Fluctuation of potential zinc status biomarkers throughout a reproductive cycle of primiparous and multiparous sows

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Abstract

Fluctuations in Zn metabolism throughout gestation and lactation might affect Zn requirements. However, scientific data on Zn requirements for breeding sows are limited. The objective of the present study was to assess the Zn status of primiparous and multiparous sows using different Zn status biomarkers, to identify periods of critical Zn status throughout the reproductive cycle at different parities. Blood samples were taken after overnight fasting before feeding in the morning from five primiparous and ten multiparous sows at fixed time intervals during gestation (days −5, 0 (insemination), 21, 42, 63 and 84), around parturition (days 108, 112, 115 (parturition) and 118) and during lactation (days 122, 129 and 143 (weaning)). At parturition, blood samples were collected from two randomly selected piglets per sow before colostrum intake. Plasma was analysed for Zn and Cu contents, whereas serum was analysed for alkaline phosphatase, metallothionein and albumin concentrations. Independently of parity, all biomarkers fluctuated differently during gestation and lactation ($P < 0.050$). This reflects their different roles in Zn metabolism, and suggests that the choice of a Zn status biomarker necessitates careful consideration. Low average plasma Zn concentration at the end of gestation and throughout lactation seem to be replenished towards weaning.

Key words: Serum alkaline phosphatase: Serum metallothionein: Plasma zinc: Serum albumin

Fluctuations in Zn metabolism throughout the reproductive cycle of sows are a result of physiological processes during gestation and lactation to support fetal growth and development and milk synthesis[1–4].

In particular, placental Zn transport, luminal Zn absorption and Zn mobilisation from the bone and liver are increased at the end of gestation and lactation[2–4]. This results in varying Zn transport and a changed role of transport proteins in both the fetus and the dam[5], thereby increasing the dietary Zn requirement.

Inadequate intake of dietary Zn during gestation and lactation may cause reproductive failures, without clinical signs of maternal Zn deficiency[6–8]: the sow depletes her body Zn reserves in tissues and fluids before litter size, fetal development or milk composition are affected[1,6–8]. Scientific data of Zn requirements for sows are limited. Therefore, adequately measuring Zn status is critical, albeit difficult[9–11].

Zn status may be reflected by biochemical markers (i.e. biomarkers), which are related to the structural, regulatory and catalytic role of Zn. Plasma Zn is the most frequently used biomarker for Zn status. However, plasma Zn alone shows limitations if study conditions are not controlled or if confounding factors are present[12]. These confounding factors may be infection or diseases, an acute-phase response, stress, feeding state (fasted or postprandial measures), reproductive phase (gestation), and also the method of blood collection, and concomitant blood sample transport, storage and analysis[11,13,14]. Other biomarkers such as albumin, metallothionein (MT) and alkaline phosphatase (ALP) play a role in Zn metabolism and homeostasis: Zn is bound to albumin after absorption[15,16]; MT (a small cysteine-rich protein) is involved in regulating the quantity of Zn absorbed[15,17,18]; ALP is a Zn-dependent enzyme in which Zn ions are present in the active centre of the enzyme[19]. Together, they may be

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Abbreviations: ALP, alkaline phosphatase; MT, metallothionein.

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useful to assess the body's response to Zn supplementation or depletion. To our knowledge, the normal fluctuation of these biomarkers throughout the reproductive cycle of sows has not yet been determined.

The objective of the present study was to assess the Zn status of primiparous and multiparous sows using different Zn status biomarkers, to identify periods of critical Zn status throughout the reproductive cycle at different parities.

Materials and methods

Animals and diets

The experiment was performed on one group of twenty-three commercial hybrid sows (RA-SE Genetics) from the Institute for Agricultural and Fisheries Research (ILVO). However, one sow was too stressed during blood sampling and therefore excluded from the experiment. Furthermore, four sows were not gravid and three primiparous sows aborted at days 58, 66 and 93 of gestation, and therefore they were not included in the analysis. One primiparous sow developed an abscess under the cheek and required veterinary treatment. This sow remained in the experimental group, because her results were not considered outliers compared with other sows. Consequently, the experiment included ten multiparous and five primiparous sows from one group that were followed during one reproductive cycle. The multiparous sows (parity ranged between two and eleven) weighed 234 (SD 36) kg at weaning of the preceding lactation period (i.e. at the start of the study, day −5). The primiparous sows (gilts, 233 (SD 12) d old at insemination) weighed 142 (SD 7) kg at day −5.

The sows were all housed individually during the first 4 weeks of gestation and from 1 week before parturition until weaning. During mid- and end gestation, the sows were housed as one group using an automated feeding system with individual sow recognition through an electronic transponder in the sow's ear.

The sows were fed a gestation diet and a lactation diet according to commercial dietary standards and nutrient requirements[20] (Tables 1 and 2). Feed samples of the gestation and lactation diet were subject to proximate analysis according to international standard methods accredited by ISO 17 025. The gestation diet was provided from the start of the study until 1 week before parturition, whereas the lactation diet was provided 1 week before parturition until weaning. During the first 4 weeks of gestation, the sows were fed twice daily, in total 2·3 kg. During mid- and end gestation, the sows were fed 2·6 kg/d. During lactation, 0·25 kg of feed per piglet was gradually supplemented in addition to 3 kg feed, provided in two equal portions daily (e.g. a sow with twelve piglets received 6 kg of feed daily). There were no feed leftovers. Throughout the experiment, all sows had *ad libitum* access to drinking-water, except in the first 4 weeks of gestation, in which water was automatically provided through nipple drinkers for 15 min every hour and for 45 min while feeding in order to prohibit water spillage.

