Long-term impact of zinc supplementation in sows: Impact on claw quality

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Summary

Objectives: To evaluate the long-term impact of zinc (Zn) supplementation on claw lesions, claw conformation, and histological and mechanical claw characteristics of sows housed in groups on rubber top layer or concrete floors during gestation.

Materials and methods: Six groups of 21 ± 4 sows were allotted to group housing on different floor types for 80 days during gestation. Within each group, sows were randomly allocated to one of three diets supplementing a basal diet (46.6 and 128.9 mg Zn per kg during gestation and lactation, respectively) with 0, 50, or 100 mg Zn per kg. Claw lesion scoring, claw conformation, and horn growth and wear measurements were performed at days 50 and 140 of every cycle. Histological and mechanical characteristics were evaluated on claw samples of 36 sows after slaughter.

Results: Dietary Zn supplementation affected heel horn erosion score ($P = .01$): sows supplemented with100 mg Zn per kg diet had better scores. Distances between dermal papillae of the sagittal heel horn were larger ($P = .004$). Heel height was lower for sows supplemented with 0 and 100 mg Zn per kg than for 50 mg per kg ($P = .01$). Horn growth and wear were lower for sows housed on rubber at day 50 ($P < .001$, both variables), but not at day 140. Dermal papillae distance was shorter for sows on rubber ($P = .04$).

Implications: Unlike floor type and phase within the reproductive cycle, and under the conditions of this study, dietary zinc supplementation minimally influences claw quality.

Keywords: swine, claw conformation, claw lesion, dietary zinc concentration, rubber top layer

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Resumen - Impacto a largo plazo de suplementar zinc en hembras: Impacto sobre la calidad de la pezuña

Objetivos: Evaluar el impacto a largo plazo de suplementar zinc (Zn por sus siglas en inglés) sobre las lesiones, conformación, y características mecánicas e histológicas de la pezuña de hembras alojadas en grupos en pisos con una capa de hule o pisos de concreto durante la gestación.

Materiales y métodos: Se asignaron seis grupos de 21 ± 4 cerdas en alojamiento en grupo en diferentes tipos de piso por 80 días durante la gestación. Dentro de cada grupo, las hembras fueron asignadas aleatoriamente a una de tres dietas suplementando una dieta básica (46.6 y 128.9 mg de Zn por kg durante la gestación y lactancia, respectivamente) con 0, 50, ó 100 mg de Zn por kg. Se realizó un puntaje de lesión, conformación, crecimiento, y desgaste de la pezuña los días 50 y 140 de cada ciclo. Se evaluaron las características histológicas y mecánicas en muestras de pezuña de 36 hembras después del sacrificio.

Resultados: El suplemento con Zn en la dieta afectó el puntaje de erosión del talón de la pezuña ($P = .01$): las hembras suplementadas con 100 mg de Zn por kg de dieta tuvieron mejores puntajes. La distancia entre la papila dérmica de la pezuña del talón sagital fue

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mayor ($P = .004$). La altura del talón fue más baja en las hembras suplementadas con 0 y 100 mg de Zn por kg que con 50 mg por kg ($P = .01$). El crecimiento y desgaste de la pezuña fueron menores en las hembras alojadas sobre hule en el día 50 ($P < .001$, para variables), pero no en el día 140. La distancia de la papilla dérmica fue más corta en las hembras sobre hule ($P = .04$).

Implicaciones: A diferencia del tipo de piso y de la fase dentro del ciclo reproductivo, y bajo las condiciones de este estudio, suplementar zinc en la dieta tiene una influencia mínima en la calidad de la pezuña.

Résumé - Impact à long terme d'un supplément de zinc chez les truies: Impact sur la qualité des onglons

Objectifs: Évaluer l'impact à long terme d'un supplément de zinc (Zn) sur les lésions aux onglons, la conformation des onglons, et les caractéristiques histologiques et mécaniques des onglons de truies logées en groupe sur un plancher de ciment ou avec revêtement en caoutchouc durant la gestation.

Matériels et méthodes: Six groupes de 21 ± 4 truies furent assignés à un hébergement de groupe sur différents types de plancher pour 80 jours durant la gestation. À l'intérieur de chaque groupe, les truies ont été assignées à l'un des trois régimes alimentaires supplémentant une diète de base (46,6 et 128,9 mg Zn par kg durant la gestation et la lactation, respectivement) avec 0, 50, ou 100 mg de Zn par kg. Le pointage des lésions aux onglons, la conformation des onglons, et les mesures de la croissance et de l'usure de la corne ont été effectués aux jours 50 et 140 de chaque cycle. Les caractéristiques histologiques et mécaniques ont été évaluées sur des échantillons d'onglons de 36 truies suite à l'abattage.

Résultats: Le supplément alimentaire de Zn a affecté le pointage de l'érosion de la corne du talon ($P = 0{,}01$): les truies ayant reçu un supplément de 100 mg de Zn par kg avaient de meilleurs pointages. Les distances entre les papilles dermiques de la corne du talon sagittal étaient plus larges ($P = 0{,}004$). La hauteur du talon était plus basse pour les truies ayant reçu 0 et 100 mg de Zn que celles ayant reçu 50 mg par kg ($P = 0{,}01$). La croissance et l'usure de la corne étaient plus faibles pour les truies hébergées sur un tapis en caoutchouc au jour 50 ($P < 0{,}001$ pour les deux variables) mais pas au jour 140. La distance entre les papilles dermiques était plus courte pour les truies sur le caoutchouc ($P = 0{,}04$).

Implications: Contrairement au type de plancher et à la phase au cours du cycle de reproduction, et dans les conditions propres à la présente étude, un supplément alimentaire de zinc influence de façon minimale la qualité de l'onglon.

Claw quality is an important factor that influences the welfare and productivity of sows. Claw quality is evaluated by visual scoring for claw shape, shape dimensions, lesion scoring, and measurement of structural, physical, and biochemical properties of the claw horn.[1,2] In sows, mainly claw lesion scores are evaluated to define claw quality, but other measurements, including claw conformation, horn growth and wear, and mechanical claw characteristics, are rarely evaluated. More recently, effect of diet on histological claw characteristics were assessed with or without partially substituting inorganic zinc (Zn), copper (Cu), and manganese (Mn) sources with their organic forms.[3,4] Claw lesions are a common multifactorial disorder in sows, with malnutrition and floor type, among others, noted as predisposing factors.[5,6] Claw lesions influence claw quality, but claw quality also influences the occurrence and severity of claw lesions. Furthermore, claw quality depends on the internal characteristics of the claw, including optimal horn production, which is influenced by a diffuse nutrient supply from the dermis to the avascular epidermis.[2,7,8] An insufficient nutrient supply results in a disturbed diffusion of nutrients to the avascular epidermis. This negatively affects horn production, thereby increasing the susceptibility of the claw to damage from the environment.[7,8] The structural, regulatory, and catalytic functions of Zn are related to horn production.[7] However, results from previous studies, mainly in cattle, are inconclusive: reports range from no effect or a reduction in claw lesion and lameness scores with varying dietary Zn concentrations.[9-11] In weaned pigs, claw quality was affected by dietary Zn level.[12] Studies in sows did not assess the impact of increased dietary Zn concentration, but showed similar claw lesion scores, neither deterioration nor better scores, or better scores with (partial) substitution of inorganic Zn, Cu, and Mn sources by organically bound Zn, Cu, and Mn, [3,13-18] except in one study.[19] Study duration may be important for detecting differences between treatment groups. A lack of effect of dietary Zn may have been related to an excessively short study duration: 12 months is the threshold reported in the literature.[10,11,20] Claw quality may be affected internally by dietary Zn concentration, while floor type is an external factor in development of claw lesions.[6] An increased occurrence of claw lesions is observed when sows are housed on fully or partly slatted concrete floors, whereas straw bedding seems to be positively associated with fewer and (or) less severe claw lesions.[6,11,21] A rubber top layer on concrete floors appears to protect claws due to a cushioning effect.[22,23] In a long-term study in sows, however, the risk of more severe claw lesions increased when sows were housed on a rubber floor.[24] Therefore, it was hypothesized that both dietary Zn concentration and floor type would influence claw quality in sows. The objective of this longitudinal study conducted over three reproductive cycles was to evaluate the effect of dietary Zn supplementation on claw quality characteristics in sows housed on two different floor types during gestation.

Materials and methods

All experimental procedures involving these animals were approved by the Institute for Agricultural and Fisheries Research (ILVO) 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 and management

Six groups of non-lame primiparous sows (n = 131 gilts, RA-SE Genetics, RA-SE Genetics NV, Ooigem, Belgium; `http://www.ra-se.com/nl/`) entered the study when their locomotion score was ≤ 60 mm on a 150-mm tagged visual analogue scale (tVAS) developed by Nalon et al.[25] These gilts were purchased per group (21 ± 4 sows per group) and quarantined for 4 to 6 weeks before their first insemination at days 233 ± 12 of age. The experimental period started 10 days before the first insemination (day 0) and the six successive production groups were monitored during three reproductive cycles (3-week interval between groups). When a sow was removed before the end of the experiment (n = 36), a new gilt replaced her.

Body weight, backfat thickness, and body condition score (BCS) at the start of the study were 149 ± 21 kg, 16 ± 4 mm, and 3.0 ± 0.5, respectively (mean ± SD). Sows were vaccinated at day 55 of gestation against porcine reproductive and respiratory syndrome virus (Porcilis; MSD Animal Health, Boxmeer, The Netherlands), 7 and 4 weeks before parturition against neonatal diarrhea caused by *Escherichia coli* (Neocolipor; Merial, Lyon, France), and 1 week postpartum against parvovirus and erysipelas (Parvoruvax; Merial). The sows were dewormed 17 days before parturition.

After weaning of the third reproductive cycle (end of the study), sows that had participated for at least 12 months in the experiment (ie, sows that had completed at least two reproductive cycles; n = 95) were transported to a commercial abattoir after the left and right front claw were marked with a color-coded tie wrap. After slaughter the following morning, both front claws were removed at the carpal joint and collected before the sows entered the scalding vat to preserve the metacarpal bones and claw structures. After the claw structures were collected for examination, the remaining parts of the front claws, including metacarpal bones, were frozen at -20°C.

Housing facilities

During the quarantine period (4 to 6 weeks), each purchased sow group (21 ± 4 sows per group) was housed as a static production group on concrete floors with straw bedding. The quarantine unit consisted of two pens with natural ventilation, no climate control, and artificial light.

Sows were housed in individual gestation crates during the insemination period (eg, shortly before the first insemination [day -10] to day 28 [insemination day 0] and from weaning [day -7] until 4 weeks after insemination [day 28] in their successive reproductive cycles). Housing and management conditions were similar for all sows. Housing was naturally ventilated, with artificial light and with temperature adjusted according to the outdoor temperature. In the first week after weaning, lights were on between 8:00 AM and 8:00 PM. Crates had partly slatted concrete floors (14.4%; 1.38 m^2 per sow).

