alimentaires de base du Zn n'a pas influencé les concentrations sériques de MT ($P = 0,77$) et les concentrations de Zn dans le plasma ($P = 0,13$), le foie ($P = 0,54$), l'os ($P = 0,26$), et la paroi de l'onglon ($P = 0,39$). Les truies ayant reçu le supplément de 100 mg par kg avaient des valeurs inférieures pour le poids corporel, le pointage de condition corporelle, et l'épaisseur du gras dorsal ($P < 0,001$). Le manque d'impact du supplément de Zn pourrait (en partie) être attribué à une quantité élevée inattendue de Zn via un pré-mélange dans la diète de lactation.

Implications: Dans les conditions de la présente étude, les truies adultes ne nécessiteraient pas de supplémentation en Zn durant la gestation lorsque leur diète de base contient du Zn avec de la phytase.

Zinc (Zn) metabolism changes throughout the reproductive cycle of sows to support fetal growth and development and milk production, as found in women.[1-3] The underlying physiological processes seem to regulate these adaptations, but other factors, such as dietary Zn concentration, may affect the capacity to adapt. Across species, insufficient dietary Zn intake during gestation and lactation may result in reproductive failure.[4-6]

In sows, dietary Zn intake may change concentrations of Zn status biomarkers, such as plasma Zn and body-tissue Zn concentrations.[7-10] In weaned piglets, plasma Zn concentration was affected by dietary Zn level.[11] However, another study in sows showed increased plasma Zn concentrations only when Zn was added to the diet at levels above 500 mg Zn per kg.[12,13]

Most of the studies in sows that observed changes in plasma Zn concentrations are dated (published between 1967 and 1996), whereas the reproductive capacity of sows increased over time, and these studies used a small number of sows.[14] None of these studies evaluated the responses of the cysteine-rich protein metallothionein (MT), which is important for absorption and storage of Zn. In addition, these studies each tested only one Zn supplementation level (between 42 and 5000 mg added Zn per kg) against a non-supplemented control group with a dietary Zn concentration between 10 ([semi] purified) and 35 mg Zn per kg to induce Zn deficiency. Furthermore, these studies did not evaluate the pattern of (changing) concentrations throughout gestation and lactation over multiple reproductive cycles. It is therefore unknown whether differences among dietary treatment groups are dependent on the reproductive phase and (or) cycle.

Therefore, the objective of the present study was to evaluate the impact of dietary Zn concentration on the profile (concentration and fluctuation) of Zn status biomarkers and performance characteristics in sows over three reproductive cycles. The chosen dietary range covered the array between low (marginal) and maximum allowed dietary Zn concentration in the European Union (maximum 150 mg Zn per kg). This longitudinal study of dietary Zn supplementation was performed on two different floor types during gestation, allowing a broader conclusion across housing conditions.

Materials and methods

All experimental procedures involving these animals were approved by the Institute for Agricultural and Fisheries Research's Ethics Committee for animal experiments.

This longitudinal 2 × 3 factorial experiment was conducted according to the institutional and national guidelines for the care and use of animals.

Animals, housing, and management

Six groups of non-lame primiparous sows (total n = 131 gilts; RA-SE Genetics) were sequentially enrolled in the study when their locomotion score was ≤ 60 mm on a 150-mm tagged visual analogue scale (tVAS). First, during a quarantine period of 4 to 6 weeks, these purchased gilts were group-housed on concrete floors with straw bedding. The experimental period started 10 days before the first insemination (233 ± 12 days old at insemination). The six successive groups (n = 21 ± 4 sows per group; 3-week interval between groups) were monitored during three reproductive cycles. Body weight, backfat thickness, and body condition score (BCS, scale from 0 to 5) at the start of the study were 149 ± 21 kg, 16 ± 4 mm, and 3.0 ± 0.5, respectively (mean ± standard deviation [SD]) and did not differ between treatment groups (data not shown). A sow that had to be removed (n = 36) before the end of the experiment was replaced by a new gilt.

During the experimental period, sows were housed in individual gestation crates 10 days before their first insemination (day -10, start of the study; insemination at day 0) and from weaning (day -7) until 4 weeks after insemination (day 28) in their successive reproductive cycles. During mid- and end of gestation (day 28 to day 108), the sows were housed in static production groups. An automated feeding system was used with individual sow recognition through an electronic transponder in the sow's ear. The group-housing facility consisted of four pens (4.45 m × 18.70 m); more detailed information on housing conditions is described in Bos et al.[15] Two of the four pens, oriented diagonally to each other, had a similar floor type: either concrete slats and solid concrete lying areas or concrete slats covered with a rubber top layer (EasyFix; Rubber Products Ltd, Galway, Ireland) and rubber lying mats

(Gummiwerk Kraiburg Elastik GmbH & Co Kg, Tittmoning, Germany) on 50% of the solid concrete floor area ("concrete" or "rubber floor" type, respectively). Each group was alternately assigned to the concrete or rubber floor type during group housing for the entire experiment. Per pen, two rubber balls and a static and dynamic rotating grooming brush were provided as environmental enrichment. The sows were housed individually in farrowing crates from 1 week before expected parturition until weaning (day 108 to day 143).

After weaning of the third reproductive cycle (end of the study), sows that had participated for at least 12 months (ie, sows that had completed at least two cycles, n = 95) in the experiment were slaughtered in a commercial slaughterhouse. This threshold was chosen on the basis of literature on dairy cows, postulating that the effect of dietary Zn on claw quality is dependent on study duration.[16-18] The impact of dietary Zn on claw quality is presented in a previous publication.[19] The sows were transported to the abattoir on the afternoon of the day before slaughter. Both front claws were marked with a coloured tie wrap to distinguish between left and right front claws. The next morning, the sows were slaughtered and both front claws were removed at the carpal joint before they entered the scalding vat to preserve the metacarpal bones and claw structures, and the whole liver was collected. The claw structures for examination, the liver, and the remaining part of the front claws, including metacarpal bones, were subsequently frozen at -20°C.

Dietary treatment

All purchased primiparous sows (gilts) were fed ad libitum during the quarantine period. The pre-experimental gestation diet was formulated according to National Research Council (NRC) recommendations[20] and commercial standards for gestating sows, containing 895 g per kg dry matter (DM), 127 g per kg crude protein, 302 g per kg neutral detergent fibre (NDF), 155 g per kg acid detergent fibre (ADF), 24 g per kg acid detergent lignin (ADL), 28 g per kg crude fat, 70 g per kg crude ash, 129 g per kg starch, 66 g per kg sugar, 121 mg per kg Zn (originating from ingredients and from 100 mg Zn per kg added as ZnO via premix), and 4.7 g apparent ileal digestible lysine per kg diet.

Throughout the experimental period, sows were fed a gestation and lactation diet formulated according to NRC recommendations[20] and commercial standards (Table 1 and Table 2) except for Zn. Phytase was added via the premix to simulate practical conditions. The gestation diet was offered 7 days before the first insemination or after weaning of the preceding reproductive cycle (day -7) until 1 week before parturition (day 108). The sows were fed twice daily from day -7 to the first 4 weeks of gestation (day 28), in total 2.3 kg per day, whereas during mid- and end of gestation (day 28 to day 108), sows were fed 2.6 kg per day. The lactation diet was provided from 1 week before parturition until weaning (day 108 to day 143). The sows were fed twice daily and received 3 kg feed provided in two equal portions the week before parturition (day 108 to day 115). After parturition, per suckling piglet, 0.25 kg of feed was gradually supplied in addition to 3 kg feed (eg, a sow with 12 piglets received 6 kg of feed per day), also provided in two equal portions per day.

Throughout the experiment, all sows had ad libitum access to drinking water, except in the first 4 weeks of gestation, during which water was automatically provided through nipple drinkers for 15 minutes every hour and for 45 minutes while feeding in order to reduce water spillage.

Within each of the six static production groups, equal numbers of sows were randomly allocated to three dietary treatment groups, depending on the number of sows. The dietary treatments differed in Zn concentration: Zn not supplemented; Zn originated from ingredients only, 50 mg Zn per kg supplemented; and 100 mg Zn per kg supplemented. The Zn supplement comprised 50% inorganic Zn as ZnO (75% Zn) (33.3 or 66.6 g ZnO per 1000 kg feed, INVE Belgium N.V., Baasrode, Belgium), and 50% organic Zn as Availa Zn containing 10% Zn in an amino acid complex: single

Table 1: Ingredient composition of the gestation and lactation diets

Ingredients (g/kg as fed)	Gestation	Lactation
Wheat	180	213
Barley	180	100
Maize	152	250
Wheat middling	150	23
Beet pulp	120	43
Soybean meal	89	166
Soybeans heated	NA	12
Soybean oil	21	NA
Alfalfa meal	47	94
Beet molasses	30	30
Premix 3%*	30	NA
Premix 2.75%†	NA	27.5
Lard	NA	30
Limestone	NA	9.4
L-Valine	NA	0.9
L-Threonine	0.8	0.7
DL-Methionine	0.7	0.3
L-Lysine HCL	0.1	0.5
L-Tryptophan	NA	0.1
Salt	0.05	NA

* Premix 3% included per kg total gestation diet (analyzed Zn concentration in premix is 260 mg/kg) are presented in Supplementary materials.[19]

† Premix 2.75% included per kg total lactation diet (analyzed Zn concentration in premix is 4366 mg /kg) are presented in Supplementary materials.[19]

NA = not applicable (ingredients not added to the gestation or lactation diet).

Table 2: Analyzed and calculated* nutrient composition of the gestation and lactation diets†

	Gestation			Lactation		
Chemical analysis (g/kg)	**0**	**50**	**100**	**0**	**50**	**100**
DM	877.4	876.9	877.1	880.0	878.3	879.6
Crude ash	56.9	56.9	56.7	62.8	63.0	63.0
Crude protein	136.7	136.9	136.8	160.8	161.0	160.7
Crude fat	41.2	41.7	41.6	51.6	52.0	51.3
Crude fiber	64.5	65.0	66.3	58.1	61.1	58.7
Starch	277	270	268	313	304	314
Sugar	55.8	56.2	55.2	55.4	55.2	53.6
Acid detergent fiber	72.0	72.4	68.5	54.8	54.1	60.3
Neutral detergent fiber	167	162	159	121	118	116
Acid detergent lignin	9.9	10.6	11.5	6.8	6.3	7.0
Ca	8.1	8.6	9.1	12.3	12.1	10.8
P	4.4	4.3	4.3	5.0	4.9	5.0
Cu (mg/kg)‡	18.6 (15-25)	14.1 (13-15)	13.8 (13-15)	20.9 (19-22)	20.8 (19-22)	19.9 (19-22)
Zn (mg/kg)‡	46.6 (45-49)	81.9 (77-91)	124.4 (119-132)	128.9 (116-137)	184.3 (167-209)	229.0 (206-256)
ID Lysine	6.0			7.9		
ID Methionine	2.3			2.9		
ID Methionine + cysteine	4.0			4.2		
ID Threonine	4.3			5.5		
ID Tryptophan	1.2			1.6		
ID Arginine	6.6			8.3		
ID Leucine	7.6			10.1		
ID Isoleucine	3.8			5.1		
ID Histidine	2.7			3.3		
ID Valine	4.5			6.6		
ID Phenylalanine	4.7			6.2		
NEv (MJ/kg)§	9.0			9.4		

* Apparent ID amino acids and NEv for pigs were calculated according to the feed tables of the Centraal Veevoederbureau (CVB, the Netherlands), 2007.

† Dietary treatment is presented as 0-, 50-, or 100-mg Zn/kg supplemented to the basal diet containing 46.6 mg Zn/kg and 128.9 mg Zn/kg during gestation and lactation, respectively.

‡ Zn and Cu concentration are the average value of multiple feed sample analyses. Ranges for concentrations of both minerals in the gestation and lactation diets over time are presented between parentheses. The analyzed Zn concentration of the premix in the gestation diet was 260 mg/kg, which represent 7.8 mg Zn/kg in the final diet with 3% premix. The analyzed Zn concentration of the premix in the lactation diet was 4366 mg/kg, which represents 120 mg Zn/kg in the final diet with 2.75% premix.

§ Net energy for pigs is expressed as MegaJoules (MJ) per kg.

DM = dry matter; Ca = calcium; P = phosphorus; Cu = copper; Zn = zinc; ID = ileal digestible; NEv = net energy.

amino acids from hydrolysed soy protein (molar ratio 1:1, 250, or 500 g Availa Zn per 1000 kg feed, Zinpro Corporation, Eden Prairie, Minnesota).

Sample collection and measurements

Performance characteristics (body weight [BW], backfat thickness, body condition score [BCS], and reproductive performance) and Zn status biomarkers (liver and bone mineral concentration) were determined in all sows. For plasma Zn and copper (Cu) concentration, serum MT concentration and horn wall Zn concentration, 36 sows (12 of each dietary treatment group and at least one of each static production group) were selected. These sows were selected according to three criteria: three reproductive cycles completed, remained in their group of origin (ie, the group the sow was allocated to at the start of the experiment; repeat breeders that were transferred to another group receiving the same feed treatment and floor type, were not selected); and housed in their group during the entire gestation period (eg, sow was not separated from the group during group-housing period).

Performance characteristics

Body weight, backfat thickness, and BCS were determined to monitor sows' health at day -10 (baseline, start of the study) and then on day 0 (insemination), day 20 (only backfat thickness and BCS), and (or) day 28 (only BW and BCS), day 108, and day 140 to day 143 (around weaning) of every reproductive cycle. Backfat thickness was determined between the 3rd and 4th last ribs, 7 cm from the left and right side of the vertebrae (P2 position). After lubricant was applied to P2, backfat measurements were determined, alternating between the left and right sides (Renco Lean Meater-12 60566; Renco Corporation, Minneapolis, Minnesota). If the difference between left and right was 2 mm or more, the measurements were repeated up to three times. The average thickness was used for further calculations. Body condition score was determined according to Evans[21] using an ordinal scale including five categories from score 1 (emaciated) to score 5 (overly fat). A BCS of three represents the ideal condition.[21]

Reproduction performances (number of piglets born alive, average bodyweight [kg] of piglets born alive, number of stillborn piglets, number of weaned piglets, and average bodyweight [kg] of weaned piglets) were recorded. Cross-fostering between dietary treatments and provision of creep feed (transitional feed) to the piglets from day 10 postpartum were standard practice within the farm.

Zinc status biomarkers

Zinc status biomarkers were determined after blood collection at day 0 (insemination), day 50, day 108, and day 143 (weaning) every reproductive cycle, and tissue collection after slaughter.

Blood samples (20 mL) were taken from all sows within a group before feeding in the morning (between 8:30 AM and 9:00 AM) after overnight fasting of at least 18 hours. Blood samples were collected from the jugular vein using stainless steel needles and plastic syringes, and deposited in one heparin and one serum vacuum tube (Terumo Europe, Leuven, Belgium). One mL of heparinized blood was used to determine hematocrit (centrifuged 2749*g*, 30 minutes, 20°C) partly to monitor the sows' health. The remainder was centrifuged (1500*g*, 10 minutes, 4°C) and plasma was divided between two 5-mL disposable polystyrene tubes. The tubes were stored for 24 hours at -20°C and then transferred to storage at -80°C until analysis of plasma Zn and Cu concentration. The vacuum serum tubes were centrifuged (1500*g*, 10 minutes, 4°C) after standing overnight at 4°C to allow clotting. Serum samples were divided between two 5-mL disposable polystyrene tubes, stored for 24 hours at -20°C and then at -80°C until analysis of serum MT concentration.

Plasma samples were deproteinized (Randox ZN2607; Randox Laboratories Ltd, Crumlin, United Kingdom) and the remaining supernatant was used within 2 hours to determine plasma Zn or Cu concentrations as described in Van Riet et al.[22] Plasma Zn concentration was determined spectrophotometrically (EZ reader 400; Biochrom Ltd, Cambridge, United Kingdom) using a commercial colorimetric diagnostic kit (Randox kit, ZN2341; Randox Laboratories Ltd, Crumlin, United Kingdom). The observed plasma Zn concentration was interpolated from the multipoint standard calibration curve. The inter- and intra-assay CVs were 1.3% and 1.1%, respectively. The minimum and maximum recovery was 99.5% and 119.8%, respectively.[22] Plasma Cu concentrations were determined spectrophotometrically (Ultrospec IIE; LKB Biochrom Ltd) using a commercial colorimetric diagnostic kit (Randox kit, CU2340, Randox Laboratories Ltd). The plasma Cu concentration was interpolated from the multipoint standard calibration curve. The inter- and intra-assay CVs were 1.8% and 1.1%, respectively. The minimum and maximum recoveries were 96.4% and 103.7%, respectively.[22] Serum MT concentration was determined spectrophotometrically (EZ reader 400; Biochrom Ltd) using competitive ELISA (Porcine Metallothionein [MET] ELISA kit, E07M0030; BlueGene Biotech Co, Shanghai, China). Serum MT concentration was interpolated from the multipoint standard calibration curve. Serum MT concentrations below the detection limit (0.1 ng per mL) were corrected using the equation: detection limit $\div \sqrt{2}$. The inter- and intra-assay CVs were 3.3% and 2.0%, respectively. The certificate of analysis reported a recovery between 94% and 103%. For quality control, serum samples were spiked with 5 ng per mL and 10 ng per mL MT. The recoveries of spiked MT were 96.6% and 94.3%, respectively.