Experimental design

Blood samples were taken before feeding in the morning (between 08.00–10.00 hours), after overnight fasting (17·5 h), at fixed time intervals throughout one reproductive cycle: day −5 (weaning of the preceding cycle for multiparous sows; 'start of the study in December 2011'); throughout gestation (days 0 (insemination), 21, 42, 63 and 84); around parturition (days 108, 112, 115 (parturition) and 118); during lactation (days 122, 129 and 143 (weaning)). The days until parturition were predicted from the expected date of parturition (based on a gestation period of 115 d), whereas from parturition onwards, the sows were sampled based on the date of parturition. Blood samples were collected from the jugular vein using stainless-steel needles and plastic syringes, and added to one heparin and one serum vacuum tube (Terumo Europe). At parturition, two piglets per sow were randomly selected for blood sampling from the jugular vein before colostrum intake, and the blood sample was added to one heparin vacuum tube (Terumo Europe).

Within 1 h after the first piglet was born, colostrum was collected. At 1 week after parturition, milk samples were also collected using 1 ml oxytocin (17 μg oxytocin/ml; VMD N.V.) to stimulate milk release. The colostrum and milk samples were collected from all nipples from either the left or right side of the udder, repeatedly from front to back nipples, until 20 ml were collected. At weaning, reproductive performances were documented, taking into account

Table 1. Ingredient composition of the gestation and lactation diets

Ingredients (g/kg)	Gestation diet	Lactation diet
Beet pulp	150·0	30·0
Wheat	150·0	–
Wheat bran	120·0	49·0
Barley	150·0	107·6
Maize	125·7	350·0
Rye	–	100·0
Alfalfa meal	60·8	80·0
Soyabeans heated	104·2	23·3
Soyabean meal	–	156·7
Soyabean oil	1·7	–
Rapeseed meal	70·0	–
Beet molasses	40·0	30·0
Premix 1 %*	10·0	10·0
Benzoic acid	–	5·0
Limestone	8·5	12·2
Calcium monophosphate	3·3	13·2
Lard	–	22·3
Salt	5·0	4·3
L-Lys HCl	0·7	2·8
L-Thr	0·1	1·6
L-Val	–	1·2
DL-Met	–	0·4
L-Trp	–	0·3
Phytase	0·1	0·1

* Premix 1 % included per kg diet: vitamin A, 4·08 mg; vitamin D_3, 0·05 mg; vitamin E, 75·0 mg; vitamin K_3, 1·01 mg; vitamin B_1, 1·5 mg; vitamin B_2, 5·0 mg; vitamin B_5, 18·0 mg; vitamin B_6, 4·0 mg; vitamin B_{12}, 0·03 mg; vitamin B_3, 25·0 mg; vitamin B_{11}, 3·0 mg; biotin, 0·3 mg; choline, 432·5 mg; Fe as $FeSO_4$. H_2O, 150·0 mg; Cu as $CuSO_4 \cdot 5H_2O$, 16·43 mg; Mn as MnO, 50·0 mg; Zn as ZnO, 92·97 mg; I as $Ca(IO_3)_2$, 2·0 mg; Se as Na_2O_3Se, 0·40 mg; Ca, 0·89 mg; P, 0·05 mg; Mg, 0·16 mg; Na, 3·02 mg; Cl, 0·12 mg; K, 0·08 mg; S, 0·11 mg; lysine, 0·04 mg; methionine, 0·01 mg; threonine, 0·03 mg; tryptophan, 0·01 mg; butylhydroxytoluene, 0·50 mg; ethoxyquine, 0·55 mg; butylated hydroxyanisol, 0·05 mg.

Table 2. Analysed and calculated* nutrient composition of the gestation and lactation diets

Chemical analysis (g/kg)	Gestation diet	Lactation diet
DM	885·6	893·2
Crude ash	58·1	62·1
Crude protein	151	164·9
Crude fat	51·3	55·7
Starch	295·6	355·1
Sugar	63·1	58·6
ADF	86·3	55·9
NDF	182·7	122·6
ADL	14·3	8·3
Ca	9·5	12·1
P	4·7	6·0
Cu (mg/kg)	19·9	24·8
Zn (mg/kg)	124·2	124·5
ID Lys	5·2	7·9
ID Met	1·8	2·3
ID Thr	3·4	5·5
ID Trp	1·2	1·6
ID Arg	6·7	8·1
ID Leu	7·8	10·0
ID Ile	3·9	4·8
ID His	2·8	3·2
ID Val	4·7	6·7
ID Phe	4·7	6·0
NEv (MJ/kg)	8·6	9·2

ADF, acid-detergent fibre; NDF, neutral-detergent fibre; ADL, acid-detergent lignin; ID, ileal digestible; NEv, net energy for pigs.
* Chemical analyses of ID amino acids and NEv (NEv is calculated according to the feed tables of the Centraal Veevoederbureau (the Netherlands), 2007).

cross-fostering and provision of creep feed (transitional feed) to the piglets from day 10 postpartum.