During mid- to late gestation (day 28 to day 108), the sows were housed again in their static production groups. An electronic feeding system was used, with individual sow recognition through an electronic transponder in the sow's ear. Ventilation was maintained at 73 m^3 d^{-1}·sow^{-1} and indoor temperature was set at 20°C. Light was artificial, and in each pen, one light source was illuminated during the night so that sows could easily access the feeding system. The group-housing facility consisted of four pens (4.45 m × 18.75 m; 40.4% slatted floor). 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 rubber (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).[26] At the start of the study, production groups were alternately assigned to the concrete or rubber floor type during group housing and returned to the same floor type for the duration of the experiment. Per pen, two rubber balls and a static and dynamic rotating grooming brush were provided as environmental enrichment.

From 1 week before the expected parturition date until weaning (day 108 to day 143), sows were housed individually in units with 10 farrowing crates. Housing and management conditions were similar for all sows. Ventilation for the farrowing units was adapted to the temperature. The indoor temperature was set to 23°C and light was artificial. The crates within the pen had a steel slatted floor (crate size 1.33 m^2), which had a nonslip section, and the pen had a PVC-slatted floor (pen size 3.60 m^2). Floor heating (0.60 m × 0.45 m) and heat lamps were provided in the pen for the piglets at the beginning of lactation.

Dietary treatment

All primiparous sows (gilts) in quarantine (4 to 6 weeks) were fed a pre-experimental gestation diet formulated according to NRC recommendations[27] and commercial standards for gestating sows. The pre-experimental diet was fed ad libitum and contained 895 g per kg dry matter (DM), 127.3 g per kg crude protein, 301.5 g per kg neutral detergent fiber (NDF), 155.4 g per kg acid detergent fiber (ADF), 23.5 g per kg acid detergent lignin (ADL), 27.9 g per kg crude fat, 70.2 g per kg crude ash, 128.6 g per kg starch, 66 g per kg sugar, 121 mg per kg total Zn (ie, ± 21 mg Zn per kg originating from ingredients and 100 mg per kg added Zn 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[27] and commercial standards (tables 1 and 2), except for Zn. Phytase was added via the premix to simulate practical conditions. The gestation diet was provided 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 end of the first 4 weeks of gestation (day 28), in total 2.3 kg per day, whereas during mid-gestation and up until the 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 during the week before parturition (day 108 to day 115). After parturition, 0.25 kg of feed per suckling piglet was gradually supplemented in addition to 3 kg feed, also provided in two equal portions daily.

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

Within each static production group, equal numbers of sows were randomly allocated to one of three dietary treatment groups, depending on the number of sows. The dietary treatments differed in Zn concentration: Zn not supplemented, originating from ingredients only; 50 mg Zn per kg supplemented; and 100 mg Zn per kg supplemented. Thus, total dietary Zn concentration was expected to remain below the European Union (EU) upper limit of 150 mg per kg (EU regulation 2016/1095). The Zn supplement comprised 50% inorganic Zn as ZnO (75% Zn) (33.3 or 66.6 g ZnO per 1000 kg feed, INVE Belgium

Table 1: Ingredient composition of the gestation and lactation diet fed to sows (n = 131 at start of study) through three reproductive cycles to assess the effect of dietary Zn supplementation on claw quality measurements when sows were housed on different floor types during group housing

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% analysis found in Supplementary materials.
† Premix 2.75% analysis found in Supplementary materials.
NA = not applicable (ingredients not added to the gestation or lactation diet).

NV, Baasrode, Belgium), and 50% organic Zn as Availa Zn containing 10% Zn in an amino-acid complex: single amino acids from hydrolysed soy proteins (molar ratio 1:1, 250 or 500 g Availa Zn per 1000 kg feed, Zinpro Corporation, Eden Prairie, Minnesota).

Claw quality measurements

On all sows, claw lesion scores, claw conformation, and horn growth and wear were determined. For some claw quality measurements (horn wall Zn concentration and histological and mechanical claw characteristics), 36 sows (12 from each dietary treatment group and at least one from 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 allocated to the same treatment group and floor type did not meet this criterion); and housed in their group during the entire gestation period (eg, not separated from the group during the group housing period).

Claw lesion scoring, measurements of claw conformation and horn growth and wear were performed at the start of the experiment (day -10, baseline) and then on day 50 and day 140 of every cycle. For these measurements, sows were placed in a sow chute (FeetFirst sow chute; Zinpro Corporation, Eden Prairie, Minnesota), and lifted off the ground for maximum 15 to 20 minutes. At day 140, the supporting beam of the sow chute was disinfected between lactating sows to prevent pathogen transmission. After cleaning (water, brush and [or] hoof knife) and drying the claws with paper towels, lateral and medial claw digits of front and hind claws were scored for eight types of claw lesions using a tagged visual analogue scale (tVAS) of 160 mm (Figure 1). This scoring system was based on the "Zeugenklauwen check" (Wageningen University) and the method of FeetFirst by Zinpro Corporation.[28] All claw digits were scored for seven claw conformation measurements[12] using a digital calliper (Mitutoyo Belgium NV, Kruibeke, Belgium) following a methodology adapted from Calabotta et al[29] and Vermunt and Greenough.[2] These dimensions were subsequently used to calculate the distal toe angle, sole area, claw volume, claw horn size, and toe:heel ratio as described in van Riet et al.[12] For horn growth and wear, a superficial reference point was incised into the dorsal horn wall of both claw digits of the left front and right hind claw by carving a small indentation with a hoof knife 0.5 cm below the periople and colored with Indian ink.[12] At the subsequent evaluation (day 50 or day 140), the displacement above and below this reference point was measured using a digital calliper to determine horn growth, wear, and net horn growth.[12] A new superficial reference point was incised into the dorsal horn wall and colored with Indian ink. For each above-mentioned claw quality variable, a mean score per sow per parameter per scoring day (day 50 or day 140) was calculated and used for further analysis.

Histological and mechanical horn characteristics were determined after slaughter. For histological examination, a claw horn wall (abaxial) sample, including the periople, and heel horn sample closest to the heel horn-sole junction (containing both the epidermal and dermal layer) of each claw digit of the front claws was collected using a scalpel. The left lateral and right medial claw digit samples were fixed in a 3.5% buffered formaldehyde solution. Subsequent preparation steps are described in van Riet et al.[12] After H&E staining, standardized photographs (n = 5 positions per section; 10× magnification) of the sagittal heel horn sections (perpendicular to the bearing surface) and transverse horn wall and heel horn sections (parallel to the bearing surface) were examined. Each photograph of the transverse horn wall and sagittal heel horn sections was alternately assessed once by one of two observers. All transverse heel horn sections were assessed once by one

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

	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 fibre	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		

* Chemical analyses of ileal digestible (ID) amino acids and net energy (NEv) for pigs are calculated according to the feed tables of the Centraal Veevoederbureau (CVB, the Netherlands), 2007. Net energy for pigs is expressed as MegaJoules per kg.

† Dietary treatment is presented as 0, 50, or 100 mg Zn/kg, supplementing a basal diet that contained 46.6 mg Zn/kg and 128.9 mg Zn/kg during gestation and lactation, respectively.

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

observer. To calculate the histological claw characteristics, 93.4% of the transverse horn wall sections, 98.7% of the transverse heel horn sections, and 71.1% of the sagittal heel horn sections were included. These characteristics included the number of dermal papillae for the sagittal heel horn or dermal lamellae for the transverse horn wall sections per 1000 µm, width, distance, and length of longest dermal papillae or lamellae, and horn tubules density.[12] The remaining sections were excluded because of broken samples and absence of the dermis layer. To assess differences between the two observers, histological measurements of 14 sagittal heel horn sections were conducted by both observers, using an average of five photographs per section. A paired *t* test and Pearson correlation were used to analyze the differences and correlations between observers.

For mechanical horn examination, an abaxial horn wall sample of the lateral and medial digits of the right front claw (mean

Figure 1: Tagged visual analogue scale (tVAS) for claw lesion scoring in sows, created on the basis of scoring guides of Wageningen University and FeetFirst (Zinpro Corp, Eden Prairie, Minnesota). To score the claw area for claw lesions, a vertical bar was drawn on the line and the distance from 0 mm determined. The average distance of lateral and medial digits from front and hind claws per claw lesion type was used for further analyses. For skin lesion scoring, only skin lesions around the claw and dewclaw were included. Hemorrhages were included in scoring the horn wall for horizontal cracks. If hemorrhages are present, but no cracks, the score is 40 mm. The length of the dewclaw was determined by pushing the dewclaw against the claw to determine whether the dewclaw exceeds heel height.

0 — 40 — 80 — 120 — 160 mm

	0-40	40-80	80-120	120-160
Heel horn	Healthy	Slight overgrowth and/or erosion	Moderate overgrowth and/or erosion with moderate cracks	Severe overgrowth and/or erosion with cracks
Heel/sole junction	Healthy	Slight detachment of the heel-sole junction	Extensive detachment of the heel-sole junction	Long, deep detachment of heel-sole junction
White line	Healthy	Shallow and/or short detachment along white line	Clear and/or long detachment along white line	Long, deep detachment along white line
Skin lesions	None	Mild injury	Moderate/substantial injury	Severe, inflammation, infection of periople
Horizontal horn wall cracks	None	Hemorrhage and short shallow horizontal crack	Long shallow horizontal crack	Multiple and/or deep horizontal crack(s)
Vertical horn wall cracks	None	Short shallow vertical crack	Long shallow vertical crack	Multiple and/or deep vertical crack(s)
Claw length	Normal, ± 50 mm	One or both toes slightly longer	One or both toes significantly longer	Long toes that complicate locomotion
Dewclaw length	Normal, ± 20 mm	Dewclaw slightly longer	Dewclaw touches floor when standing	Dewclaw is cracked or (partially) missing

length, width, thickness: 27 × 17 × 4.5 mm) was sawn using an oscillating saw, after which the underlying tissues were removed with a scalpel. The angle adjacent to the dorsal border and periople was marked. The horn wall samples were weighed and individually stored in a vacuum (to prevent fluctuations in moisture content) at -20°C until analysis. Horn wall samples of the left front claw could not be used to test the mechanical horn characteristics because of the incised superficial reference point for horn growth and wear measurements. The lateral or medial abaxial horn wall samples (n = 36 sows, 1.7 ± 0.4 g) were defrosted during 24 hours at 4°C and weighed (1.8 ± 0.4 g). The horn wall samples were cut into the required dimensions: two subsamples of 20 mm length and 6 mm width with a variable thickness using a mitre cutter with lever transmission (LOWE 3140/HÜ, Original LOWE, Gebr. Schröder GmbH, Kiel, Germany) and were weighed. On the first day, the two subsamples of the lateral or medial sample were tested. The subsample with the marked angle adjacent to the dorsal border and periople was tested first, followed by the subsample without marking. The two subsamples of the other lateral or medial sample from the same sow were tested the next day in the opposite order to that used the first day. The horn wall samples were tested with a three-point bending test (Texture Analyzer; Stable Micro Systems Ltd, Surrey, United Kingdom) according to the methodology described by Franck et al.[30] The test characteristics (stress area and strain height) were adjusted for each sample because the sample thickness and weights differed. The span between the two supports was set to 15 mm and the sample was compressed over a distance of 5.5 mm (eg, maximal deformation) using a force transducer (load cell, 30 kg) exerted in the middle of the span distance of 15 mm. Each sample was tested with a loading velocity of 1 mm per minute and 15 mm per minute to determine visco-elastic properties of the claw horn. The time between the velocities ranged between 1 and 1.5 hours. A force-deformation curve was generated and converted (Exponent Software; Stable Micro Systems Ltd) to a stress-strain diagram. Then Young's modulus, yield stress, and maximal stress were determined.[30] Young's modulus is a measure of the rigidity and stiffness of the horn and is represented as the slope of the linear phase of the initial line. Yield stress is the point on the stress-strain diagram in which the material starts to lose its mechanical function and material properties begin to change at further loading. Yield stress is represented as the point of the line where the line becomes nonlinear, using a parallel straight line with the same slope as the initial line (strain equal to 1%), where the intersect with the stress-strain diagram is defined as the yield stress.[30] Maximal stress is represented as the maximal load a sample can withstand.[30] The abaxial horn wall samples were stored in a vacuum at -20°C post testing until further analysis. Abaxial horn wall samples were dried at 103°C to a constant weight and analyzed for Zn content.

