Effects of maternal dietary nitrate supplementation during the perinatal period on piglet survival, body weight, and litter uniformity

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ABSTRACT: The objective of this study was to evaluate effects of different dosages of dietary nitrate supplementation to sows from d 108 of gestation until d 5 of lactation on reproductive performance of sows and piglet performance from birth until weaning. Dietary nitrate supplementation leads to nitric oxide (NO) formation that can potentially increase blood flow to the fetuses (by the vasodilative effect of NO), leading to a decrease in the loss of potential viable piglets in the form of stillbirth and preweaning mortality. Three hundred and five gilts and sows were allocated to one of six diets from d 108 of gestation until d 5 of lactation, containing 0.00% (Control), 0.03%, 0.06%, 0.09%, 0.12%, or 0.15% of dietary nitrate. The source of nitrate used was calcium nitrate double salt. Calcium levels were kept the same among diets by using limestone. Gilts and sows were weighed and backfat was measured at arrival to the farrowing room (d 108 of gestation) and at weaning (d 27 of age). Data included number of piglets born alive, born dead, and weaned, as well as individual piglet weights at d 0, 72 h of age and weaning. Preweaning mortality was determined throughout lactation. Body weight d 0 ($P = 0.04$) as well as BW at 72 h of age ($P < 0.01$) increased linearly with increasing dosages of nitrate in the maternal diet. Litter uniformity (SD) at birth was not affected by maternal nitrate supplementation level ($P > 0.10$), but tended to be higher at 72 h of age in the control treatment than in all nitrate-supplemented treatments ($P = 0.07$), and SD decreased linearly (increased uniformity) at weaning with increasing dosages of nitrate ($P = 0.05$). BW at weaning ($P > 0.05$) and average daily gain of piglets during lactation ($P > 0.05$) were not affected by maternal nitrate supplementation. A tendency for a quadratic effect ($P = 0.10$) of the dosage of maternal dietary nitrate was found on preweaning mortality of piglets with the lowest level of mortality found at 0.09% to 0.12% of maternal nitrate supplementation. We conclude that the use of nitrate in the maternal diet of sows during the perinatal period might stimulate preweaning piglet vitality. Exact mode of action and optimal dose of nitrate still need to be elucidated.

Key words: birth weight, farrowing, litter uniformity, nitrate, preweaning mortality, sow

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INTRODUCTION

In the Netherlands, on average 1.2 piglets per litter are stillborn and 2 piglets per litter die before weaning (Agrovision, 2016), resulting in a 20.4% total loss of potential viable piglets before

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weaning. This loss might be due to selection for larger litter sizes, leading to prolonged farrowing duration and consequently stillbirth or a reduced vitality at birth (van Dijk et al., 2005), a high number of low birth weight piglets, and high variation in birth weight among litter mates (Milligan et al., 2002; Fix et al., 2010).

A potential way to improve piglet viability at birth is the use of maternal dietary nitrate. Dietary nitrate is a nitric oxide (NO) precursor (Lundberg and Govoni, 2004). NO, is an endothelium-derived relaxing factor leading to vasodilation (Lundberg and Govoni, 2004; Webb et al., 2008), which plays an important role in regulating placental–fetal blood flow and transfer of nutrients and O_2 from mother to fetus (Bird et al., 2003). Restricted blood flow to the uterus reduces fetal development and survival (Molina et al., 1985). Although placental blood flow increases significantly as pregnancy progresses, uterine blood flow per fetus decreases when litter size increases (Père and Etienne, 2000), which might explain why piglets from larger litters are lighter at birth (Molina et al., 1985). This suggests that a larger blood flow (by improved vasculogenesis and/or angiogenesis, better placental development, or otherwise), especially in the last stages of gestation when fetal growth increases tremendously (McPherson et al., 2004), could stimulate piglet birth weight and may affect litter uniformity and, therefore, piglet survival. In addition, a larger blood flow toward piglets during farrowing could potentially decrease the risk for asphyxiation and might therefore decrease stillbirth and increase vitality at birth. It is thus hypothesized that maternal dietary nitrate supplementation can increase piglet birth weight, litter uniformity, and survival of piglets both during and after birth.