Body weight and back-fat thickness were measured at the start of the study (i.e. weaning of the preceding lactation period for the multiparous sows (day −5)), at days 63, 84 and 108 of gestation, and at weaning (day 143). Back-fat thickness was measured between the 3rd and 4th last rib, 7 cm at the left and right side of the vertebrae (P2). After P2 was lubricated, back-fat measurements were repeated three times, alternately at the left and right side (PIGLOG 105; SFK Technology). The average thickness was used for further calculations.

The present observational study was conducted according to the institutional and national guidelines for the care and use of animals, and all experimental procedures involving these animals were approved by the ILVO's ethical committee for animal experiments.

Preparation of blood samples

A heparinised blood sample (1 ml) was used for haematocrit assay and centrifuged at 2749 g for 30 min at 20°C. The remainder was centrifuged at 1500 g for 10 min at 4°C, divided over different 5 ml disposable polystyrene tubes, and stored for 24 h at −20°C, thereafter at −80°C until analysis. Plasma was analysed for Zn and Cu concentrations.

After overnight incubation at 4°C, serum was separated by centrifugation at 1500 g for 10 min at 4°C and stored for 24 h at −20°C, thereafter at −80°C until analysis. Serum was analysed for ALP, albumin and MT concentrations. Colostrum,

milk and the piglets' plasma were analysed for Zn and Cu concentrations. Plasma Cu concentration was not measured as a Zn status biomarker; however, because it uses some of the same transporter mechanisms, it may provide information on the affected pathways[15,18,21,22].

Analysis of zinc status biomarkers

Plasma samples were deproteinated (Randox ZN2607; Randox Laboratories Limited) by mixing with an equal volume of TCA and centrifuged at 10 000 g for 10 min. The remaining supernatant was used within 2 h to determine plasma Zn and Cu concentrations.

For plasma Zn analysis, the deproteinated plasma was diluted five times with a colour reagent (Randox Kit, ZN2341; Randox Laboratories Limited), and incubated for 5 min at 25°C. Absorbance was measured at a wavelength of 570 nm with a reference wavelength of 620 nm using a microplate reader (EZ reader 400; Biochrom Limited). Plasma Zn concentration was interpolated from the standard multipoint calibration curve. The inter- and intra-assay CV were 2·19 and 3·61 %, respectively. For quality control, pooled porcine plasma samples were spiked using $ZnCl_2$ to calculate the rate of Zn recovery. The regression line of the calibration curve to compare the measured and expected Zn concentrations was as follows: $y = 0·0003x + 0·0252$, R^2 0·9966. The minimum and maximum recovery rates were 99·5 and 119·8 %, respectively.

For plasma Cu analysis, a reagent (Randox Kit, CU2340; Randox Laboratories Limited) was added to the deproteinated plasma, and this solution was incubated for 60 s at 37°C (Memmert Elanco incubation oven; Memmert GmbH). Absorbance was measured at a wavelength of 580 nm (Ultrospec IIE; LKB Biochrom Limited). A colour reagent was added and the solution was mixed. After 5 min of incubation at 37°C,

Table 3. Body weight and back-fat thickness throughout the reproductive cycle of sows (n 15; five primiparous and ten multiparous sows)

(Mean values with their standard errors)

	Body weight (kg)				Back-fat thickness (mm)			
	Primiparous		Multiparous		Primiparous		Multiparous	
Cycle Day*	Mean	SE	Mean	SE	Mean	SE	Mean	SE
−5	142[a,A]	3	234[a,B]	11	18[a]	2	18[a,b]	1
63	188[b,A]	4	250[b,B]	11	21[a,b]	2	19[a]	2
84	203[c,A]	6	269[c,B]	11	21[a,b]	1	19[a]	1
108	223[d,A]	6	279[d,B]	11	23[b]	1	18[a]	1
143	169[e,A]	6	237[a,B]	10	14[c]	1	15[b]	2
P								
Day	<0·001				<0·001			
Parity	<0·001				0·373			
D × P†	<0·001				0·117			

[a,b] Mean values within a column with unlike superscript letters were significantly different between different days of the reproductive cycle (P<0·050).
[A,B] Mean values within a row with unlike superscript letters were significantly different between the primiparous and multiparous sows for the specific day of the reproductive cycle (P<0·050).
* Day −5 represents the start of the study, days 63, 84 and 108 represent gestation and day 143 represents weaning.
† Interaction between day (D) and parity (P).

