# 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

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(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 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<sup>2</sup>, P < .001), a greater claw volume (+5357 mm<sup>3</sup>, P < .001) and a greater claw horn size (+272 mm<sup>2</sup>, 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		Р	
	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 (mm²)	1298 [1288, 1308]	1515 [1505, 1525]	1539 [1529, 1549]	1275 [1265, 1284]	< .001	< .001
Claw volume (mm <sup>3</sup> )	11177 [10859, 11495]	16534 [16217, 16852]	16706 [16382, 17029]	11017 [10710, 11324]	< .001	< .001
Claw horn size (mm <sup>2</sup> )	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		Р	
	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].</li>

#### 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  $\mu$ m (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  $\mu$ m (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<sup>2</sup>, 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<sup>2</sup>, P < .001), a smaller claw volume (-5689 mm<sup>3</sup>, P < .001) and a smaller claw horn size (-312 mm<sup>2</sup>, 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).

	Clav	v digit	SEM	Р	
Claw quality measurement	Medial	Lateral	JEIM	P	
listological 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	
Aechanical 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	

**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).

\* 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),  $C_5H_{14}CINO$  (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<sub>3</sub>)<sub>2</sub> (I: 2 mg/kg), Na<sub>2</sub>O<sub>3</sub>Se (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<sub>5</sub>H<sub>14</sub>CINO (748 mg), FeSO<sub>4</sub> • H<sub>2</sub>O (Fe: 150 mg/kg), CuSO<sub>4</sub> • 5H<sub>2</sub>O (Cu: 15 mg/kg), MnO (Mn: 50 mg/kg), anhydrous Ca(IO<sub>3</sub>)<sub>2</sub> (I: 2 mg/kg), Na<sub>2</sub>O<sub>3</sub>Se (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).