Chemical analysis

Feed samples of the gestation and lactation diets were collected from each batch and ground to pass through a 1-mm sieve for Near Infrared Spectroscopy evaluation, then pooled per dietary treatment group every 3 months for proximate analysis according to international standard methods accredited by ISO 17025.[31] 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[32] were used to determine ADF, NDF, and ADL.

The homogenized feed sample was further ground to pass through a 0.5-mm sieve, and three of five samples per dietary treatment were subjected to Zn and Cu analysis. Copper was analyzed 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, then transferred to a 50-mL flask. The Zn and Cu concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Vista MPX; Varian Inc, Palo Alto, California).

The Zn concentration in abaxial horn wall samples (± 0.8 g) was determined by ICP-OES, Vista MPX after the samples were diluted in 10 mL 6N HNO_3 for 12 hours, heated on a hot plate at 150°C for approximately 2 hours, and transferred to a 50-mL flask.

Statistical analysis

Linear mixed models were used for the outcome variables (Y) mean claw-lesion scores, claw conformation, horn growth, horn wear, and net horn growth. Dietary Zn supplementation (X_{Zn}), floor type (X_{FT}), phase within the reproductive cycle (X_{phase}), parity (X_{par}), digit (X_{dig}, lateral or medial), claw (X_{claw}, front or hind), the two-way interactions between dietary Zn supplementation or floor type and phase within the reproductive cycle and interaction between dietary Zn supplementation and floor type were included as fixed effects. Reproductive cycle (B_{cycle}), sow (B_{sow}) and group (B_{group}) were included in the models as random effects to correct for the repeated measurements.

An example of a model is given in the following equation:

$$Y = \beta_0 + \beta_1 X_{Zn} + \beta_2 X_{FT} + \beta_3 X_{phase} + \beta_4 X_{par} + \beta_5 X_{dig} + \beta_6 X_{claw} + \beta_7 X_{Zn} X_{phase} + \beta_8 X_{FT} X_{phase} + \beta_9 X_{Zn} X_{FT} + B_{cycle} + B_{sow} + B_{group}$$

Similar linear mixed models were used to analyze the histological and mechanical claw characteristic data. Fixed and random effects included in the models differed according to the sampled structures (eg, only right front claw was used for mechanical claw characteristic versus all claws included for claw-lesion scoring) and time of sampling (eg, claw structures collected after slaughter versus multiple observations throughout the reproductive cycle). For the histological claw-characteristic data, dietary Zn supplementation, floor type, leg (left or right front), digit, and interaction between dietary Zn supplementation and floor type were included as fixed effects. Sow and group were included in the model as random effects. For the mechanical claw-characteristic data, dietary Zn supplementation, floor type, digit, and interaction between dietary Zn supplementation and floor type were included in the linear mixed model as fixed effects, and sow and group as random effects per test velocity. Differences between test velocities and between two samples of the same digit of the same sow were analyzed using a paired sample *t* test.

Non-significant interactions 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. Partitioned post-hoc tests are 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 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 ($P > .05$). Effects of parity, claw (front and hind), and claw digits (lateral and medial) on claw quality measurements are presented in the supplementary data and supported by Suppl tables 1 and 2.

Throughout three reproductive cycles, 36 sows (27.5% of 131 gilts that entered the study), of which 9, 16, and 11 sows from the 0-, 50-, and 100-mg supplemented Zn per kg treatment group, respectively, were removed from the experiment, and 21 of them were replaced by non-lame 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). In total, 70 sows remained in their group of origin, whereas 26 sows (repeat breeders) were transferred to another group allocated to the same dietary treatment and floor type. In total, 92 of 95 sows were slaughtered. Two sows died after their third parturition and front claws from one sow were collected post mortem after euthanasia at the ILVO experimental farm directly after the other sows of the group were loaded for transport.

Claw lesion scores

Dietary Zn supplementation influenced the mean heel horn erosion score ($P = .01$), showing a 3.48-mm higher (worse) mean heel horn erosion score for the non-supplemented sows compared to the 100-mg Zn per kg supplemented sows (Table 3). Other types of claw lesions did not differ between dietary treatment groups ($P > .05$). Floor type did influence some claw lesion scores.[26] Mean claw lesion scores were lower at day 50 than at day140 of the reproductive cycle, except for horizontal wall cracks (Table 3).[26]

Claw conformation

Claw dimension measurements. An interaction was found between dietary Zn supplementation and phase within the reproductive cycle for the mean sole (base) length ($P = .03$), showing shorter sole lengths for the non-supplemented sows than for the 50-mg Zn per kg supplemented sows at day140 (Table 3). Mean heel height was influenced by dietary Zn supplementation ($P = .01$), with lower heel heights for the non-supplemented and 100-mg Zn per kg supplemented sows compared with the 50-mg Zn per kg supplemented sows (Table 3).

Independent of dietary Zn supplementation, the mean toe height and mean claw length of sows were lower and shorter, respectively, for the rubber floor than for the concrete floor ($P = .001$ and $P = .04$, respectively) at day 50 (Table 4). Mean heel height tended to be lower for sows housed on the concrete floors at day 50 ($P = .06$) and was lower for sows housed on the rubber floors ($P = .04$) at day 140. The interaction between floor type and phase within the reproductive cycle was not significant for claw width. Mean claw width did not differ between floor types.

Mean length of the dorsal border, diagonal claw length, toe height, heel height, and claw length were all shorter at day 50 than at day 140; claw width was the only exception (Table 3).

Claw morphology calculations. The mean claw volume was less ($P = .03$) for non-supplemented sows and tended to be less ($P = .06$) for the 100-mg Zn supplemented sows than for 50-mg Zn per kg supplemented sows. The mean toe:heel ratio was influenced by dietary Zn supplementation ($P = .02$), with a higher ratio for the non-supplemented sows compared with the ratio of the 50-mg Zn per kg supplemented sows. Distal toe angle, sole area, and claw horn size did not differ (Table 3).

The mean distal toe angle, sole area, and toe:heel ratio were lower for sows housed on rubber floors than for sows housed on the concrete floors at day 50 (Table 4). Mean claw volume was lower for the rubber floor type compared with the concrete floor type at day 140 ($P = .01$). The mean claw horn size did not differ (Table 4).

Mean distal toe angle and toe: heel ratio were lower, while mean sole area, claw volume, and claw horn size were higher at day 140 than at day 50 of the reproductive cycle (Table 3).

Horn growth and wear. Horn growth and wear did not differ between dietary treatment groups (Table 3). At day 50, horn growth and wear were lower for sows housed on rubber floors than on concrete floors. At day 140, horn growth and wear did not differ between floor types (Table 4). Net horn growth (horn growth minus wear) did not differ between dietary treatment groups nor between floor types at day 50 and day 140 (tables 3 and 4).

Net horn growth differed between day 50 (-4.2 mm, horn wear dominated) and day 140 (+4.4 mm, horn growth dominated) ($P < .001$).

Histological claw characteristics. Length of the longest dermal papillae ($P = .83$) and width of papillae ($P = .13$) did not differ between observers. Differences between observers were found for sample length ($P = .03$; confidence interval [CI] 3.3-57.8 µm, mean difference 30.5 µm), number of dermal papillae ($P = .003$; CI -0.9 to -0.2, mean difference 0.6), number of dermal papillae per 1000 µm ($P = .003$, CI -1.0 to -0.2, mean difference -0.6), and distance between papillae ($P = .02$, CI 9.1-100.3 µm, mean difference 54.7 µm). Correlation coefficients of inter-observer reliability were 0.95 for sample length, 0.79 for dermal number, 0.96 for longest length, 0.43 for distance between papillae, and 0.94 for papillae width. On the basis of the high inter-observer reliability (except distance between papillae) and the numerically irrelevant (but significant) differences, observer was not included in the final statistical models.

The number of dermal lamellae per 1000 µm, distance between lamellae, width of the lamellae, or length of the longest lamellae of the transverse horn wall did not differ between dietary treatment groups nor between floor types (Table 5).

The distance between the dermal papillae of the sagittal heel horn tended to be shorter for the non-supplemented sows ($P = .08$) and was shorter for the 50-mg Zn per kg supplemented sows ($P = .003$) compared with the 100-mg Zn per kg supplemented sows (Table 5). The number of dermal papillae per 1000 µm, width of the papillae, or length of the longest papillae of the sagittal heel horn did not differ ($P > .05$) between dietary treatment groups.

Independent of dietary Zn supplementation, the length of the longest papillae of the sagittal heel horn tended to be shorter and the distance between dermal papillae was shorter for sows housed on rubber floors than sows housed on concrete floors (Table 5). The number of dermal papillae per 1000 µm or width of the papillae did not differ between floor types (Table 5).

The density of the heel horn tubules of the transverse heel horn, expressed as the number of horn tubules within a defined surface area of 1 mm^2, did not differ between dietary treatment groups nor between floor types (Table 5).

Mechanical horn characteristics. Horn wall Zn concentration did not differ between dietary treatment groups (Zn concentration in DM was 128, 122, 120 mg Zn per kg for non-supplemented, 50-mg per kg- and 100-mg per kg supplemented sows, respectively; $P = .39$) nor between floor type treatments (Zn concentration in DM was 123 and 124 mg Zn per kg for concrete and rubber floor types, respectively; $P = .86$).

Thickness of the abaxial horn wall sample did not differ with dietary Zn supplementation ($P = .94$) nor with floor type ($P = .77$). None of the mechanical abaxial horn wall characteristics were significantly affected by dietary Zn supplementation or floor type (Table 6). Young's modulus differed between 1 mm per minute and 15 mm per minute test loading velocities ($P = .01$); tended to differ for maximal stress ($P = .07$); and did not differ for yield stress ($P = .22$), showing visco-elastic properties of the horn wall. Differences between the two horn wall samples per claw digit per sow for both test velocities were found for Young's modulus, yield stress, and maximal stress ($P < .001$).