Table 4. Reproductive performance of primiparous (*n* 5) and multiparous (*n* 10) sows*

(Mean values with their standard errors)

	Primiparous		Multiparous		
	Mean	SE	Mean	SE	*P*
Piglets born alive (*n*)	13·0	1·2	13·0	0·9	0·741
Stillborn piglets (*n*)	1·0	0·4	1·0	0·4	0·537
Average body weight of piglets born alive (kg)	1·3	0·1	1·5	0·1	0·210
Piglet mortality (%)	13·0	5·8	10·0	2·4	0·537
Weaned piglets (*n*)	11·0	0·9	12·0	0·9	0·480
Average body weight of weaned piglets (kg)	8·9	0·2	8·4	0·2	0·153

* No significant differences were found between the primiparous and multiparous sows (*P* > 0·050).

absorbance was measured at the same wavelength. Plasma Cu concentration was interpolated from the standard multipoint calibration curve. The inter-assay CV was 2·83 %. For quality control, pooled porcine plasma samples were spiked using $CuCl_2.2(H_2O)$ to calculate the rate of Cu recovery. The regression line of the calibration curve to compare the measured and expected Cu concentrations was as follows: $y = 0.0006x + 0.0806$, R^2 0·9912. The minimum and maximum recovery rates were 96·4 and 103·7 %, respectively.

Albumin was determined colorimetrically (Cobas Integra Albumin Gen.2; Roche Diagnostics GmbH), by binding to an anionic dye (bromocresol green) to form a blue–green complex. The total albumin concentration was proportional to the colour intensity and determined by an increase in absorbance at 583 nm. Serum albumin concentrations were within the expected range of the kit (3–96 g/l), and the recovery rate was within 10 % of the initial value at an albumin concentration of 35 g/l. The observed albumin concentrations were daily controlled using an independent human control serum with a high and low known concentration.

MT was determined using competitive ELISA (Porcine Metallothionein (MET) Elisa Kit, E07M0030; BlueGene Biotech Company), including duplicate standards and controls from human origin for each run. The product of the enzyme–substrate reaction forms a blue-coloured complex and turns into yellow after the reaction was stopped by adding a stop solution. The intensity of the colour was measured spectrophotometrically at 450 nm (BEP 2000; Siemens AG). MT concentration was interpolated from the standard curve. The certificate of analysis reported an inter- and intra-assay CV of 6·6–8·2 % and 4·4–5·6 %, respectively. The recovery rates ranged between 94 and 103 %.

ALP concentration was determined colorimetrically (Cobas c systems ALP2; Roche Diagnostics GmbH). In the presence of Mg and Zn, *p*-nitrophenyl phosphate was cleaved into phosphate and *p*-nitrophenol. The released *p*-nitrophenol was directly proportional to the catalytic ALP activity, which is determined by an increase in absorbance at 480/450 nm. The device was calibrated with a known concentration before the analysis to measure the concentration of ALP. Serum ALP concentrations were within the expected range of the kit (5–1200 units/l), and the recovery rate was within

10 % of the initial value at an ALP concentration of 100 units/l. The observed ALP concentrations were daily controlled using an independent human control serum with a high and low known concentration.

Concentration of zinc and copper in colostrum and milk

Colostrum and milk samples (1·5 g) were digested in a microwave oven (MarsX; CEM), using HNO_3/H_2O_2, and analysed for Zn and Cu concentrations by inductively coupled plasma optical emission spectrometry (ICP-OES, Vista MPX; Varian, Inc.). For quality control, two cows' milk samples were spiked, both with 50 and 100 µg/l of Cu and Zn. The recovery rates of spiked Cu and Zn varied between 81·2 and 88·9 %, and between 83·7 and 91·2 %, respectively.

Statistical analysis

The evolution over time during gestation (period between day 0 (insemination) and day 115 (parturition)) and lactation (period between day 118 and day 143 (weaning)) of all biomarkers were analysed using a linear mixed model for each reproductive phase (gestation and lactation), with the biomarker as the outcome and parity, haematocrit, time, interaction between parity and time, and a quadratic time effect as fixed effects. Inclusion of a quadratic time effect allows the identification of a non-linear evolution over time. In case of repeated measures, a random effect for sows was introduced in the model.

Biomarkers in colostrum, milk and the piglets' plasma were analysed using a linear mixed model, with the biomarker as the outcome and all sows' biomarkers at parturition or the average during gestation, and parity as fixed effects. The non-significant biomarkers were removed from the model. Sow was introduced as a random effect to correct for clustering of piglets within sows.
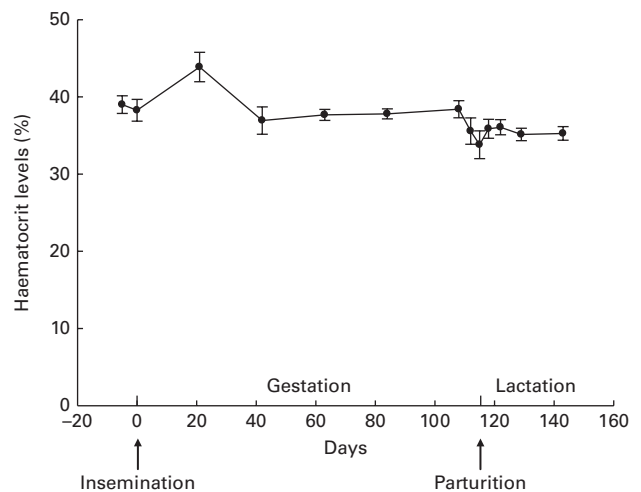

