# Supporting the hyper prolific sow and her litter through the perinatal period by dietary nitrate supplementation

Moniek van den Bosch

# Propositions

1. Maternal dietary nitrate supplementation in the perinatal period decreases piglet losses. (This thesis)

2. The optimal duration of farrowing of sows in terms of piglet losses depends on litter size. (This thesis)

3. In polytocous species, mortality is inevitable to obtain stronger offspring.

4. Animal welfare can never be more important than human safety.

5. Never kill a good idea by doing a literature search on it.

6. If you can't explain it simply, you don't understand it well enough.

Propositions belonging to the thesis, entitled;
*"Supporting the sow the hyper prolific sows and her litter through the perinatal period by dietary nitrate supplementation"*

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# Chapter 1

**General Introduction**

# Introduction

The number of piglets reared per year by a sow is a determining factor for the profitability of pig production. This number can be increased by either increasing litter size, decreasing the farrowing interval and/or decreasing the number of piglets lost per litter. Farrowing is a crucial event in terms of piglet losses. Three to nine percent of all piglets born are stillborn (Borges *et al.*, 2005; Vanderhaeghe *et al.*, 2010; van den Bosch *et al.*, 2021) and when litter sizes are higher than 20 piglets born total, this percentage can increase to 15% (Nielsen *et al.*, 2021; van den Bosch *et al.*, 2021). Stillborn piglets either die shortly before (10%), during (75%) or immediately after farrowing (15%) (Leenhouwers *et al.*, 1999), meaning that parturition is of large importance. An increase in litter size is associated with an increased duration of farrowing (van Rens and van der Lende, 2004; Oliviero *et al.*, 2019), which poses an increased risk for hypoxia due to a limited or complete cut off of the blood flow in the umbilical cord (Curtis, 1974; Christianson, 1992; Rootwelt *et al.*, 2012), which will be reviewed and discussed in *Chapter 2*. Piglets that are born alive have to recover from the stress during farrowing and get to the udder where they have to compete with their siblings for colostrum (Muns *et al.*, 2016). Piglet vitality at birth is directly linked to level of asphyxia during birth (Zaleski and Hacker, 1993; Trujillo-Ortega *et al.*, 2007). Being born wet, with a high surface/volume ratio and with a lack of brown adipose tissue (which is used for thermoregulation) (Berthon *et al.*, 1993), piglets are at high risk for hypothermia, lethargy, and therefore a high latency to suckle. Consequently, they have a lower ability to move quickly and the risk of being crushed by the sow is increased (Weary *et al.*, 1996). It is therefore crucial that piglets are born vital, are able to get to the piglet nest for warmth or get to the udder to consume colostrum and increase its own heat production.

Since farrowing is a crucial event in piglet survival, and stillbirth and pre-weaning mortality are linked, it can be hypothesized that supporting the sow through this event, can reduce piglet losses. By increasing the stamina of the sow and thereby decreasing the total farrowing duration, the incidence of stillbirth could be decreased, piglet vitality could be increased and the incidence of pre-weaning mortality could be decreased. In addition,

supporting the piglets during the farrowing process by increasing the blood and therefore oxygen flow to them, might mitigate the effects of asphyxia.

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**Infobox 1.** The scale of the issue of stillbirth and pre-weaning mortality in the Netherlands and Denmark

In 2020 in the Netherlands, the number of stillborn piglets per litter was 1.3 (8.0%) and another 1.8 (12.2%) piglet per litter died before weaning (AgroVision, 2021). With an average farrowing index of 2.34, an average herd size of 548 sows, and the average loss of 3.1 piglets per sow per litter (stillbirths plus mortality before weaning), the average farmer losses 3975 potential viable piglets per year before weaning. These piglets do not only represent an economical loss, but also a welfare and ethical problem, which is damaging to the image and potential "licence to produce" of the swine industry.