Discussion

The welfare and productivity of sows can be affected by poor claw quality. In the present study, multiple claw characteristics were determined to evaluate claw quality in response to dietary Zn supplementation. Minor effects on claw quality measurements were found irrespective of floor type. Possible factors that overruled the potential influence of Zn in the present study are the overall good claw lesions score – with a mean lesion score close to the upper threshold of 40 mm for healthy claws – and the unexpectedly high background Zn supply via premix in the

Table 3: Effect of dietary Zn supplementation on mean claw lesion scores, claw conformation (claw dimensions and calculation) and horn growth and wear* at day 50 and day 140 from sows housed on different floor types during group housing and followed through three reproductive cycles (n = 131 at start of study)

	Reproductive cycle						*P*†	
	Day 50			Day 140			Zn	Cycle
Dietary treatment‡	0	50	100	0	50	100		
Claw lesion type (mm)								
Heel horn erosion	48.2 [46.6, 49.8]	47.3 [45.6, 49.1]	44.3 [42.8, 45.8]	60.1 [58.7, 61.5]	58.1 [56.8, 59.5]	57.2 [55.7, 58.6]	.01	< .001
Heel/sole junction separation	41.8 [40.4, 43.1]	39.4 [38.0, 40.8]	40.0 [38.6, 41.3]	53.5 [52.1, 54.9]	52.1 [50.7, 53.4]	50.6 [49.2, 52.1]	.14	< .001
White line separation	48.5 [47.1, 50.0]	45.5 [43.9, 47.0]	45.7 [44.2, 47.1]	55.2 [53.5, 56.8]	53.8 [52.1, 55.6]	53.3 [51.6, 55.0]	.13	< .001
Skin lesions	18.9 [17.8, 20.1]	22.9 [21.5, 24.3]	22.2 [21.0, 23.5]	26.3 [25.0, 27.6]	26.5 [25.2, 27.8]	26.6 [25.3, 27.9]	.11	< .001
Horizontal wall cracks	41.9 [40.5, 43.4]	42.1 [40.6, 43.7]	42.1 [40.6, 43.6]	42.4 [41.0, 43.8]	44.1 [42.6, 45.6]	43.2 [41.7, 44.6]	.61	.26
Vertical wall cracks	26.2 [24.6, 27.9]	28.2 [26.6, 29.8]	28.4 [26.8, 29.9]	29.9 [28.5, 31.4]	32.2 [30.6, 33.9]	29.9 [28.3, 31.4]	.36	.002
Overgrown claw	30.9 [29.8, 32.1]	28.3 [27.1, 29.5]	29.1 [28.0, 30.2]	38.8 [37.7, 39.9]	38.7 [37.5, 39.8]	37.9 [36.7, 39.0]	.66	< .001
Overgrown dewclaw	34.5 [33.1, 36.0]	34.1 [32.5, 35.8]	35.6 [34.1, 37.2]	41.0 [39.5, 42.5]	40.9 [39.2, 42.5]	41.0 [39.5, 42.5]	.88	< .001
Claw dimensions (mm)								
Sole (base) length	24.8 [24.6, 25.1]	24.9 [24.6, 25.2]	24.4 [24.1, 24.6]	26.3 [26.0, 26.6]	27.2 [26.8, 27.5]	26.8 [26.5, 27.1]	¶	¶
Claw width	27.5 [27.2, 27.7]	27.9 [27.6, 28.1]	27.3 [27.1, 27.6]	27.2 [26.9, 27.4]	27.6 [27.3, 27.8]	27.5 [27.2, 27.7]	.67	.18
Length dorsal border	43.1 [42.8, 43.4]	42.5 [42.2, 42.7]	42.7 [42.4, 43.0]	48.3 [48.0, 48.6]	48.1 [47.8, 48.5]	48.2 [47.9, 48.6]	.90	< .001
Diagonal claw length	55.0 [54.6, 55.4]	54.9 [54.5, 55.3]	55.2 [54.8, 55.5]	59.5 [59.1, 59.9]	59.4 [59.0, 59.8]	59.3 [58.9, 59.7]	.56	<.001
Toe height	35.4 [35.1, 35.7]	35.3 [35.1, 35.6]	35.1 [34.8, 35.4]	36.2 [35.9, 36.6]	35.9 [35.5, 36.3]	36.3 [36.0, 36.6]	.71	.001
Heel height	8.0 [7.6, 8.3]	8.2 [7.8, 8.5]	8.3 [8.0, 8.7]	11.2 [10.9, 11.6]	12.2 [11.8, 12.5]	11.1 [10.7, 11.4]	.01	< .001
Claw length	50.2 [49.8, 50.6]	50.1 [49.7, 50.4]	50.1 [49.7, 50.5]	52.0 [51.6, 52.4]	51.5 [51.1, 51.9]	52.2 [51.7, 52.6]	.91	< .001
Claw calculations								
Distal toe angle (°)	56.7 [56.0, 57.4]	57.3 [56.6, 58.0]	56.5 [55.8, 57.2]	49.7 [49.0, 50.4]	49.3 [48.5, 50.0]	50.2 [49.5, 50.8]	.88	< .001
Sole area (mm^2)	1384 [1365, 1403]	1400 [1380, 1419]	1373 [1355, 1391]	1418 [1399, 1437]	1424 [1403, 1445]	1441 [1421, 1461]	.97	< .001
Claw volume (mm^3)	11091 [10585, 11598]	11532 [11013, 12052]	11540 [11025, 12056]	15961 [15385, 16538]	17248 [16639, 17858]	15823 [15233, 16413]	.03	< .001
Claw horn size (mm^2)	1520 [1499, 1540]	1537 [1516, 1558]	1515 [1495, 1534]	1624 [1603, 1645]	1641 [1619, 1663]	1638 [1617, 1660]	.64	< .001
Toe: heel ratio	3.9 [3.6, 4.3]	3.8 [3.7, 3.9]	3.7 [3.6, 3.8]	3.0 [2.9, 3.1]	2.9 [2.8, 2.9]	3.0 [3.0, 3.1]	.02	< .001

Table 3: Continued

Dietary treatment‡	Reproductive cycle Day 50 0	50	100	Day 140 0	50	100	P† Zn	Cycle
Horn growth and wear (mm)								
Horn growth	12.4 [12.0, 12.8]	12.7 [12.3, 13.1]	12.3 [11.9, 12.6]	20.9 [20.3, 21.4]	20.8 [20.2, 21.3]	21.7 [21.2, 22.2]	.67	< .001
Wear rate	16.0 [15.6, 16.5]	17.0 [16.5, 17.5]	16.9 [16.5, 17.4]	16.4 [15.9, 17.0]	16.5 [15.9, 17.1]	17.2 [16.7, 17.8]	.28	.93
Net horn growth§	-3.8 [-4.2, -3.3]	-4.4 [-4.9, -3.9]	-4.7 [-5.1, -4.3]	4.4 [3.9, 4.9]	4.3 [3.8, 4.8]	4.4 [3.9, 4.9]	.55	< .001

* Mean claw lesion score (mm) is the average score per lesion type for all sows including front and hind claws, lateral and medial digits. Higher values represent worse scores. Mean claw conformation measurements and calculations (mm) is the average score measurement for all sows including front and hind claws, lateral and medial digits. Horn growth and wear (mm) was determined from both lateral and medial claw digits of the left front and right hind claws. Values are mean with [95% CI].

† There were no interactions between dietary Zn supplementation and floor type or between dietary Zn supplementation and phase within the reproductive cycle, except for sole (base) length. *P* values are presented for the main effect of dietary Zn supplementation (Zn) and for the main effect of phase within the reproductive cycle (Phase). The effect of floor type on claw lesion scores are presented by Bos et al.[26] Level of significance is $P < .05$.

‡ 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.

§ Net horn growth is horn growth minus wear and represents the balance between horn growth and wear throughout the reproductive cycle.

¶ The post-hoc portioned *P* values for sole (base) length are $P = .34$ for day 50 and $P = .03$ for day 140. Differences were observed between the non-supplemented sows and 50 mg/kg supplemented sows.

basal diet during lactation. The impact is, however, not quantifiable. Differences in claw quality for floor type were observed under these similar study conditions, but floor type is documented as an external predisposing factor for claw lesions, whereas dietary Zn concentration may affect claw quality internally[6,18] by influencing horn production, which may be more time dependent. Therefore, study duration is an important factor to take into account. Claws require a minimum duration for the horn capsule to be produced. In cattle, this was determined as 12 months.[10,11,20,33] On the basis of the length of the dorsal border and horn growth in the present study, the horn capsule in sows may have been produced in 5 to 6 months. The present study lasted 14.8 months, thus it seems that study duration is unlikely to have influenced the lack of response to Zn observed here.

Claw quality is determined by multiple claw characteristics and depends on an optimal horn production.[1,2,7,8] In the literature, Zn has been shown to have an important function in horn production.[7] If Zn supplementation influences horn production, changes in multiple claw quality measurements should become visible. In the present study, however, only heel horn erosion, heel height, claw volume, toe:heel ratio, and the distance between dermal papillae were influenced. These differences were not linear and not constant between different treatment groups for all characteristics. For instance, the distance between dermal papillae was longer for the 100-mg per kg supplemented sows, indicative of a lower quality of the structure, whereas the heel horn erosion score was better. In accordance with the present study, Anil[17] observed differences only for vertical wall cracks in group-housed sows and only for heel-sole junction lesions in stall-housed sows. Other claw lesion scores assessed in their study were not improved over time. Some differences in claw quality measurements were observed only at day 50 or only at day 140 of the reproductive cycle, suggesting that phase within the reproductive cycle is important for claw quality. This is supported by the substantial fluctuation in net horn growth (horn growth minus wear) between gestation (-4.2 mm between day 140 and day 50, horn wear dominant) and lactation (+4.4 mm between day 50 and day 140, horn growth dominant). This difference in net horn growth between gestation and lactation can be caused by housing conditions and differences in physiology. Sows were housed in groups for 80 days during gestation and were more able to move around, whereas during lactation the sows were individually housed.

Comparing results of the present study with other studies in sows is difficult, because those studies did not assess the impact of Zn supplementation but did investigate (partially) substituted inorganic Zn, Cu, and Mn sources with organically bound sources at high mineral levels. Similar or better claw lesion scores were reported with organically bound Zn, Cu, and Mn supplementation compared with inorganic sources.[13-18] Bradley[19] found no difference between mineral sources for claw size and shape, however. Results of the present study do not agree with those of some other studies. This may reflect differences in breeds and test conditions, but also the difference in background levels of Zn in the basal diet. While the results derived from the chosen test conditions in our study should be extrapolated with caution, yet the detail

Table 4: Effect of floor type on mean claw conformation (claw dimensions and calculation) and horn growth and wear* at day 50 and day 140 from sows housed on different floor types during group housing and followed for three reproductive cycles (n = 131 at start of study).

