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No indications that zinc and protein source affect Zn bioavailability in sows during late gestation fed adequate dietary Zn concentrations

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ABSTRACT

Previous *in vitro* research has shown the possibility of spontaneous chelation of Zn in the presence of easily digestible protein sources. The objective of this study was to investigate the possible interaction between zinc (Zn) source and protein source on the *in vivo* Zn bioavailability in sows during late gestation that were fed adequate dietary Zn concentrations. Fifty-six sows were randomly allocated to one of four dietary treatment groups during a 20-day experimental period: (1) organic Zn + soybean meal, (2) inorganic Zn + soybean meal, (3) organic Zn + hydrolysed feather meal, and (4) inorganic Zn + hydrolysed feather meal. Zinc was provided at adequate dietary Zn concentrations, in which organic Zn was added as a Zn amino acid complex and inorganic Zn as ZnO. Blood samples were collected at the start (day 1) and at the end (day 20A) of the experimental period before feeding and 3 h after feeding (day 20B) to determine plasma Zn and serum metallothionein (MT) concentration. Faecal samples were collected rectally, alternately in the morning (day 15, 17, and 19) and afternoon (day 16, 18, and 20) directly after feeding to calculate apparent nutrient digestibility and apparent Zn absorption. Neither Zn nor protein source affected Zn status (plasma Zn: $P = 0.288$ and $P = 0.237$, respectively, Serum MT: $P = 0.161$ and $P = 0.193$, respectively) or apparent Zn absorption ($P = 0.360$ and $P = 0.527$, respectively). Hydrolysed feather meal showed lower crude protein, crude fat, and crude ash digestibility compared to soybean meal ($P < 0.001$). Faecal Zn concentration was not affected by Zn source ($P = 0.442$). This study did not confirm the earlier observed *in vitro* effect of protein source on Zn bioavailability and shows that, at adequate levels commonly used in practice, the choice of Zn or protein source does not influence Zn status.

Abbreviations: Zn, zinc; ZnO, zinc oxide; MT, metallothionein; AIA, acid insoluble ash; SB, soybean meal; HF, hydrolysed feather meal; ID, ileal digestible; Ca, calcium; Cu, copper; Mn, manganese; P, phosphorus; ADF, acid detergent fibre; NDF, neutral detergent fibre; ADL, acid detergent lignin; OZ, organic Zn source; IZ, inorganic Zn source; Zn Abs, apparent Zn absorption; P, protein source.

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1. Introduction

Zinc (Zn), a micromineral, is important for a number of biochemical processes such as enzyme function, protein synthesis, hormone regulation, bone mineralisation, cell growth and differentiation, cell mediated immunity, and gene expression (McDowell, 2003; Lowe et al., 2009). To assure normal biochemical processes, Zn is tightly regulated by the processes of absorption and (endogenous) excretion to maintain homeostasis (King et al., 2000; McDowell, 2003; Hill and Link, 2009). Zinc absorption in the intestines is claimed to be influenced by the amount and source of Zn in the diet and by the interaction with other nutrients (Ammerman et al., 1995; McDowell, 2003; Suttle, 2010).

In practice, Zn is usually added to the diet at levels near the European legal maximum allowance (150 mg Zn/kg diet). Adding Zn above the animal's requirements (100 mg Zn/kg diet; (NRC, 2012) might negatively influence the environment (soil and water conditions), if animal excretion of excessive Zn is continuously applied to the soil in excess of crop requirements (Jongbloed and Lenis, 1998; Revy et al., 2004; Jongbloed, 2010). A possible strategy to reduce Zn excretion to the environment is to use Zn sources that are more easily absorbed by the animal, resulting in lower Zn supplementation (Spears, 1996; Jongbloed, 2010; Paulicks et al., 2011).

Organic Zn sources are claimed to have a higher bioavailability[1] compared to inorganic Zn sources. However, the underlying mechanisms are not well understood (Spears, 1996; Wright and Spears, 2004) and results from previous research are inconsistent (Jongbloed, 2010; Paulicks et al., 2011; Nitrayova et al., 2012).