MATERIALS AND METHODS

The experiment was performed at the Swine Innovation Centre Sterksel of Wageningen University and Research, the Netherlands, from May until October 2015. All experimental procedures were approved by the institutional animal use and care committee of Wageningen University and Research.

Animals and Diets

In eight consecutive batches, 305 crossbred (Yorkshire × Dutch Landrace; Topigs 20) sows were allocated to one of six diets containing 0.00%, 0.03%, 0.06%, 0.09%, 0.12%, or 0.15% of nitrate. Dosages were based on the work of Bouwkamp and Counotte (1988) who looked at elevated levels of nitrate in drinking water for growing finishing pigs and found no negative effects on performance, nor signs of toxicity. Allocation to diets was balanced for parity (3.8 ± 2.1; mean ± SD). All parities were included in the experiment (parity range 1 to 9). The source of nitrate used was calcium nitrate ($5Ca(NO_3)_2.NH_4NO_3.10H_2O$; containing 63.1% of nitrate; commercial name Bolifor CNF [Yara Phosphates Oy, Helsingborg, Sweden]). Calcium levels in the diets were kept constant by using limestone. Diet compositions are shown in Table 1. Diets were produced at ABZ Animal Feeds (Leusden, the Netherlands). One basal diet was produced, this contained 90% of ingredients that was split in six homogenous batches. Varying ingredients per experimental diet were added by mixing them into one of the six batches of the basal diet. Diets were produced as 4-mm pellets at 70 to 80 °C. Experimental diets were fed twice a day (7.30 h and 16.30 h) from the moment sows entered the farrowing room (d 108 ± 1 of gestation; mean ± SD) until 5 d after farrowing (based on the individual farrowing date of the sow). Diets were fed restrictedly at 3.25 kg/sow/d from d 108 to 112 of gestation, 2.7 kg/sow/d from d 113 of gestation onward, and 2.0 kg/sow/d on the day of farrowing. After farrowing, diets were provided at 2.5, 3.0, 3.0, and 3.5 kg/sow/d at d 1, 2, 3, and 4 after the day of farrowing, respectively. Feeds were weighed and provided manually. From d 5 of lactation to weaning (d 27.0 ± 1.7 postpartum; mean ± SD; range 22 to 32) a commercially available pelleted lactation diet was fed (14.9% crude protein [CP], 9.5 MJ net energy [NE]/kg) at 4.0, 4.5, 4.5, 5.0, 5.5, 6.0, 6.5, 6.5, 7.0, and 7.5 kg/sow/d for d 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and from d 15 after farrowing onward, respectively. Feed refusals of sows were removed and weighed before the next feeding. Wet feed refusals were oven-dried at 100 °C until weight of the sample did not decrease anymore to determine dry matter content. Sows had ad libitum access to water. Piglets received a commercial available pre-starter in a feeding bowl from d 3 of age till weaning (17.4% CP, 11.6 MJ NE/kg; Top Wean, Agrifirm, Apeldoorn, the Netherlands).

Animal Housing and Management

Approximately 1 wk (d 7.2 ± 1.8) before the expected date of farrowing, the pregnant sows were transported to individual pens (180 × 240 cm) with farrowing crates (55 × 185 cm) in 1 of 10 farrowing units. Pens were fully slatted with plastic and steel slats under the farrowing crate, located over a manure pit. Each pen contained a piglet nest with a heating lamp set at 30 °C. Farrowing was never induced, no

Table 1. Composition of the experimental lactation diets (per kg of diet)[a]