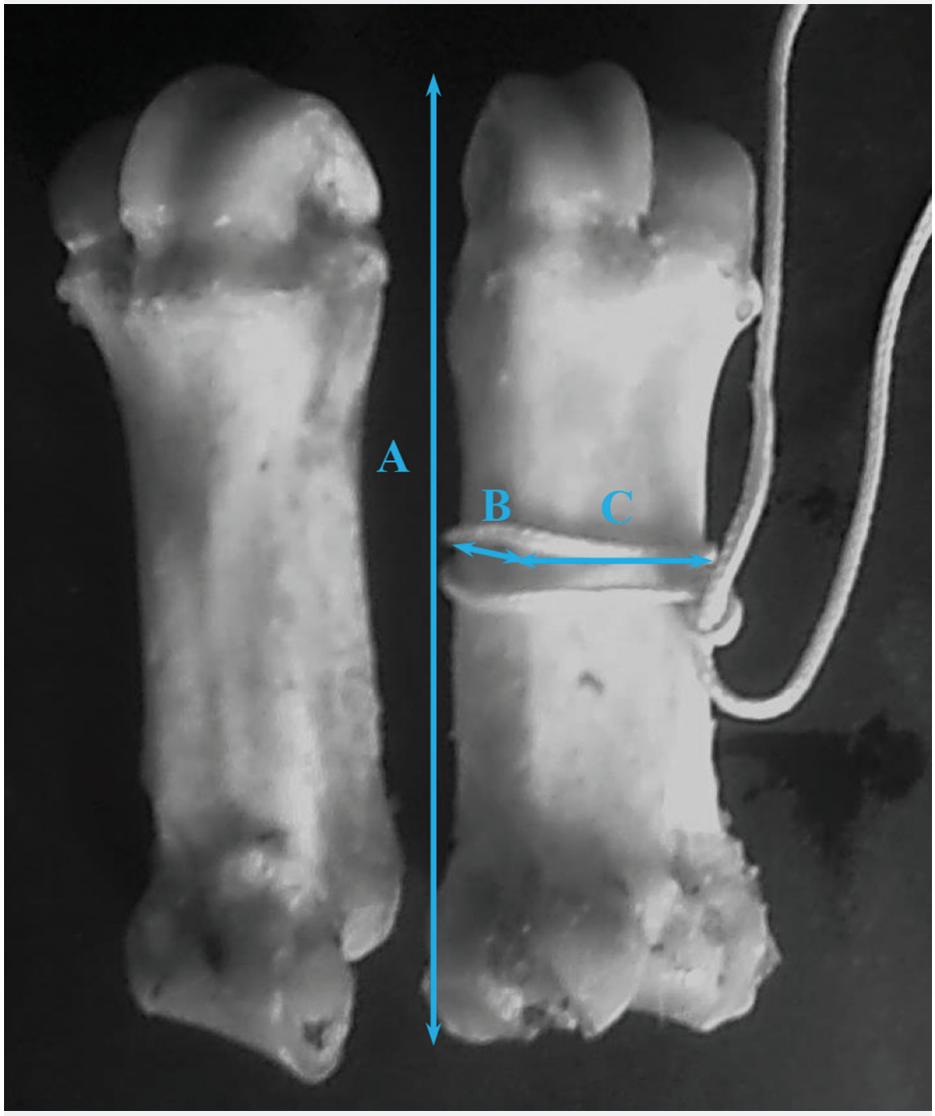
Body tissues (liver, bone, horn wall) were collected after slaughter for mineral analyses. Livers were cooled during transport. The left lateral and medial lobe (not including the right lobe with gall bladder) were sliced and ground using a mincer with 4.5 mm sieve (Kenwood kMix stand mixer with food grinder; Kenwood Ltd, Woking, United Kingdom). Then a representative homogenized sample (mean ± SD; 189 ± 23 g) was collected in a petri dish and stored at -20°C until lyophilisation. After lyophilisation, liver samples were oven dried at 103°C to a constant weight, and Zn and Cu concentrations were analyzed.

An abaxial horn wall sample of the lateral and medial digits of the right front claw was collected using an oscillating saw and the underlining tissues were removed. The remaining right front claw was stored at -20°C for collection of metacarpals. The horn wall samples were weighed and individually stored under vacuum at -20°C until testing for mechanical characteristics; procedures described in a previous publication.[19] Post testing, the lateral and medial abaxial horn wall samples of 36 sows (dimensions: two samples of 20 mm length and 6 mm width with a variable thickness) were stored under vacuum at -20°C until analysis. Abaxial horn wall samples were dried at 103°C to a constant weight and analysed for Zn and Cu content.

The frozen right front claws with tie wrap were placed in a beaker (500 mL) filled with warm water with the claws pointing upwards to prevent charring of the metacarpal bones. The beaker was then placed in a warm water bath (75°C). After 24 hours, metacarpals 3 and 4 were collected and surrounding tissue removed. The metacarpals were weighed, oven dried for 16.45 hours at 65°C (Drying oven Binder APT line series ED; Binder GmbH Tuttlingen, Germany), and weighed again (Sartorius CP 324S, Göttingen, Germany). The lengths of metacarpals 3 and 4 (standardised as the length of each bone at the interior side located between metacarpals 3 and 4) was determined using a digital calliper. Metacarpals 3 and 4 were crushed in a vise lined with paper towels and stored at -20°C until fat extraction. The crushed metacarpals were defatted by extraction with petroleum ether (boiling point 40° to 60°C, ISO 6492A), dried at 103°C to a constant weight, and ashed at 825°C to a constant weight according to Bikker et al.[23,24] The ash content of the fat-free dry matter was calculated on the basis of the weight of the bones before and after ashing. Metacarpals 3 and 4 were then ground according to a validated protocol (data not shown) that does not affect Zn and Cu concentrations using a ball mill (Retsch PM100; Led Techno NV; Heusden-Zolder, Belgium) with a 125-mL stainless steel grinding jar, seven stainless steel balls, and five droplets of ethanol (reagent grade) to obtain a homogenized sample. Grinding jar and balls were cleaned with sequentially distilled water (Type II), methanol (reagent grade), and distilled water (Type II) to prevent contamination. Equal aliquots (10 g) of the ground metacarpal 3 and 4 samples were combined to obtain one sample per sow and were analysed for calcium, phosphorus, Zn, and Cu content.

The left front claws were thawed for 24 hours and surrounding tissues were removed using a surgical knife. The weight and dimensions (length, thickness, and width) of metacarpals 3 and 4 were measured according to Combs et al[25] (Figure 1).

Figure 1: Length (A), thickness (B), and width (C) dimensions of metacarpals 3 and 4 of left front claw. Measurements determined according to Figure 1 of Combs et al.[25]



Chemical analysis

Feed samples of the gestation and lactation diets were collected from every batch and ground to pass through a 1-mm sieve for Near Infrared Spectroscopy evaluation and composited per dietary treatment group every 3 months for proximate analysis according to international standard methods accredited by ISO 17025.[26] Dry matter, crude ash, crude protein, crude fat, calcium, and phosphorus content were determined according to 71/393/EEC, ISO 5984, ISO 5983-2, ISO 6492, ISO 6490/1, and ISO 6491, respectively. The American Oil Chemists' Society (AOCS) approved procedure Ba 6a-05 was used to determine crude fiber content and the procedures described in Van Soest et al[27] were used to determine ADF, NDF, and ADL.

The homogenized sample was further ground to pass a 0.5-mm sieve and three of five samples per dietary treatment were subjected to Zn and Cu analysis. Copper was analysed to assess possible antagonistic effects of Zn on Cu metabolism. Feed samples (1 g) were ashed and digested with HNO_3 on a hot plate (150°C) for at least 30 minutes and transferred to a 50-mL flask. Liver samples (0.25 g) were diluted with HNO_3:H_2O_2 for 12 hours and digested using microwave-assisted matrix digestion (MarsX; CEM, Matthews, North Carolina). Bone samples (1 g) were digested with 10 mL 6N HNO_3 in a flask and diluted to 50 mL, and horn wall samples (approximately 0.8 g) were diluted in 10 mL 6N HNO_3 for 12 hours, heated on a hot plate (150°C) for approximately 2 hours, and transferred to a 50-mL flask.

The Zn, Cu, and calcium concentration in the feed and tissue samples were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Vista MPX,

Varian Inc Palo Alto, California), and bone phosphorus concentration was determined spectrophotometrically after matrix digestion.

Statistical analysis

Performance characteristics. Sow performance (BW, BCS, backfat thickness, reproduction) was analysed using a linear mixed model. Dietary Zn supplementation, floor type, phase within the reproductive cycle, parity, and all two-way interactions were included as fixed effects. Reproductive cycle, sow, and group were included in the models as random effects to correct for repeated measurements. A similar Poisson mixed model was used for the reproductive performance characteristics related to the number of born piglets (ie, number of piglets born alive, number of stillborn piglets, and number of weaned piglets).

Zn status biomarkers. Similar to the models for sow performance, linear mixed models were used to analyse the blood biomarkers and tissue characteristic data. Fixed and random effects included in the models differed according to time of sampling (eg, tissue collected at slaughter versus multiple blood collections throughout the reproductive cycle). For Zn status biomarkers in blood, dietary Zn supplementation, floor type, phase of the reproductive cycle, parity, and all two-way interactions were included as fixed effects, and reproductive cycle, sow, and group were included as random effects to correct for the repeated measurements. Observations for serum MT were transformed to a natural logarithm to obtain a normal distribution. For the mineral concentration in liver, bone, and horn wall and bone characteristic data, dietary Zn supplementation, floor type, parity, and all two-way interactions were included as fixed effects and sow and group were included as random effects.

Non-significant interactions and non-significant parity effects were excluded from all final models and *P* values of the main effects are presented. In case of a significant interaction, partitioned post-hoc *P* values are presented as tests of the simple effects of one variable for each level of the other variable. In the case of non-significant interactions, an all pairwise comparisons post-hoc test was performed. The *P* values of all post-hoc tests were corrected for multiple comparisons using the Tukey-Kramer method.

The analyzed data (except reproductive performance characteristics related to the number of born piglets) were considered to be sufficiently normally distributed, on the basis of the graphical evaluation (histogram and QQ-plot) of the residuals. All analyses were performed using SAS 9.4 (SAS Institute Inc, Cary, North Carolina).

Results

The interaction between dietary Zn supplementation and floor type was not significant for any of the outcome variables ($P > .07$) except for bone width dimension ($P = .02$). Therefore, main effects of dietary Zn supplementation and floor type, irrespective of the other main effects, are presented. Regarding the dietary Zn concentration, an unexpected high supply of Zn through premix in the non-supplemented lactation diet was analyzed that met NRC requirements during the last week of gestation until weaning (day 108 to day 143). However, the impact is unquantifiable.

Sows' characteristics and farrowing performance

Throughout three reproductive cycles, 36 sows (27.5%), of which 9, 16, and 11 sows from the 0, 50, and 100 mg added Zn per kg treatment group, respectively, were removed from the experiment, and 21 of them were replaced with primiparous sows. Sows were removed for several reasons: spontaneous death (n = 10), euthanasia (rectal or uterine prolapse, severe locomotion disorders, n = 7), or reproductive failure after multiple attempts (n = 19). Seventy sows remained in their group of origin, whereas 26 sows were transferred to another group allocated to the same dietary treatment and floor type due to reproductive failure. In total, 92 of 95 sows that completed a minimum of two reproductive cycles were slaughtered at the end of the experiment. Of the other three sows, two died after their third parturition and the third sow was euthanized at the ILVO experimental farm immediately after the rest of the group was loaded for transport. The front claws and liver of this third sow were collected post mortem.

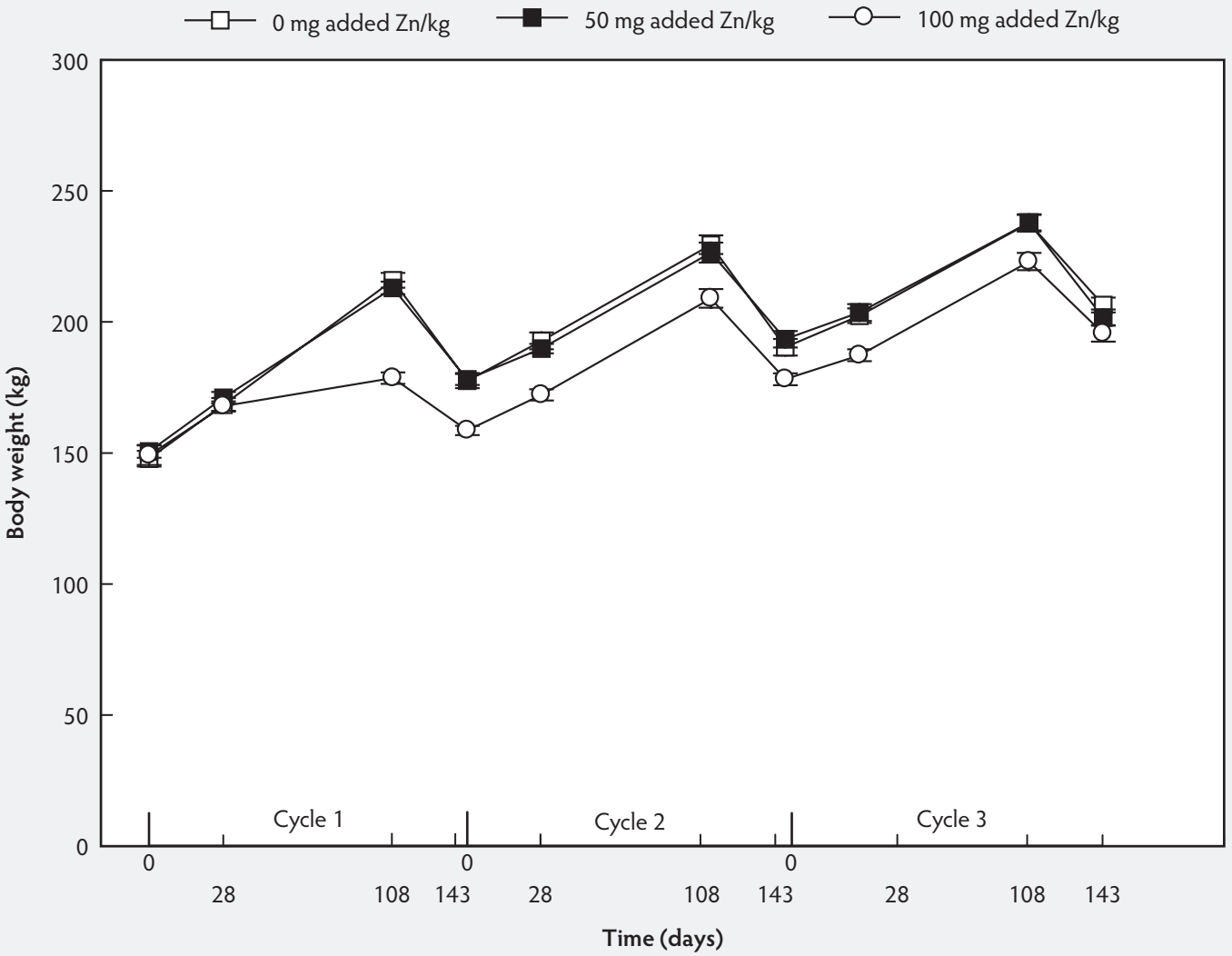
For the sows' BW, an interaction between phase of reproductive cycle and dietary Zn supplementation was found irrespective of floor type. The mean BW of 100-mg Zn per kg supplemented sows was lower than that of the non-supplemented and 50-mg Zn per kg supplemented sows at day 28 ($P = .001$ and $P < .001$, respectively), day 108 ($P < .001$ and $P < .001$, respectively), and day 143 ($P < .001$ and $P < .001$, respectively), but not at the start of the study (Figure 2). No differences in sow BW were found between the non-supplemented and 50 mg added Zn per kg dietary treatment groups throughout the reproductive cycle ($P = 1.0$). Body condition score and backfat thickness were also lower for the 100 mg Zn per kg diet supplemented sows (Table 3). Body condition score remained constant between day 28 and day 50 ($P = .13$), increased towards day 108 ($P < .001$), and decreased towards day 143 ($P < .001$) of the reproductive cycle (Table 4). The BCS between day 28 and day 108 did not differ ($P = .24$) and BCS at day 143 was lower ($P < .001$) compared with other phases of the reproductive cycle. Backfat thickness increased from day 28 to day 108 ($P = .008$) and decreased towards day 143 ($P < .001$) of the reproductive cycle (Table 4). At day 108, backfat thickness was higher compared with other phases of the reproductive cycle ($P < .001$). A parity effect was found for BW ($P = .005$), BCS ($P < .001$), and backfat thickness ($P < .001$), showing higher BW and lower BCS and backfat thickness in the third parity (Table 4).

Dietary treatment was not associated with number of piglets born alive, average BW of piglets born alive, number of stillborn piglets, or number of weaned piglets (Table 3). Piglets from sows that receieved 100-mg added Zn per kg diet had a lower BW at weaning than did those of non-supplemented and 50-mg added Zn per kg supplemented sows (Table 3). A parity effect was found for the number of stillborn piglets, which was lower in the second parity ($P < .001$), and for the average BW of piglets born alive, which was lower in the first parity ($P < .001$) (Table 4). The average BW of weaned piglets was lower in the third parity ($P < .001$) (Table 4).

Hematocrit (Hct; %) did not differ between dietary treatment groups (Table 3). The Hct remained constant between day 0 and day 50 ($P = .91$), decreased towards day 108 ($P = .03$), and then decreased until day 143 ($P = .003$) (Table 4). Hematocrit was lower in reproductive cycles 2 and 3 than in reproductive cycle 1 ($P = .003$ and $P < .001$, respectively) (Table 4).

Independent of dietary Zn supplementation, floor type had only minor influences on reproductive performance ($P > .47$ for number of piglets born alive, number of weaned piglets and average BW of weaned piglets; $P = .095$ for average BW of piglets born

Figure 2: Fluctuations in body weight (kg) throughout three reproductive cycles in non-supplemented, 50-mg per kg, and 100-mg per kg supplemented sows (n = 36; 12 of each dietary treatment group). Values presented are means and their standard errors. Sows were group-housed between day 28 and day 108 of gestation on different floor types. The body weight of 100-mg Zn per kg supplemented sows was lower than those of the non-supplemented and 50-mg added Zn per kg supplemented sows at day 28, day 108, and day 143 (eg, interaction between phase of reproductive cycle and dietary Zn concentration, $P < .001$, linear mixed model). There were no differences between the non-supplemented and 50-mg added Zn per kg diet dietary treatment groups throughout the reproductive cycle ($P = 1.0$).



alive; and $P = .044$ for number of stillborn piglets).

Zn status biomarkers

Plasma Zn concentration did not differ between dietary treatment groups irrespective of floor type ($P = .13$). Generally, plasma Zn concentration decreased from insemination (day 0) to day 50 of gestation ($P < .001$), remained constant to day 108 of gestation ($P = .999$), and decreased until weaning (day 143) ($P = .003$). Plasma Zn concentration was lower in reproductive cycle 3 than in reproductive cycles 1 and 2 ($P < .001$ and $P < .001$, respectively) (Figure 3).

Plasma Cu concentration did not differ between dietary treatment groups ($P = .37$), but was lower at day 143 than at day 0 and day 50 ($P = .005$ and $P = .03$, respectively). Plasma Cu concentration was lower in reproductive cycle 3 than at reproductive cycles 1 and 2 ($P = .04$ and $P = .008$, respectively) (Figure 4).

Serum MT concentration did not differ between dietary treatment groups ($P = .77$). It decreased from day 0 towards day 50 and day 108 and increased towards day 143 ($P = .01$, $P < .001$, and $P = .04$, respectively), but the MT concentration did not differ between day 50 and day 143 ($P = .29$). The serum MT concentration was lower in reproductive cycle 2 than in reproductive cycle 3 ($P = .03$) (Figure 5).

Floor type did not affect plasma Zn and Cu concentrations ($P = .28$ and $P = .69$, respectively). Serum MT concentration was lower for sows housed on rubber floors ($P = .003$) (Table 5).