Figure 1 shows the trends of number of piglets born alive, stillborn, weaned and percentage of pre-weaning mortality as registered by AgroVision (1995-2021) in the Netherlands. The steep increase in litter size that has been observed over the last decades seems to plateau over the last 5 years. Stillborn maintained stable at 1.3 piglets per litter from 2016 until 2021. A decrease in pre-weaning mortality percentage of 1.7% is observed between 2016 and 2021. Number of piglets weaned is linearly increased, mainly driven by the drop in pre-weaning mortality seen since 2016.
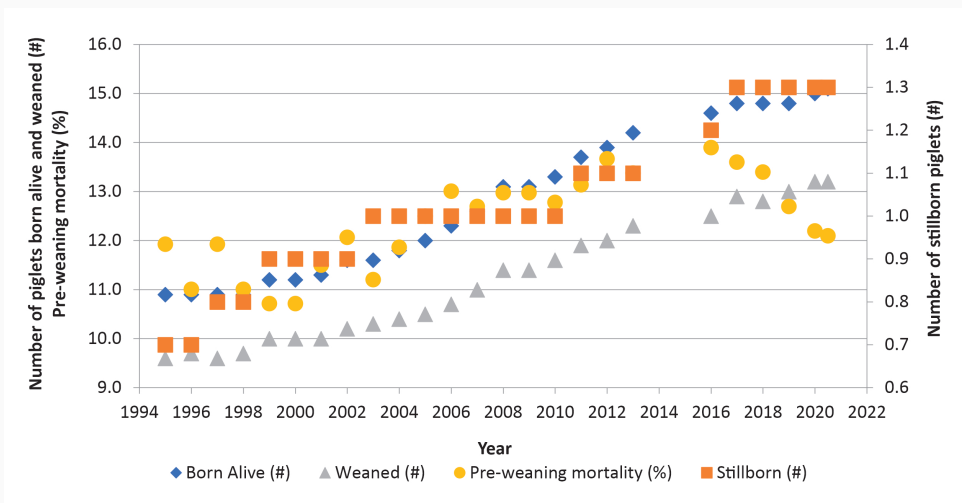


**Figure 1.** Number of piglets born alive, stillborn, weaned and percentage of pre-weaning mortality between 1995 and 2021 in the Netherlands based on approximately 295 farms in 2021 (AgroVision Kengetallenspiegel 1995-2021).

The swine industry in Denmark is known for its high production levels. Figure 2 shows the number of piglets born total, born alive, stillborn and percentage of pre-weaning mortality over the last decade in Denmark. Litter size has continuously increased over the last 10 years. Stillbirth and pre-weaning mortality levels lowered until 2016, but increased after to 1.9 piglets per litter being stillborn and an a pre-weaning mortality percentage of 14.9% in 2020. In 2020, in total 4.5 piglets per litter were lost out of 19.6 piglets born total.
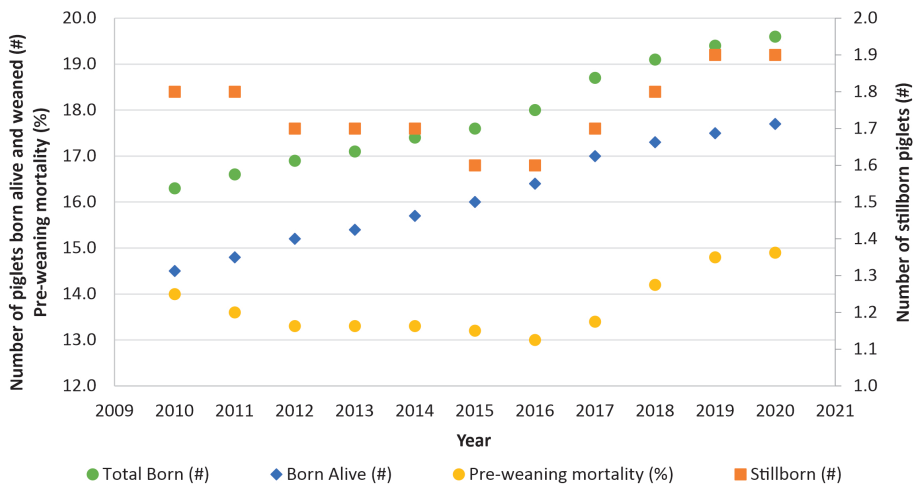


**Figure 2.** Number of piglets born total, born alive, stillborn and percentage of pre-weaning mortality between 2009 and 2020 in Denmark based on approximately 821 farms in 2020 (Landsgennemsnit for produktivitet i produktionen af grise i 2010-2020).

Several factors on a sow (e.g. parity, mothering ability, body condition (Le Cozler *et al.*, 2002; Canario *et al.*, 2006; Cecchinato *et al.*, 2008; Vanderhaeghe *et al.*, 2010; Muns *et al.*, 2016)), piglet (e.g. birth weight and vitality (Le Cozler *et al.*, 2002; Canario *et al.*, 2006; Baxter *et al.*, 2008; Rootwelt *et al.*, 2013; Muns *et al.*, 2016)), and farm level (e.g. housing, management and climate (Holyoake *et al.*, 1995; Le Cozler *et al.*, 2002; Mota-Rojas *et al.*, 2002; Vanderhaeghe *et al.*, 2010)) influence the incidence of stillbirth and pre-weaning mortality. Maternal feeding strategies and nutritional solutions have the potential to help reduce piglet losses as well (Theil *et al.*, 2014; Theil, 2015; Feyera *et al.*, 2018; Feyera *et al.*, 2021). Sports nutrition in humans is a good source of inspiration for nutritional