	Experimental lactation diet, %					
Item	Control	0.03% Nitrate	0.06% Nitrate	0.09% Nitrate	0.12% Nitrate	0.15% Nitrate
Ingredient						
Wheat				20.00		
Corn				18.00		
Wheat middlings				15.00		
Soybean meal CP > 48%				7.21		
Soybean hulls crude fiber (CF) < 32%				4.93		
Rapeseed meal CP < 37%				4.00		
Palm kernel expeller CF < 18%				4.00		
Sunflower seed meal				3.50		
Sugarcane molasses				2.50		
Sugarbeet pulp (dehydrated)				1.50		
Palm oil				1.50		
Vitamin and mineral premix[b]				1.00		
Sodium chloride				0.36		
L-Lysine HCL (78.0%)				0.31		
Sodium bicarbonate				0.16		
Monocalcium phosphate				0.12		
L-Threonine (98.5%)				0.10		
L-Tryptophan (98.0%)				0.007		
Phyzyme XP 10,000 thermo-stable protected technology				0.005		
Barley	11.71	11.70	11.66	11.63	11.60	11.57
Soybean oil	2.097	2.086	2.098	2.104	2.113	2.122
Limestone[c]	2.000	1.976	1.952	1.929	1.905	1.881
Bolifor CNF[d]	0.000	0.048	0.095	0.143	0.190	0.238
Chemical composition (calculated)[e]						
DM, %	87.41	87.42	87.42	87.42	87.43	87.43
NE, MJ	9.24	9.24	9.24	9.24	9.24	9.24
CP, %	15.28	15.32	15.37	15.41	15.45	15.49
SID Lys, %	0.78	0.78	0.78	0.78	0.78	0.78
SID Met + Cys, %	0.45	0.45	0.45	0.45	0.45	0.45
SID Trp, %	0.16	0.16	0.16	0.16	0.16	0.16
SID Thr, %	0.52	0.52	0.52	0.52	0.52	0.52
Ca, %[c]	0.95	0.95	0.95	0.95	0.95	0.95
Digestible *P*, %	0.31	0.31	0.31	0.31	0.31	0.31
Total *P*, %	0.51	0.51	0.51	0.51	0.51	0.51

[a]Lactational diets were provided in two meals (7.30 a.m. and 4.30 p.m.) at 3.25 kg/sow/d from d 108 to 112 of gestation, 2.7 kg/sow/d from d 113 of gestation onward, and 2.0 kg/sow/d on the day of farrowing. After farrowing, diets were provided at 2.5, 3.0, 3.0, and 3.5 kg/sow/d at d 1, 2, 3, and 4 after the day of farrowing, respectively.

[b]The vitamin and mineral premix provided the following per kg of complete feed: 40 mg of Mn as manganous oxide; 160 mg of Fe as iron sulfate; 65 mg of Zn as zinc sulfate; 15 mg of Cu as copper sulfate; 4 mg of I as potassium iodide; 0.4 mg of Se as sodium selenite; 10,000 IU vitamin A as vitamin A; 1 mg vitamin B1, 3.75 mg vitamin B2; 1 mg of vitamin B6; 0.03 mg of vitamin B12; 2,000 IU vitamin D3; 30 mg of vitamin E; 20 mg vitamin E equivalent of Proviox nucleus; 0.5 mg of vitamin K3 as menadione nicotinamide bisulfite; 15 mg of niacin; 400 mg choline as choline chloride 70%; 15 mg of pantothenic acid; 3 mg of folic acid.

[c]Calcium levels in the diets were kept constant by using limestone.

[d]The source of nitrate used in this experiment was calcium nitrate ($5Ca(NO_3)_2.NH_4NO_3.10H_2O$; containing 63.1% of nitrate; commercial name Bolifor CNF available from Yara Phosphates Oy).

[e]No synthetic L-arginine was added to the diets. SID Arg = 0.845% for all diets.

DM = dry matter.

medicine was administered during farrowing, and no birth assistance was given. Sows that received any form of birth assistance in case of emergency were excluded from the experiment. Split suckling was not allowed. Cross-fostering was allowed only between sows receiving the same treatment, having the same farrowing date, and between 24 and 48 h after birth (d 1). Litters were standardized to 14 or 15 piglets per sow. Piglets

to cross-foster on or off a sow were selected randomly. Number of litters to which cross-fostering was applied was similar between treatments (n = 35, 33, 30, 27, 33, and 27 for diets containing 0.00%, 0.03%, 0.06%, 0.09%, 0.12%, or 0.15% of nitrate, respectively). After 48 h after birth, no cross-fostering was applied. Researchers and other staff were not allowed to interfere with piglet survival (e.g., prevent crushing or by placing them under the heating lamp or at the udder).