Liver weight, liver Zn and Cu concentrations, bone Zn, Cu, calcium, and phosphorus concentrations, and horn wall Zn and Cu concentrations did not differ between dietary treatment groups nor between floor type treatments (Table 6). An interaction for the bone width dimension was found between

Table 3: Effect of dietary Zn supplementation on sows' characteristics and farrowing performance when group housed on different floor types between day 28 and day 108 of gestation (n = 21 ± 4 sows per group)

	Dietary treatment* × Floor type							
Characteristic	**0 × C**	**50 × C**	**100 × C**	**0 × R**	**50 × R**	**100 × R**	**SEM**	***P*†**
BCS	3.2	3.1	2.9	3.1	3.1	2.8	0.02	< .001
Backfat (mm)	14.8	14.0	12.7	14.8	15.0	12.5	0.20	< .001
Hct (%)	37.1	36.6	36.1	37.2	36.5	37.5	0.47	.65
Farrowing performance								
Piglets born alive (n)	13.1	14.3	13.3	13.7	14.1	13.7	0.31	.16
Ave BW, piglets born alive (kg)	1.43	1.44	1.38	1.39	1.31	1.37	0.02	.72
Stillborn piglets (n)	1.1	1.1	1.3	0.8	1.0	0.7	0.15	.77
Weaned piglets (n)	10.8	11.7	10.7	11.2	11.0	11.0	0.28	.45
Ave BW, weaned piglets (kg)	7.45	7.63	7.06	7.48	7.46	7.15	0.10	.01

* Dietary treatment is presented as 0, 50, or 100 mg Zn/kg supplemented to the basal diet containing 46.6 mg Zn/kg and 128.9 mg Zn/kg during gestation and lactation, respectively.

† As no interaction between phase of the reproductive cycle and dietary treatment for BCS, backfat thickness, or Hct was observed, the average BCS, backfat thickness, and Hct are presented. Level of significance is $P < .05$, linear mixed models, except for farrowing performance characteristics related to the number of born piglets for which a similar Poisson mixed model was used.

BCS = body condition score; C = concrete floor; R = rubber floor; Backfat = Backfat thickness; Hct = hematocrit; BW = body weight; Ave = average.

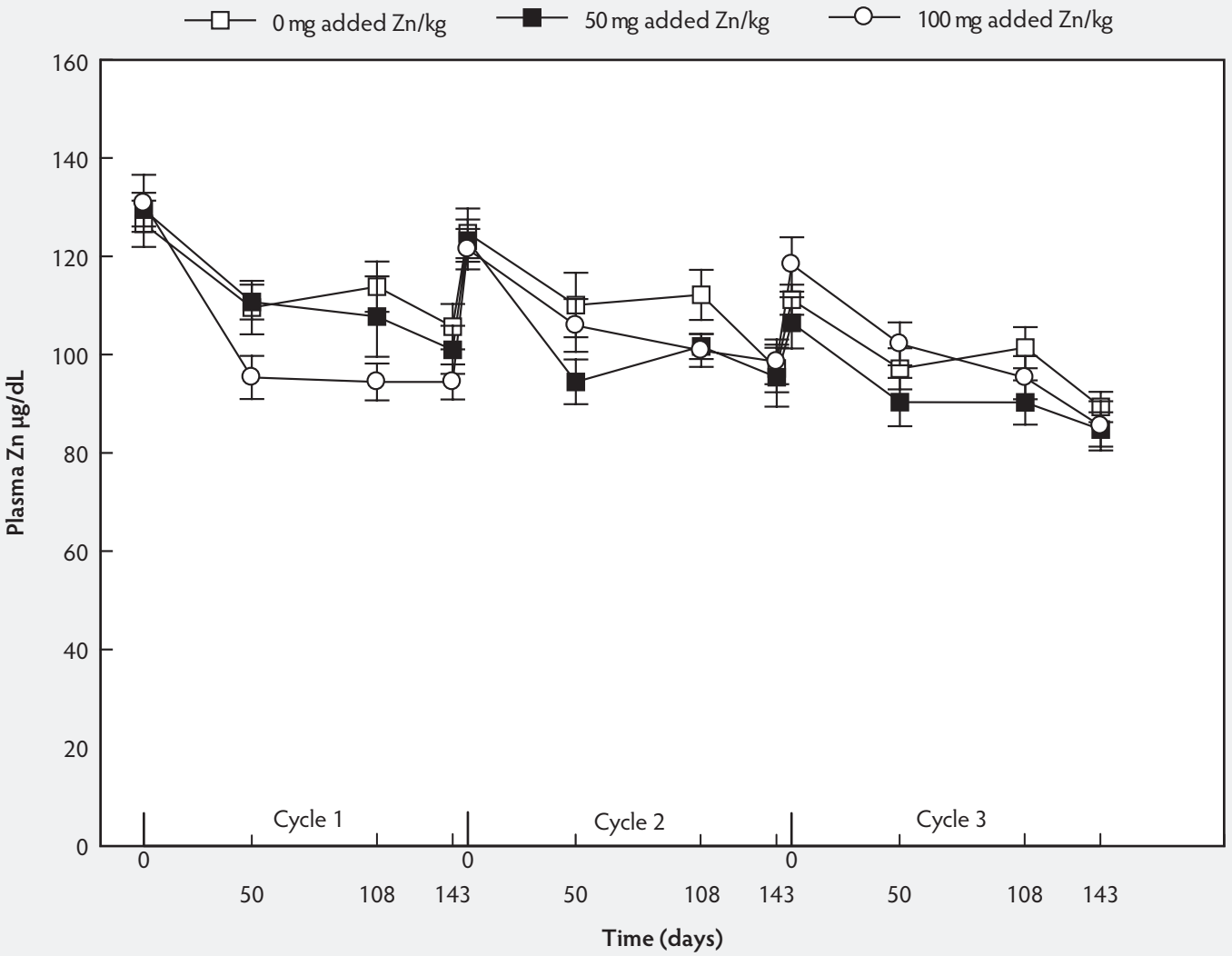
Table 4: Fluctuations in sows' characteristics and farrowing performance throughout three reproductive cycles and over parity (n = 21 ± 4 sows per group)

						P*	
Characteristic	**Reproductive phase (days)**	**Parity 1**	**Parity 2**	**Parity 3**	**SEM**	**Parity**	**Phase**
BCS	28	3.4	3.1	2.9	0.10	< .001	< .001
	108	3.2	3.3	3.2	0.07		
	143	2.7	2.7	2.9	0.03		
Backfat (mm)	20	16.1	11.9	11.1	0.30	< .001	< .001
	108	17.7	16.2	16.0	0.40		
	143	12.4	11.0	11.5	0.33		
Hct (%)	0	40.9	37.9	37.5	0.70	< .001	< .001
	50	40.8	37.9	36.2	1.23		
	108	37.4	36.1	35.5	0.63		
	143	34.5	33.6	33.4	0.73		
Farrowing performance							
Piglets born alive (n)		13.3	13.8	14.1	0.33	.16	NA
Ave BW of piglets born alive (kg)		1.3	1.5	1.4	0.00	< .001	NA
Stillborn piglets (n)		1.1	0.6	1.2	0.17	< .001	NA
Weaned piglets (n)		10.7	11.5	11.4	0.30	.09	NA
Ave BW of weaned piglets (kg)		7.3	7.7	7.1	0.10	< .001	NA

* *P* values are presented for parity and phase within the reproductive cycle. Level of significance is $P < .05$ using linear mixed models, except for farrowing performance characteristics related to the number of born piglets for which a similar Poisson mixed model was used.

BCS = body condition score; Backfat = Backfat thickness; Hct = hematocrit; BW = body weight; Ave = average; NA = not applicable.

Figure 3: Fluctuations in plasma Zn concentrations (μg/dL) throughout three reproductive cycles in non-supplemented, 50-mg/kg and 100-mg/kg supplemented sows (n = 36, 12 of each dietary treatment group). Values presented are means and their standard errors. Sows were group-housed between day 28 and day 108 of gestation on different floor types. Plasma Zn concentration did not differ between dietary treatment groups ($P = .13$) or between floor types ($P = .28$). Plasma Zn concentration decreased from insemination (day 0) to day 50 of gestation, remained constant to day 108 of gestation, and decreased further towards weaning (day 143) ($P < .001$; linear mixed model). Plasma Zn concentration was lower in reproductive cycle 3 than in reproductive cycles 1 and 2 ($P < .001$).



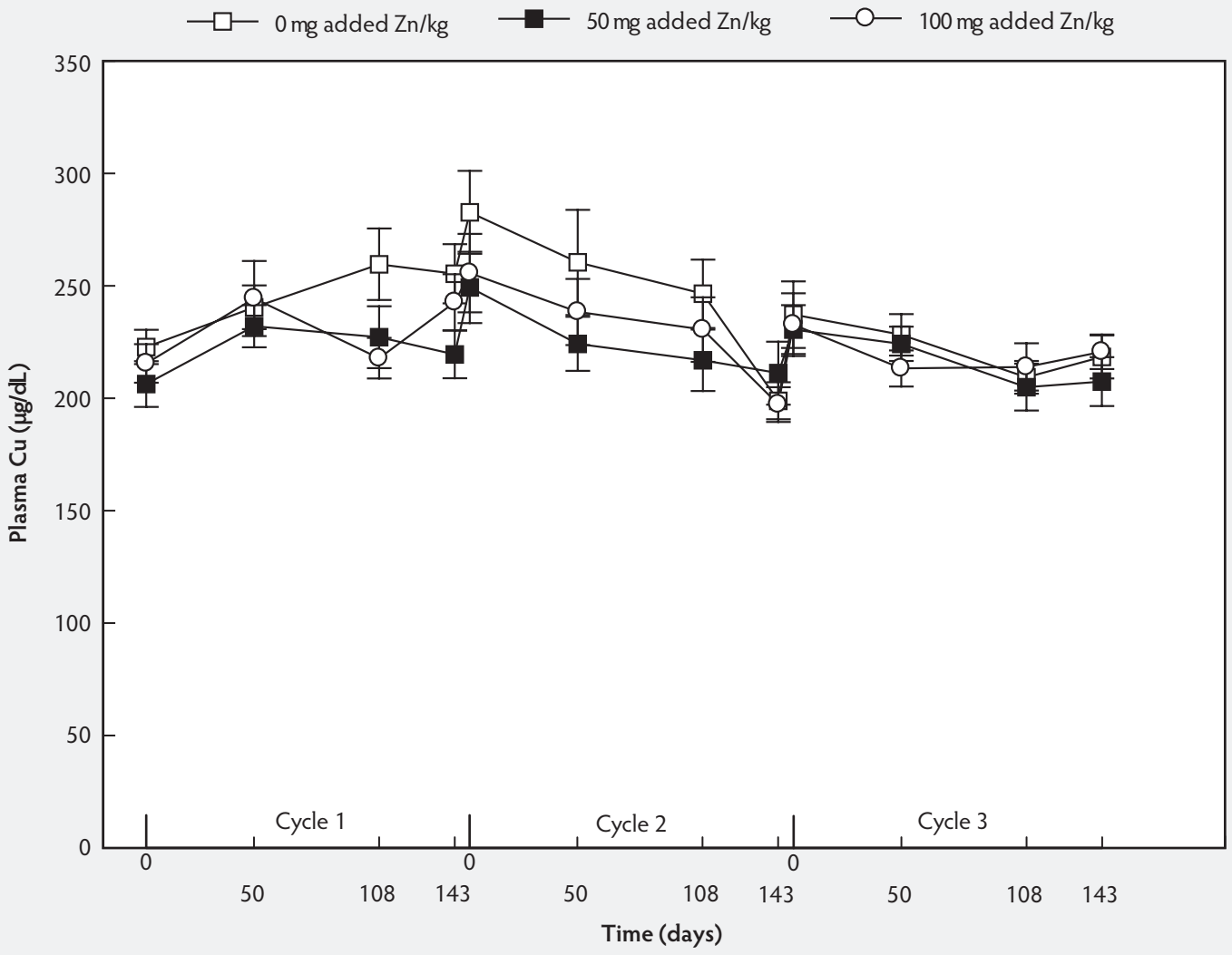
dietary Zn supplementation and floor type (Table 7). Other bone characteristics did not differ between dietary or floor type treatment groups (Table 7).

Discussion

Despite the considerable range in dietary Zn concentration among the treatments, the serum MT concentrations and concentrations of Zn in blood plasma, liver, bone, and horn wall did not respond to increased dietary Zn inclusion levels beyond basal dietary Zn concentrations in the present study. The lack of impact of Zn supplementation may have been (partly) attributed to the unexpected high supply of Zn through premix in the non-supplemented lactation diet that met NRC requirements during the last week of gestation until weaning (day 108 to day 143). Although sows received Zn above NRC requirements during this period, these results still suggest that the tested dietary range did not disturb Zn homeostasis, including the non-supplemented sows during gestation, as compared to earlier studies in rats.[28-31] Because Zn homeostasis is very important to ensure that all body processes are optimally regulated, Zn flow is tightly regulated. Adjustments in the processes of Zn absorption and excretion as shown in rats and weaned piglets,[14,28,30,32-34] may have also occurred in the present study. Although we did not measure absorption, it can be assumed that a higher Zn intake did not result in a proportionally higher absorption and utilisation of Zn, for example, due to the down-regulation of the transport protein ZIP4 in the lumen.[35] Simultaneously, the amount of Zn in feces might increase due to reduced absorption and increased excretion when the transport protein ZnT1 and MT are upregulated in the lumen.[35] Because MT binds Zn within the enterocyte, Zn is excreted when the enterocyte is sloughed off.[34,36]

Most other studies on the effect of dietary Zn levels found differences between treat-

Figure 4: Fluctuations in plasma Cu concentrations (μg/dL) throughout three reproductive cycles in non-supplemented, 50-mg per kg and 100-mg/kg supplemented sows (n = 36; 12 of each dietary treatment group). Values presented are means and their standard errors. Sows were group-housed between day 28 and day 108 of gestation on different floor types. Plasma Cu concentration did not differ between dietary treatment groups (*P* = . 37) or between floor types (*P* = .69), but was lower at day 143 than at day 0 or day 50 (*P* = .004, linear mixed model). Plasma Cu concentration was lower in reproductive cycle 3 than in reproductive cycles 1 and 2 (*P* = .006).



ment groups in plasma Zn[7-10] and liver and bone Zn concentration in sows[7,12,13,37] and piglets.[23,38-42] Most of these studies, especially the studies on sows, compared the responses between a non-supplemented and supplemented treatment group without addition of phytase, though diets were (semi) purified or used to induce Zn deficiency and do not represent practical conditions. The dietary Zn concentration of the control group was lower (< 35 mg Zn per kg) in these studies than in the concentration of the non-supplemented diet with phytase in the present study during gestation (47 mg Zn per kg). The lower dietary Zn concentration of the other studies suggests that the non-supplemented sows were not able to maintain homeostasis and that those dietary Zn concentrations were insufficient. Phytase was added in the present study to simulate practical conditions, but this may have increased Zn status more than Zn supplementation without phytase, as found in piglets.[23,40-42] However, in sows, addition of phytase did not affect the digestibility of Zn during gestation, but it did during lactation.[43] The long-term impact of the addition of phytase on Zn status is unknown in sows during reproduction. Nonetheless, these findings suggest that the basal dietary Zn concentrations with phytase were adequate for sows in the present study. Similar observations were found in rats, where lowered distribution between tissues, decreased fecal Zn excretion, and decreased concentration of Zn in some tissues (digestive organs, hair, plasma, and skeleton) at insufficient dietary Zn concentrations were found, whereas differences between tissue Zn concentrations were negligible between adequate and excessive dietary Zn intake.[30] At adequate and excessive dietary Zn concentrations, whole-body Zn concentration in rats seems to be unaffected.[28-31] This indicates that excessive Zn is not absorbed but excreted, or that Zn is redistributed between tissues and that this redistribution is enhanced with increasing dietary Zn concentrations to protect tissues.[14,44,45] However, disturbed homeostasis does not necessarily

indicate that a sow is (marginally) deficient, because despite the differences between treatment groups for plasma Zn concentrations in most other studies in sows, the concentrations reported in these studies are within the range of 50 to 150 µg per dL. It is possible that the remarkable fluctuations throughout the reproductive cycles, independent of dietary Zn concentration, had already overruled the effects of different dietary Zn intake. In the present study, plasma Zn and serum MT concentrations fluctuated during the reproductive cycle, which is in agreement with observations in other sow studies,[9,10,13,22,46] in women,[1] and in sheep.[47]

Liver and bone are major storage organs for Zn in pigs. Especially bone seems to accumulate Zn with increasing dietary Zn concentrations compared to other Zn status biomarkers, which plateau at lower inclusion levels.[23,41] Possibly in sows, liver and bone will accumulate Zn only when dietary Zn intake is substantially beyond Zn requirements, with or without addition of phytase, in an attempt to maintain Zn homeostasis. This suggests that in our study, the absorbed fraction was still at a level that did not require Zn accumulation in storage tissues. At excessive dietary Zn concentrations, Hill et al[12,13] found a linearly increased liver Zn concentration in sows fed diets reaching pharmacological dietary Zn concentrations (> 500 mg added Zn per kg diet). At these concentrations, Zn absorption may have changed from a carrier-mediated to a passive-transport pathway as found in piglets.[35] As a response, Zn absorption and Zn (re)distribution between body tissues are disturbed, resulting in increased Zn concentration in the liver.[12,13,35] The diet provided during lactation had a considerable Zn concentration (129 mg Zn per kg diet), which might have prevented a potential lack during gestation, meaning that, in general, the Zn concentration in the lactation diet needs to be considered in evaluating the impact of dietary Zn supplementation during gestation.

Figure 5: Fluctuations in serum metallothionein (MT) concentrations (ng/mL) throughout three reproductive cycles in non-supplemented, 50-mg/kg and 100-mg/kg supplemented sows (n = 36, 12 of each dietary treatment group). Values presented are means and their standard errors. Sows were group housed between day 28 and day 108 of gestation on different floor types. Serum MT concentration did not differ between dietary treatment groups ($P = .77$), but was lower for sows housed on the floor covered with a rubber top layer ($P = .003$). Serum MT concentration decreased from day 0 towards day 50 and day 108 and increased towards day 143 ($P = .01$, $P < .001$, and $P = .04$, respectively), but the MT concentration did not differ between day 50 and day 143 ($P = .29$; linear mixed model). Serum MT concentration was lower in reproductive cycle 2 than in reproductive cycle 3 ($P = .03$).