Moreover, the Zn requirements of breeding sows and the effect of other nutrients on Zn absorption such as proteins have not been fully determined. Previous *in vitro* research results demonstrated the possibility of spontaneous chelation of Zn in the presence of easily digestible protein sources (Van paemel and Janssens, 2008), which was also observed with calcium (Ca), copper (Cu), and manganese (Mn) (Zhu et al., 2013). Spontaneous chelation may enhance Zn absorption. We therefore hypothesised that the protein source, and its concomitant profile of amino acid release during digestion, can affect the level of spontaneous chelation with Zn, thus altering the bioavailability of Zn. The present study investigated the possible interaction between Zn source and protein source on the *in vivo* Zn bioavailability in sows during late gestation fed adequate dietary Zn concentrations (*e.g.* above estimated requirements and below legal maximum allowance).

2. Materials and methods

2.1. Animals and management

Fifty-seven sows (RA-SE Genetics) from three succeeding groups in a 3-week interval from the experimental herd of the Institute for Agricultural and Fisheries Research (ILVO) were selected during late gestation ($n = 57$: 55 gravid sows at 86 days $\pm$ 1.3; 1 non-gravid sow, and 1 gravid sow at 20 days at the start of the experiment). The total duration of the experiment was 60 days, 20 days for each sow group. Per sow group, all sows were randomly divided into four dietary treatment groups. One multiparous sow aborted during the experiment and was subsequently excluded. Consequently, the experiment included 56 sows (parity: 3.7 ± 2.5). Parity was equally distributed among dietary treatment groups (parity per dietary treatment group: 3.5 ± 2.7 ($n = 13$), 3.7 ± 2.4 ($n = 14$), 4.0 ± 2.8 ($n = 15$), and 3.5 ± 2.4 ($n = 14$)). Sows' average bodyweight at the start of the study was 250 ± 43 kg. These sows were selected during late gestation, because previous results showed low Zn status (low plasma Zn concentration) during late gestation (van Riet et al., 2015).

The sows were housed in groups in free access stalls (maximum eight sows per compartment, three compartments). In each compartment, sows from the four treatment groups were present. The stalls were naturally ventilated and the surface area per sow was 2.78 m^2, including 1.17 m^2 for the individual stalls.

During feeding and faecal sampling sows were separated and the feeding stalls were locked. Each sow was randomly assigned to one of four experimental gestation diets throughout a 20-day experimental period. The feed allowance was 2.6 kg, provided in two equal portions at 8:00 a.m. and 2:30 p.m. Feed leftovers were collected after feeding and recorded per sow at the end of each week. Drinking water was provided automatically through individual nipple drinkers in the feeding troughs for 15 min every hour to avoid water spillage. One drink nipple per compartment opposite of the stalls ensured *ad libitum* access to water. One hour before feeding, water provision in the individual feeding troughs was suspended to facilitate cleaning. Water was provided again 30 min after feeding and after removing feed leftovers.

All sows were fed a gestation diet according to commercial dietary standards and nutrient requirements for gestating sows ((NRC, 2012). A pre-experimental diet was fed for at least 3 weeks and contained 880.5 g/kg DM, 144.9 g/kg crude protein, 137 mg/kg Zn (originating from ingredients and Zn added as ZnO), and 5.2 g ileal digestible lysine per kg diet.

2.2. Dietary treatment

The treatment groups differed in the combination of Zn and protein source used in their gestation diet (provided between day 84 and 108 of gestation) (Tables 1 and 2), yielding four possible combinations: (1) organic Zn + soybean meal, (2) inorganic Zn + soybean meal, (3) organic Zn + hydrolysed feather meal, and (4) inorganic Zn + hydrolysed feather meal.

[1] Bioavailability is defined as the proportion of a nutrient capable of being absorbed and available for use or storage (Heaney, 2001; Srinivasan, 2001).