Measurements

Sow body weight and P2 backfat thickness (BFP2 on the last rib, 6 cm down the dorsal middle line) were determined at arrival to the farrowing room and at weaning. For each litter, gestation length (GL), total number of piglets born (TNB), total number of piglets born alive (TBA), and total number of stillborn piglets (TSB) were recorded. GL was calculated as the difference between the day of first insemination and the day of parturition. To make sure a stillborn piglet was a true stillborn, a small piece of lung tissue (± 2 cm^2) was removed after dissection on the day of birth and placed in a bowl of water. When lung tissue floated, the piglet was scored as a preweaning death instead of a stillborn. A stillborn piglet was defined as a piglet born without any respiration, potentially with a heartbeat. Mummified and degenerating piglets were excluded from TNB. All piglets, born alive or stillborn, were individually weighed and identified within 24 h after birth (BW d 0). Individual piglet weight was determined again at 72 h of age (BW 72 h), i.e., 48 h after the first weighing and at weaning. Uniformity of the litter was expressed by the SD of the individual piglet weights per litter. Number of dead piglets, reason for death (e.g., crushing, splay legs, starvation, lameness, weak, low birth weight, and unknown), and weight of dead piglets were registered on a daily basis. Preweaning mortality was calculated by the following equation:

$$\text{Preweaning mortality} = \left(\frac{\text{Number of preweaning deaths (excl. TSB)}}{\left(\begin{array}{l} \text{TBA} + \text{number of piglets added} \\ -\text{number of piglets removed} \\ \text{at cross fostering} \end{array} \right)} \right) \times 100\%$$

Livability was calculated by the following equation:

$$\text{Livability} = 1 - \left(\frac{\left(\begin{array}{l} \text{number of TSB} \\ +\text{number of preweaning deaths} \end{array} \right)}{\left(\begin{array}{l} \text{TNB} + \text{number of piglets added} \\ -\text{number of piglets removed at} \\ \text{cross fostering} \end{array} \right)} \right) \times 100\%$$

Statistical Analyses

Data from sows that received birth assistance (n = 3), were sick, or died around farrowing (n = 3) were removed from the dataset. One other sow was removed from the experiment, because she expressed extreme aggressive behavior toward the piglets (biting them to death) and was treated with Stresnil (active component: Azaperonum; Janssen Animal Health) during farrowing. All variables were checked for normality on both means and residuals before analysis. TSB (ordinal data) was found to be non-normally distributed even after transformation and was expressed as a percentage of TNB. Because numerical differences in TNB were found between treatments, TBA was also analyzed as a percentage of TNB.

Variables were analyzed with mixed models using the PROC GLIMMIX procedure in SAS (version 9.3, 2011; SAS Institute Inc., Cary, NC, United States) according to the following statistical model:

$$Y_{ijklmn} = \mu + \alpha_i + b_j + c_k + d_l + f_m + \varepsilon_{ijklmn}$$

where: Y_{ijklmn} = dependent variable, μ = overall mean, α_i = fixed treatment effect (i = 0.00, 0.03, . . . , 0.15% dietary nitrate), b_j = random batch effect (i = 1,2, . . . , 8), c_k = random parity effect (j = 1, 2, . . . , 9), d_l = random farrowing unit effect (l = 1, 2, . . . , 10), f_m = random effect of days on experimental diet before farrowing (m = 3, 4, . . . , 13), and ε_{ijklmn} = residual error term. Sow was considered as the experimental unit. TNB was included as a covariate for BW d 0 and litter uniformity d 0. Number of piglets after cross-fostering was included as a covariable for BW 72 h and weight at weaning. Contrasts were used to determine significant relationships for linear and quadratic effects of increasing nitrate contents, and to assess the effect of no nitrate vs. nitrate (0.00% nitrate vs. 0.03%, 0.06%, 0.09%, 0.12%, and 0.15% nitrate).

