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Marginal dietary zinc concentration affects claw conformation measurements but not histological claw characteristics in weaned pigs

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ABSTRACT

The aim of the present study was to explore whether marginal dietary zinc (Zn) concentrations affect claw quality measurements in weaned pigs. Twenty-four weaned pigs were randomly assigned to two dietary treatment groups: (1) 42 mg Zn/kg diet from ingredients only (unsupplemented, marginal dietary Zn concentration below Zn requirements of 80 mg Zn/kg feed); and (2) 106 mg Zn/kg diet, where Zn was added as ZnO (common commercial dietary Zn concentration). Claw conformation characteristics were measured at the start (day 0, 4 weeks of age) and at the end (day 36) of the study, and the histological claw characteristics of horn wall and heel horn were examined on samples collected at 9 weeks of age. Non-supplemented pigs had narrower claw widths ($P = 0.028$) and lower toe heights ($P = 0.010$) at 9 weeks. The length of the dorsal border tended to be lower for the non-supplemented piglets ($P = 0.092$). Claw volume and claw horn size were lower ($P = 0.003$ and $P < 0.001$, respectively) for the non-supplemented pigs at 9 weeks of age. Horn growth and wear were lower for the non-supplemented pigs ($P = 0.044$ and $P < 0.001$, respectively), but net horn growth (horn growth minus wear) was not different ($P = 0.406$). No changes in the histological claw characteristics were observed. Differences in claw quality measurements were found between lateral and medial claw digits and between fore and hind claws. It was concluded that marginal dietary Zn concentration affected various claw quality measurements. Marginal dietary Zn concentrations may not be sufficient to maintain claw quality in pigs.

Introduction

Claw quality is determined as the product of horn characteristics to support the inner structure of the digits and assist in the dispersal of weight and stress during locomotion (Vermunt and Greenough, 1995; Lethbridge, 2009). It is evaluated by visual scoring for claw shape, claw shape dimensions, claw scoring, and measurement of structural, physical and biochemical properties of the claw horn (Politiek et al., 1986; Vermunt and Greenough, 1995). The claw quality of pigs depends largely on the quality of horn production, which is influenced by the nutrient supply to the avascular epidermis (Tomlinson et al., 2004; Muelling, 2009; Torrison, 2010). Poor horn quality can lead to development of claw lesions, such as separations along the white line, haemorrhages and cracks at the horn wall. Lower horn quality may be a result of trauma or impaired horn production, along with excessive or inadequate wear (Ossent, 2010; Torrison, 2010). Lameness is evident in 5–20% of sows with claw lesions (Anil et al., 2007) and they have considerable economic and welfare consequences (Heinonen et al., 2013).

Malnutrition is an important factor in impaired horn production, especially if the nutrient supply, for example of amino acids, vitamins and minerals, is insufficient (Butler and Hintz, 1977; Tomlinson et al., 2004; Muelling, 2009). This causes perturbation of nutrient diffusion from the dermis to the avascular epidermis, thereby reducing claw quality, which in turn increases the susceptibility to damage from the environment (Tomlinson et al., 2004; Muelling, 2009). The catalytic, structural and regulatory functions of zinc (Zn) may all influence the processes required for horn production (Tomlinson et al., 2004; Andrieu, 2008; van Riet et al., 2013).

Despite the demonstrated effect of Zn on claw quality in previous studies in cows (Moore et al., 1988; Nocek et al., 2000, 2006; Enjalbert et al., 2006), few studies have been performed in pigs evaluating differences between Zn sources (Bradley, 2010; Anil, 2011). The pig industry has previously focused on intensifying body growth,

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Table 1
Ingredient composition of the meal diet fed to weaned pigs with and without Zn supplementation ($n = 24$ pigs) during a 5 week experimental period.