Fig. 1. Haematocrit levels throughout gestation and lactation in sows (*n* 15; five primiparous and ten multiparous sows). Values are means, with their standard errors represented by vertical bars. During gestation and lactation, no fluctuation was observed (*P* = 0·955 and *P* = 0·581, respectively).
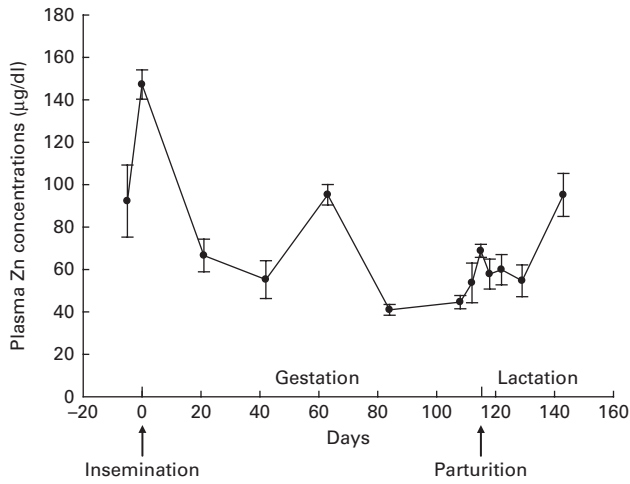
Fig. 2. Plasma zinc concentrations throughout gestation and lactation in sows (*n* 15; five primiparous and ten multiparous sows). Values are means, with their standard errors represented by vertical bars. During gestation, plasma zinc concentration decreased linearly after insemination (*P*<0·001) and increased quadratically towards parturition (*P*<0·001). During lactation, plasma zinc concentration tended to increase quadratically at the end of lactation (*P*=0·063). To convert values from μg/dl to μmol/l, multiply by 0·153.

The analysed data were considered sufficiently normally distributed based on the graphical evaluation (histogram and *QQ* plot) of the residuals. In case of *post hoc* pairwise testing, *P* values were corrected with the Tukey–Kramer adjustment for multiple comparisons. All analyses were performed using SAS 9.4 (SAS Institute, Inc.).

Results

The body weight of multiparous sows increased during gestation and decreased after parturition to similar values at weaning of the preceding cycle (Table 3). Primiparous sows showed a lower body weight compared with multiparous sows throughout the reproductive cycle, and had a higher body weight at weaning compared with the start of the study (Table 3).

Back-fat thickness decreased during lactation (*P*<0·001), independently of parity.

No significant parity effect was found in reproductive performances (Table 4).

The haematocrit level (Fig. 1) did not fluctuate significantly throughout gestation ($\beta = 0.019$, *P*=0·955) and lactation ($\beta = -0.107$, *P*=0·581), despite the numerically increased percentage at day 21 of gestation. There was also no parity effect for haematocrit during gestation (*P*=0·458) and lactation (*P*=0·955). The concentrations of plasma Zn (*P* = 0·173 for gestation and *P* = 0·389 for lactation), serum MT (*P*=0·127 for gestation and *P*=0·514 for lactation), plasma Cu (*P*=0·314 for gestation and *P*=0·259 for lactation) and serum ALP (*P*=0·346 for gestation and *P*=0·738 for lactation) were not associated with the haematocrit level. Fluctuation in albumin levels tended to be positively associated with the haematocrit level during gestation (*P*=0·086) and lactation (*P*=0·049).

Despite the parity effect for body weight and a tendency for an interaction between parity and serum albumin concentration during gestation, the fluctuations of the other biomarkers did not differ between the primiparous and multiparous sows. Therefore, average biomarker fluctuations are presented in the figures.

Plasma Zn concentration decreased linearly after insemination ($\beta = -1.934$, *P*<0·001) and increased quadratically towards parturition ($\beta = 0.012$, *P*<0·001) (Fig. 2). After parturition, plasma Zn concentration seemed to first increase, then decrease and increase again during the last 14 d of the lactation period. However, there was only a tendency for a quadratic time effect for the last 14 d of lactation ($\beta = 0.118$, *P*=0·063). The variation between the sows was high, especially at days −5, 42 and 112 of gestation, and at weaning (day 143).

A distinct fluctuation in plasma Cu concentration during gestation was not found ($\beta = 0.408$, *P*=0·127; Fig. 3). Plasma Cu concentration decreased linearly during lactation ($\beta = -6.716$, *P*<0·001) and increased quadratically towards weaning ($\beta = 0.126$, *P*=0·024).

The total serum albumin concentration tended to decrease linearly during the beginning of gestation, though it tended to decrease less for primiparous sows (tendency for an interaction between parity and day of gestation, *P*=0·055). A quadratic time effect was found towards the end of gestation ($\beta = 0.000$, *P*<0·001; Fig. 4). During lactation, serum albumin concentration decreased linearly ($\beta = -0.039$, *P*=0·018).

During gestation, serum MT concentration did not change significantly ($\beta = -0.016$, *P*=0·162) (Fig. 5). Shortly after parturition, MT concentration decreased linearly ($\beta = -0.244$, *P*=0·001) and increased quadratically towards the end of lactation ($\beta = 0.007$, *P*<0·001).