solutions or supplements that could potentially support the sows through the process of farrowing. A recent trend in sport nutrition is the use of red beetroot (Beta vulgaris rubia) juice to enhance athletic performance or to increase endurance. One of the main active compounds in beetroot juice causing these effects is nitrate ($NO_3^-$). Nitrate itself is not known for specific physiological functions, but in vivo it is reduced to nitric oxide (NO), a multivarious messenger molecule with important vascular functions (Machha and Schechter, 2011; Hobbs *et al.*, 2013), which particularly occur in environments of hypoxia and acidosis, like farrowing.

**Nitrate Metabolism**

To my knowledge, no studies investigated the metabolism of nitrate ($NO_3^-$) via nitrite ($NO_2^-$) to nitric oxide (NO) in pigs. Studies described in the following paragraph thus refer to studies done in humans. It has been hypothesized that the metabolism is very similar between pigs and humans, since the digestive tracts of pigs and humans are largely comparable (Heinritz *et al.,* 2013). The hypothesized nitrate metabolism in the pig based on studies done in humans, which will be explained below, is shown in Figure 3.

The two major sources for $NO_3^-$ in the body are; 1) the endogenous L-arginine-NO synthase pathway, by which NO produced via the NOS-enzymes (Nitric Oxide Synthases) can be oxidized in the blood to $NO_2^-$ or $NO_3^-$; and 2) dietary $NO_3^-$ (Moncada and Higgs, 1993; Lundberg *et al.*, 2008), which is rapidly absorbed after ingestion from the proximal part of the gastrointestinal tract (GIT) into the bloodstream, where a basal level of $NO_3^-$ is already present, coming from the NOS pathway (Wagner *et al.*, 1984). Most of the dietary $NO_3^-$ is excreted via urine (~60%), while approximately 25% of plasma $NO_3^-$ is taken up by the salivary glands and excreted in the saliva (Spiegelhalder *et al.*, 1976). Saliva containing $NO_3^-$ is secreted in the oral cavity, where approximately 20% of the $NO_3^-$ is reduced to $NO_2^-$ via $NO_3^-$ reductase enzymes by commensal facultative anaerobic bacteria that live in the crypt of the tongue (Spiegelhalder *et al.*, 1976; Duncan *et al.*, 1995). These oral bacteria use $NO_3^-$ as an alternate terminal electron acceptor to generate ATP when oxygen is scarce, leaving $NO_2^-$, which is further processed in the stomach (Lundberg *et al.*, 2011). Due to the acid environment (pH of 3 – 4) in the stomach, $NO_2^-$ in the swallowed saliva is

non-enzymatically converted to NO by protonation by first yielding nitrous acid ($HNO_2$). Nitrous acid is converted into di-nitrogen trioxide (N2O3), also yielding H2O, which is eventually converted into NO and $NO_2^-$ (Benjamin *et al.*, 1994; Lundberg *et al.*, 1994). After the stomach, the remaining $NO_2$ is either converted by anaerobic gut flora in the lower parts of the GIT to NO (Sobko *et al.*, 2005) or absorbed into the blood. Studies on NO production from faecal bacteria are limited. It seems that mainly lactobacilli and bifidobacteria generate NO when $NO_3^-$ or $NO_2^-$ is added to the diet in humans (Sobko *et al.*, 2005). $NO_3^-$ and $NO_2^-$ are absorbed from the intestine and can be converted into bioactive NO in blood and tissue (Benjamin *et al.*, 1994; Lundberg *et al.*, 1994; Lundberg *et al.*, 2008). In tissues, NO can be generated from nitrite involving 1) Deoxyhaemoglobin (HbFe$^{2+}$ in blood) or myoglobin (iron and oxygen binding protein in muscle tissue), 2) Xanthine oxidoreductase, 3) Protons 4) Ascorbate and 5) Polyphenols (Lundberg *et al.*, 2008).

| Where | What | How |
|---|---|---|
| Mouth | 25% of $NO_3^-$ is taken up by salivary glands and recycled in the body | Bacteria reduce $NO_3^-$ to $NO_2^-$ |
| Stomach | $NO_2^-$ is transformed into NO due to low pH | |
| Gut | $NO_3^-$ and remaining $NO_2^-$ is absorbed in the intestine | • $NO_3^-$ reduction to $NO_2^-$ by commensal bacteria <br> • $NO_2^-$ reduction to NO by anaerobic bacteria |
| Blood and tissue | • In human blood $NO_3^-$ has a half-life of 5-8 hours <br> • $NO_3^-$ originates from feed and systematic NO production | $NO_2^-$ reduction to NO via 5 different reactions involving haemoglobin, protons, xanthine oxidoreductase, ascorbate and polyphenols |
| Urine | | Nitrate is excreted in saliva and urine. |