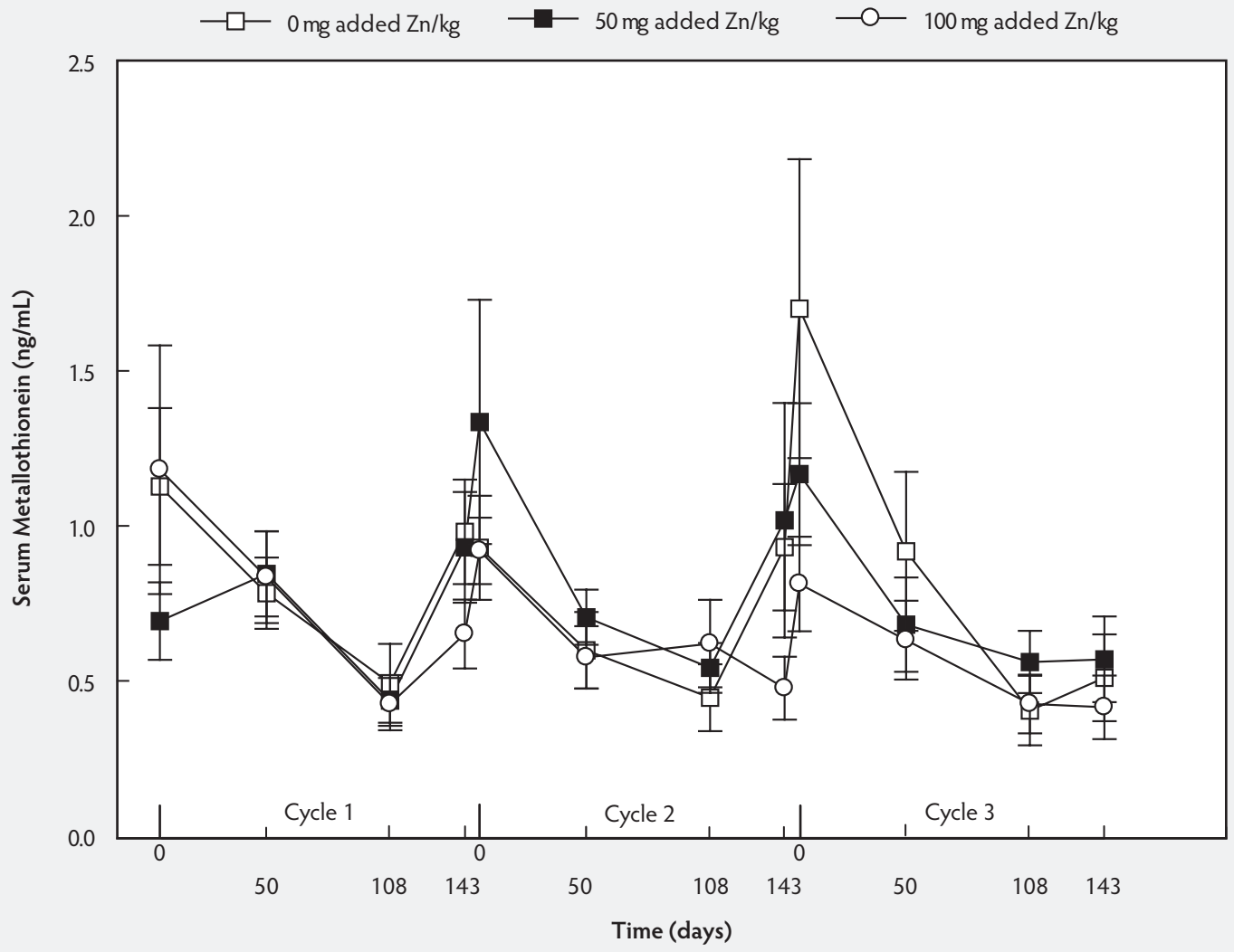


Table 5: Effect of floor type on Zn status biomarkers (n = 21 ± 4 sows per group)

	Dietary treatment* × Floor type							
Zn status biomarker	**0 × C**	**50 × C**	**100 × C**	**0 × R**	**50 × R**	**100 × R**	**SEM**	***P*†**
Plasma Zn	107.2	99.6	102.6	109.2	106.5	104.6	0.96	.28
Plasma Cu	238.2	234.3	221.6	237.7	207.1	231.4	2.22	.69
Serum MT	1.10	0.99	0.94	0.54	0.60	2.15	0.03	.003

* Dietary treatment is presented as 0, 50, or 100 mg Zn/kg supplemented to the basal diet containing 46.6 mg Zn/kg and 128.9 mg Zn/kg during gestation and lactation, respectively.

† As no interaction between phase of the reproductive cycle and floor type for Zn status biomarkers was observed, the average values are presented. Level of significance is $P < .05$, linear mixed models.

Zn = zinc; Cu = copper; MT = metallothionein; C = concrete floor; R = rubber floor.

Table 6: Effect of dietary Zn supplementation on mineral concentration of body tissues from slaughtered sows group-housed on different floor types between day 28 and day 108 of gestation (n = 92 sows for liver and bone, n = 36 sows for horn wall)

	Dietary treatment* × Floor type							***P*†**	
Body tissues	**0 × C**	**50 × C**	**100 × C**	**0 × R**	**50 × R**	**100 × R**	**SEM**	**Zn**	**F**
Liver‡									
Total liver weight (g)	2790.0	2912.5	2738.0	2892.2	2736.9	2601.3	54.9	.43	.59
Zn in FM (mg/kg)	56.2	57.6	53.7	53.4	51.4	51.3	1.7	.72	.30
Total liver Zn (mg)	155.0	162.5	147.2	152.1	137.0	133.4	5.2	.54	.22
Cu in FM (mg/kg)	26.5	30.6	36.1	43.0	47.9	30.0	3.6	.93	.22
Total liver Cu (mg)	75.2	81.7	99.4	121.3	142.1	81.6	10.7	.87	.21
Metacarpals 3 and 4									
Ash (g/kg FFDM)	625.0	620.0	618.0	631.0	621.0	624.0	0.2	.28	.25
Zn in ash (mg/kg)	57.6	50.6	56.6	63.5	54.1	64.0	2.2	.28	.19
Cu in ash (mg/kg)	9.9	9.1	8.5	8.8	8.1	10.1	0.3	.49	.80
Ca in ash (mg/kg)	394.9	395.6	397.3	395.1	394.6	394.1	0.8	.86	.40
P in ash (mg/kg)	179.5	181.8	180.4	181.0	182.7	179.4	0.5	.15	.64
Zn in FFDM (g/kg)	36.2	31.4	35.0	40.2	33.7	40.0	1.4	.26	.18
Cu in FFDM (g/kg)	6.2	5.6	5.3	5.6	5.0	6.3	0.2	.40	.90
Ca in FFDM, (g/kg)	246.6	245.2	245.5	249.2	245.2	245.8	0.9	.43	.55
P in FFDM (g/kg)	112.1	112.7	111.5	114.2	113.5	111.9	0.5	.38	.24
Horn wall Zn									
Dry matter (g/kg)	761.2	757.0	774.6	768.3	779.6	775.0	3.6	.46	.39
Zn in DM (mg/kg)	126.6	118.6	124.6	129.2	126.4	116.7	2.3	.39	.86
Cu in DM (mg/kg)	4.0	4.2	4.1	3.8	3.8	3.6	0.2	.94	1

* Dietary treatment is presented as 0, 50, or 100 mg Zn/kg supplemented to the basal diet containing 46.6 mg Zn/kg and 128.9 mg Zn/kg during gestation and lactation, respectively

† No interactions between dietary Zn supplementation and floor type were observed ($P > .30$, except for metacarpal Cu concentrations [$P > .07$]). These non-significant interactions were excluded from the final models and *P* values are presented for the main effect of dietary Zn supplementation (Zn) and for the main effect of floor type.

‡ Total liver weight may deviate due to losses during liver collection in the slaughterhouse. Metabolic weight is the $BW^{0.75}$ of sows before transport to slaughterhouse and did not differ between treatment groups ($P = .11$) and floor type ($P = .24$). Level of significance is $P < .05$, linear mixed models.

C = concrete floor; R = rubber floor; Zn = zinc; F = floor type; Ca = calcium; P = phosphorus; FFDM = fat free dry matter; FM = fresh matter; CU = copper; DM = dry matter.

Table 7: Effect of dietary Zn supplementation on metacarpal characteristics from slaughtered sows group-housed on different floor types between day 28 and day 108 of gestation (n = 54 for left front claw and n = 36 for right front claw).

	Dietary treatment† × Floor type							*P*‡	
Metacarpals*	**0 × C**	**50 × C**	**100 × C**	**0 × R**	**50 × R**	**100 × R**	**SEM**	**Zn**	**F**
Left front claw									
Length (mm)	90.6	89.8	88.1	89.7	92.0	91.2	0.4	.57	.15
Thickness (mm)§	19.7	20.7	19.8	19.8	20.3	20.1	0.1	.10	.46
Width (mm)§	17.3	17.3	16.7	16.2	16.7	17.7	0.1	**	
Weight (g)	43.6	45.3	41.4	43.3	45.8	45.6	0.7	.29	.24
Right front claw									
Length (mm)	88.6	88.2	88.7	89.3	90.2	88.8	0.3	.94	.26
Weight (g)¶	37.2	38.1	37.4	38.6	38.9	38.5	0.3	.88	.21

* Average of metacarpals 3 and 4 for length, weight, and (or) thickness and width.
† Dietary treatment is presented as 0, 50-, or 100-mg Zn/kg supplemented to the basal diet containing 46.6 mg Zn/kg and 128.9 mg Zn/kg during gestation and lactation, respectively.
‡ No interactions between dietary Zn supplementation and floor type were observed ($P = .23$, excluding $P = .07$ for bone thickness), except for bone width dimension. Non-significant interactions were excluded from the final models and *P* values are presented for the main effect of dietary Zn supplementation (Zn) and for the main effect of floor type. Level of significance is $P < .05$, linear mixed models.
§ Thickness and width dimensions of average metacarpal 3 and 4 of left front claw (Figure 1) were determined according to Combs et al.[25]
¶ Average metacarpal 3 and 4 of the right front claw were weighed after 24 hours at 75°C.
** Interaction of dietary Zn supplementation and floor type for bone width dimension, post-hoc partitioned *P* values are $P = .41$ (Concrete × dietary Zn supplementation) and $P = .02$ (rubber × dietary Zn supplementation).
C = concrete floor; R = rubber floor; Zn = zinc; F = floor type.

In the present study, sows' BW, backfat thickness, and BCS and the average BW of their weaned piglets were negatively affected for the 100-mg Zn per kg supplemented sows. Hill et al[12,13] observed a similar effect in sows, but only at much higher levels (5000 mg added Zn per kg diet), possibly because phytase was not included in their diets. Studies in cattle also found decreased performance characteristics in their supplementation group (Fagari-Nobijari et al,[48] 150 mg added Zn per kg DM; and Dermauw et al,[49] 540 mg added Zn per cow per day). It is not yet fully understood why greater Zn supplementation would reduce performance. The response seems to be mainly caused by events in the first reproductive cycle. Feed intake, Zn reserves, and Zn requirements cannot explain the lowered performance in the present study, and no antagonistic effects on Cu, calcium, or phosphorus were observed, which is in agreement with other studies in sows[12,13] and weaned piglets.[34] Furthermore, no noteworthy situations were reported throughout the experiment that could have influenced the observed results. Possibly, although not evaluated in the present study, protein expression in the liver and pancreas may be altered, which is related to cellular stress responses, transport, metabolism, and signal transduction, without influencing liver or pancreas Zn concentration or hepatic mRNA MT expression as found in piglets.[50,51] Further research is required. The poorer performance of the piglets seems a logical consequence of poorer sow performance.

No differences in the concentration of Zn status biomarkers were observed between dietary treatment groups in the present study, suggesting that Zn homeostasis was maintained and that Zn requirements were met, even for the non-supplemented sows. Floor type did not affect the observed responses of Zn status biomarkers. The reproductive phase had a more important effect on the measured parameters for Zn status with fluctuations noted throughout the cycle. The negative effect of higher dietary Zn supplementations on body weight of sows and piglets as apparently observed in other species suggests the need for additional mechanistic studies.

Implications

- Under the conditions of this study, commercially grown sows might not need supplemental Zn in their gestation diet beyond basal dietary Zn concentrations with phytase. On the basis of the assessment of Zn status biomarkers, there are no indications that Zn homeostasis is disturbed.
- During gestation, basal dietary Zn concentrations with phytase seem adequate for sows to maintain Zn homeostasis, but there is a need for mechanistic studies to evaluate the negative effect of higher dietary Zn supplementation on body weight of sows and piglets.
- Fluctuations of Zn status biomarkers throughout the reproductive cycle need to be taken into account when assessing Zn status.

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Conflict of interest

None reported.

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Brief communication

Alkaline stabilization of manure slurry inactivates porcine epidemic diarrhea virus

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Summary

Hydrated lime manure treatment was evaluated to determine porcine epidemic diarrhea virus (PEDV) susceptibility to alkaline stabilization. At pH 10, PEDV decreased (quantitative polymerase chain reaction) and lost infectivity (swine bioassay). Although ammonium decreased above pH 9 (up to 25%), alkaline stabilization managed to control potential infection from manure sources.

Keywords: swine, manure, porcine epidemic diarrhea, pH, hydrated lime

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Resumen – La estabilización alcalina del estiércol líquido desactiva al virus de la diarrea epidémica porcina

Se evaluó el tratamiento del estiércol con cal hidratada para determinar la susceptibilidad del virus de la diarrea epidémica porcina (PEDV por sus siglas en inglés) a la estabilización alcalina.

A un pH de 10, el PEDV disminuyó (reacción en cadena de la polimerasa cuantitativa) y perdió infectividad (bioensayo porcino). Aunque el amonio disminuyó arriba de un pH de 9 (hasta 25%), la estabilización alcalina logró controlar la infección potencial de las fuentes de estiércol.

Résumé – Inactivation du virus de la diarrhée épidémique porcine par stabilisation alcaline du purin

Un traitement du purin à l'aide de chaux hydratée a été évalué afin de déterminer la sensibilité du virus de la diarrhée épidémique porcine (VDEP) à une stabilisation alcaline. À une valeur de pH de 10 on nota une diminution du VDEP (réaction d'amplification en chaîne par la polymérase quantitative) et une perte d'infectivité (bio-essai sur des porcs). Bien que l'ammonium diminuait à pH au-dessus de 9 (jusqu'à 25%), la stabilisation alcaline a permis de limiter l'infection potentielle à partir du purin.

The emergence of the porcine epidemic diarrhea virus (PEDV) in the United States in 2013 resulted in billions of dollars in annual losses in the US swine industry.[1,2] Infection with PEDV causes severe diarrhea and vomiting in swine, spreads rapidly through ingestion of infected manure, and in naïve herds produces nearly 100% mortality in piglets less than 1 week old. Although the virus persists in feces for several days and may transport several miles from infected production sites as bioaerosol,[3,4] recent research indicates that management strategies can limit the virus' spread between production sites on transportation equipment.[5] However, concerns about virus persistence in various types of manure storage (ie, deep pit, lagoon, or slurry tank) remain a major barrier to proper manure management.

Because swine manure slurry is a valuable source of nitrogen and phosphorus, manure typically is utilized in agricultural fields for crop production. Proper manure handling and application practices are necessary to control the risk of pathogen re-infection at affected production sites, or infecting new sites through virus-contaminated manure-handling equipment. A variety of treatment options have been proposed and evaluated for their capacity to inactivate viruses in swine manure slurry.[6] Hydrated lime [$Ca(OH)_2$] has been demonstrated to inactivate porcine enterovirus types 2 and 3,[7] and alkaline stabilization is an approved treatment for septage prior to land application when a pH of 12 is maintained for at least 30 minutes.[8] However, increasing manure slurry pH may decrease its value as a fertilizer, since ammonia losses through volatilization would be enhanced. It was hypothesized that alkaline stabilization of manure would decrease infectious PEDV in swine production and in manure-handling systems. Laboratory studies were conducted to assess the abundance and survival of PEDV in stored swine manure slurry treated with hydrated lime and to quantify potential ammonia volatilization losses during hydrated lime treatment.

Materials and methods

The experimental protocol was approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee prior to the initiation of any research activity.

Manure for the first pH incubation study (conducted in 2015) was collected from swine that had been experimentally infected with PEDV strain CO/13 at the Life Sciences Annex at the University of Nebraska-Lincoln School of Veterinary Medicine and Biomedical Sciences (UNL VMBS).[9] Manure for the second study was collected in 2016 at a commercial breed-to-wean operation in south central Nebraska. At the commercial location, freshly excreted swine manure solids were collected into sterile sample containers from the floor surfaces in

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four separate farrowing room sites showing clinical signs of suspected porcine epidemic diarrhea, and transported on ice to the University of Nebraska-Lincoln. Prior to use in incubation studies, manure samples were confirmed as PEDV-positive using a reverse transcriptase polymerase chain reaction protocol (RT-qPCR). The quantification cycle (Cq) value for these manure sources was 23, equivalent to approximately 10^5 virus genomes per PCR reaction.

The first alkaline stabilization incubation had triplicate manure slurries consisting of fresh manure (UNL VMBS) and deionized water (final composition: 18.5% solids content, 38.4% "volatile" by combustion loss at 550°C). The three slurries were mixed and sampled prior to any treatment (time = 0 hours, no hydrated lime added). Each slurry was then distributed (250 mL) into two glass beakers (six total). Each pair received 1.5 g and 2.5 g of hydrated lime per L to achieve a final pH of 10 or 12, respectively. Aliquots (10 mL) were collected from each beaker at 1 and 12 hours following hydrated lime addition, immediately neutralized with 10 mM HCl, and frozen at -80°C for subsequent analysis.

In the second alkaline stabilization incubation, manure samples were collected at four replicate sites at the commercial swine operation. To better mimic the typical consistency of stored manure slurry, each manure sample was mixed in equal portion with deionized water (1 kg manure:1 L H_2O) prior to treatment (final composition: 21.6% total solids, 80.2% "volatile" by combustion loss). Each 250-mL replicate of slurry received stepwise (0.25 g) additions of hydrated lime with continuous stirring to gradually increase manure slurry pH to 12. After each addition of hydrated lime, pH was determined (FiveEasy Plus; Metter-Toledo AG, 8603 Schwerzenbach, Switzerland) and duplicate 2-mL samples of each manure slurry were collected, immediately neutralized (10 mM HCl), and stored at -80°C for subsequent PEDV RNA copy enumeration and infectivity in a pig bioassay.