Data are expressed as LSMeans and SEM unless reported otherwise. Differences were assumed to be significant if P value ≤ 0.05 and a P value > 0.05, but <0.10 was considered a trend.

RESULTS

At arrival to the farrowing unit, average sow BW was 265.6 ± 36.3 kg and BFP2 was 16.3 ± 3.4 mm (both mean ± SD). Average GL was 115.1 ± 1.6 d, meaning sows received the experimental diets for 7.2 ± 1.8 d before farrowing (both mean ± SD). At weaning, average sow BW was 221.0 ± 33.1 kg and BFP2 was 12.7 ± 2.7 mm (both mean ± SD). Weight loss (45.1, 44.7, 45.2, 46.7, 45.9, and 45.1 kg for 0.00%, 0.03%, 0.06%, 0.09%, 0.12%, and 0.15% nitrate, respectively) and BF loss (3.6, 3.7, 3.7, 4.2, 3.9, and 3.6 mm for 0.00%, 0.03%, 0.06%, 0.09%, 0.12%, and 0.15% nitrate, respectively) of sows during lactation were not affected ($P > 0.05$) by maternal nitrate supplementation. Maternal nitrate supplementation did not affect average daily feed intake of sows pre-farrowing (2.79, 2.76, 2.74, 2.77, 2.70, and 2.70 kg/sow/d for 0.00%, 0.03%, 0.06%, 0.09%, 0.12%, and 0.15% nitrate, respectively; $P > 0.05$), between farrowing and d 4 post-farrowing (2.96, 2.96, 3.04, 3.00, 3.06, and 2.97 kg/sow/d for 0.00%, 0.03%, 0.06%, 0.09%, 0.12%, and 0.15% nitrate, respectively; $P > 0.05$) and from d 5 post-farrowing till weaning (6.45, 6.47, 6.32, 6.49, 6.42 and 6.24 kg/sow/d for 0.00%, 0.03%, 0.06%, 0.09%, 0.12%, and 0.15% nitrate, respectively; $P > 0.05$).

Results on sow reproduction and litter performance are presented in Table 2. Mean BW d 0 (for both TNB and TBA) and BW 72 h increased linearly ($P < 0.05$) as maternal dietary nitrate levels increased, with notably weights being lower in the control compared to the nitrate-supplemented groups ($\Delta = 56$, 54, and 72 g, all Ps < 0.05 for TNB, TBA, and BW 72 h, respectively). SD of weight at d 0 (for both TNB and TBA) was not affected by treatment. SD of BW 72 h tended to be lower when comparing the control to the nitrate-supplemented groups ($\Delta = 23$ g, $P = 0.07$). Weaning weights were not affected by maternal dietary nitrate supplementation, whereas SD of weaning weights decreased linearly as maternal dietary nitrate level increased ($P = 0.05$). Nitrate supplementation did not affect TBA, TSB, or number of piglets at weaning, although preweaning mortality (% of TBA) tended to be lower in the nitrate-supplemented groups compared to the control (2.8%, $P = 0.06$; Figure 1a). For both preweaning mortality and livability (Figure 1b), a trend for a quadratic effect of dosage of maternal dietary nitrate supplementation ($P = 0.10$ and 0.09, respectively) was found, with a lower preweaning mortality and higher livability at intermediate levels (0.09% to 0.12%) of nitrate supplementation. Between treatments there was no difference in registered cause of death.