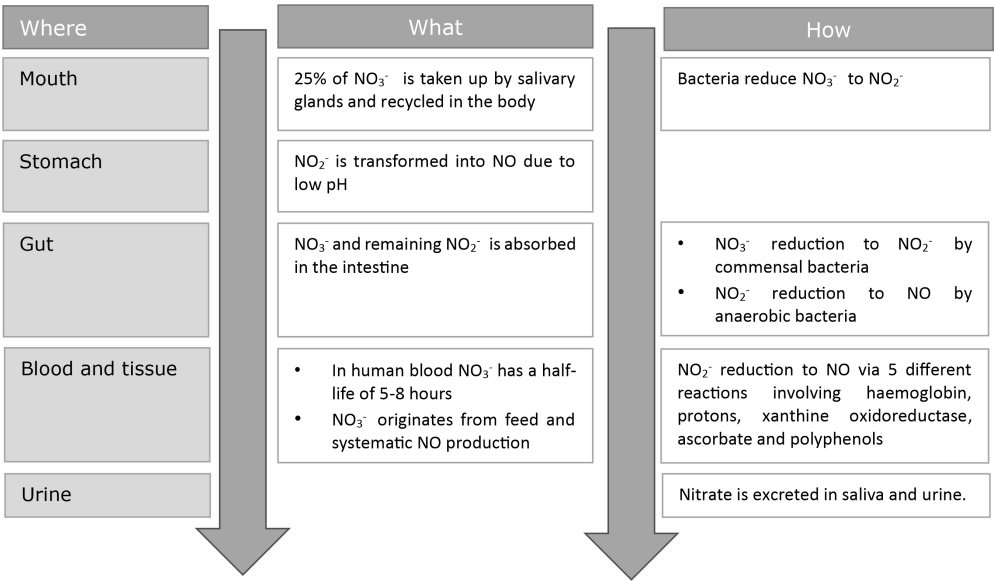**Figure 3.** Hypothesized nitrate metabolism in the pig based on studies done in humans.

Several studies reported a rapid increase in plasma $NO_3^-$ levels within the first 30 minutes after ingestion, with a peak occurring 1.5 - 2 hours after ingestion (Lundberg and Govoni,

2004; Webb *et al.*, 2008; Miller *et al.*, 2012). Plasma $NO_3^-$ levels stay elevated for several hours after ingestion. In human blood, $NO_3^-$ has a half-life of 5-8 hours (Lundberg and Weitzberg, 2005; Bryan and Grisham, 2007) and $NO_2^-$ of 1-5 minutes (Lundberg and Weitzberg, 2005). NO is a highly reactive free radical with a half-life of only a few seconds. NO (or one of the reaction products) is quickly oxidized to make higher nitrogen oxides, like $NO_3^-$ and $NO_2^-$ (Lundberg *et al.*, 2008). How fast plasma $NO_3^-$ levels are increased and what the half-life of $NO_3^-$ is in the body combined after ingesting a meal in sows and which feeding strategies on farms are used (meal size, time of feeding and feeding amount) are important information to determine the dosage of maternal nitrate supplementation in the diet. This to ensure sows have sufficient $NO_3^-$ levels and therefore continuous synthesize $NO_2^-$ and NO at the moment of farrowing to ensure an effect on endurance and vasodilatation, which may benefit a reduction in stillbirth and an increase in vitality. This is, however, not investigated yet.

## Differences Between the $NO_3$-$NO_2$-NO Pathway and the NOS Pathway

Since the process of birth can be considered as moderate to heavy exercise, during which oxygen levels in the body might be low and tissue acidification may occur, it can be hypothesized that the $NO_3$-$NO_2$-NO pathway is used as the main pathway for NO production during this event. In this paragraph, NO syntheses from the conventional NOS-pathway will be compared to the $NO_3$-$NO_2$-NO pathway and insights will be given in why $NO_3^-$ seems more suited to be supplemented around farrowing compared to the L-Arginine.