A PCR approach was used to quantify PEDV genomes in manure samples. The RNA in each manure slurry sample was extracted using TRIzol reagent following manufacturer's suggested protocol for biological liquids and hard to lyse samples (Life Technologies, Carlsbad, California). Bead mill homogenization using 0.1-mm glass beads in an Omni Bead Ruptor (Omni International, Kennesaw, Georgia) at 4.5 meters per second for 45 seconds was included in the protocol to aid in cell lysis. An RT-PCR product was generated from RNA extracted from reference PEDV (CO/13) using primers and conditions as previously described.[10] Run-off transcripts were generated from the T7 promoter on the PEDV forward primer using the MEGAshortscript T7 kit (Invitrogen, Carlsbad, California). Transcripts were quantified by RiboGreen fluorometry (Turner BioSystems, Sunnydale, California), and then 10-fold serial dilutions of the transcripts were prepared at concentrations ranging from 1×10^1 to 1×10^6 copies of PEDV (as RNA targets) per µL for subsequent RT-qPCR. Quantification of PEDV genomes in the purified manure slurry RNA extracts was accomplished using an Applied Biosystems StepOnePlus thermal cycler (ThermoFisher Scientific, Waltham, Massachusetts), primers, probes, and amplification conditions as previously described,[10] with the exception that internal PCR probe contained both 3' Iowa Black fluorescence quencher and an internal ZEN quencher (Integrated DNA Technologies, Coralville, Iowa) located nine bases from the 5' end. Briefly, one step RT-qPCR was carried out in a 20-µL reaction containing 1 µL of RNA extract or RNA standard, 0.1 µL of both PEDV forward and reverse primer, 0.25 µL of PEDV internal PCR probe, 12.5 µL of QIAGEN QuantiTect Probe Taq enzyme mix, 0.25 µL QIAGEN QuantiTect Probe reverse transcriptase mix, and 5.8 µL of water. Thermal cycler conditions: initial reverse transcription at 50°C for 30 minutes, followed by initial denaturation at 95°C for 15 minutes, 40 cycles of denaturation at 94°C for 15 seconds, annealing at 60°C for 1 minute, and extension at 72°C for 30 seconds. All RT-qPCR runs had reported efficiencies > 80% and $R^2 > 0.997$.

Two swine bioassays were conducted with the alkaline stabilized and non-stabilized PEDV-infected manure slurry samples in order to relate RT-qPCR results with disease infectivity (Figure 1). For the first study, 15 pigs (approximately 21 days old) were sourced from a high-health facility whose dams tested negative for PEDV antibodies and virus by PCR. Pigs were tested for PEDV upon arrival and confirmed negative by fecal swab RT-qPCR. Pigs were each randomly assigned to individual housing in one of three BSL-2 animal rooms at the University of Nebraska-Lincoln Life Sciences Annex, grouped as follows, and allowed to acclimate for 3 days: control (three pigs), pH 10 manure (six pigs), and pH 12 manure (six pigs). Each pig was then administered a 10-mL oral gavage of diluted manure slurry from the first alkaline stabilization incubation (1 part manure slurry: 9 parts sterile buffer): three pigs in the control room each received one of the three un-limed slurry samples; six pigs in the pH 10 room received one of the six limed (pH 10) slurry samples (three limed for 1 hour and three limed for 12 hours); and six pigs in the pH 12 room received one of the six limed (pH 12) slurry samples (three limed for 1 hour and three limed for 12 hours). Pigs were monitored for fecal shedding of PEDV for 4 days until control animals began to demonstrate clinical signs of PEDV infection, at which time all pigs were humanely euthanized. Fecal swabs and ileum, jejunum, and mesenteric lymph node tissue samples were collected from each animal and fixed in formalin. Fecal and tissue samples were analyzed for the presence of PEDV by immunohistochemistry(IHC)[9] and RT-PCR (Cq only).

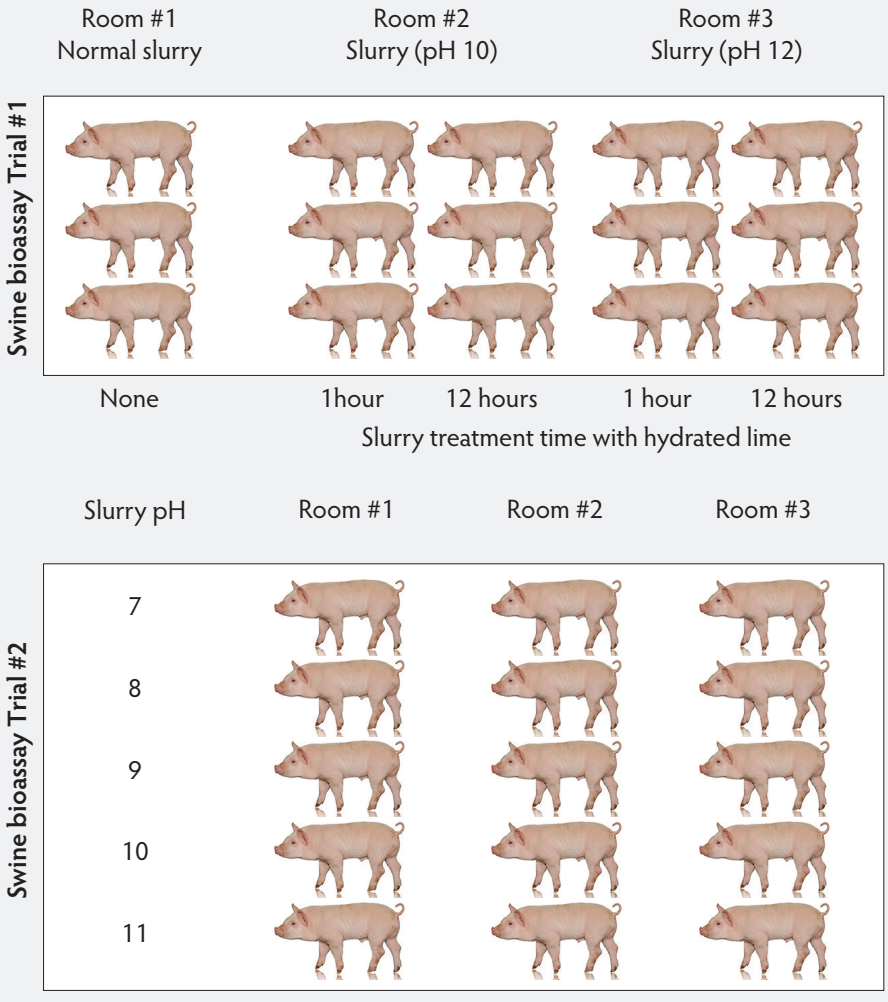
The second bioassay used a similar design, including pig source, history, age, housing, inoculation, and processing to assess PEDV infectivity in the various samples from the second incubation study. Manure slurry samples were selected from three of the manure slurries at points where pH was closest to 7, 8, 9, 10, or 11. Fifteen pigs were housed in three rooms (five per room) with one animal in each room receiving one of the five pH-diluted manure slurries by oral gavage. Pigs were monitored for signs of disease for a week prior to euthanasia. Fecal swabs and tissue samples were collected and tested for the presence of PEDV.

A third manure slurry incubation was conducted to assess changes in nitrogen content, since alkaline stabilization may enhance ammonia volatilization from treated manure during simulated storage in a deep pit or transport in a manure tank wagon. Fresh manure samples were collected from three replicate locations at the commercial site, diluted to create manure slurry (1 kg manure:1 L H_2O), and distributed into ten 250-mL bottles. Five bottles were each randomly assigned to one of two treatments: simulated storage in a manure pit (PIT) or simulated transport in a manure tank wagon (TANK), and hydrated lime additions were randomly applied to each manure slurry (n = 3) within PIT or TANK

blocks to achieve one of five pH endpoints: 8.0, 8.5, 9.0, 9.5 and 10.0. To mimic deep pit storage at a swine production site, PIT bottles were left uncapped while the trial was conducted. To mimic storage in a tank, the TANK bottles were tightly capped during the experiment. PIT samples (1 mL) were sampled initially and 24 hours following hydrated lime application (simulated overnight treatment). Samples (1 mL) from the TANK block were collected initially and 2 hours following hydrated-lime application (simulated short-term treatment). All samples were acidified with 20 µL of 10% sulfuric acid to adjust the pH to < 3 and refrigerated until analysis for ammonium using the Phenate method.[11]

ANOVA (SAS version 9.2, SAS Institute, Cary, North Carolina) was used to analyze log-transformed PEDV abundance in the first two manure alkaline stabilization incubations and to analyze ammonium percentage increase or decrease ($1\text{-}C_{final} \div C_{initial}$) in the third manure slurry incubation. For the first incubation, five treatments were compared (control, pH 10 for 1 hour, pH 12 for 1 hour, pH 10 for 12 hours, and pH 12 for 12 hours) with treatment as the main effect comparing log PEDV. In the second incubation, ANOVA was conducted using target pH (7, 8, 9, 10, and 11) as the main effect, comparing log PEDV. For the third incubation, manure storage and manure pH were the main effects, comparing the ammonium percentage increase or decrease.

Figure 1: For the swine bioassay, pigs were randomly assigned to multiple rooms and housed in individual crates. The pigs were administered diluted, PEDV-positive manure slurry (untreated and treated with hydrated lime) and monitored for several days for signs of disease (including PEDV-specific PCR of fecal swabs). After euthanasia, additional gastrointestinal tissue samples were collected for PCR and immunohistochemistry tests. PEDV = porcine epidemic diarrhea virus; PCR = polymerase chain reaction.



Results

In the first manure slurry incubation, RT-qPCR analysis of samples detected PEDV RNA sequences in all treatments (hydrated lime or untreated) except at pH 12 after a 12-hour incubation (Table 1). A clear trend for lower PEDV abundance with hydrated-lime addition (pH 10 versus 12) and with increased hydrated-lime exposure time (1 versus 12 hours) was observed. In the swine bioassay, pigs receiving limed manure treatments (pH 10 or 12 incubated for 1 or 12 hours) via oral gavage displayed none of the clinical signs of PEDV infection (eg, diarrhea, dehydration, or vomiting) and did not shed PEDV in the feces (as determined by PCR). All control pigs (n = 3) receiving un-limed manure displayed clinical signs of disease, tested positive for PEDV infection via IHC, and shed PEDV in the feces (ie, had a low Cq by RT-PCR).

In the second manure slurry incubation, stepwise addition of hydrated lime gradually increased the pH of the manure slurries (Figure 2). Quantitative PCR analysis of samples revealed a rapid decline in the number of PEDV copies above pH 10, but no change in the abundance of PEDV targets below pH 10 (10^9 PEDV targets per gram of manure slurry). Swine bioassay results on a subset of those samples were consistent with RT-qPCR results: IHC and RT-PCR detections of PEDV were observed only in pigs exposed to manure slurry when the pH was less than 10 (Table 2).

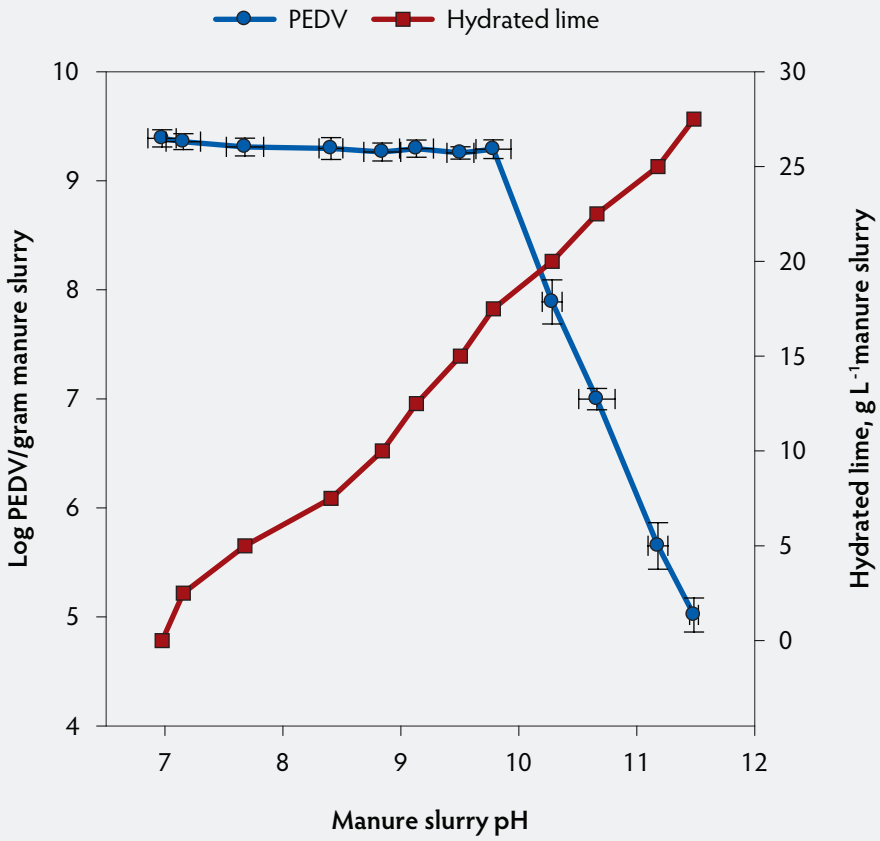
For the final manure slurry incubation, initial ammonium concentrations varied considerably between the three replicate locations at the commercial site (0.90 ± 0.06, 1.89 ± 0.17, and 2.49 ± 0.24 g NH_4^+ per L). Prior to statistical analysis, final concentrations were normalized to initial concentration for each manure slurry container yielding a percentage increase or decrease ($1\text{-}C_{final} \div C_{initial}$). Of the two main effects (manure storage and manure pH) and their interaction term, only manure pH proved to be significant ($P < .05$). During manure storage, the average ammonium content increased by 6.6%. The largest differences in manure slurry ammonium content were found between low pH (8, 8.5, and 9) and high pH (9.5 and 10) manure samples ($P < .01$). Ammonium in the low pH group increased an average of 15.7% ± 3.9% relative to initial concentrations. In comparison, ammonium in the high pH group decreased by 7.1% ± 3.5%.

Table 1: Effect of hydrated lime manure treatment exposure (1 or 12 hours) at pH 10 or 12 on porcine epidemic diarrhea virus (PEDV) abundance and potential to cause disease

Treatment	Time (hours)	Slurry PEDV* log/gram	Pig bioassay†	
			IHC (%)	Rectal swab (Cq)
None	0	9.16 ± 0.02[a]	3/3 (100)	20.4, 23.4, 20.7
pH 10	1	7.00 ± 0.36[b]	0/3 (0)	All > 40
	12	5.38 ± 0.33[c]	0/3 (0)	All > 40
pH 12	1	4.5 ± 0.01[c]	0/3 (0)	All > 40
	12	BD	0/3 (0)	All > 40

* Log RNA targets/g wet manure slurry determined by reverse transcriptase quantitative PCR ± 1 SE; (Cq > 40 ≈ 10^4 per gram in manure slurry or 25 copies/PCR reaction).
† IHC was performed as previously described.[9] Cq = quantification cycle for rectal swab at necropsy. Cq values ≤ 35 were considered positive and > 35 were considered negative.
[abc] Values within a column with different superscripts are significantly different ($P < .05$; ANOVA).
BD = below detection; IHC = immunohistochemistry.

Figure 2: Effect of increasing hydrated lime amendment during alkaline stabilization on swine manure slurry pH and PEDV genome abundance assessed using reverse transcriptase quantitative PCR. Error bars = 1 SE; PEDV = porcine epidemic diarrhea virus; PCR = polymerase chain reaction.



Discussion

Alkaline stabilization was achieved in manure initially containing 10^9 PEDV targets per gram of slurry at and above pH 10 (ie, infectivity was eliminated). Comparing the pig bioassay results with RT-qPCR results, an interesting relationship emerges. Although reduced by more than 100-fold above pH 10, PEDV target genomes could still be detected at 10^5 to 10^7 per gram of slurry. Alkaline stabilization impeded virus infection but did not destroy all past evidence of the presence of the virus (ie, some remnant RNA persisted for a short period of time). Alkaline pH likely altered virus envelope integrity, which released PEDV RNA into the manure slurry where RNA was quickly hydrolyzed. Not all animals exposed to PEDV-contaminated manure treated below pH 10 became infected with PEDV, particularly animals in the second study. It was noted that the pigs in the second manure slurry trial were slightly larger than those in the first trial, and this may account for the lower incidence of disease in pigs exposed to manure slurry below pH 10.

Ammonium increased by a substantial fraction in the third manure slurry incubation, particularly in the lower pH treatments (8, 8.5, and 9). Decomposition processes in the lower pH fresh manure (urea hydrolysis and organic matter decomposition) likely account for the increase, while higher pH may have inhibited these decomposition processes. Additionally, in the manure samples of higher pH (9.5 and 10), the dissociation of ammonium to ammonia (pK_a 9.25) would shift ammonium to ammonia, which is more

Table 2: Effect of incremental hydrated lime addition on manure slurry pH and PEDV abundance and potential to cause disease

			Pig bioassay†	
Hydrated lime (g/L)	**Average pH**	**Slurry PEDV* log/g**	**IHC (%)**	**Rectal swab (Cq)**
0.0	6.92	9.36[a]	1/3 (33)	> 40; 25.93; > 40
6.7	8.14	9.26[a]	1/3 (33)	25.49; > 40; > 40
12.5	9.13	9.23[a]	2/3 (67)	23.25; >40; 23.31
18.3	9.96	8.66[a]	0/3 (0)	All > 40
24.2	11.07	6.15[b]	0/3 (0)	All > 40

* Log RNA targets/g wet manure slurry determined by reverse transcriptase quantitative PCR.
† IHC performed as previously described.[9] Cq = quantification cycle for rectal swab at necropsy. Cq values ≤ 35 were considered positive and > 35 were considered negative.
[ab] Values within a column with different superscripts are significantly different ($P < .05$, ANOVA).
PEDV = porcine epidemic diarrhea virus; IHC = immunohistochemistry.

easily lost via volatilization. Although simulated storage (PIT versus TANK) showed no difference, slurry pH had a dramatic effect on ammonium concentrations (up to 25% difference between low and high pH) after a short incubation period.

While "lime" is a term broadly used to describe calcium-containing inorganic materials, "quicklime" applies to the chemical compound calcium oxide (CaO), which is unstable and highly reactive to moisture. To reduce the reactivity of quicklime and make it more stable, water is often added to quicklime to convert all oxides of calcium and magnesium to hydroxides. The resulting compound, calcium hydroxide [$Ca(OH)_2$], is sold under a number of different names, including hydrated lime, slaked lime, caustic lime, and others. Among other applications, hydrated lime is commonly used during the cleanup phase after a disease outbreak in livestock production systems. Despite being more stable than quicklime, hydrated lime is still caustic and quick to react with water, so it must be handled with care. Precautions should be taken to protect against inhalation or contact with skin and eyes. In addition to keeping arms and legs covered, gloves, safety goggles and a dust mask should be worn during handling.

To accomplish alkaline treatment of manure slurry at a swine production site using hydrated lime, a dosing rate of approximately 23 kg (50 lb) of hydrated lime per 3800 L (1000 gal) of manure is recommended. At a cost of around $40 for a 50-lb bag, treatment of a full 5000-gal slurry tank spreader can be accomplished for approximately $200 (USD). Addition of the hydrated lime to the tank wagon prior to it being filled with slurry is recommended to facilitate mixing. Addition of lime while a slurry tank is being filled with manure is not recommended, since the concentration of ammonia gas emanating from the tank wagon fill port could be high enough to cause asphyxiation for the person adding lime at the tank port. While the research presented included an analysis of ammonia loss during treatment of stored slurry, the addition of hydrated lime to deep pit manure storages is not recommended. The substantial amount of ammonia gas generated during alkalization of an entire manure pit containing several thousand gallons of manure slurry may pose a significant health risk to workers and animals in and near the production facility.