DISCUSSION

The linear effects of dosage found (on BW d 0, BW 72 h and litter uniformity at weaning, all linear $P < 0.05$) in this study may suggest that higher dosages of maternal nitrate supplementation could increase the response further. However, it must also be stated that the effect of nitrate dosage on preweaning mortality tended to slightly increase again at the highest dosage (quadratic $P = 0.10$), which might suggest that there is an optimal nitrate dose. Dietary intervention started approximately 7 d before farrowing, which seems a relatively short time span to influence fetal gain. This study is, to the best of our knowledge, the first study conducted looking at the effect of maternal dietary nitrate supplementation on reproductive performance of sows and piglet performance. More research has been done on the use of maternal L-arginine supplementation. Although oxidized in a reaction catalyzed by the NO synthase family (Moncada and Higgs, 1993), and not via the NO_3–NO_2–NO pathway, like dietary nitrate (Lundberg et al., 2008), arginine is also a precursor of several important metabolites, including NO (Wu et al., 2004). This suggests that similar effects can be expected from arginine and dietary nitrate supplementation in late gestation on piglet birth weight. Bass et al. (2017) compared feeding a +1.0% of L-arginine diet (46.6 g standardized ileal digestibility [SID] Arg/d) or a control diet (19.8 g SID Arg/d) from d 93 to 110 of gestation, but found no effect of arginine supplementation on birth weights of piglets. In addition, Quesnel et al. (2014) also found no effect on piglet birth weight when providing +0.77% of L-arginine (25.5 g/sow/d) compared to a control diet for a longer period of time in late gestation (d 77 of gestation until term). Wu et al. (2012) compared a diet supplemented with +1.0% of L-arginine to a control diet from d 90 until 114 of gestation and found higher birth weights of piglets born alive (+160 g/live-born piglet, $P < 0.05$) after L-arginine supplementation. Exact NO production resulting from arginine vs. nitrate feeding is not known and, therefore, results cannot be compared directly. Summarizing, maternal nitrate supplementation appears to have the potential to affect piglet birth weight, but involved pathways are still unknown.

Birth weight is driven by placental nutrient supply, which is determined by both the placental blood flow and the size of the placenta (van

Table 2. Effect of maternal dietary nitrate supplementation from d 108 of gestation until d 5 of lactation on sow reproduction and litter performance

	Level of nitrate (%)								Contrasts		
Item	0.00	0.03	0.06	0.09	0.12	0.15	SEM	*P* value	Linear	Quadratic	Control vs. all levels of nitrate
n	52	51	49	48	47	51					
Parity before farrowing	3.8	3.7	4.0	3.6	3.9	3.8					
Number of days on feed before farrowing	7.3	7.1	6.9	7.1	7.5	7.1					
TNB											
TNB, *n*[a]	16.8	16.3	17.5	16.4	17.0	17.0	0.08	0.55	0.49	0.82	0.76
Mean BW d 0, kg[b]	1.272	1.323	1.313	1.321	1.339	1.345	0.033	0.31	0.04	0.61	0.03
SD BW d 0, kg[b]	0.293	0.277	0.271	0.293	0.267	0.280	0.011	0.36	0.39	0.47	0.16
TBA											
TBA, *n* (% of TNB)	15.7 (94.2)	15.3 (94.1)	16.4 (94.6)	15.4 (94.4)	15.9 (93.9)	15.9 (93.9)	1.0	0.98	0.73	0.60	0.98
Mean BW d 0, kg[b]	1.288	1.338	1.323	1.336	1.350	1.364	0.033	0.29	0.03	0.78	0.04
SD BW d 0, kg[b]	0.286	0.269	0.266	0.286	0.263	0.267	0.011	0.42	0.30	0.75	0.15
TSB											
TSB, *n* (% of TNB)	1.1 (5.8)	1.1 (5.9)	1.0 (5.4)	1.0 (5.6)	1.1 (6.1)	1.1 (6.1)	1.0	0.98	0.73	0.60	0.98
Litter characteristics											
Number of piglets after cross-fostering, *n*	14.7	14.7	15.2	14.4	14.7	14.7					
Number of piglets weaned, *n*	12.2	12.4	12.5	12.4	12.7	12.2	1.9	0.51	0.46	0.27	0.33
BW 72 h, kg[b]	1.557	1.618	1.594	1.626	1.639	1.668	0.035	0.06	0.00	0.96	0.01
SD BW 72 h, kg[b]	0.318	0.301	0.284	0.304	0.292	0.292	0.013	0.42	0.18	0.34	0.07
ADG between d 0 and 72 h of age, g/pig/d[b]	96.6	103.2	100.8	101.1	102.6	105.8	7.16	0.85	0.28	0.97	0.26
ADG between 72 h of age and weaning, g/pig/d[c]	258.4	258.0	249.4	260.1	261.2	255.9	6.38	0.90	0.40	0.87	0.30
BW at weaning, kg[c]	7.967	8.003	7.798	8.132	8.056	8.021	0.271	0.45	0.42	0.88	0.78
SD BW at weaning, kg[c]	1.455	1.477	1.366	1.413	1.377	1.318	0.064	0.38	0.05	0.85	0.30