Serum ALP concentration increased linearly at the beginning of gestation ($\beta = 0.395$, *P*=0·002) and decreased quadratically
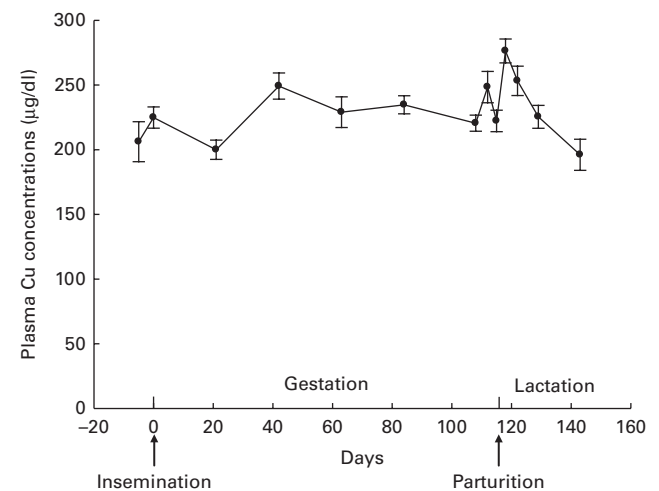

Fig. 3. Plasma copper concentrations throughout gestation and lactation in sows (*n* 15; five primiparous and ten multiparous sows). Values are means, with their standard errors represented by vertical bars. During gestation, no fluctuation was observed for plasma copper concentration (*P*=0·127). During lactation, plasma copper concentration decreased linearly (*P*<0·001) and increased quadratically towards weaning (*P*=0·024). To convert values from μg/dl to μmol/l, multiply by 0·157.
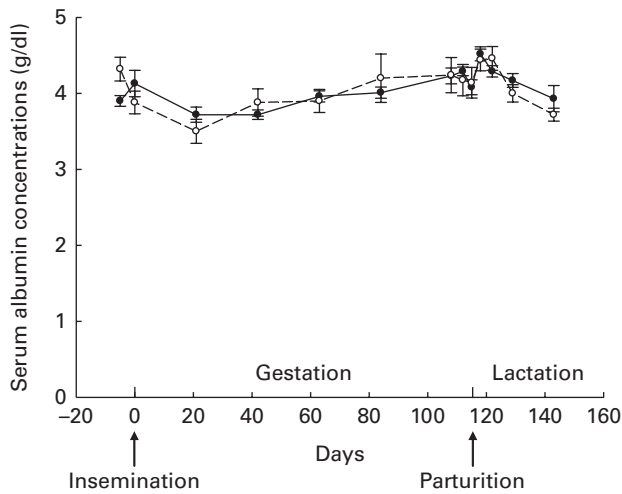
Fig. 4. Total serum albumin concentrations throughout gestation and lactation in sows (n 15; five primiparous (−○−) and ten multiparous (−●−) sows). Values are means, with their standard errors represented by vertical bars. During gestation, serum albumin concentration tended to decrease linearly at the beginning of gestation, though tended to decrease less for primiparous sows (tendency for an interaction between parity and day of gestation, $P = 0.055$) and increased quadratically towards the end of gestation ($P < 0.001$). During lactation, serum albumin concentration decreased linearly ($P = 0.018$). To convert values from g/dl to µmol/l, multiply by 10.

towards parturition ($\beta = -0.003$, $P = 0.001$) (Fig. 6). During the lactation period, no significant fluctuations were observed in ALP concentration ($\beta = 0.859$, $P = 0.140$). No parity effect was found, despite the numerically higher serum ALP concentrations of primiparous sows throughout the reproductive cycle ($P = 0.102$ for gestation and $P = 0.473$ for lactation).

Biomarkers in piglets' plasma, colostrum and milk

No significant parity effects were found for piglet body weight, haematocrit level, plasma Zn and Cu concentrations, placental weight, colostrum Zn and Cu concentrations, and milk Zn concentration ($P > 0.050$; Table 5). Milk Cu concentration tended to be lower for primiparous sows than for multiparous sows ($\beta = -0.378$, $P = 0.099$).

At parturition, serum albumin concentration of the sow tended to be associated with the average body weight of her piglets born alive ($\beta = 0.297$, $P = 0.060$).

Plasma Zn concentration of the piglets was associated with serum MT concentration of the sow during gestation and at parturition ($\beta = 11.891$, $P = 0.036$ and $\beta = 14.261$, $P = 0.028$, respectively) and associated with the haematocrit level of piglets ($\beta = -1.732$, $P = 0.032$).

Discussion

Fluctuations of Zn status biomarkers during gestation and lactation differed distinctly between biomarkers and were independent of parity (except for serum albumin). These differences are probably related to the respective roles of these biomarkers in Zn homeostasis throughout the different phases of the reproductive cycle, as has been shown in other animal species and human subjects. However, the

observed evolution over time might have been partly influenced by random period effects, such as temperature.

During gestation, Zn requirements are increased to support embryogenesis and fetal growth and development[1–4,23,24], resulting in homeostatic adjustments to (re)distribute more Zn to maternal tissues (e.g. placenta, liver and bone) and to the fetus[23–26]. This Zn sequestration to maternal tissues and the fetus may decrease the total circulating Zn concentration[24].

In the present study, plasma Zn concentration decreased during gestation as hypothesised based on other studies in sows[27–29]. This conceivably reflects the increased intracellular Zn need to protect maternal erythrocytes from oxidative stress, thereby increasing erythrocyte Zn and MT concentrations (due to the synthesis of carbonic anhydrase) and decreasing plasma Zn concentrations[24,25,30].