Implications

- Alkaline stabilization through hydrated lime addition to achieve a threshold pH 10 for 1 hour is sufficient to deactivate the porcine epidemic diarrhea virus in manure slurry on the basis of bioassay outcomes. Although PEDV was still detectable above pH 10 by RT-qPCR (10^5 to 10^7 genomes per gram manure slurry), no disease risk was observed.
- Important questions remain regarding the minimum treatment time needed for alkaline stabilization and whether longer treatment periods at < 10 pH are as efficacious as briefer, higher pH treatment.
- Raising manure slurry pH above 9.25 will likely enhance ammonia losses by volatilization and decrease fertilizer N value. Alkaline stabilization of manure slurry could present a risk for ammonia asphyxiation during manure treatment and pumping if proper air flow is inadequate.

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Conflict of interest

JDL, co-author, has served as a consultant for, and thus has disclosed a significant financial interest in, Harrisvaccines, Inc. In accordance with its Conflict of Interest policy, the University of Nebraska-Lincoln's Conflict of Interest in Research Committee has determined that this must be disclosed.

Disclaimer

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Conversion tables

Weights and measures conversions

Common (US)	Metric	To convert	Multiply by
1 oz	28.35 g	oz to g	28.4
1 lb (16 oz)	453.59 g	lb to kg	0.45
2.2 lb	1 kg	kg to lb	2.2
1 in	2.54 cm	in to cm	2.54
0.39 in	1 cm	cm to in	0.39
1 ft (12 in)	0.31 m	ft to m	0.3
3.28 ft	1 m	m to ft	3.28
1 mi	1.6 km	mi to km	1.6
0.62 mi	1 km	km to mi	0.62
1 in^2	6.45 cm^2	in^2 to cm^2	6.45
0.16 in^2	1 cm^2	cm^2 to in^2	0.16
1 ft^2	0.09 m^2	ft^2 to m^2	0.09
10.76 ft^2	1 m^2	m^2 to ft^2	10.8
1 ft^3	0.03 m^3	ft^3 to m^3	0.03
35.3 ft^3	1 m^3	m^3 to ft^3	35
1 gal (128 fl oz)	3.8 L	gal to L	3.8
0.264 gal	1 L	L to gal	0.26
1 qt (32 fl oz)	946.36 mL	qt to L	0.95
33.815 fl oz	1 L	L to qt	1.1

Temperature equivalents (approx)

°F	°C
32	0
50	10
60	15.5
61	16
65	18.3
70	21.1
75	23.8
80	26.6
82	28
85	29.4
90	32.2
102	38.8
103	39.4
104	40.0
105	40.5
106	41.1
212	100

°F = (°C × 9/5) + 32
°C = (°F - 32) × 5/9

Conversion chart, kg to lb (approx)

Pig size	Lb	Kg
Birth	3.3-4.4	1.5-2.0
Weaning	7.7	3.5
	11	5
	22	10
Nursery	33	15
	44	20
	55	25
	66	30
Grower	99	45
	110	50
	132	60
Finisher	198	90
	220	100
	231	105
	242	110
	253	115
Sow	300	135
	661	300
Boar	794	360
	800	363

1 tonne = 1000 kg
1 ppm = 0.0001% = 1 mg/kg = 1 g/tonne
1 ppm = 1 mg/L

Peer reviewed

Literature review

Shoulder lesions in sows: A review of their causes, prevention, and treatment

Fiona C. Rioja-Lang, MSc, PhD; Yolande M. Seddon, MSc, PhD; Jennifer A. Brown, MSc, PhD

Summary

Severe shoulder lesions in sows are manifested as ulcers comparable to pressure ulcers in humans. In sows, shoulder lesions appear on the skin overlying the bony prominence of the scapula, and are most commonly observed in the first weeks of lactation. Shoulder ulcers arise due to prolonged compression of blood vessels around the tuber of the scapular spine when the sow is lying, leading to insufficient blood circulation, necrosis, and subsequent ulceration. Due to the nature of shoulder lesions and their estimated occurrence (5%-50% of breeding sows worldwide), they represent an obvious welfare concern. There is also an economic impact due to labor time for treatment, medication, and premature culling of sows. While multiple factors contribute to ulcer development, maintaining optimum body condition in sows appears to be a key factor in prevention. This review summarizes the literature on sow shoulder ulcers, including the causes, prevention, and treatment. Regular monitoring of lesions is recommended, as this will help to identify individual farm causes and prevention measures. While much is known about shoulder ulcers, we conclude that there are significant gaps in the scientific literature regarding the mechanisms of development and healing, pain caused, and effective means for treatment and prevention.

Keywords: swine, sows, shoulder lesions, review, welfare

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Resumen – Lesiones de hombro en hembras: Una revisión de sus causas, prevención, y tratamiento

Las lesiones severas de hombro en hembras se manifiestan como úlceras comparables a las úlceras de presión en humanos. En las hembras, las lesiones de hombro aparecen en la piel sobre la prominencia de hueso de la escápula, y se observan más comúnmente en las primeras semanas de lactancia. Las úlceras de hombro surgen debido a la compresión prolongada de vasos sanguíneos alrededor del tubérculo de la espina escapular cuando la hembra está acostada, llevando a una circulación de sangre insuficiente, necrosis, y la ulceración subsecuente. Debido a la naturaleza de las lesiones de hombro y su ocurrencia estimada (5%-50% de las hembras de cría en todo el mundo), estas representan una obvia preocupación de bienestar. Hay también un impacto económico debido al tiempo de trabajo utilizado para su tratamiento, medicación, y el desecho prematuro de hembras. Si bien, múltiples factores contribuyen al desarrollo de la úlcera, mantener una condición corporal óptima de las hembras parece ser un factor clave en su prevención. Esta revisión resume la literatura de las úlceras de hombro en hembras, incluyendo las causas, prevención y tratamiento. Se recomienda el monitoreo regular de las lesiones ya que esto ayudará a identificar las causas individuales en la granja y las medidas de prevención. Aunque se sabe mucho de las úlceras de hombro, concluimos que hay una falta significativa de datos en la literatura científica sobre los mecanismos de desarrollo y curación, dolor causado, y medios efectivos de tratamiento y prevención.

Résumé – Lésions aux épaules chez les truies: Revue des causes, de la prévention et du traitement

Les lésions sévères aux épaules chez les truies se manifestent comme des ulcères comparables aux ulcères de décubitus chez les humains. Chez les truies, les lésions aux épaules apparaissent sur la peau recouvrant la proéminence osseuse de l'omoplate, et sont le plus fréquemment observées durant la première semaine de lactation. Les ulcères de l'épaule surviennent suite à la compression prolongée des vaisseaux sanguins autour de la tubérosité de l'épine scapulaire lorsque la truie est couchée, entrainant une circulation sanguine insuffisante, de la nécrose, et une ulcération subséquente. Étant donné la nature des lésions aux épaules et leur fréquence estimée (5%-50% des truies reproductrices mondialement), elles représentent un souci évident relativement au bien-être. Il y a également un impact économique étant donné le temps passé pour traiter, la médication, et la réforme prématurée des truies. Bien que de multiples facteurs contribuent au développement des ulcères, le maintien de la condition corporelle optimale des truies semble être un facteur clé dans la prévention. Cette revue résume la littérature sur les ulcères de l'épaule chez les truies, incluant les causes, la prévention et le traitement. Une surveillance régulière des lésions est recommandée étant donné que ceci aidera à identifier les causes dans les élevages de manière individuelle et les mesures de prévention. Bien que plusieurs choses soient connues sur les ulcères de l'épaule, nous avons conclu qu'il y a des lacunes importantes dans la littérature scientifique en ce qui concerne les mécanismes de développement et la guérison, la douleur causée et des moyens efficaces de traitement et de prévention.

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Introduction

Several terms are used to describe skin sores in the shoulder region of sows. These include (but are not limited to) shoulder lesions, shoulder ulcers, decubital shoulder ulcers (stemming from the Latin word "decumbre" meaning to lie down),[1] shoulder sores, and abrasions. The terms "shoulder lesions" and "shoulder ulcers" are often used, erroneously, as synonyms. Jensen[2] rightfully pointed out that when considering skin sores in the shoulder region of sows, it is essential to differentiate between shoulder ulcerations and non-ulcerating shoulder lesions. Shoulder lesions can take any form, from mild with intact epithelium or simple abrasions, to severe. Shoulder ulcers are a more severe subset of shoulder lesions where there is necrosis of epidermis, loss of basement membrane, and effacement of superficial adnexal structures, manifested and commonly referred to as "open sores."[3] Decubital shoulder ulcers in sows are comparable with pressure ulcers in humans (bed sores).

In sows, shoulder ulcers often appear over the underlying bony prominences, in which the amount of soft tissue (eg, muscular and [or] adipose tissue) between the skin and bone is insufficient to distribute external pressure.[4] The reduced body condition of sows during lactation, combined with the prolonged recumbency during nursing, increases the incidence of shoulder ulcers.[5] However, the precise mechanism behind the development of shoulder lesions is not well understood. There remain several opinions as to how and why pressure leads to tissue breakdown.[6] It is thought that the ischemia (restriction of blood flow) results in insufficient blood circulation, causing necrosis and subsequent ulceration. Severity depends on the force and duration of the pressure, but is also influenced by the robustness of the skin.[4] Prospective and cross-sectional studies have determined that these wounds typically develop in the first week after farrowing.[7,8] It is estimated that the majority of shoulder lesions are present for at least 2 to 3 weeks and that some lesions will develop into ulcers during this period.[6] The severity of shoulder lesions can vary greatly, ranging from superficial lesions to deep subcutaneous ulcers.

For this review, the term "shoulder lesion" will be used to broadly refer to abnormal structure of skin, and "shoulder ulcer" will be used to specifically identify a wound with loss of overlying epithelium.

The occurrence of shoulder lesions as established by cross-sectional studies on-farm and in abattoirs reveals a large between-herd variation in lesion prevalence, from 4.6%[9] to 50%.[5] Where studies have taken repeated data from herds, a large range of within-herd prevalence has also been found (eg, Cleveland-Nielsen et al 2004[10]), which reflects sow management decisions. A summary of studies recording the prevalence of shoulder lesions is presented in Table 1. These studies cover a variety of housing types, genotypes, and stages of gestation, and include both ante- and post-mortem observations, which must be considered alongside results. Regardless, the large between-herd variation in prevalence highlights the influence of farm facilities and sow management on the development of lesions. Surveys may underestimate the prevalence of shoulder ulcers because sows with severe ulcers may be euthanized, and thus not recorded. On the other hand, survey summaries may overestimate the prevalence of shoulder ulcers because lesions (eg, abrasions) that are not ulcerated may be included in the definition. While this research suggests that shoulder lesions likely have an economic impact on pork production, there is currently no information on the overall cost of this problem, or the cost-benefit of treatment options.

The degree of pain caused by shoulder lesions is poorly understood, however human patients with pressure ulcers self-report pain.[19,20] On the basis of human literature, sows may also experience varying degrees of pain at different stages of severity.[21] Presently, no pain relief is typically given for the treatment of shoulder lesions or shoulder ulcers. Ulcers also provide a portal of entry for pathogens that may cause local or systemic infection.[4]

Materials and methods

The objective of this review was to collect and review the literature related to sow shoulder lesions from a range of sources, to explore the major causes leading to their development, treatment, and prevention, and to identify potential areas for future research. The information presented is excerpted from a comprehensive report that was funded by the National Pork Board. This review is targeted towards pork producers, veterinarians, researchers, and students.

The main databases used were AGRICOLA, CAB International, Scopus, and Science Direct. Because of the limited literature available, both peer-reviewed and non-peer-reviewed resources were evaluated for inclusion. The non-peer-reviewed information was largely published by industry or academics (eg, National Pork Board, British Pork Executive, or conference abstracts). They were still based on science, but did not go through the same rigorous process as a peer-reviewed journal article. Very old studies, > 30 years old, were unlikely to be relevant due to the changing nature of the swine industry (eg, heavier animals, larger litters). After the initial collection of material, the literature selection was refined to remove older studies. References from as early as the 1980s were included if they provided useful information that is relevant to present-day sow management.

Causes of shoulder lesions

Anatomy of the sow shoulder

The scapula or shoulder blade of the pig is a large, flat bone located over the rib cage with muscle attachment by *M infraspinatus, M supraspinatus*, and *M deltoidius*. From the cranial aspect (front), a ridge or spine is present on the side of the scapula that terminates dorsally in a large bump, known as the prominent tuber. When the sow lies laterally, the anatomy and location of the prominent tuber results in pressure being exerted on the overlying tissue, and predisposes this area to pressure ulcers.

Sow-related risk factors

Numerous pig-related risk factors have been identified as contributing to the development of shoulder lesions, including (but not limited to) body condition post farrowing,[14,22] parity,[18,23] health status (underlying disease),[18] lameness,[11,23] previous history of shoulder lesions,[24] weaning weight of the litter,[18] lactation length,[25] sow behavior (unrelieved pressure),[6] breed,[18] and genetics.[5,26] Studies have found that sows with a body condition score (BCS) < 3 at weaning,[17] or ≤ 2 during gestation,[18] have a three-fold greater likelihood of developing shoulder lesions than do those with BCS ≥ 3. A low BCS reduces the cushion of fat covering the tuber of the scapula,[17] increasing the likelihood of lesion development.

Anil et al[11] studied 162 sows from four US farms and reported that longer lactation periods presented a risk for increased likelihood of lesion development. Similarly, a

Table 1: Studies reporting on the prevalence of shoulder lesions in sows including study location and key findings or associations*

Authors	Year	Country	No. of sows in study	% of sows observed with lesions	Method: Post mortem/ante mortem	Findings/association with lesion prevalence
Anil et al[11]	2006	United States (USA)	162	33%, of which 19% bilateral lesions	On-farm (4 herds), before weaning	↑ Longer lactation length ↑BCS ≤ 2 ↑ Lameness ↔ Parity ↔ Farrowing performance
Bausted and Fredriksen[12]	2006	Norway	3048	10%	Post mortem (4 abattoirs)	↓ BCS ↑ Body size
Cleveland-Nielsen et al[10]	2004	Denmark	23,794	0% to 40% within herd prevalence	Post mortem (207 herds, sampled in 4 abattoirs)	Large within and between herd prevalence: indicates varying management and farm factors
Davies et al[7]	1996	USA	1916	8%, of which 4% bilateral	On-farm, (one herd)	↓ BCS
Davies et al[8]	1997	USA	147	16% to 48%	On-farm (prospective study)	↑Parity ↓ Scapular tuber depth significantly associated with ulcers and ulcer size
Deen[13]	2010	USA	157	33%	On-farm	↓ Increased backfat at 109 days gestation ↑ Cast iron slats ↓ Rubber mats ↔ Lameness ↔ Time in lateral recumbency
Havn and Poulsen[14]	2004	Denmark	429	14% to 37%	On-farm	↑ In farrowing area ↓BCS ↑ Parity
KilBride et al[15]	2009	United Kingdom	344	10%	On-farm (4 lactating sows from 86 herds)	↓ Outdoor housed sows ↑ Fully slatted floors ↓ > 20 cm between tail and back of crate
Knauer et al[16]	2007	USA	3146	18%	Post mortem	↓ BCS
Dahl-Pederson et al[17]	2013	Denmark	2733	5%	On-farm (37 herds)	↔ Condition of concrete floors in farrowing pen ↓ BCS
Ritter et al[9]	1999	USA	1751	5%	Post mortem	↓BCS
Zurbrigg[18]	2006	Canada	312	34%	On-farm	↓BCS ↑ Flank-to-flank at weaning, breed, parity, farrowing room section, weaning weight of litter

* Arrows denote the relationship between risk factor and development of shoulder lesions: ↑ = positive association; ↓ = negative association; ↔ = no relationship.

BCS = body condition score.

heavier litter weaning weight was identified as a significant risk factor[18] on one Canadian farm, and Ocepek et al[27] identified purebred maternal sow lines, and high-producing first-parity sows in particular, as being at greater risk. These studies link shoulder lesions to high maternal investment by the sow.[5] Ultimately, these factors can be mitigated by appropriate management of the sow during the lactation period. Therefore, competent management of high-producing sows during lactation, or lack thereof, may have the greatest impact on lesion development. Three conditions that create inappetence in the sow, namely, disease, injury, and climatic extremes,[18] create challenges to maintaining sow condition, especially during lactation. Furthermore, these factors can also influence sow activity and lying patterns, which may further increase the risk of lesion development. Hence, rapid identification of the cause of inappetence and prompt interventions to rectify are keys to reducing the risk of lesion development.

Sow behavior

Sow behavior can impact the occurrence of shoulder lesions. Factors within the environment influence the behavior of the sow, including the floor surface, the environmental temperature, and the health and comfort of the sow. However, individual sow characteristics also influence sow behavior. The duration of lateral recumbency has been identified as a major contributing cause of shoulder lesion development. Lateral recumbency is the predominant posture of post-parturient sows and is necessary for nursing piglets. Shoulder ulcers are also more common in diseased or lame sows,[7] which may be linked to increased lateral recumbency (as reported in sows in which lameness was induced).[1,25] A Danish study by Larsen et al[28] compared the behavior of 19 sows with shoulder ulcers and 19 sows without ulcers. They found that sows with shoulder ulcers spent less time lying and nursing, and more time standing still. Affected sows performed a greater amount of shoulder rubbing and tended to perform a greater number of postural changes than did sows with no ulcers,[28] which may indicate discomfort. There is little research on the relationship between sow behavior and development of shoulder lesions. However, research to identify relationships between sow lying postures and duration, movement in relation to shoulder ulcer development, and correlations with other sow and management factors, would provide a better understanding of the problem.