	Reproductive cycle				*P*†	
	Day 50		Day 140		Day 50	Day140
Floor type	**Concrete**	**Rubber**	**Concrete**	**Rubber**		
Claw dimensions (mm)						
Sole (base) length	25.2 [24.9, 25.4]	24.2 [24.0, 24.4]	26.8 [26.5, 27.0]	26.7 [26.4, 27.0]	.19	.94
Claw width	27.8 [27.6, 28.0]	27.3 [27.1, 27.5]	27.4 [27.2, 27.6]	27.4 [27.2, 27.6]	§	§
Length dorsal border	43.0 [42.7, 43.2]	42.6 [42.4, 42.8]	48.8 [48.6, 49.1]	47.5 [47.3, 47.8]	.84	.22
Diagonal claw length	55.3 [54.9, 55.6]	54.8 [54.5, 55.1]	60.0 [59.7, 60.3]	58.7 [58.4, 59.0]	.87	.38
Toe height	36.4 [36.2, 36.6]	34.1 [33.8, 34.3]	35.9 [35.6, 36.2]	36.4 [36.1, 36.7]	.001	.49
Heel height	7.7 [7.4, 8.0]	8.6 [8.3, 8.8]	12.1 [11.8, 12.4]	10.7 [10.4, 11.0]	.056	.04
Claw length	51.2 [50.9, 51.6]	49.1 [48.8, 49.4]	52.3 [51.9, 52.6]	51.5 [51.1, 51.8]	.04	.57
Claw calculations						
Distal toe angle (°)	58.6 [58.1, 59.0]	55.0 [54.3, 55.6]	48.5 [47.9, 49.0]	51.1 [50.6, 51.7]	.04	.16
Sole area (mm^2)	1430 [1413, 1447]	1344 [1330, 1357]	1441 [1424, 1457]	1413 [1398, 1429]	.03	.52
Claw volume (mm^3)	11136 [10709, 11562]	11616 [11203, 12030]	17429 [16969, 17889]	15031 [14530, 15533]	.25	.01
Claw horn size (mm^2)	1543 [1526, 1561]	1502 [1487, 1518]	1654 [1636, 1672]	1612 [1595, 1629]	§	§
Toe:heel ratio	4.1 [3.9, 4.4]	3.5 [3.4, 3.6]	2.9 [2.8, 3.0]	3.1 [3.0, 3.1]	.02	.75
Horn growth and wear (mm)						
Horn growth	13.7 [13.4, 14.0]	11.3 [11.0, 11.6]	21.1 [20.6, 21.6]	21.2 [20.8, 21.6]	.001	.63
Wear rate	17.7 [17.3, 18.0]	15.7 [15.3, 16.1]	16.6 [16.1, 17.1]	16.9 [16.4, 17.3]	.001	.68
Net horn growth‡	-4.1 [-4.4, -3.7]	-4.5 [-4.9, -4.1]	4.5 [4.1, 4.9]	4.3 [3.9, 4.7]	§	§

* Mean claw conformation measurements and calculations (mm) is the average score measurement for all sows including front and hind claws, lateral and medial digits. Horn growth and wear (mm) was determined from both lateral and medial claw digits of the left front and right hind claws. Values are mean with [95% CI].

† There were no interactions between dietary Zn supplementation and floor type, but there were interactions between floor type and phase within the reproductive cycle, except for claw width, claw horn size, and net horn growth. *P* values are presented as post-hoc portioned *P* values for day 50 and day 140. The effect of floor type on claw lesion scores are reported by Bos et al.[26] Level of significance is $P < .05$.

‡ Net horn growth is horn growth minus wear and represents the balance between horn growth and wear throughout the reproductive cycle.

§ *P* values for the main effect of floor type for claw width, claw horn size, and net horn growth are $P = .49$, $P = .31$, and $P = .94$, respectively, and for the main effect of phase within the reproductive cycle are $P = .18$, $P < .001$, and $P < .001$, respectively.

Table 5: Effect of dietary Zn supplementation on histological claw characteristics* from sows housed on different floor types during group housing after slaughter at the third reproductive cycle (n = 36)

	Dietary treatment (mg Zn/kg)†			Floor type		*P*‡	
Histologic characteristics	**0**	**50**	**100**	**Concrete**	**Rubber**	**Zn**	**F**
Transverse horn wall							
Dermal lamellae (n)	6.6 [5.8, 7.4]	7.4 [6.4, 8.4]	7.0 [6.2, 7.7]	6.9 [6.3, 7.6]	7.0 [6.3, 7.7]	.46	.77
Distance (µm)	153.7 [136.9, 170.5]	139.9 [121.4, 158.4]	145.5 [130.9, 160.1]	146.3 [134.2, 158.4]	146.2 [131.5, 160.9]	.56	.86
Width (µm)	56.4 [47.2, 65.6]	51.9 [39.4, 64.5]	50.0 [43.1, 57.0]	51.9 [45.9, 58.0]	53.6 [44.1, 63.0]	.63	.90
Length (µm)	233.3 [207.0, 259.5]	222.2 [189.3, 255.0]	201.4 [181.4, 221.5]	215.7 [194.2, 237.2]	221.9 [199.7, 244.1]	.35	.77
Sagittal heel horn							
Dermal papillae (n)	3.0 [2.7, 3.3]	2.9 [2.5, 3.3]	2.5 [2.2, 2.9]	3.0 [2.7, 3.3]	2.7 [2.4, 3.0]	.18	.22
Distance (µm)	315.7[ab] [289.3, 342.2]	282.6[a] [253.5, 311.7]	390.3[b] [331.9, 448.7]	340.4 [308.7, 372.1]	315.1 [281.8, 348.4]	.004	.04
Width (µm)	126.6 [110.6, 142.6]	131.8 [111.3, 152.3]	150.1 [130.2, 169.9]	130.9 [115.9, 145.9]	137.6 [122.8, 152.3]	.22	.48
Length (µm)	500.0 [386.9, 613.0]	461.3 [364.2, 558.4]	443.7 [370.7, 516.6]	537.7 [438.6, 636.7]	423.6 [363.1, 484.0]	.63	.05
Transverse heel horn							
Horn tubules (n)	7.4 [6.7, 8.0]	6.5 [5.9, 7.1]	6.7 [6.2, 7.2]	6.9 [6.4, 7.4]	6.8 [6.4, 7.2]	.11	.75

* Dermal papillae/lamellae = number of dermal papillae/lamellae per 1000 µm, visible at their full width; Distance = distance between the axis lines of the papillae/lamellae at their base (µm); Width = width of the dermal component halfway and perpendicular to the dermal papillae/lamellae (µm); Length = length of the longest papillae measured from the top of the dermal papillae/lamellae to the origin at the base (µm); Horn tubules = heel horn tubules density expressed as number of horn tubules within a defined surface area of 1 mm^2. Horn tubules that were only partially visible from two of the four sides of the defined surface area were also included. Values are means with [95% CI].

† 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.

‡ There were no interactions between dietary Zn supplementation and floor type. *P* values are presented for the main effect of dietary Zn supplementation (Zn) and for the main effect of floor type (F). Level of significance is $P < .05$.

[a,b] Mean values within a row and main effect lacking common superscript letters differ significantly; $P < .05$.

of our measurements, together with the three-cycle duration of the study, rendered important insights that are new to the field. Nevertheless, the type of Zn supplement used may be an interfering factor, as other studies in sows did find positive results of Zn source on claw lesion scores.[13-17] Perhaps Zn alone does not trigger the processes required to optimise claw quality, and other minerals or dietary components are required as well.

Other studies in sows defined claw quality mainly by claw lesion scores, but claw conformation, horn growth and wear, and mechanical horn characteristics are rarely evaluated. Histological claw characteristics have been assessed more recently.[3,4] Claw conformation did not differ between treatment groups except for heel height, claw volume, and toe:heel ratio, which is in accordance with Bradley.[19] Horn growth and wear rate also did not differ in the present study. Lisgara et al[18] also found no effect of partially substituted mineral sources on toe and dewclaw length in one herd, whereas the score did improve over time in each of the other two herds. Herd-specific characteristics might have influenced the outcome in that study. In the same study,[18] claw (front or hind) did interfere with the results. In the present study, horn growth and wear rate differed between front and hind claws as well as for other claw lesion scores and claw conformation. Net horn growth did not differ between claws, however. There is a need to further determine the impact of these factors. For histological claw characteristics, Varagka et al[3,4] found that lamellar hyperplasia was most frequently observed and sows had a higher claw lesion score with lamellar hyperplasia. This histological condition is also described in bovine and equine laminitis and may cause low quality horn production. Partially substituting inorganic Zn, Cu, and Mn sources with organic sources resulted in fewer histological changes than in the control group with inorganic mineral sources.[3] These interesting findings were deduced from a sample at the dorsal wall halfway between the periople and tip of the toe, whereas in the present study a

Table 6: Effect of dietary Zn supplementation on mechanical horn characteristics* from sows housed on different floor types during group housing after slaughter at the third reproductive cycle (n = 36)

Mechanical characteristics	Test velocity†						*P*‡	
	1 mm/min			15 mm/min			1	15
Dietary treatment§	**0**	**50**	**100**	**0**	**50**	**100**		
Young's modulus (MPa)	68.3 [60.1, 76.5]	70.2 [59.5, 80.9]	70.0 [61.1, 78.8]	96.6 [86.2, 106.9]	99.2 [84.3, 114.0]	97.0 [85.1, 109.0]	.99	1.00
Yield stress (MPa)	10.8 [9.7, 11.8]	10.5 [9.3, 11.8]	10.1 [9.3, 11.0]	13.1 [11.9, 14.3]	13.5 [12.0, 15.1]	12.9 [11.7, 14.2]	.72	.98
Maximum stress (MPa)	14.8 [13.6, 16.1]	15.3 [13.9, 16.7]	15.1 [13.9, 16.3]	19.5 [18.0, 21.0]	20.2 [18.3, 22.1]	19.7 [18.1, 21.3]	.96	.98
Floor type	**Concrete**		**Rubber**	**Concrete**	**Rubber**			
Young's modulus (MPa)	66.2 [58.5, 73.8]		72.2 [65.1, 79.3]	93.3 [83.1, 103.5]	101.1 [91.6, 110.7]		.74	.73
Yield stress (MPa)	9.9 [9.1, 10.8]		11.0 [10.1, 11.8]	12.6 [11.5, 13.8]	13.6 [12.6, 14.6]		.68	.76
Maximum stress (MPa)	14.8 [13.6, 15.9]		15.3 [14.4, 16.3]	19.4 [18.0, 20.9]	20.0 [18.8, 21.3]		.96	.96

* Young's modulus is a measure of the rigidity and stiffness of the horn; yield stress represents the point on the stress-strain diagram in which the material starts to lose its mechanical function and material properties start to change at further loading, and maximal stress represents the maximum compression (Franck et al.[30])

† Mechanical claw characteristics were tested on two test velocity of the right front claw, 1 and 15 mm/min, to test if the abaxial horn wall had visco-elastic properties. The abaxial horn wall does have these properties, because test velocities differ ($P < .05$). Values are means with [95% CI].

‡ There were no interactions between dietary Zn supplementation and floor type. *P* values are presented for the main effect of dietary Zn supplementation (Zn) and for the main effect of floor type (F) at test velocity 1 mm/min (1) and 15 mm/min (15). Level of significance is $P < .05$.

§ 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.

MPa = MegaPascals

sample from the periople was collected, where horn production is initiated. In the present study, no differences were found in histological characteristics of the horn wall or in mechanical horn strength, although the sample for mechanical horn strength was more closely located to the sample location of Varagka et al.[3] Testing for mechanical horn characteristics has not been conducted previously and was based on test conditions used in bovine horn wall without dietary interventions.[30] For sows, the extrapolation of this test and its conditions needs to be further explored.