Ingredients	g/kg fresh matter
Barley	258.2
Maize	186.6
Wheat	174.8
Soybean meal	150.0
Premix 6% without Zn[a]	60.0
Soybeans heated	58.1
Starpro 40[b]	40.0
Beet molasses	30.0
Soy oil	14.3
Nutrisure[c]	10.0
Limestone	3.8
Monocalcium phosphate	1.7
Salt	1.7
L-Lysine HCL	4.4
L-Threonine	2.0
L-Valine	1.1
DL-Methionine	2.1
L-Tryptophan	0.8
Phytase	0.1

[a] Premix 6% without Zn, included per kg diet: vitamin A (15,000 IU), vitamin D3 (2000 IU), vitamin E (100 mg), vitamin K3 (20 mg), vitamin B1 (2.5 mg), vitamin B2 (7.5 mg), vitamin B5 (20 mg), vitamin B6 (5 mg), vitamin B12 (0.04 mg), vitamin C (100 mg), vitamin B3 (30 mg), vitamin B11 (3 mg), biotin (0.2 mg), choline (324 mg), $FeSO_4 \cdot H_2O$ (Fe: 100 mg), $CuSO_4 \cdot 5H_2O$ (Cu: 160 mg), $MnSO_4 \cdot H_2O$ (Mn: 60 mg), $Ca(IO_3)_2$ (I: 2 mg), Na_2O_3Se (Se: 0.4 mg), Ca (483 mg), P (423 mg), Mg (165 mg), Na (326 mg), Cl (1514 mg), K (1183 mg), S (235 mg), lysine (341 mg), methionine (77 mg), threonine (227 mg), tryptophan (68 mg), butylhydroxytoluene (13 mg), ethoxyquine (13 mg), propyl gallate (3 mg), citric acid (13 mg).
[b] Protein concentrate (DSM nutritional products, Basel, Switzerland).
[c] DSM nutritional products: A mixture of calcium salts of the following organic acids: lactic acid, formic acid, citric acid monohydrate, orthophosphoric acid, propionic acid.

Table 2
Analysed nutrient composition of the meal diet fed to weaned pigs with and without Zn supplementation ($n = 24$ pigs) during a 5 week experimental period.

Chemical analysis	g/kg[a]
Dry matter	896.8
Crude ash	48.5
Crude protein	196.0
Crude fibre	27.7
Crude fat	58.2
Starch	362.7
Sugar	78.8
ADF	34.9
NDF	90.7
ADL	2.4
Ca	6.3
P	5.3
Cu (mg/kg)	159.0
Zn (mg/kg)[b]	41.9/106.0
ID Lysine	11.5
ID Methionine	4.9
ID Threonine	7.2
ID Tryptophan	2.5
ID Arginine	9.4
ID Leucine	11.4
ID Isoleucine	6.0
ID Histidine	3.7
ID Valine	7.8
ID Phenylalanine	7.3
NEv (MJ/kg)	9.8

ADF, acid detergent fibre; NDF, neutral detergent fibre; ADL, acid detergent lignin.
[a] The chemical analyses of the apparent ileal digestible (ID) amino acids and net energy (NEv) are calculated according to CVB (2007).
[b] The Zn concentration was 41.9 mg/kg for the non-supplemented diet (marginal dietary Zn concentration, below Zn requirements) and 106.0 mg/kg for the Zn-supplemented diet (Zn added as ZnO).

with less priority given to leg and claw conformation (Kroneman et al., 1992). Nonetheless, claw lesions and lameness are an increasing economic and welfare concern, especially in sows (Anil et al., 2005; Heinonen et al., 2013).

Horn is continuously produced and requires Zn (Vermunt and Greenough, 1995; Winkler, 2005); therefore, it is worth questioning whether a marginal dietary Zn supply is sufficient to maintain claw quality. The maximum dietary Zn allowance for weaned pigs in Europe to minimise Zn excretion to the environment is 150 mg Zn/kg, although reported Zn requirements to avoid Zn deficiency are lower (80 mg Zn/kg) (McDowell, 2003; Van Paemel et al., 2010; EFSA, 2014). The aim of the present study was to explore whether a 5 week period of marginal dietary Zn provision (i.e. below Zn requirements) results in observable claw quality differences, assessed by several measurements for claw conformation and histological claw characteristics.