The $NO_3$-$NO_2$-NO pathway is independent from the conventional pathway in which L-arginine is oxidised in a reaction catalysed by the nitric oxide synthase (NOS) family (Moncada and Higgs, 1993). L-arginine in combination with oxygen and NOS forms NO and L-citrulline when nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) is present. During this reaction, 5 electrons of the guanidine nitrogen of L-arginine are oxidized. The L-citrulline that is formed during the reaction can be recycled back to L-arginine by arginine synthase (Ghalayini, 2004). There are three isoforms of NOS; 1)

neural NOS (nNOS or NOS1; expressed in nerve cells, skeletal- and heart muscle), 2) inducible NOS (iNOS or NOS2; expressed in many cell types as a response to the immune system) and endothelial NOS (eNOS or NOS3; expressed in cells lining blood vessels). nNOS and eNOS are both Ca2+ dependent for NO production and produce only small amounts of NO, in contrast to iNOS (Wu and Meininger, 2009; Wu *et al.*, 2013). Essential cofactors for enzymatic activities of all NOS isoforms are NADPH, calmonulin (calcium-modulating protein – calcium binding messenger protein), flavin adenine dinucleotide (FAD) or mononucleotide (catalyser in redox reactions) and tetrahydrobiopterin (BH4) (Sooranna *et al.*, 1995; Wu and Meininger, 2009). In the porcine placenta, NOS activity and NO production are increased by arginine, which increases BH4 synthesis and bioavailability (Wu and Meininger, 2009).

A difference between the NOS pathway and the $NO_3$-$NO_2$-NO pathway is that NO production from $NO_2^-$ is enhanced in the $NO_3$-$NO_2$-NO pathway when tissue pH reduces (e.g. lactate production), like during heavy exercise (Cosby *et al.*, 2003; Ferguson *et al.*, 2016), whereas the NOS pathway is oxygen dependent (Moncada and Higgs, 1993). When myoglobin gets deoxygenated (for example in exercising skeletal muscle), it will rapidly convert $NO_2^-$ to NO (Shiva *et al.*, 2007; Lundberg *et al.*, 2008). NO formed by myoglobin can bind to cytochrome-c-oxidase of the mitochondrial electron transport chain, reducing electron flow and oxygen utilization, which suggests that myoglobin and $NO_2^-$ play an important role in energetics and oxygen utilization under hypoxia (Shiva *et al.*, 2007; Lundberg *et al.*, 2008). Xanthine oxidoreductase can reduce $NO_2^-$ to NO when there is a low oxygen tension and pH, again indicating an increased NO formation under hypoxia (Lundberg *et al.*, 2008). Lastly, $NO_2^-$ reduction also occurs via acidic disproportionation, indicating that a reduction in pH will promote NO generation from $NO_2^-$ (Lundberg and Weitzberg, 2005). This suggest that the $NO_3$-$NO_2$-NO pathway may be particular important for NO production during exercise (Bailey *et al.*, 2012).

**How Could Nitrate Support the Sow Through Farrowing?**

$NO_3^-$ ingestion influences exercise efficiency (amount of effort vs. the output to work) by reducing $O_2$ cost of exercise as shown by a decreased $VO_2$ max[1] (-3%), without increasing blood lactate values (indicating that oxidative energy production is not increased) as reviewed by Bailey *et al.* (2012). This effect is likely depending on the training status of the subjects, with larger effects observed in untrained subjects (Bailey *et al.*, 2012). Exercise tolerance (time till exhaustion during a constant-work-rate exercise) was also increased by 16-25% after ingestion of dietary $NO_3^-$ (Bailey *et al.*, 2009; Bailey *et al.*, 2011; Lansley *et al.*, 2011). However, a large increase in exercise tolerance does not automatically lead to a similarly large exercise performance (time to cover a set distance). Hopkins *et al.* (1999) found that a ~20% improvement in time to exhaustion corresponds to an exercise performance increase of 1-2%. Based on beneficial effects of NO on exercise efficiency in humans, it can be hypothesized that $NO_3^-$ supplementation might increase stamina of sows during farrowing, thus decreasing farrowing duration, reduce incidence of stillbirth, levels of asphyxia and early mortality in piglets. This hypothesis is based on the following assumptions; 1) $NO_3^-$ levels already circulating in the body, originating from the NOS-pathway are limiting for sufficient NO formation, limiting the beneficial effects of NO during farrowing. 2) Dietary $NO_3^-$ supplementation will lead to an increase in NO formation. 3) The beneficial effects of NO on exercise performance and/or endurance can be extrapolated to process of farrowing/uterine contractions.