In the present study, most sows had one or more claw lesions, but only the mean heel horn erosion score was better for the 100-mg Zn per kg supplemented sows compared with the non-supplemented sows at day 50 of the reproductive cycle. This result may favor Zn supplementation; however, it is questionable whether the measured difference is relevant for the sows' welfare and performance and whether this difference is distinguishable during visual claw scoring. Furthermore, it is remarkable that the non-supplemented sows were able to maintain claw quality at the same level as the supplemented sows. While the non-supplemented group had a high background Zn supply during lactation, overall dietary Zn concentration was well below commercial practice and EU regulation of 150 mg per kg total Zn (EU regulation 2016/1095), without negative results, which questions whether the systematic supplementation of Zn, in addition to the amount of Zn (and phytase) present in the basal diet, should be revisited. Further reductions of the EU upper limit for total dietary Zn concentration when using phytase, as suggested by the European Food Safety Authority in 2014, should be tested in relation to claw quality in future studies.

The effect of floor type on claw quality measurements in the present study appeared to be substantial, irrespective of dietary Zn supplementation. Sows housed on a rubber floor had better scores for some claw lesion types, but worse scores for vertical horn wall cracks, white line separation, and overgrown claw at day 50 of gestation, which changed to better scores on rubber at day 140.[26] At day 140, scores for white line and claw length were better for sows housed on a rubber floor during gestation.[26] Other types of claw lesions did not differ between floor types. This finding is in contrast to results of another study[24] in sows that found an increased risk of poor scores for toe overgrowth, heel sole cracks, and horn wall cracks in first-parity sows housed on rubber slat mats, as compared to sows housed on a concrete slatted floor. In the second parity of that study, sows housed on rubber slat mats had an increased risk of poor scores for toe overgrowth, heel sole cracks, and white line damage.[24]

Discrepancies between studies may relate to the proportion of solid floor area, as the present study had a higher proportion of solid floor area as compared to a fully slatted floor covered with rubber in the study of Calderón Díaz et al.[24] Another explanation may be the quality of the floor as characterised by the slip resistance, abrasiveness, hardness, wear resistance, and age of the floor.[6] Rubber slat mats may be less abrasive [24,34] and may be softer than concrete slats.[24,35-39] The influence of floor type on claw conformation in the present study can be a result of this softer rubber floor. Softer floors can reduce natural horn wear,[6,40-42] which was also found in the present study in which sows housed on rubber floors had less horn wear at day 50 compared with sows housed on concrete floors. However, some claw lesion scores were lower (better) on rubber. It seems possible that a higher risk for claw lesions does not depend on insufficient wear alone: the balance between horn growth and wear and load bearing (eg, the pressure the floor exerts on the claw and how the load is distributed over the weight-bearing surface) may also be important factors.[43] In the present study, horn growth was lower for sows housed on rubber floors at day 50 and net horn growth was not affected, indicating that the balance between horn growth and wear could be maintained and that therefore claws were less prone to develop lesions. Similarly, the distal toe angle was lower on rubber floors, which indicates that there was less wear, less growth, and a smaller sole area.[44] Load bearing may have been better in the sows housed on rubber floors in the present study, because the histological claw characteristics showed a shortened distance between dermal papillae in the sagittal heel horn, indicating a stronger structure.

Slat properties are also important.[6,45-47] The rubber top layer in the present study was attached to the concrete floor in a fashion similar to that reported in Calderón Díaz et al.[24] One advantage of the rubber top layer may be a reduction in the risk of claws getting entrapped between the slats. An important disadvantage in the study of Calderón Díaz et al[24] was that the manure could not pass through the slats easily. This observation was not included in the present study. Floors covered with liquid and manure have been reported to soften and irritate the claw, resulting in diminished claw strength.[6] This may not have been the case in the present study, where moisture content of the horn wall and horn wall strength were not affected by floor type.

In conclusion, no interaction effects between dietary Zn supplementation and floor type were found for claw quality measurements. Dietary Zn supplementation to a typical basal diet had only minor influences on claw quality in sows. Floor type affected multiple claw quality measurements positively. The rubber top layer does improve claw quality and can be implemented for prevention of claw lesions, but more research is warranted. Apart from dietary Zn supplementation and floor type, other factors affected claw quality measurements: differences between lateral and medial claw digits and between front and hind claws were observed, and the reproductive phase had an important effect on claw quality. Worse claw lesion scores were found at day 140, a period in which horn growth dominates and claw conformation characteristics changes, compared with day 50.

Implications

- Dietary Zn supplementation seems not to be the major factor affecting claw quality in the sows in this study.
- Floor type affects claw quality in sows.
- Phase within the reproductive cycle influences claw quality measurements and needs to be considered when claw quality is assessed.

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Conflict of interest

None reported

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References

1. Politiek RD, Distl O, Fjeldaas T, Heeres J, McDaniel BT, Nielsen E, Peterse DJ, Reurink A, Strandberg P. Importance of claw quality in cattle: review and recommendations to achieve genetic improvement. Report of the EAAP working group on "claw quality in cattle." *Livest Prod Sci*. 1986;15:133–152.

2. Vermunt JJ, Greenough PR. Structural characteristics of the bovine claw: horn growth and wear, horn hardness and claw conformation. *Brit Vet J.* 1995;151:157–180.

3. Varagka N, Lisgara M, Skampardonis V, Psychas V, Leontides L. Partial substitution, with their chelated complexes, of the inorganic zinc, copper and manganese in sow diets reduced the laminitic lesion in the claws and improved the morphometric characteristics of the hoof horn of sows from three Greek herds. *Porcine Health Manag.* 2016;2:26.

4. Varagka N, Lisgara M, Skampardonis V, Psychas V, Leontides L. Pathological evaluation of claw lesions in culled sows from a Greek herd. *J Swine Health Prod*. 2016;24:72–80.

5. Heinonen M, Peltoniemi O, Valros A. Impact of lameness and claw lesions in sows on welfare, health and production. *Livest Sci*. 2013;156:2–9.

6. Pluym L, Van Nuffel A, Maes D. Treatment and prevention of lameness with special emphasis on claw disorders in group-housed sows. *Livest Sci*. 2013;156:36–43.

7. Tomlinson DJ, Mulling CH, Fakler TM. Invited Review: Formation of keratins in the bovine claw: Roles of hormones, minerals, and vitamins in functional claw integrity. *J Dairy Sci.* 2004;87:797–809.

*8. Muelling CKW. Nutritional influences on horn quality and hoof health. *WCDS Advances in Dairy Technology*. 2009;21:283–291.

9. Enjalbert F, Lebreton P, Salat O. Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: retrospective study. *J Anim Physiol Anim Nutr*. 2006;90:459–466.

10. Griffiths LM, Loeffler SH, Socha MT, Tomlinson DJ, Johnson AB. Effects of supplementing complexed zinc, manganese, copper and cobalt on lactation and reproductive performance of intensively grazed lactating dairy cattle on the South Island of New Zealand. *Anim Feed Sci Tech*. 2007;137:69–83.

11. Lethbridge LA. Lameness of dairy cattle: factors affecting the mechanical properties, haemorrhage levels, growth and wear rates of bovine claw horn. PhD dissertation. Massey University, New Zealand. 2009.

12. van Riet MMJ, Janssens GPJ, Cornillie P, Van Den Broeck W, Nalon E, Ampe B, Tuyttens FAM, Maes D, Du Laing G, Millet S. Marginal dietary zinc concentration affects claw conformation measurements but not histological claw characteristics in weaned pigs. *Vet J.* 2016;209:98–107.

*13. Aae H. Danish experience with claw lesions and mineral nutrition. *Zinpro Feet First Symposium*. Minneapolis, Minnesota. 2008;58–63.

*14. Anil SS, Deen J, Anil L, Baidoo SK, Wilson ME, Ward TL. Analysis of the effect of complex trace minerals on the prevalence of lameness and severity of claw lesions in stall-housed sows. *ADSA-ASAS Joint Annual Meeting*, Denver, Colorado. 2010;127.

*15. Anil SS, Deen J, Anil L, Baidoo SK, Wilson ME, Ward TL. Evaluation of the supplementation of complexed trace minerals on the number of claw lesions in breeding sows. *Manipulating Pig Production XII, Australasian Pig Science Association*, Cairns, Australia. 2010;108.

*16. Da Silva A, Anil SS, Deen J, Baidoo SK. Effect of the supplementation of complexed trace minerals on the healing of claw lesions in two sow herds. *Proc IPVS Cong.* Vancouver, Canada. 2010;1169.

17. Anil SS. Epidemiology of lameness in breeding female pigs. PhD dissertation. University of Minnesota; 2011.

18. Lisgara M, Skampardonis V, Leontides L. Effect of diet supplementation with chelated zinc, copper and manganese on hoof lesions of loose housed sows. *Porcine Health Manag*. 2016;2:6.

19. Bradley CL. Evaluating the impact of dietary inorganic or organic trace mineral supplementation on gilt development and sow reproduction, lameness, and longevity. PhD dissertation. University of Arkansas, Fayetteville, Arkansas; 2010.

20. Hedges VJ, Blowey R, Packington AJ, O'Callaghan CJ, Green LE. A longitudinal field trial of the effect of biotin on lameness in dairy cows. *J Dairy Sci.* 2001;84:1969–1975.

21. Tuyttens FAM. The importance of straw for pig and cattle welfare: a review. *Appl Anim Behav Sci.* 2005;92:261–282

22. Tuyttens FAM, Wouters F, Struelens E, Sonck B, Duchateau L. Synthetic lying mats may improve lying comfort of gestating sows. *Appl Anim Behav Sci.* 2008;114:76–85.

23. Elmore MRP, Garner JP, Johnson AK, Richert B. A flooring comparison: The impact of rubber mats on the health, behaviour, and welfare of group-housed sows at breeding. *Appl Anim Behav Sci.* 2010;123: 7–15.

24. Calderón Díaz JA, Fahey AG, KilBride AL, Green LE, Boyle LA. Longitudinal study of the effect of rubber slat mats on locomotory ability, body, limb and claw lesions, and dirtiness of group housed sows. *J Anim Sci.* 2013;91:3940–3954.

25. Nalon E, Maes D, Van Dongen S, van Riet MMJ, Janssens GPJ, Millet S, Tuyttens FAM. Comparison of the inter- and intra-observer repeatability of three gait scoring scales for sows. *Animal.* 2014;8:650–659.

26. Bos E-J, van Riet MMJ, Maes D, Millet S, Ampe B, Janssens GPJ, Tuyttens FAM. Effect of rubber flooring on group-housed sows' gait, claw and skin lesions. *J Anim. Sci.* 2016;94:2086–2096.

27. National Research Council. *Nutrient Requirements of Swine*. 11th rev ed. *Natl Acad Press*: Washington DC; 2012.

*28. Deen J, Schuttert M, van Amstel S, Ossent P, van Barneveld R. Lesion Scoring Guide. In: Feet-First Zinpro Corporation. Eden Prairie, Minnesota; 2009.

29. Calabotta DF, Kornegay ET, Thomas HR, Knight JW, Notter DR, Veit HP. Restricted energy intake and elevated calcium and phosphorus intake for gilts during growth. I. Feedlot performance and foot and leg measurements and scores during growth. *J Anim Sci.* 1982;54:565–575.