Materials and methods

Animals, housing and diets

Twenty-four weaned pigs (Piétrain boar × hybrid sow, 27.8 ± 1.6 days old, bodyweight at weaning of 9.3 ± 0.3 kg) from the herd at the Institute for Agricultural and Fisheries Research (ILVO) were selected for homogeneous groups and allocated randomly to one of two treatment groups ($n = 12$ pigs). Each pen, comprising one treatment group, held six non-sibling pigs (three barrows and three sows). The pigs were vaccinated against *Mycoplasma hyopneumoniae* (Ingelvac Mycoflex, Boehringer Ingelheim) at around 11 days postpartum and against porcine circovirus type 2 (Ingelvac Circoflex, Boehringer Ingelheim) at around 19 days postpartum.

The pigs were housed in a conventional pen (floor area 205 cm × 180 cm) with a plastic slatted floor, a feeding trough along the full length of the pen and two drinking nipples at the opposite sides of the feeding trough. Two stainless steel chains per pen were provided as environmental enrichment.

All pigs were fed a meal diet according to commercial standards and met the nutrient requirements for pigs (NRC, 1998) (Tables 1 and 2). To prevent diarrhoea, colistin (1 g/pig) was added to the diet for 5 days after weaning and then, after a 2 day break, for an additional 5 days. The non-supplemented pigs in the control group ($n = 12$ pigs) received 42 mg Zn/kg diet, originating from the ingredients only (marginal dietary Zn concentration; below Zn requirements). The Zn-supplemented pigs ($n = 12$ pigs) received in total 106 mg Zn/kg diet (analysed), in which Zn was added as ZnO via premix (adequate dietary Zn concentration; median Zn concentration in conventional commercial diets is 137 mg Zn/kg; EFSA, 2014). This dosage was chosen based on conventional commercial diets.

Water and feed were provided ad libitum. Feed samples were subject to proximate analysis (crude nutrient analysis) according to ISO 17025 (2005) and performed by the accredited laboratory of the Animal Sciences Unit of ILVO. Dry matter (DM) content was determined according to 71/393/EEC. Other procedures were ISO 5984 (crude ash), ISO 5983-2 (crude protein), AOCS approved procedure Ba 6a-05 (crude fibre), ISO 6492 (crude fat), Van Soest et al. (1991) (ADF, NDF, ADL), ISO 6490/1 (Ca) and ISO 6491 (P). All experimental procedures were approved by the ILVO ethical committee (approval number 2012/174, date of approval 17 February 2012).

Samples and measurements

Bodyweight was measured at 4 (day 0, weaning; start of the study), 6 and 9 (day 36, end of experiment) weeks of age. After the 5 week experimental period, the pigs were sedated with a 7 mL/pig mixture of Xyl-M 2% (20 mg xylazine/mL; VMD N.V.) and Zoletil 100 (250 mg Tiletamine base and 250 mg Zolazepam base; Virbac S.A.). After sedation, claw dimensions were measured and blood was collected via cardiac puncture (20 mL), then the pigs were euthanased. Blood was analysed for haematocrit, and plasma Zn and copper (Cu) concentration.

Claw conformation, expressed as dimensions, was determined using a digital calliper (Mitutoyo Belgium N.V.) at 4 weeks (day 0) and at 9 weeks (day 36) of age. The claw dimensions (Fig. 1) included sole (base) length, claw width, length of the dorsal border, diagonal claw length, toe height, heel height and claw length, following a methodology adapted from Calabotta et al. (1982) and Vermunt and Greenough (1995). These dimensions were subsequently used to calculate the distal toe angle (sine of the length of the dorsal border and toe height), sole area (claw length × claw width), claw volume (sole area × heel height), claw horn size (claw width × diagonal claw length) and toe:heel ratio (toe height:heel height), these values representing nominal dimensions (Calabotta et al., 1982; Vermunt and Greenough, 1995; Manske, 2002; Bradley et al., 2008; Van Amstel and Doherty, 2010).

At 4 weeks (day 0), a superficial reference point was incised into the dorsal horn wall by carving a small indentation (estimated depth ≤ 0.5 mm) with a hoof knife, 0.5 cm below the coronary band (periople). This indentation was coloured with Indian ink at the time of incision and again after 15 days. At 9 weeks (day 36) the displacement above and below this reference point was measured using a digital calliper to determine horn growth (distance between periople and reference point at 9 weeks