NO also has a vasodilative effect by being a major endothelium-derived relaxing factor (Bird *et al.*, 2003; Bailey *et al.*, 2012), and influences vasculogenesis and angiogenesis in placental tissues (Wu *et al.*, 2004). Adequate vasculogenesis and angiogenesis of the placenta are not only important during gestation to ensure the required blood supply for foetal development (Liu *et al.*, 2012), but can also be of importance during the farrowing process. It might be that, by ensuring a larger blood, and thereby oxygen and nutrient, flow in the placenta and the umbilical cord during farrowing, the risk for asphyxiation

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[1] $VO_2$ max is a measure for the maximum oxygen consumption per time unit (e.g. l/min or ml/kg/min) which is a measurement of physical fitness. The lower a $VO_2$ max the higher the endurance capacity of an individual. $O_2$ intake and output is measured under increasing physical exercise. $VO_2$ max is reached when $O_2$ levels are not rising anymore despite of increasing intensity of exercise.

is reduced. Since, to our knowledge, no other studies are done supplementing dietary nitrate to sows in the perinatal period, studies in which L-Arginine was supplemented are the best comparison. Wu *et al.* (2013) reviewed twelve studies investigating effects of maternal supplementation of L-Arginine (dose ranging between 0.4 - 1.23%) during gestation (most of them applying L-Arginine between approximately day 14 and 28 of gestation). The use of arginine supplementation during early gestation aims at an improved vascularisation of the placenta due to its action on angiogenesis (Wu *et al.*, 2004) via NO formation. The increased placenta quality will result in a higher rate of survival of embryo's and eventually a higher litter size (Mateo *et al.*, 2007; Bérard and Bee, 2010; Gao *et al.*, 2012; Wu *et al.*, 2013). Arginine supplementation during later stages of pregnancy mainly aims at an increased blood flow to the piglets, which will result in higher piglet birth weights (Mateo *et al.*, 2007; Gao *et al.*, 2012; Wu *et al.*, 2012; Wu *et al.*, 2013). Only a few studies investigated effects of arginine supplementation till right before the moment of farrowing, which also evaluated the effect on stillbirth and vitality of piglets. Che *et al.* (2013) evaluated the supplementation of 1% of L-Arginine to sows from day 30 of gestation to day 114 of gestation (so not until the moment of farrowing) and showed a significant decrease in absolute number of stillborn piglets (-0.6 pigs, $P < 0.05$) compared to the control. Additionally, Mateo *et al.* (2007) and Gao *et al.* (2012) evaluated stillbirth when L-Arginine was supplemented to sows until day 114 of gestation. Mateo *et al.* (2007) supplemented 1.0% of L-arginine to gilts from day 30 to 114 of gestation and showed a significant reduction in stillbirth (-1.2 pigs, $P < 0.05$). Gao *et al.* (2012) found no significant difference in the absolute number of stillborn piglets (1.21 vs 1.42, for the control and Arginine, respectively), but this lack of effect is probably affected by the difference in total born piglets (12.46 vs 13.77, for the control and Arginine, respectively). Che *et al.* (2013) hypothesized that the reduction in stillbirth can be due to increased utero-placental blood flow and maternal nutrient transfer, which supports a more efficient uterine capacity for fetal growth and development. However, since L-Arginine was not supplemented until the moment of farrowing, it seems unlikely that blood flow was increased during farrowing. None of the authors linked the beneficial effects of NO to farrowing duration (potentially due to an increased stamina of sows).

In the current thesis, $NO_3^-$ supplementation was used instead of L-Arginine because of two main reasons. 1) Inorganic dietary $NO_3^-$ sources are relatively cheap compared to synthetic L-Arginine sources, which will give a profitable and affordable solution for the industry. 2) Dietary $NO_3^-$ is a good source of NO under hypoxic situations (as explained before), which most likely occurs during farrowing. Calcium nitrate was selected as the source of nitrate to use, since it is a safe (non-explosive) source and a registered feed ingredient within the EU. Feeding nitrites were not considered due to a higher risk of toxicity (methemoglobinemia), since they are 6-10 times more toxic compared to nitrates.

**The Aim of This Thesis**

The aim of this thesis is to evaluate effects of maternal dietary nitrate supplementation during the perinatal period on farrowing duration, piglet characteristics related to vitality, the incidence of stillbirth and pre-weaning mortality.

Based on this aim the following hypotheses are developed:

1. NO production from dietary nitrate will induce vasodilation in the placenta and by increasing oxygen and nutrient flow to the piglets, it will decrease the risk for asphyxia.
2. Sow stamina will be increased, which could shorten the duration of farrowing.

These hypotheses are visualised in Figure 4, including the main parameters of interest and potential interfering factors which are taken into account in the current thesis.