30. Franck A, Cocquyt G, Simoens P, De Belie N. Biomechanical properties of bovine claw horn. *Biosystems Eng.* 2006;93:459–467.

31. ISO 17025. General requirements for the competence of testing and calibration laboratories. International Standards Organization, Geneva, Switzerland; 2005.

32. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci.* 1991;74:3583–3597.

33. Toni F, Grigoletto L, Rapp CJ, Socha MT, Tomlinson DJ. Effect of replacing dietary inorganic forms of zinc, manganese, and copper with complexed sources on lactation and reproductive performance of dairy cows. *Prof Anim Sci.* 2007;23:409–416.

34. Telezhenko E, Bergsten C, Magnusson M, Ventorp M, Nilsson C. Effect of different flooring systems on weight and pressure distribution on claws of dairy cows. *J Dairy Sci.* 2008;91:1874–1884.

35. Mouttotou N, Hatchell FM, Green LE. Foot lesions in finishing pigs and their associations with the type of floor. *Vet Rec.* 1999;144:629–632.

36. Scott K, Chennells DJ, Campbell FM, Hunt B, Armstrong D, Taylor L, Gill BP, Edwards SA. The welfare of finishing pigs in two contrasting housing systems: Fully-slatted versus straw-bedded accommodation. *Livest Sci*. 2006;103:104–115.

37. Gillman CE, KilBride AL, Ossent P, Green LE. A cross-sectional study of the prevalence of foot lesions in post-weaning pigs and risk factors associated with floor type on commercial farms in England. *Prev Vet Med.* 2009;91:146–152.

38. KilBride AL, Gillman CE, Ossent P, Green LE. A cross sectional study of prevalence, risk factors, population attributable fractions and pathology for foot and limb lesions in preweaning piglets on commercial farms in England. *BMC Vet Res.* 2009;5:31.

39. KilBride AL, Gillman CE, Green LE. A cross sectional study of the prevalence, risk factors and population attributable fractions for limb and body lesions in lactating sows on commercial farms in England. *BMC Vet Res*. 2009;5:30.

40. McKee CI, Dumelow J. A review of the factors involved in developing effective non-slip floors for pigs. *J Agric Eng Res*. 1995;60:35–42.

41. Kremer PV, Nueske S, Scholz AM, Foerster M. Comparison of claw health and milk yield in dairy cows on elastic or concrete flooring. *J Dairy Sci.* 2007;90:4603–4611.

42. Platz S, Ahrens F, Bahrs E, Nuske S, Erhard MH. Association between floor type and behaviour, skin lesions, and claw dimensions in group-housed fattening bulls. *Prev Vet Med*. 2007;80:209–221.

43. Winkler B. Mechanical properties of hoof horn, sole haemorrhage and lameness in dairy cattle. PhD dissertation. University of Plymouth, United Kingdom; 2005.

*44. Kroneman A, Vellenga L, Vermeer HM, van der Wilt FJ. Claw health in pigs. Research Report 1.78. Faculty of Veterinary Medicine, Utrecht, The Netherlands. 1992;1–32.

45. Webb NG. Compressive stresses on, and the strength of the inner and outer digits of pigs' feet and the implications for injury and floor design. *J Agric Eng Res.* 1984;30:71–80.

46. Boon CR, Wray C. Building design in relation to the control of diseases of intensively housed livestock. *J Agric Eng Res*. 1989;43:149–161.

47. Anil SS, Anil L, Deen J, Baidoo SK, Walker RD. Factors associated with claw lesions in gestating sows. *Swine Health Prod*. 2007;15:78–83.

* Non-refereed references.

Supplementary material

Long-term impact of zinc supplementation in sows: Impact on claw quality

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Observed differences between parity, claw digits (lateral and medial), and claws (front and hind) in sows related to claw quality measurement will be presented below.

Parity

Claw lesions score

Parity had no effect on heel horn erosion scores ($P = .43$), but tended to influence separations along the heel-sole junction ($P = .10$) in which sows in their third parity showed worse scores compared with the first parity. Parity did influence scores for separations along the white line ($P < .001$), skin lesions scores ($P < .001$), horizontal wall cracks ($P < .001$), overgrown claw length ($P < .001$), and overgrown dewclaw length ($P < .001$) with sows in their third parity showing worse scores.

Vertical wall cracks scores were worse for sows in their third parity compared with the first parity but better than the second parity ($P < .001$).

Claw conformation

Claw dimension measurements. Base (sole) length was longer for sows in their third parity compared with the first parity but shorter than the second parity ($P < .001$). Dorsal border length and claw length were longer in third-parity sows compared with the first parity ($P < .001$ for both variables). Toe height was higher for sows in their third parity compared with the second parity ($P < .001$). Diagonal claw length was longer ($P < .001$) and heel height higher ($P < .001$) for the third parity.

Claw morphology calculations. Distal toe angle was lower for sows in their third parity compared with the first parity, but a higher distal toe angle compared with the second parity ($P < .001$). Sole area and toe:heel ratio were greater in the third parity compared with the first parity ($P < .001$ and $P = .005$, respectively). Claw volume and claw horn size was greater in the third parity ($P < .001$ for both variables).

Horn growth and wear

Horn growth was lower for sows in their third parity compared with the first parity ($P < .001$). Wear was lower for sows in their third parity compared with the first and second parity ($P < .001$). Net horn growth (horn growth minus wear) was influenced by parity ($P < .001$), in which sows in their third parity had a higher net horn growth compared with the second parity.

Claw digits

Claw lesion score

Lateral claw digits had a worse score for all types of claw lesion compared with the medial claw digits: heel horn erosion (+ 21.7 mm, $P < .001$), separations along the heel sole junction (+14.6 mm, $P < .001$) and white line (+22.8 mm, $P < .001$), skin lesion scores (+7.6 mm, $P < .001$), horizontal wall cracks (+6.2 mm, $P < .001$), vertical wall cracks (+10.1 mm, $P < .001$), overgrown claw length (+7.7 mm, $P < .001$), and overgrown dewclaw length (+4.9 mm, $P < .001$) (Table S1).

Claw conformation

Claw dimension measurements. All claw dimension measurements had higher values for the lateral claw digits. Lateral claw digits had a longer sole (base) length (+3.1 mm, $P < .001$), a wider claw width (+3.3 mm, $P < .001$), a longer dorsal border length (+2.0 mm, $P < .001$), a longer diagonal claw length (+3.3 mm, $P < .001$), higher toe height (+2.6 mm, $P < .001$), higher heel height (+2.4 mm, $P < .001$) , and longer claw length (+1.8 mm, $P < .001$) compared with the medial claw digits (Table S1).

Claw morphology calculations

Lateral claw digits had a higher distal toe angle (+1.9°, $P < .001$), a greater sole area (+217 mm^2, $P < .001$), a greater claw volume (+5357 mm^3, $P < .001$) and a greater claw horn size (+272 mm^2, $P < .001$) compared with the medial claw digits (Table S1). The lateral claw digits had a lower toe:heel ratio (-0.1, $P = .07$) compared with the medial claw digits.

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Table S1: Differences in claw quality between lateral and medial claw digits from sows followed for three reproductive cycles (n = 131 at start of study).

Claw quality measurement	Claw digit		Claw		*P*	
	Medial	Lateral	Front	Hind	Digit	Claw
Claw lesion type (mm)*						
Heel horn erosion	41.4 [40.7, 42.2]	63.1 [62.3, 64.0]	50.7 [49.8, 51.5]	53.9 [52.9, 54.8]	< .001	< .001
Heel/sole junction separation	38.7 [38.0, 39.4]	53.3 [52.4, 54.2]	44.1 [43.3, 44.9]	47.9 [47.1, 48.7]	< .001	< .001
White line separation	38.8 [38.1, 39.5]	61.6 [60.7, 62.5]	49.3 [49.4, 50.2]	51.1 [50.1, 52.0]	< .001	.001
Skin lesions	20.0 [19.4, 20.6]	27.6 [26.8, 28.4]	23.3 [22.6, 23.9]	24.3 [23.5, 25.2]	< .001	.02
Horizontal wall cracks	39.5 [38.7, 40.3]	45.7 [44.9, 46.6]	43.1 [42.3, 44.0]	42.1 [41.2, 43.0]	< .001	.045
Vertical wall cracks	24.0 [23.3, 24.7]	34.1 [33.1, 35.1]	27.2 [26.3, 28.0]	31.0 [30.0, 31.9]	< .001	< .001
Overgrown claw	29.9 [29.3, 30.6]	37.6 [36.9, 38.3]	34.4 [33.7, 35.1]	33.2 [32.5, 33.8]	< .001	< .001
Overgrown dewclaw	35.3 [34.4, 36.2]	40.2 [39.3, 41.1]	46.5 [45.6, 47.3]	29.0 [28.2, 29.8]	< .001	< .001
Claw dimensions (mm)†						
Sole (base) length	24.1 [24.0, 24.3]	27.2 [27.0, 27.4]	26.6 [26.4, 26.8]	24.8 [24.6, 24.9]	< .001	< .001
Claw width	25.8 [25.7, 26.0]	29.1 [29.0, 29.3]	29.4 [29.3, 29.5]	25.6 [25.4, 25.7]	< .001	< .001
Length of dorsal border	44.4 [44.2, 44.6]	46.4 [46.2, 46.6]	44.8 [44.6, 45.0]	46.0 [45.8, 46.2]	< .001	< .001
Diagonal claw length	55.5 [55.2, 55.7]	58.8 [58.5, 59.0]	58.9 [58.7, 59.1]	55.4 [55.1, 55.6]	< .001	< .001
Toe height	34.4 [34.2, 34.5]	37.0 [36.8, 37.2]	35.4 [35.2, 35.6]	35.9 [35.8, 36.1]	< .001	< .001
Heel height	8.6 [8.4, 8.8]	11.0 [10.8, 11.2]	11.0 [10.8, 11.2]	8.7 [8.4, 8.9]	< .001	< .001
Claw length	50.1 [49.9, 50.3]	51.9 [51.7, 52.2]	52.3 [52.1, 52.5]	49.7 [49.5, 50.0]	< .001	< .001
Claw calculations						
Distal toe angle (°)	52.4 [52.0, 52.9]	54.3 [53.9, 54.7]	53.7 [53.3, 54.2]	53.0 [52.6, 53.4]	< .001	< .001
Sole area (mm^2)	1298 [1288, 1308]	1515 [1505, 1525]	1539 [1529, 1549]	1275 [1265, 1284]	< .001	< .001
Claw volume (mm^3)	11177 [10859, 11495]	16534 [16217, 16852]	16706 [16382, 17029]	11017 [10710, 11324]	< .001	< .001
Claw horn size (mm^2)	1441 [1430, 1452]	1713 [1702, 1724]	1733 [1722, 1744]	1421 [1411, 1432]	< .001	< .001
Toe:heel ratio	3.4 [3.3, 3.4]	3.3 [3.2, 3.5]	3.2 [3.1, 3.3]	3.5 [3.5, 3.6]	.07	< .001

Table S1 Continued: Differences in claw quality between lateral and medial claw digits from sows followed for three reproductive cycles (n = 131 at start of study).