Heritability of shoulder lesions

Several researchers have estimated the heritability of shoulder lesions[5,26,29] or have observed breed differences in the prevalence of shoulder lesions.[18,27] Lundeheim et al[26] reported that shoulder ulcers are a heritable trait, specifically, the heritability was estimated at 0.13, which was based on a population of Swedish Yorkshire sows (including 4336 farrowings in 2634 sows). Hedebro Velander et al[29] reported a similar figure for heritability of shoulder ulcers ($h^2 = 0.18$) and also calculated the heritability of the size of ulcers ($h^2 = 0.09$) in Landrace × Yorkshire crossbred sows. Lundgren et al[5] estimated the heritability of shoulder ulcers and the genetic correlations between shoulder ulcers, mean piglet weight, and sow body condition. Data were extracted from the Norwegian litter recording scheme, and the genetic analysis included 5549 Norwegian Landrace sows (7614 lactations) in 45 herds. Their results estimated the heritability of shoulder ulcers to be 0.25. Lundgren et al[5] also found a genetic correlation between shoulder ulcers and mean piglet weight. The correlation was low but positive ($r^2 = 0.23$), indicating that the sow's ability to raise heavy piglets is associated with a higher risk of shoulder ulcers. The authors concluded that high-producing sows are at greater risk of developing shoulder lesions than are low-producing sows. This conclusion is supported by recent work by Ocepek and colleagues,[27] who compared productivity and prevalence of shoulder lesions in sows from purebred maternal (Norsvin Landrace) and crossbred (Norsvin Landrace and Swedish Yorkshire) lines. Shoulder lesions were most common in first-parity sows from purebred maternal lines ($P < .001$), and were associated with higher litter weights at birth ($P = .003$) and weaning ($P = .05$), and greater weight loss during lactation ($P = .016$). Zurbrigg[18] compared Duroc, Landrace, and Yorkshire sows in a commercial herd in Ontario, Canada, and found that Landrace and Duroc sows were 3 and 4.6 times (respectively) more likely to develop shoulder lesions than Yorkshire sows ($P < .05$).

It can be concluded the propensity for a sow to develop shoulder ulcers is heritable, at least in so far as greater prevalence can be found in specific lines. On this basis, it should be possible to reduce their prevalence using appropriate selection and breeding programs. However, the heritability of ulcers is likely to be linked to selection for other production traits, as indicated by the findings of Lundgren et al,[5] which may hinder the ability to reduce their prevalence through selection.

Environmental risk factors

Several environmental risk factors have been identified as contributing to the occurrence of shoulder lesions, including flooring type,[23,30] pen location,[18] temperature and humidity,[31,32] type of sow housing,[33] and friction properties of the floor.[34]

Environmental risk factors can be described at both individual sow and herd level. In farrowing pens (crates), flooring type has been associated with the risk of developing limb and body lesions.[30] Metal slatted flooring is a risk factor for having more sows with shoulder lesions when compared with those housed on solid concrete, because slatted floors support the sow's body weight over a smaller surface area.[17] KilBride et al[15] found there was an increased risk for body lesions when the lying surface was either damaged or soiled when compared to clean, dry, and (or) undamaged floors.

Farrowing crate floors should provide a comfortable surface for lying, sufficient space for comfortable nursing, a non-slip surface for rising and standing, and separation from excreta, and must be sufficiently robust for the sow's size and weight.[15] In the human medical literature it is believed that kinetic friction forces rubbing the skin, possibly in combination with increased skin moisture, contribute to the development of pressure ulcers.[34] Friction, along with other flooring properties, such as abrasiveness, hardness, surface profile, and thermal properties,[35] may all contribute to the development of shoulder lesions in sows and should be explored further.

The location of sows within a farrowing room can also contribute to the development of shoulder lesions because of variation in climatic conditions associated with room temperature fluctuation, location of ventilation units, and the use of drip coolers. No data are currently available regarding the direct effect of temperature on the prevalence of shoulder ulcers in sows.[4] Sow movement is likely reduced at higher temperatures, and could thus be a contributing factor in the development of shoulder ulcers. Citations from human medical literature often conclude that moisture, humidity, and temperature are likely to play a role in the development of bed sores.[34,36,37]

There is an increasing global trend to reduce close confinement management of sows. Many countries have placed a ban or partial ban on the use of gestation stalls, and alternate indoor farrowing systems are being explored, from farrowing pens to group lactation systems.35,38 The greater freedom of movement provided to sows in these systems may increase muscle tone and encourage more frequent postural changes, which may help to reduce the incidence of shoulder lesion development in sows. The use of bedding or alternative flooring types (ie, solid flooring, rubber-coated flooring) in these systems may also influence the development of shoulder lesions. Assumptions driving these changes in system design and their outcomes on sow welfare and longevity are worthy of investigation.

Interventions and treatment

The primary intervention for sows with shoulder ulcers is to move them into pens with softer flooring. Deep straw bedding provides the correct properties for improving comfort by providing wider distribution of pressure for lying sows. However, in many modern pig production facilities, the use of straw is not feasible because of incompatibility with liquid manure disposal systems.[39,40] In Denmark, a pathoanatomical scale from 0 to 4 is used to grade shoulder ulcers, where grade 0 is no lesion and grade 4 is a lesion (ulcer) involving all three layers of the skin and underlying bone. On Danish farms, sows with grade 3 or 4 lesions must be kept loose and have access to soft bedding.[40] Rubber mats can provide a means for increasing the comfort of flooring in unbedded systems.[41]

For established lesions, there is evidence that rubber mats can be beneficial. In the study by Zurbrigg, sows provided with a mat had shorter healing times (25 days) than did sows housed in a conventional farrowing crate (32 days to heal), or those provided with solid stainless steel plates under the shoulder region.[18]

Few commercially available products exist for the topical treatment of shoulder lesions. A study testing AluShield Aerosol Bandage (Neogen, Lexington, Kentucky), a food-animal labelled product specifically for the treatment of wounds, was found to be ineffective. There was no difference in the reduction of lesion size between control and treatment groups (reductions of 66% and 60% respectively), nor a difference in the change of lesion diameter or time to lesion healing between the control and treatment groups.[41,42]

A study by Kaiser et al[42] compared the effectiveness of a combination treatment of rubber mats and zinc ointment (25% zinc oxide) with a local antibiotic treatment (chlortetracycline spray) on healing of shoulder ulcers in three sow herds. Sows were paired according to the grade of their ulcer (Danish pathoanatomical scale: 0 to 4) on the first observation and were randomly divided into treatment groups: mats and zinc ointment (Apotekets Baby Zinksalve, Denmark), or antibiotic spray (Cyclo Spray Vet, Eurovet Animal Health B.V., Netherlands). The rubber mat plus zinc oxide treatment had a statistically significant effect, reducing the size of the ulcer on days 14 and 21 of treatment compared to antibiotic spray. For lean sows provided with rubber mats and zinc oxide, the average shoulder ulcer size on day 14 was 3.8 cm^2, versus 9.5 cm^2 when antibiotic spray was used. This treatment appeared to be equally effective in all three herds studied. Therefore, the authors recommended rubber mats as a means to reduce the number of sows that needed to be euthanized, culled, or weaned early due to this type of lesion, and suggested that rubber mats be used preventatively for sows at risk.[42]

As an alternative to providing a rubber mat, some Danish producers use a padded shoulder protector (eg, Maxi Pork, designed by Danish company Unitron Scandinavia A/S). The device consists of two layers of foam rubber coated with nylon netting, and straps which allow it to be fastened to the sow. This device is best for treating sows in the early stages of lesion development, before the ulcer has formed, and not for treating the open wound. Producers install the pads on sows as soon as they observe any redness of the skin on the shoulder.

In general, products used to treat decubital ulcers on sows are few and have not been well-evaluated.[42] Nevertheless, individual farms should implement procedures for identification, surveillance, and treatment of shoulder ulcers. Future research should focus more on preventative management of sows, as this is a far more effective approach. Traumatic neuromas found in healed ulcerations suggests that sows continue to experience discomfort after ulcer healing;[41] however, robust strategies to deal with shoulder ulcers must also be developed, as ulcers will persist until effective means of prevention can be implemented.

Prevention

Maintaining an optimum BCS is a critical factor in the prevention of shoulder lesions. Sows need sufficient backfat at farrowing (ideally BCS 3 on a 1 to 5 scale) to maintain sufficient levels throughout lactation. In a study investigating the effect of softer flooring in the farrowing crate on subsequent sow performance,[43] fifty-two of 140 sows developed shoulder ulcers (17 were from farrowing crates with rubber mat floors and 35 from farrowing crates without rubber mats). Analysis revealed that lower backfat thickness at day 109 of gestation was associated with increased likelihood of having shoulder ulcers at weaning. Backfat thickness is affected by a combination of sow genetics and diet. Maximizing feed intake during lactation may be hindered by a variety of factors, including hot temperatures in summer[11] or the onset of illness.[43] Regular, objective assessment of body condition can assist stockpersons to identify low BCS in individual sows and take appropriate action. New technologies for individual sow feeding during gestation and lactation, which provide feed on demand rather than all at once, may also help to optimize sow body condition. The cause of decreased feed intake needs to be identified promptly to avoid a reduction in body condition. Earlier management interventions to rectify problems will go a long way to preventing development of shoulder lesions and ulcers.

Regular monitoring of early signs of skin insults, such as redness, abrasion, or irritation, are paramount, since early detection and intervention are important and effective in prevention of sow suffering. Lesions may be as subtle as slight redness; the observation of flies on the shoulder crest can be an early indication of an incipient shoulder lesion.[42] If these early signs are observed, the floor surface should be checked for roughness. It would be appropriate to place a pad over the affected area of the sow's shoulder to relieve pressure, or to move the affected sow to a comfort pen with a softer lying surface, such as a rubber mat or deep bedding.[40] Lesions should be cleaned and treated with an antiseptic (according to veterinary advice and farm health protocols).

One reason that sows may be reluctant to stand or change position during lactation is locomotor problems. In evaluation of sow lesions at slaughter, Stalder and Karriker[44] reported that open shoulder lesions were significantly and positively associated with rear foot abscesses. This suggests sow-herd leg and foot health should be evaluated

in combination with the goal of reducing shoulder lesions. Uninterrupted lying bouts increase the risk of developing shoulder lesions;[23] therefore, the authors suggest that it may be beneficial to stimulate sow activity by making them stand or move about on a daily basis, particularly in the first weeks post farrowing.

Flooring is an important risk factor. The common use of fully slatted floors increases pressure due to the distribution of body weight over a smaller surface area.[9,45] The odds of a sow housed on a slatted floor developing shoulder ulcers during lactation was three times higher in sows not provided with rubber mats than in those with rubber mats extending to their hind limbs.[4,13] The use of alternative flooring in the farrowing pen may benefit the sow by reducing occurrences of a painful condition, while benefiting the producer by subsequent productivity improvement.[45] Regardless of treatment and prevention options, in severe cases sows should be culled or euthanized.

Future research

Numerous gaps exist in our knowledge of shoulder lesions and ulcers, providing a basis for future research, summarized as follows:

- Pathogenesis: Identify the mechanisms by which shoulder lesions and ulcers develop and heal. A better understanding of lesion pathogenesis will help towards identifying effective prevention measures.
- Feeding practices, nutrition, and body condition: Investigation of feed quality, feed delivery, and feeding management (eg, automated or on-demand lactation feeders) for lactating sows and the occurrence of shoulder lesions and ulcers.
- Housing and use of alternate flooring: Studies on alternative flooring for farrowing crates are warranted, with consideration for sow comfort and pressure-relieving properties, while maintaining drainage and cleaning properties. Rubber matting has shown promise, but further evaluation of the physical characteristics, durability, and effectiveness for lesion prevention is required.
- Lying behavior and time in recumbency: Identify whether changes in sow behavior (eg, lying posture and duration) are predictive of lesion development and can be used as risk indicators.
- Sow productivity: Identify the contribution of increasing litter size, milking ability, and duration of lactation on the development of shoulder lesions and related management strategies to reduce the risk of ulcer development.
- Cost and financial analysis: Quantify the economic cost of shoulder ulcers to the swine industry, considering prevalence, reductions in performance, treatment costs, and sow retention.
- Impact on animal welfare: Quantify the level of pain experienced by sows during the development, presence, and healing of shoulder lesions and ulcers. Identify appropriate wound treatment and pain mitigation strategies for shoulder ulcers. Identify the time when pain control provision is most beneficial and whether this influences recovery time or production outcomes.
- Environmental temperature and humidity: While higher temperatures are believed to influence occurrence of shoulder lesions, no data are available regarding the effects of temperature and possible interaction with humidity on the occurrence of shoulder ulcers.[3]

Perhaps just as important as these research topics is the need for greater on-farm monitoring of this condition. The old maxim, "you can only manage what you measure" surely applies to shoulder lesions and ulcers. Lesions can easily be recorded on-farm, either during lactation or as sows exit from farrowing. The increasing use of computerized and automated systems for data collection will make this a simpler task in the future, and can provide an important first step towards increasing recognition of the problem and identification of appropriate treatments.

Conclusions

Estimates of prevalence of shoulder lesions, including ulcers, are reported at 5% to 50% in breeding sows; however, the true incidence of shoulder lesions and (or) shoulder ulcers is not known. Lesions in sows are a painful condition, represent a welfare concern, and benefit from timely interventions. Treatment success of sow shoulder lesions is enhanced by early recognition and intervention.

Lesions typically develop in the weeks following farrowing, when sows spend the majority of their time lying and nursing. Other factors, such as low body condition score, hard or abrasive flooring, genetic predisposition, and a host of other environment and management factors increase sows' susceptibility to developing shoulder lesions.

Most of the literature on shoulder ulcers has been in surveys and is epidemiological in nature. The process of wound development and healing is poorly understood, hence research aimed to better understand the underlying causes, progression, effects of pain on productivity, and development of more effective treatment and preventative interventions is warranted.

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Conflict of interest

None reported.

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US pig farmers receive 25:1 return on Pork Checkoff investments

US pork producers receive a positive return on their Checkoff investment, according to a 2017 study conducted and released by Harry Kaiser, the Gellert Family Professor in the Dyson School of Applied Economics and Management, Cornell University. Additionally, 91% of pig farmers who took part in the Pork Checkoff's annual producer survey in November 2017 acknowledged their overwhelming support of the Pork Checkoff, with a record-low opposition of just 3%.

The National Pork Board commissions an economic analysis of Pork Checkoff programs every five years. The study quantifies the returns generated by Pork Checkoff investments in research, pork promotion and producer education programs. The latest results, published in 2017, cover 2011 to 2016 programs.

"It's important to producers – those who directly fund the Pork Checkoff – to understand and quantify the value of their investments," said Terry O'Neel, National Pork Board president and a pig farmer from Friend, Nebraska. "The results indicate a positive impact of all aspects of the Pork Checkoff, from conducting production-focused research to growing consumer and export demand for pork."

Specifically, the study documented a growing return on investment through defined benefit-cost ratios across several key program areas from 2011 to 2016:

- Production research: Each dollar invested in production research to benefit on-farm practices yielded $83.30 in producer value.
- Foreign market development: Each dollar invested in developing foreign markets yielded $24.70 in producer benefits.
- Advertising and non-advertising promotion: Other pork promotion resulted in benefits of $14.20 for advertising and $12.40 for non-advertising promotion.
- Research to grow demand: Research on market drivers returned $8.30 for each $1 invested.
- Net result: Collectively, the overall return of Checkoff program activities is $25.50 for each dollar invested.

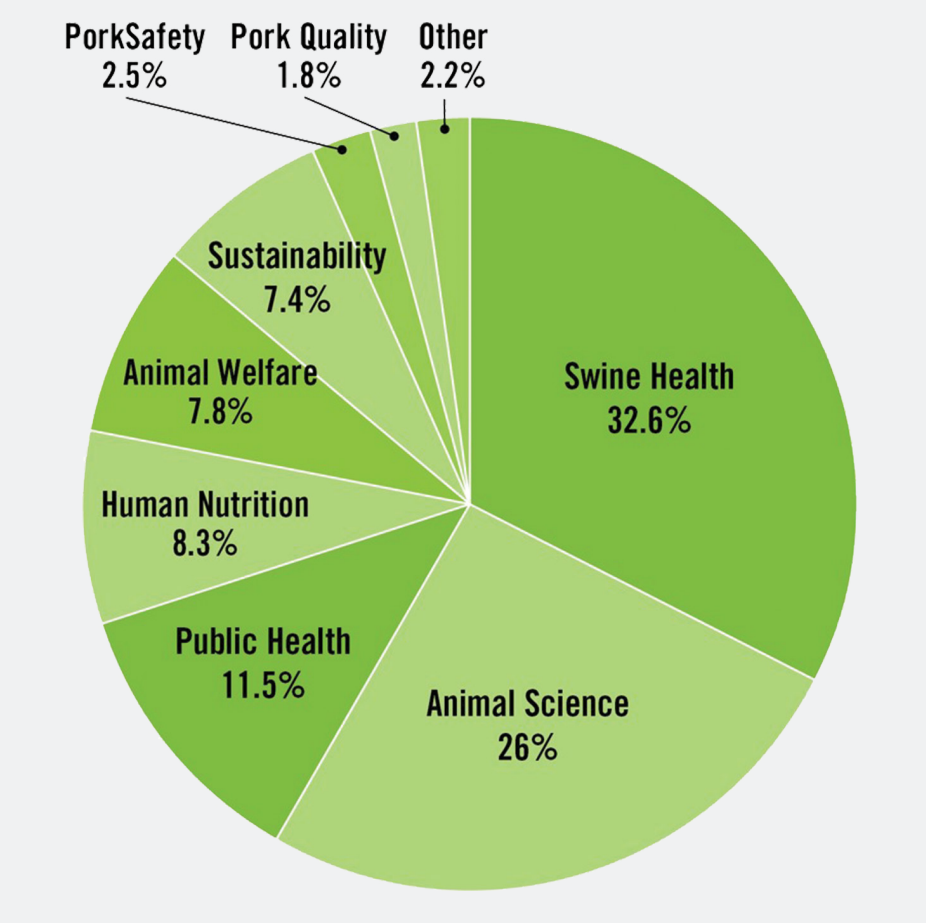
The US Department of Agriculture requires a return on investment analysis every five years. The 2001 to 2006 study showed an overall return of $13.80 to $1 invested, and the most previous study, released in 2012 for the period of 2006 to 2011, found a return of $17.40 to $1 invested.

For more information, contact Kevin Waetke at KWaetke@pork.org or 515-223-2638.

Investment breakdown of Checkoff's scientific studies in 2017

In 2017, of the various science-based research studies recommended for funding by the producer-led committees, the National Pork Board approved 57 of them for a total Checkoff investment of $4.73 million. These studies focus primarily on seeking on-farm solutions to today's production challenges. This graphic shows the share of the total investment for each research area for the year.

For more information, contact Dave Pyburn, DPyburn@pork.org or 515-223-2634.



New pork.org offers improved functionality

With the recent overhaul of its look and feel, the National Pork Board's **pork.org** website offers users an enhanced experience with better capabilities. Most notably, the ability to find research items has been improved and now more than 20 individual National Pork Board sites fall under the domain of **pork.org**.

The launch topped off work throughout 2017 by the Pork Checkoff's digital strategy team on the consolidation project. The result? For the first time, **pork.org** now serves as a single online resource for pork producers and all of the Pork Checkoff's key audiences.

"The overarching goal is to provide a single source of information at **pork.org** for all pork website users and to give them an outstanding user experience," said Kevin Waetke, vice president of strategic communications for the Pork Checkoff and co-leader of the digital strategy team.