**Outline of This Thesis**

*Chapter 2* reviews current knowledge on uterine contractions, placental blood and the role of the umbilical cord in the sow and the relationship of these parameters with stillbirth and pre-weaning mortality. In *Chapter 3,* a study is described that focussed on effects of maternal dietary nitrate supplementation in the perinatal period under commercial circumstances in hyper prolific sows. In this study (referred to as Study I), 0.1% of

calcium nitrate was supplemented to sows from approximately 5 days before until 4 days after farrowing. Incidence of stillbirth, birth weight, placental redness and pre-weaning mortality were the main parameters of interest. In *Chapter 4 and 5,* a study is described (referred to as Study II) in which calcium nitrate was supplemented approximately 7 days before until 4 days after farrowing in a dose response approach (diets contained 0.00%, 0.03%, 0.06%, 0.09%, 0.12% and 0.15% of nitrate). Effects on incidence of stillbirth, birth weight, litter uniformity, weight development and pre-weaning mortality (*Chapter 4*) and on farrowing and placental characteristics, level of asphyxia at birth and piglet vitality (*Chapter 5*) are evaluated.
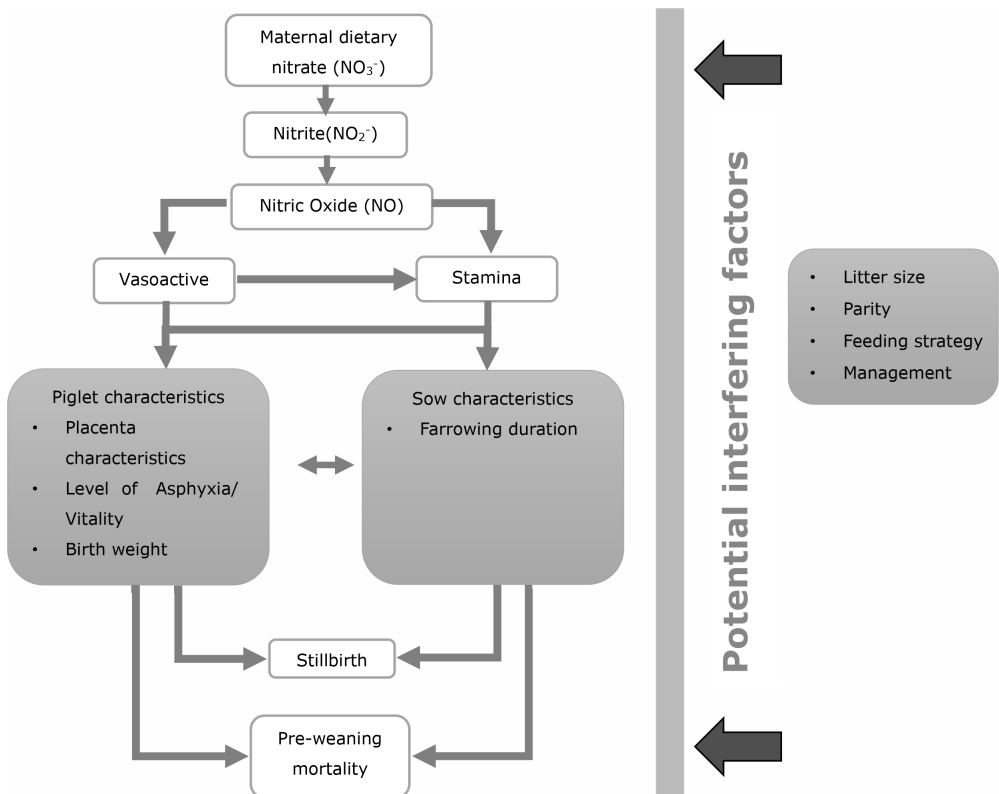


**Figure 4.** Hypotheses, parameters of interest and potential interfering factors evaluated within this thesis.

In *Chapter 6,* the effects of both an increasing litter size as well as an increasing farrowing duration on incidence of stillbirth, level of asphyxia at birth, piglet vitality and pre-weaning mortality are evaluated. In addition, effects of both litter size and farrowing duration were disentangled and it was evaluated whether or not the optimal duration of farrowing depends on litter size (*Chapter 6*). In *Chapter 7*, the results obtained from the studies described in *Chapters 3* till *6* are combined and discussed.