Claw quality measurement	Claw digit		Claw		P	
	Medial	Lateral	Front	Hind	Digit	Claw
Horn growth and wear (mm)‡						
Horn growth	16.1 [15.7, 16.4]	16.5 [16.2, 16.9]	14.9 [14.5, 15.2]	17.6 [17.2, 18.0]	< .001	< .001
Wear rate	16.1 [15.8, 16.4]	17.3 [17.0, 17.6]	15.5 [15.2, 15.8]	17.8 [17.5, 18.0]	< .001	< .001
Net horn growth	0.02 [-0.4, 0.4]	-0.8 [-1.2, -0.3]	-0.6 [-1.0, -0.2]	-0.2 [-0.6, 0.2]	< .001	.22

* Mean claw lesion score (mm) is the average score per lesion type for all sows for lateral and medial claw digits and for front and hind claws.
† Mean claw conformation measurements and calculations (mm) is the average score measurement for all sows for lateral and medial claw digits and for front and hind claws.
‡ Horn growth and wear (mm) was determined from both lateral and medial claw digits of the left front and right hind claws. Net horn growth is horn growth minus wear and represents the balance between horn growth and wear throughout the reproductive cycle.
Level of significance is *P* < .05. Values are mean with [95% CI].

Horn growth and wear

Horn growth and wear were higher for the lateral claw digits (+0.4 mm and +1.2 mm respectively, *P* < .001) compared with the medial claw digits. Net horn growth was lower for the lateral than for the medial claw digits (-0.8 mm, *P* < .001). The net horn growth of the lateral claw digits was negative and of the medial claw digits positive (Table S1).

Histological claw characteristics

Transverse horn wall. No significant differences were found between lateral and medial claw digits for the number of dermal lamellae per 1000 µm (*P* = .89), distance between lamellae (*P* = .82), width of the lamellae (*P* = .27), or length of the longest lamellae of the transverse horn wall (*P* = .23, Table S2).

Sagittal heel horn. No significant differences were found between lateral and medial claw digits for the number of dermal papillae per 1000 µm (*P* = .81), distance between papillae (*P* = .88), width of the papillae (*P* = .36), or length of the longest papillae of the sagittal heel horn (*P* = .47, Table S2).

Transverse heel horn. The density of the heel horn tubules of the transverse heel horn, expressed as the number of horn tubules within a defined surface area of 1 mm^2, was lower for the lateral digits compared with the medial digits (*P* = .03, Table S2).

Mechanical claw characteristics

Abaxial horn wall was thicker for the lateral claw digits compared with the medial claw digits (+0.3 mm, *P* < .001). Young's Modulus, yield stress and maximum stress of 1 mm/min test velocity did not differ between the lateral and medial digit of the right front claw (*P* = .18, *P* = .36, *P* = .10, respectively). Young's Modulus, yield stress and maximum stress of 15 mm/min test velocity did not differ between the lateral and medial digit of the right front claw (*P* = .11, *P* = .93, *P* = .50, respectively) (Table S2).

Claw

Claw lesion score

Hind claws had worse scores for heel horn erosion (+ 3.2 mm, *P* < .001), separations along the heel sole junction (+3.8 mm, *P* < .001) and white line (+1.8 mm, *P* = .001), skin lesion scores (+1.0 mm, *P* = .02), and vertical wall cracks (+3.8 mm, *P* < .001) compared with the front claws (Table S1). Hind claws had a better score for horizontal wall cracks lesion score (-1.0 mm, *P* = .045), overgrown claw length (-1.2 mm, *P* < .001), and overgrown dewclaw length (-17.5 mm, *P* < .001) compared with the front claws (Table S1).

Claw conformation

Claw dimension measurements. Hind claws had a longer dorsal border length (+1.2 mm, *P* < .001), and higher toe height (+0.5 mm, *P* < .001) compared with the front claws.

Hind claws had a shorter sole (base) length (-1.8 mm, *P* < .001), a narrower claw width (-3.8 mm, *P* < .001), shorter diagonal claw length (-3.5 mm, *P* < .001), lower heel height (-2.3 mm, *P* < .001), and a shorter claw length (-2.6 mm, *P* < .001) compared with the front claws (Table S1).

Claw morphology calculations. Hind claws had a higher toe:heel ratio (+0.3, *P* < .001) compared with the front claws. Hind claws had a lower distal toe angle (-0.7°, *P* < .001), a smaller sole area (-264 mm^2, *P* < .001), a smaller claw volume (-5689 mm^3, *P* < .001) and a smaller claw horn size (-312 mm^2, *P* < .001) compared with the front claws (Table S1).

Horn growth and wear

Horn growth and wear were higher for the hind claws (+2.7 mm and +2.3 mm respectively, *P* < .001) compared with the front claws. Net horn growth was not different between front and hind claws (*P* = .23, Table S1).

Table S2: Differences in histological and mechanical claw characteristics* between lateral and medial claw digits in sows after slaughter at the third reproductive cycle (n = 36).

Claw quality measurement	Claw digit		SEM	*P*
	Medial	Lateral		
Histological claw characteristics†				
Transverse horn wall				
Dermal lamellae, n	7.0 [6.3, 7.7]	7.0 [6.3, 7.6]	0.2	.89
Distance, µm	146.8 [133.1, 160.6]	145.7 [132.6, 158.7]	4.6	.82
Width, µm	54.9 [45.9, 64.0]	50.5 [44.2, 56.8]	2.7	.27
Length, µm	208.9 [188.4, 229.5]	228.9 [206.2, 251.5]	7.6	.23
Sagittal heel horn				
Dermal papillae, n	2.8 [2.6, 3.1]	2.8 [2.5, 3.1]	0.1	.81
Distance, µm	322.1 [297.7, 346.4]	329.3 [286.0, 372.6]	11.5	.88
Width, µm	139.8 [124.0, 155.6]	129.1 [115.3, 143.0]	5.2	.36
Length, µm	490.6 [402.3, 578.9]	447.1 [385.1, 509.1]	27.1	.47
Transverse heel horn				
Horn tubules	7.2 [6.7, 7.7]	6.5 [6.1, 7.0]	0.2	.03
Mechanical claw characteristics‡§				
Abaxial horn wall thickness (mm)	4.3 [4.2, 4.4]	4.6 [4.5, 4.8]	0.1	< .001
Test velocity,1 mm/min				
Young's modulus, MPa	71.9 [64.5, 79.2]	67.0 [59.7, 74.4]	4.3	.18
Yield stress, MPa	10.3 [9.5, 11.0]	10.7 [9.8, 11.6]	0.7	.36
Maximum stress, MPa	14.6 [13.6, 15.5]	15.5 [14.5,16.6]	0.9	.10
Test velocity,15 mm/min				
Young's modulus, MPa	101.4 [91.6, 111.3]	93.6 [83.7, 103.4]	3.6	.11
Yield stress, MPa	13.2 [12.2, 14.2]	13.2 [12.0, 14.3]	0.4	.93
Maximum stress, MPa	19.5 [18.2, 20.8]	20.0 [18.7, 21.4]	0.5	.50

* Histological claw characteristics determined for both front claws, mechanical claw characteristics determined for the right front claw.

† Dermal papillae/lamellae, number of dermal papillae/lamellae per 1000 µm, visible at their full width; Distance, distance between the axis lines of the papillae/lamellae at their base (µm); Width, width of the dermal component halfway and perpendicular to the dermal papillae/lamellae (µm); Length, length of the longest papillae measured from the top of the dermal papillae/lamellae to the origin at the base (µm); Horn tubules, heel horn tubules density expressed as number of horn tubules within a defined surface area of 1 mm2. Horn tubules that were only partially visible from two of the four sides of the defined surface area were also included.

‡ Young's modulus is a measure for the rigidity and stiffness of the horn, yield stress represents the point on the stress-strain diagram in which the material starts to lose its mechanical function and material properties starts to change at further loading, and maximal stress represents the maximum compression. (Franck et al., 2006).

§ Mechanical claw characteristics were tested on two test velocities, 1 and 15 mm/minute, to test if the abaxial horn wall had visco-elastic properties. The abaxial horn wall does have these properties, because test velocities differ ($P < .05$).

Level of significance is $P < .05$. Values are mean with [95% CI].

MPA = MegaPascals

Table 1 [*J Swine Health Prod*. 26(1):13]

Footnotes for the analysis of Premix 3% and Premix 2.75%

* Premix 3% included per kg total gestation diet (analysed Zn concentration in premix is 260 mg/kg): vitamin A (12,499 IU), vitamin D3 (1995 IU), vitamin E (60 mg), vitamin K3 (2.0 mg), vitamin B1 (2.0 mg), vitamin B2 (5.0 mg), vitamin B5 (20 mg), vitamin B6 (4.0 mg), vitamin B12 (0.04 mg), vitamin B3 (35 mg), vitamin B11 (3.0 mg), biotin (0.4 mg), choline (282 mg), $C_5H_{14}ClNO$ (325 mg), $FeSO_4 \bullet H_2O$ (Fe: 80 mg/kg), $CuSO_4 \bullet 5H_2O$ (Cu: 10 mg/kg), MnO (Mn: 80 mg/kg), anhydrous $Ca(IO_3)_2$ (I: 2 mg/kg), Na_2O_3Se (Se: 0.4 mg/kg), Ca (5.3 g), P (0.3 g), Mg (0.2 g), Na (1.5 g), Cl (2.8 g), K (0.1 g), 3-phytase (1000 FTU), anhydrous trimethylglycine (275 mg), sepiolite (470 mg/kg), bentonite-montmorillonite (470 mg/kg), formic acid (5.2 mg/kg), propionic acid (49 mg/kg), citric acid (1.5 mg/kg), ethoxyquine (2.4 mg/kg), butylated hydroxy anisol (1.9 mg/kg).

† Premix 2.75% included per kg total lactation diet (analysed Zn concentration in premix is 4366 mg /kg): vitamin A (15,015 IU), vitamin D3 (1501 IU), 25-hydroxycholecalciferol (0.01 mg), vitamin E (150 mg), vitamin C (100 mg), vitamin K3 (2.0 mg), vitamin B1 (2.0 mg), vitamin B2 (9.0 mg), vitamin B5 (25 mg), vitamin B6 (5.0 mg), vitamin B12 (0.03 mg), vitamin B3 (45 mg), vitamin B11 (5.3 mg), biotin (0.5 mg), choline (649 mg), $C_5H_{14}ClNO$ (748 mg), $FeSO_4 \bullet H_2O$ (Fe: 150 mg/kg), $CuSO_4 \bullet 5H_2O$ (Cu: 15 mg/kg), MnO (Mn: 50 mg/kg), anhydrous $Ca(IO_3)_2$ (I: 2 mg/kg), Na_2O_3Se (Se: 0.3 mg/kg), organic Se (0.1 mg/kg), Ca (3.6 g), P (1.6 g), Mg (0.6 g), Na (1.7 g), Cl (3.3 g), K (0.02 g), 6-phytase (1500 FTU), citric acid (2.5 mg/kg), ethoxyquine (6.7 mg/kg), butylated hydroxy anisol (1.1 mg/kg), propyl gallate (1.1 mg/kg).