For more information, contact Kevin Waetke at **KWaetke@pork.org** or 515-223-2638.

Pig survivability: Key research area in 2018

The Checkoff's Animal Science Committee has determined that improving pig survivability is a key area of research for 2018. With additional research dollars from the Checkoff Animal Welfare Committee, the committee has devoted $1 million in Checkoff funds for this effort. In addition, the committee submitted a proposal on improving pig survivability to the Foundation for Food and Agriculture Research (FFAR) that seeks matching funds. FFAR subsequently committed $1 million in matching funds for this research. Its goal is to get a better understanding of the underlying drivers of pig mortality that will lead to improved production efficiency and profitability.

For more information, contact Chris Hostetler, **CHostetler@pork.org** or 515-223-2606.

AASV NEWS

Alternate Student Delegate selected for AASV Board

The AASV Student Recruitment Committee is pleased to announce the selection of Jonathan Tubbs (Auburn, 2020) as the incoming Alternate Student Delegate to the AASV Board of Directors.

Tubbs grew up exposed to the swine industry. Throughout high school and college, he worked at his father's veterinary clinic, where his first exposure to swine medicine occurred. Tubbs spent several years working in animal welfare, biosecurity, and environmental auditing and assessment specific to the swine industry. Following this, he worked in swine genetics and production as a technical services manager. His experience in the swine industry is both extensive and well rounded.

Outside of the swine industry, Tubbs earned two degrees in education. He has taught in several venues including high school, university, and even adult education during his time in the US Peace Corps. Tubbs notes "the knowledge and experience of educating would be very beneficial in the position of alternate student delegate as it is necessary in this position to effectively disseminate information, facilitate discussion, and to make informed decisions."

As a veterinary student, Tubbs currently serves as the president-elect to the Auburn SAVMA chapter, and holds officer positions in the Students for One Health and Production Animal Medicine clubs. Tubbs believes that "service to the swine industry is best achieved through service to, and in, AASV. Therefore, as alternate student delegate for AASV, I would be dedicated to continual improvement and advancement through the specific duties required."

Tubbs assumes his duties as alternate student delegate during the 2018 AASV Annual Meeting in San Diego, California. The current alternate delegate, Jordan Gebhardt (Kansas State University, 2019), will assume the delegate position currently held by Brent Sexton, who will rotate off the board. Jordan and Jonathan will represent student interests within AASV as non-voting members of the board of directors and the Student Recruitment Committee.

Please join us in welcoming Jonathan to the AASV Board of Directors and thanking Brent for his service! And, yes, Jonathan is the son of AASV member and Past President Dr Rick Tubbs.

AASV Annual Meeting proceedings online

The proceedings of the 2018 AASV Annual Meeting are available at **www.aasv.org/annmtg/proceedings** for members to download.

The proceedings are available in the following formats:

- The "big book" of all the regular session papers in a single PDF file with a linked table of contents
- Seminar booklets: a PDF file for each seminar
- Offline web app to provide searchable access to papers on your desktop, laptop, tablet, or other mobile device (similar format to the USB drive). The web app is available only for a limited time before and after the meeting, so don't wait to download it!
- Individual papers in the Swine Information Library: **www.aasv.org/library/swineinfo/**

To access the files, make sure your AASV membership has been renewed for 2018. You'll need your AASV website username and password to log in. If they are not handy, contact the AASV office or use the "Reset Password" link in the upper right of the AASV Web site (**www.aasv.org**) to receive them by email.

Funding and online resources available for students

Veterinary students, are you looking for a summer internship or planning future externship opportunities? As student members of AASV, you have access to a database of swine-oriented opportunities available to students which can be found at: **www.aasv.org/internships/index.php**. Members of AASV who would like their internship and externship opportunities included in the directory are encouraged to contact Jordan Gebhardt, AASV student delegate (**aasvstudentdelegate@gmail.com**), for more information.

Are you looking for funding for a swine-based externship experience? The AASV Foundation provides grants of $200 to $500 to veterinary students who complete an externship of at least two (2) weeks in a swine practice or a mixed practice with a considerable swine component. Any AASV student member in veterinary school who fulfills the requirements is eligible to apply. More information can be found at: **www.aasv.org/students/externgrant.htm**.

AASV members: Want to practice better medicine? We want to help!

AASV and Texas A&M University Medical Sciences Library are teaming up to provide you with assistance to practice evidence-based veterinary medicine. The best part... there is no cost to you.

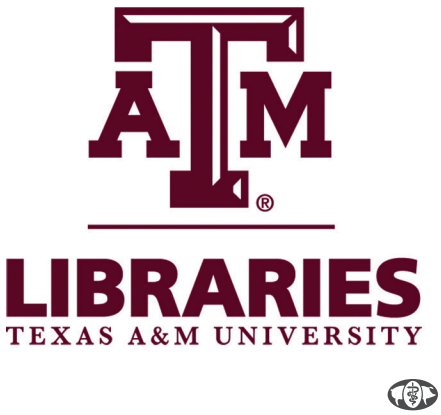
Do you have a question? Want to know what has been published about a topic? Need to have a fact verified? Need demographic information? We will help you find the answers. You have access to the searching expertise of the medical science librarians at Texas A&M University. Submit your question or literature search by email (**AskMSL@library.tamu.edu**) or phone (979-845-7428) and receive the answer via email, generally within two working days.

Do you know the specific article, chapter, or paper you want to read but don't have the full text? You may request copies of articles, chapters, and proceeding papers from the library's extensive collection. Requests are generally filled within two working days.

These benefits are available to AASV members in private practice but not to students or those already associated with an institution that provides library benefits.

More details and instructions for taking advantage of these member benefits are available at **guides.library.tamu.edu/aasv**.

Attending the annual meeting in San Diego? A Texas A&M librarian will be available Saturday and Sunday, March 3rd and 4th, at a table near registration to answer questions and assist with registering for the service. Team up with the Medical Sciences Library to enhance your practice with knowledge and information gained from colleagues. Stand on the shoulders of all those clinicians, researchers and academics who have gone before you by putting their published knowledge into your practice!



AASV Foundation Fundraising AUCTION

Held in conjunction with the AASV Annual Meeting March 5, 2018 – San Diego

THANK YOU to the individuals, veterinary practices and companies who helped us **"Rock the Boat and SEAL the Deal!"** with their generous contributions to the auction.

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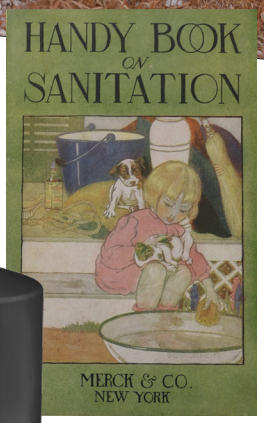

All Silent Auction bidding will be conducted via ClickBid.

Check out ALL of the items up for bid, sign up for your bidding number, and start bidding on the silent auction today at **www.cbo.io/aasvf**!

For information about the AASV Foundation, see **www.aasv.org/foundation**.

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FOUNDATION NEWS

ACAW scholarship available to members seeking welfare certification

The AASV Foundation Board of Directors continues to accept applications from AASV members seeking board certification in the American College of Animal Welfare (ACAW). The applicant must have a DVM or VMD degree and at least 5 years of continuous membership in the AASV.

To apply, the applicant must submit a curriculum vitae, an ACAW-approved program plan, and three (3) letters of reference (one of which must come from the applicant's mentor). There is no submission "due date," but there is a limit to the amount of funding available each year. A selection committee will review applications as they are received.

The scholarship will provide annual reimbursements for actual expenses related to the ACAW program, including travel, course fees, and textbooks, with a maximum reimbursement amount of $20,000. Reimbursement will not cover lost income. An additional incentive payment of $10,000 will be issued upon successful and timely completion of the ACAW Board Certification.

For more information, contact the AASV office: Tel: 515-465-5255; E-mail: **aasv@aasv.org**.

Endowment grows as past presidents rise to the challenge

The race is on! AASV Foundation Chairman Dr John Waddell has called upon each of his fellow AASV past presidents to recruit at least 3 new Leman, Heritage, or Legacy donors to build the foundation endowment to $2 million by the 2019 AASV Annual Meeting.

In terms of the number of new donors, Dr Lisa Tokach is leading the way, having already recruited 3 new Leman Fellows and 1 new Heritage Fellow. Other past presidents who have successfully recruited new endowment contributions include Drs Randy Jones, Bob Morrison, Max Rodibaugh, Alex Ramirez, and Larry Rueff. Since the challenge was announced, three new Legacy funds have been established!

How about you? Are you ready to lend your support and help build the endowment to ensure future support for the swine veterinary profession? When you enroll in one of the giving programs described here, you will be providing a perpetual source of income for foundation programs that include scholarships, swine externship grants, travel stipends for veterinary students, research grants, and more!

Leman

Named for the late industry leader and former AASV president Dr Allen D Leman, this giving program confers the title of "Leman Fellow" upon those who contribute $1000 or more to the foundation endowment. Send your check to the AASV Foundation, 830 26th Street, Perry, IA 50220, or contribute online at **ecom.aasv.org/foundation**.

Heritage

The Heritage Fellow program recognizes contributions of $5000 or more. In addition to monetary donations, other giving options such as life insurance policies, estate bequests, and retirement plan assets may be utilized. To enroll, complete the Heritage Letter of Intent available at **www.aasv.org/foundation/documents/heritageform.pdf**.

Legacy

A donor, multiple donors, or a veterinary practice may establish and name a Legacy Fund with a gift of $50,000 or more. The fund may be named after the donor or another individual or group. The donor designates which of three foundation mission categories the fund's proceeds will support: 1) research, 2) education, or 3) long-range issues. To enroll, complete the Legacy Fund form available at **www.aasv.org/foundation/documents/legacyform.pdf**.

For more information about the AASV Foundation's endowment giving programs, or to make your contribution, see **www.aasv.org/foundation**, or contact the foundation: Tel: 515-465-5255, E-mail: **aasv@aasv.org**.

Delegation explores African swine fever prevention efforts

A delegation including representatives from the American Association of Swine Veterinarians, National Pork Producers Council and USDA visited Poland, Germany and Denmark to discuss African swine fever (ASF) prevention efforts in each of those countries. The group met with pork industry and government representatives in each country to better understand each organization's perspectives and activities associated with preventing the introduction of ASF into their commercial operations.

African swine fever has been on a steady march through the Caucuses, Russia and eastern Europe since the introduction of the virus into Georgia in 2007. The virus was identified in Estonia, Latvia, Lithuania and Poland in 2014. In Latvia, Lithuania and Poland, the infected animals were located along the border with Belarus which, to date, has not reported finding any positive ASF cases. Thus far, the European experience has found the virus occasionally in domestic swine but widespread in wild pigs. Numerous reports of infected domestic swine have occurred in Russia, Ukraine and the Caucuses.

In response to the ongoing outbreak, the European Union has regionalized the affected countries into one of four statuses (Parts I – IV) based on the epidemiology of the disease. If the virus has been identified in both domestic and feral swine the region is classified as Part III (ongoing, dynamic spread) or IV (stable and endemic). Part II designates regions where the virus is only known to exist in the feral swine population. Swine located in a Part I region are not known to be infected but are at increased risk due to their proximity to positive animals.

Poland, the fourth largest pig producing member state in the European Union, represents the farthest west the virus has been detected with recent outbreaks occurring around Warsaw. In 2016, there were 9544 sows producing approximately 11.3 million pigs on 172,200 farms. Farms housing over 200 head accounted for 61.5% of the pigs produced in Poland.

According to representatives from the Polish pig producers, ASF has been detected on 107 pig farms and in 904 wild boar. Confirming previous reports that the virus is not terminal in all wild pigs, approximately 3% – 5% of the positive cases have come from hunter-killed wild boar. Most ASF-positive domestic herds have been farms with less than 20 head although one 1000 head grow-finish unit also became infected.

To prevent the introduction of ASF and control the spread of the virus, numerous control measures have been implemented by the Polish authorities and producers. These measures include testing all incoming replacements, quarantines and the implementation of control zones, enhanced cleaning and disinfection, and fencing. Positive farms are depopulated and the owners are compensated.

The wild boar population is considered a significant risk factor for the introduction and maintenance of ASF in Poland. Veterinarians are also recognized as a risk factor for viral spread as is anyone having contact with infected swine or wild boar. The number of case submissions increased dramatically in 2016 following the institution of incentive payments to hunters and forest workers for the notification of dead wild boar.

The Polish representatives we spoke with suggested that the most likely cause of viral spread in 2016 and early 2017 was illegal animal movement. In 2017, non-compliance with biosecurity rules has also been identified as a likely route of spread. This includes the use of contaminated straw bedding and grass feedstuffs. Approximately 30% of the "peasant farmers" accepted compensation and agreed to cease pig production for at least 3 years.

"Emphasis was placed on strict adherence to biosecurity practices throughout the production system as key to preventing ASF introduction and managing an outbreak."

The delegation also visited German and Danish government officials and producers. To date, no ASF cases have been detected in either Germany or Denmark. While Denmark has very few wild boar (estimated to be less than 100 head), Germany, like Poland, has a large population of feral swine. Reportedly, hunters in Germany killed approximately 600,000 wild boar in 2016.

The German swine industry is comprised of 1.9 million sows producing 48.8 million slaughter pigs annually. There are approximately 8000 sow farms. Germany has an insurance policy to cover compensation payments to affected farmers. The premiums are paid by the government and compulsory payments from every livestock farmer. The funds can be used to pay for testing and prevention activities. During an outbreak, funds may be used to compensate for dead animals, depopulation, and cleaning and disinfection. Farmers whose animals test positive for ASF, are in a control zone, or considered a "contact" premises are eligible to receive insurance payments equivalent to 50% of the market value. Public funds make up the remaining 50%. In addition, three companies currently provide optional private insurance to cover losses not covered by the compulsory program.

German officials utilize a risk-based approach to focus routine inspections. They have a stockpile of resources purchased by the industry and the insurance program available to facilitate the outbreak response should it be necessary. Each county in Germany has its own competent veterinary authority which would direct the response to an outbreak.

In Denmark, there are 8707 pig farms raising 13.4 million slaughter pigs. Danish farmers produce 2 million tons of pork annually of which 95% is exported. There are 12 slaughter plants in the country, 10 are owned and operated by one cooperative, Danish Crown.

Given the relative lack of wild boar in Denmark, the biggest threat for ASF introduction is transportation and human movements. Danish pork producers have adopted very stringent transportation restrictions and cleaning and disinfection procedures. Although voluntary, the system is reportedly followed by over 99% of producers. We visited a truck wash located along the Denmark and Germany border. Nationally, they wash approximately 26,808 trucks annually at a cost of €2.1 million per year (US $2.6 million). Every truck is tracked by GPS to verify exactly where the trucks have been. The trucks must be thoroughly cleaned and disinfected before leaving Poland and transiting Germany to return to a Danish pig farm. Upon reaching the Danish border, the truck must be inspected and disinfected again. Following disinfection, the truck driver is issued a color-coded certificate and the data is entered into a web-based database accessible by all stakeholders. The certificate indicates the risk zones visited by the truck and determines the length of time the truck is quarantined before returning to a Danish pig farm.

In summary, everyone we spoke to expressed obvious concern over the impact ASF would have on their country's pork production. It was interesting to visit three countries whose pork industries were intertwined but with varying levels of resources and risk. In all cases, the objective was to protect the domestic pig herd and a recognition of the threat posed by the wild pig population. Emphasis was placed on strict adherence to biosecurity practices throughout the production system as key to preventing ASF introduction and managing an outbreak. Access to data regarding farm locations, pig movements and transportation was considered critical in all three countries. Database management was sophisticated and controlled cooperatively between the industries and the authorities.

Harry Snelson, DVM
Director of Communications

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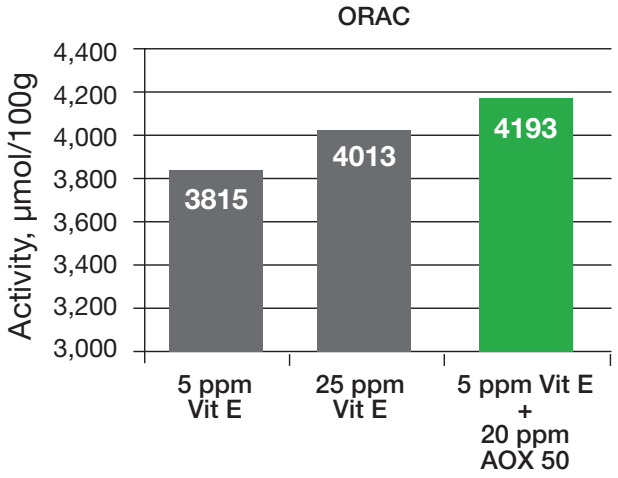

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Upcoming meetings

American Association of Swine Veterinarians 49th Annual Meeting

March 3-6, 2018 (Sat-Tue)
Manchester Grand Hyatt, San Diego, California

For more information:
American Association of Swine Veterinarians
830 26th Street, Perry, IA 50220-2328
Tel: 515-465-5255
E-mail: aasv@aasv.org
Web: www.aasv.org/annmtg

10th European Symposium of Porcine Health Management (ESPHM)

May 9-11, 2018 (Wed-Fri)
Barcelona, Spain

For more information:
Joaquim Segalés
E-mail: joaquim.segales@irta.cat
Web: www.esphm2018.org
Maria Sanmiguel
E-mail: msanmiguel@pacifico-meetings.com

6th International Symposium on Animal Mortality Management

June 3-7, 2018 (Sun-Thu)
Embassy Suites, Amarillo, Texas

For more information:
Web: animalmortmgmt.org/

World Pork Expo

June 6-8, 2018 (Wed-Fri)
Iowa State Fairgrounds, Des Moines, Iowa
Hosted by the National Pork Producers Council

For more information:
Web: worldpork.org

25th International Pig Veterinary Society Congress

June 11-14, 2018 (Mon-Thu)
Chongqing, China

For more information:
Web: www.ipvs2018.net/

11th Biennial Conference of the Association for Applied Animal Andrology

July 14-16, 2018 (Sat-Mon)
Hilton Riverside, New Orleans, Louisiana

For more information:
Dr Steven P. Lorton
E-mail: splorton04@tds.net
Web: animalandrology.org/futuremeetings.htm

Allen D. Leman Swine Conference

September 15-18, 2018 (Sat-Tue)
Saint Paul River Centre, Saint Paul, Minnesota

For more information:
Tel: 612-624-4754
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Humane Endings Symposium

November 2-4, 2018 (Fri-Sun)
Westin O'Hare, Rosemont, Illinois
Hosted by the American Veterinary Medical Association

For more information:
E-mail: humaneendings@avma.org

For additional information on upcoming meetings: www.aasv.org/meetings/

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Photo Corner

Suckling piglets on shredded newspaper in Spain

Photo courtesy of Dr Antonio Palomo Yagüe