[a]Excluding mummified piglets.

[b]Included TNB as covariate in the model.

[c]Included number of piglets after cross-fostering in the model.

ADG = average daily gain.

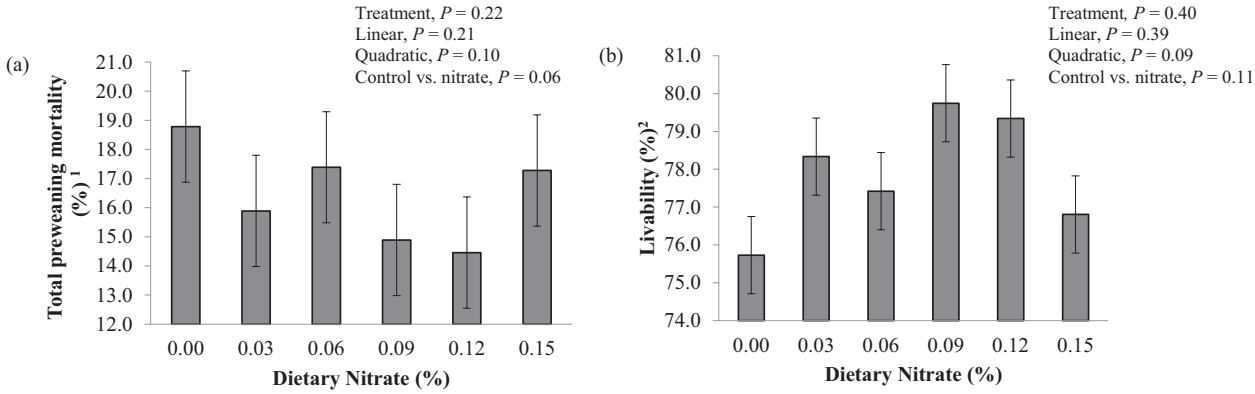

Figure 1. LSMeans ± SEM of total preweaning mortality (a) and livability (b) per maternal dietary nitrate level. [1]Preweaning mortality is calculated as the number of preweaning deaths (excl. TSB) / (TBA + number of piglets added − number of piglets removed at cross fostering). [2]Calculated as 1 – [(TSB + number of preweaning deaths) / (TNB + number of piglets added – number of piglets removed at cross fostering)] × 100%.

Rens et al., 2005). In pigs, a positive correlation between placental blood flow and fetal weight exists (Wootton et al., 1977). It might be that maternal nitrate supplementation, leading to NO production and vasodilation, increased uteroplacental blood flow per fetus, resulting in higher nutrient delivery to the developing fetuses and higher piglet birth weights. To get an estimation of the additional blood flow needed for the additional piglet gain seen in our experiment, the studies of McPherson et al. (2004) and Père and Etienne (2000) were used as a references for daily fetal gain (on average 40.3 g/d) and intrauterine fetal blood flow (on average 564.2 mL/min/fetus) between d 108 and 115 of gestation. Combining these two studies, it can be calculated that the average blood flow needed per gram of fetal growth during the time span in which the experimental diets were fed was 0.104 mL/min/fetus. It can be hypothesized that the same amount of blood flow is needed per gram of fetal gain obtained by maternal nitrate supplementation. This means in our study, with approximately 4 g of additional fetal weight per 0.01% of nitrate supplementation, 0.417 mL/min/fetus of additional blood flow is expected. However, additional studies to assess blood flow are needed to confirm or disprove whether this additional blood flow is realistic.