Zn is predominantly bound to albumin after absorption and has a high affinity to albumin[15,16]. A lower Zn fraction bound to albumin or a lower affinity of Zn for albumin was mentioned to explain the decreased plasma Zn concentration. Interestingly, the total serum albumin concentration in the present study increased during gestation, hence not reflecting the decline in Zn concentration, as expected and as has been found in other studies in women[3,31].

Whereas albumin serves as an important Zn transporter, MT is involved in Zn transfer within the intestinal mucosal cell, regulating the quantity of Zn absorbed[15,17,18]. When Zn is bound to albumin in plasma and entering the liver, MT is synthesised. MT in the liver removes Zn from the plasma and redistributes it to various tissues[15,23,32]. Hepatic MT synthesis is influenced by dietary Zn intake as well as plasma Zn concentrations[15,17,33,34], suggesting that serum MT concentration reflects Zn absorption. This is confirmed in the present study, where serum MT concentration fluctuated similarly to plasma
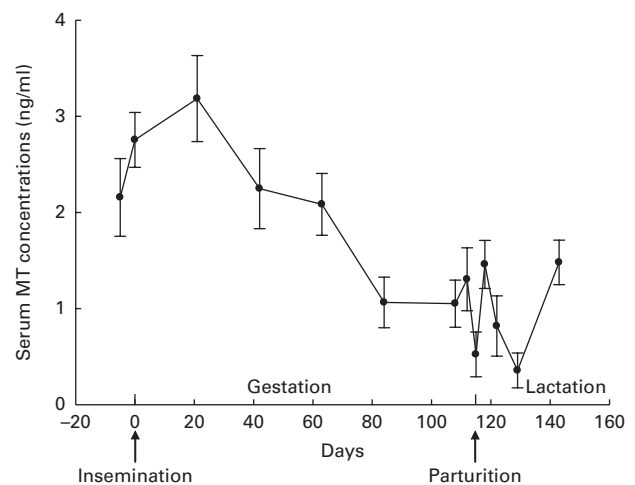

Fig. 5. Serum metallothionein (MT) concentrations throughout gestation and lactation in sows (n 15; five primiparous and ten multiparous sows). Values are means, with their standard errors represented by vertical bars. During gestation, no fluctuation was observed for serum MT concentration ($P = 0.162$). During lactation, serum MT concentration decreased linearly ($P = 0.001$) and increased quadratically towards the end of lactation ($P < 0.001$).
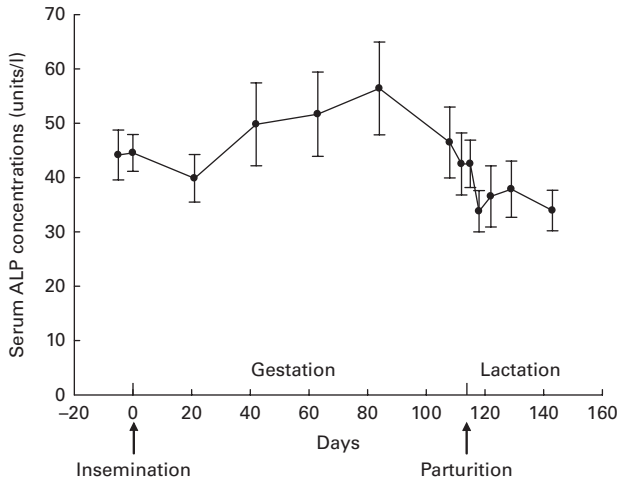
Fig. 6. Serum alkaline phosphatase (ALP) concentrations throughout gestation and lactation in sows (*n* 15; five primiparous and ten multiparous sows). Values are means, with their standard errors represented by vertical bars. During gestation, serum ALP concentration first increased linearly (*P*=0·002) and decreased quadratically towards parturition (*P*=0·001). During lactation, no fluctuation was observed for serum ALP concentration (*P*=0·140).

Zn concentrations during gestation but not at parturition when dietary Zn intake was low.

Plasma Cu can be bound to MT as well, but fluctuations in plasma Cu concentration in the present study are comparable with other studies in sows[29,33], women[35] and ewes[36], and therefore suggests that plasma Cu was not interfering with the fluctuation of serum MT. This was also observed in the study by López-Alonso *et al.*[37], where liver and kidney MT concentrations were highly dependent on the Zn status of the pig, and neither Cu nor Cd displaced Zn from MT.

ALP is found in the blood and bone and is involved in the formation of hydroxyapatite[16,38], which is important for regulating ordered mineral deposition during bone formation[38–40]. ALP concentration increased during gestation and decreased towards parturition in the present study. This fluctuation may indicate that ALP is related to bone formation in the process of bone remodelling.

During lactation, Zn requirements are increased to support milk synthesis and secretion[24], resulting in a (re)distribution of Zn to the mammary gland. This occurs by mobilising Zn from involuting tissues (uterus) and maternal blood after parturition, mobilisation from the bone, and by increased efficiency of Zn absorption in the intestine[24–26,41,42]. The distribution of Zn to the mammary gland may affect Zn-related blood parameters differently than during gestation[24].

Towards the end of lactation, plasma Zn concentrations in the present study seemed to return to initial values at early gestation, probably resulting from increased absorption or decreased nutrient requirements[24,43]. These plasma Zn fluctuations during lactation correspond to other studies in sows[29], women[2] and sheep[44], except for the study by Kalinowski & Chavez[28], who did not observe an increase in plasma Zn concentration during lactation in sows. However, in that study, no blood samples were analysed after 2 weeks of lactation.

Similarly to fluctuation in serum albumin concentrations during gestation, total serum albumin concentrations did not reflect the changes in plasma Zn concentration during lactation in the present study: the increase in plasma Zn concentrations coincided with an increase in serum albumin concentrations. Zn is then rapidly removed from albumin and transferred to other tissues, thereby ensuring the capacity to maintain Zn homeostasis. However, this was not demonstrated in the present study or other studies in women[3,31].

Serum MT concentrations increased at the end of lactation in the present study. This increase agrees with increased Zn absorption, in which more plasma Zn enters the liver where hepatic MT is synthesised to sequester Zn from the plasma to the tissues.

The fact that plasma Cu concentrations decreased towards the end of lactation, as opposed to plasma Zn concentrations, demonstrates that plasma mineral concentrations probably reflect dietary intakes. Zn is an important antagonist of Cu, hence the rise in plasma Zn concentrations towards the end of lactation combined with a decrease in plasma Cu concentrations seems logical and corresponds to other studies in sows[28,29] and ewes[36].

The mobilised and absorbed Zn will be used for milk production to provide Zn to the piglets. Plasma Zn concentration in piglets before colostrum intake may reflect the dietary Zn intake levels of sows during gestation[1,16,45–47]. However, in the present study, maternal plasma Zn concentration was not associated with the piglets' plasma Zn concentration. Interestingly, maternal serum MT concentration was significantly associated with the piglets' plasma Zn concentration: higher MT concentrations in sows during gestation and at parturition increased plasma Zn concentration in the piglets' plasma.

Additionally, fluctuations of Zn status biomarkers may identify critical periods throughout the reproductive cycle during which high Zn requirements are not met by dietary

Table 5. Piglets' indicatives* (*n* 30) and colostrum and milk zinc and copper concentrations

(Mean values with their standard errors)

	Primiparous		Multiparous		
	Mean	SE	Mean	SE	P
BW (kg)	1·5	0·1	1·6	0·1	0·172
HCt level (%)	37·9	2·0	41·6	1·2	0·676
Plasma Zn					0·268
µg/dl	73·0	8·9	60·7	5·9	
µmol/l	11·2	1·4	9·3	0·9	
Plasma Cu					0·888
µg/dl	12·6	1·2	15·6	2·1	
µmol/l	2·0	0·2	2·4	0·3	
Placental weight (kg)	2·6	0·5	2·7	0·2	0·951
Colostrum (mg/kg)					
Zn	18·4	2·0	16·2	1·2	0·529
Cu	4·5	0·2	4·4	0·3	0·564
Milk (mg/kg)					
Zn	6·7	0·2	6·6	0·2	0·948
Cu	1·1	0·1	1·4	0·1	0·099

BW, body weight; HCt, haematocrit.
* Piglets' BW, HCt, and plasma Zn and Cu concentrations were from two randomly selected piglets per sow at parturition (day 115) before colostrum intake.

Zn intake. It has been shown that Zn deficiency during gestation may affect the growth and development of the fetus, induce complications at birth, and limit the metabolic adaptation capacity of the sow[35,48–50].

In the literature, marginal values for plasma/serum Zn concentrations for pigs have been suggested[51], with a lower limit of normality of 60 μg/dl (9·2 μmol/l) and a normal marginal range for mean serum concentrations between 40 and 70 μg/dl (6·1–10·7 μmol/l). In the present study, the average plasma Zn concentration was 71·5 μg/dl (10·9 μmol/l) during gestation and 66·9 μg/dl (10·2 μmol/l) during lactation. The sows in the present study had on average sufficient plasma Zn levels. However, at days 42, 84, 108, 112, 118, 122 and 129, the average plasma Zn concentrations were below the normal range defined by Suttle[51], though sows can replace their Zn stores towards weaning. The dietary Zn concentration was above the Zn requirements, and no conclusion can be drawn for the presence of marginal Zn deficiency. Furthermore, plasma Zn concentrations varied widely among sows, which also hampers sound conclusions in individual sows.

Conclusion

Zn status shows distinct fluctuations throughout the reproductive cycle of sows, independently of parity. However, this Zn status pattern differs according to the chosen Zn status biomarker. Therefore, assessing Zn status in breeding sows should best be determined with multiple biomarkers. For the present study, a combination of plasma Zn and serum MT seemed most suitable to assess the Zn status.

Sound conclusions regarding critical periods for Zn status throughout the reproductive cycle in sows could not be distinguished in the present study. For future studies, it is good to take into account that fluctuations in plasma Zn concentrations over a reproductive cycle are noticeable. After periods of lower plasma Zn concentration at the end of gestation and throughout lactation, plasma Zn concentrations increase towards weaning.

Acknowledgements

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