Placental weight is positively related to fetal weight (Leenhouwers et al., 2002; van Rens et al., 2005; Rampersad et al., 2011), but a less clear relationship was found between placental weight and preweaning mortality (Leenhouwers et al., 2002; van Rens et al., 2005; Baxter et al., 2008; Rootwelt et al., 2013), and between placental surface area and preweaning mortality (Baxter et al., 2008; Rootwelt et al., 2013). Van den Bosch et al. (unpublished data) showed a linear increase in placental width as maternal nitrate supplementation increased from 0.00% to 0.15%. NO formation, originating from maternal nitrate supplementation, along with growth factors, could have influenced new vessel formation (vasculogenesis/angiogenesis) (Ghimire et al., 2017) in the placenta (Wu et al., 2004), indicating that a higher placental size, and therefore more exchange area, with higher dosages of nitrate could have been the driver for the effect on piglet birth weights.

In our study, average preweaning mortality (excluding stillborn) was higher than Dutch average mortality rates in the Netherlands (15.6% vs. 13.9%, respectively; Agrovision, 2016), which is likely because researchers and other staff were not allowed to interfere with survival. Nevertheless, preweaning mortality tended to be higher in the control vs. nitrate-supplemented groups (Δ = 1.8%, P = 0.06 for contrast testing control vs. 0.03%, 0.06%, 0.09%, 0.12%, and 0.15% of nitrate) and tended to be the lowest at intermediate levels of nitrate supplementation (Δ = 3.9% and 4.3% for 0.09% and 0.12% of dietary nitrate compared to the control, respectively, P = 0.10 for quadratic contrast testing). It is unclear why a tendency for a quadratic effect instead of an expected linear effect of dosage was found.

TNB, TBA, and TSB were not affected by maternal dietary nitrate supplementation. TNB is mainly determined in the peri-implantation period, because an estimated two-thirds of the embryonic death losses occur before d 30 of gestation (Bazer et al., 2010). After d 30 of gestation, many factors may contribute to fetal losses (e.g., virus infections, nonuniform development of fetuses, intrauterine crowding, or limited uterine capacity) (Wu et al., 2006). In this study, however, TNB did not include mummies, and dietary treatments were provided well beyond the critical period for embryonic and fetal

losses (i.e., 7 d before farrowing). It was thus not expected that TNB would be affected by maternal nutrition. TSB, which is determined right before or during the process of farrowing and is often associated with hypoxia during farrowing (Randall, 1972; Herpin et al., 1996; van Dijk et al., 2008), was not affected by maternal dietary nitrate supplementation. It was hypothesized that NO (synthesized after nitrate supplementation), which has a vasodilative effect (Bird et al., 2003; Bailey et al., 2012), would have increased blood and, therefore, oxygen flow in the placenta and the umbilical cord during farrowing, thus potentially reducing the risk for asphyxiation and, therefore, stillbirth. Rootwelt et al. (2012) estimated that a broken umbilical cord explained a large proportion (71%) of stillbirths. This suggests that stimulating umbilical cord blood flow has limited effect on the incidence of stillbirth when the umbilical cord breaks, which cannot be prevented by the use of maternal nitrate supplementation.

In conclusion, nitrate supplementation of sow diets from d 108 of gestation until d 5 of lactation has potential to result in higher piglet BW and litter uniformity and higher piglet survival in the intermediate to higher levels of maternal dietary nitrate. Exact mode of action and optimal dose of nitrate still need to be elucidated.

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Conflict of interest statement. M. van den Bosch, I. B. van de Linde, A.A.M. van Wesel and D. Melchior are employed at the Cargill Innovation Center Velddriel, the Netherlands. A related patent application (PCT/US2015/064293) was filed on December 7, 2015, and published as WO/2016/090366 on June 9, 2016. Research was conducted objectively and in a solid scientific way without any bias. The other authors do not have a conflict of interest.

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