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# Chapter 2

## Maternal nutrition, uterine contractions and placental blood flow in the sow: a review.

To be submitted

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## Introduction

The parturition process is challenging for both the sow and her piglets. For the sow, parturition is an energy demanding, stressful and painful event (Rutherford *et al.*, 2013), and for piglets, it is also a stressful event and the odds of dying are highest during parturition and the first days of life (Rootwelt *et al.*, 2013). The parturition process in sows has been studied mainly from a behavioral or endocrine point of view (Algers and Uvnäs-Moberg, 2007; Peltoniemi *et al.*, 2016). Surprisingly, fewer studies investigated uterine contractions and placental or umbilical blood flow, and in relation to that, the changing metabolism of the sow during the perinatal period (Mosnier *et al.*, 2010). With an increase in litter size, the challenges to the perinatal piglet have increased, related to a decrease in uterine blood flow per piglet (Pere and Etienne, 2000), a decrease in piglet (Quiniou *et al.*, 2002; Boulot *et al.*, 2008; Rootwelt *et al.*, 2012) and placental development (Knight *et al.*, 1977; Wilson *et al.*, 1999; Rootwelt *et al.*, 2013), and an increase in farrowing duration (van Rens and van der Lende, 2004). The outcome of the birth process for piglets mainly depends on fetal oxygenation, which in turn depends on farrowing duration (van Dijk *et al.*, 2005; Oliviero *et al.*, 2010), the duration and intensity of uterine contractions (Maffeo *et al.*, 1990) and placental blood flow and therefore oxygen flow (van Dijk *et al.*, 2008). The maternal diet needs to provide the right nutrients to provide energy for uterine contractions and for sufficient placental blood flow, and its role has been investigated in recent studies (Le Cozler *et al.*, 1999; Quiniou, 2005; Guillemet *et al.*, 2007; Tydlitát *et al.*, 2008; Vallet *et al.*, 2013; Feyera *et al.*, 2018). This review focuses on possible interventions in the maternal diet that may facilitate the parturition process, affecting uterine contractions and/or placental blood flow in the perinatal period.

## Uterine Contractions and Uterine Blood Flow

The parturition process in the sow can be divided in three stages; 1) Increase in myometrial activity and dilation of the cervix (approximately 6-12 hours), 2) Expulsion of the piglets with the sow lying down and abdominal straining (approximately 5-8 hours) and 3) Expulsion of the placenta (approximately 4 hours, which may already start during

stage 2) (Jones, 1966; van Rens and van der Lende, 2004). Several reviews discuss the complex endocrine changes in the peripartum period (Ellendorff *et al.*, 1979; Purohit, 2010; Peltoniemi and Oliviero, 2015), so here we only highlight the major changes. The increase in myometrial contractions in stage 1 result from a cascade of endocrine events. Fetal cortisol induces a release of endometrial PGF2α, which induces luteal regression and thereby results in a decline in progesterone. The placenta produces estrogens, and the changed progesterone/estrogen ratio stimulates myometrial contractions starting at 4-9 hours before the expulsion of the first piglet. These contractions last for 2-3 minutes each and occur at regular intervals (Taverne, 1982). Contractions keep increasing in frequency and amplitude and staining efforts of the sows start to appear the last few hours before expulsion of the first piglet (Taverne, 1982). As soon as the first fetus enters the cervix, stage 1 of parturition is considered to be completed (Senger, 2003). Then, the Ferguson-reflex is activated, releasing oxytocin from the pituitary. The increased oxytocin levels stimulate abdominal muscles straining to expel fetuses (Vallet *et al.*, 2010). The frequency of uterine contractions is highest when piglets and the placentae are being expelled (Taverne *et al.*, 1979a), but large variations occurs between sows in frequency, duration and amplitude and for an individual sow from one hour to the next of the expulsion phase (Zerobin, 1968). During this phase, on average uterine contractions last for 1-2 minutes and occur in a frequency of 18 per hour (Taverne *et al.*, 1979b). Maffeo *et al.* (1990) was the latest study found gaining insight in frequency and amplitude of contractions during different timepoint during the parturition process using a two implanted strain gauges (one in each horn) in spontaneous births. The frequency, amplitude and duration of contractions 12, 5 and 1 hours before the birth of the first piglet, during piglet expulsions and during placenta expulsion are shown in Figure 1.

During the expulsion phase, tubo-cervical contractions move the fetuses towards the cervix. In addition, cervico-tubal contractions occur, which are likely meant to shorten the uterine horns and to prevent accumulation of fetuses at the caudal ends of the uterine horns (Taverne, 1982) and/or to keep fetuses at a fixed place to keep the umbilical cord functionality before expulsion (Taverne *et al.*, 1979a). Contractions are initiated at the two ends of the horns and convey (either as a tubo-cervical or cervico-tubal contraction)

| Timepoint |
| --- |



**12h pre-partum**
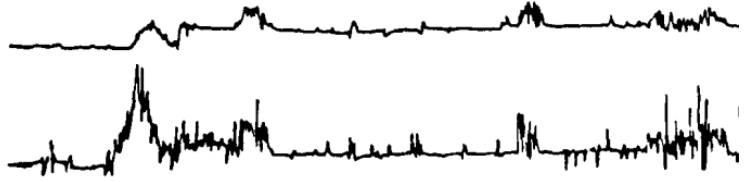
**5h pre-partum**

**1h pre-partum**
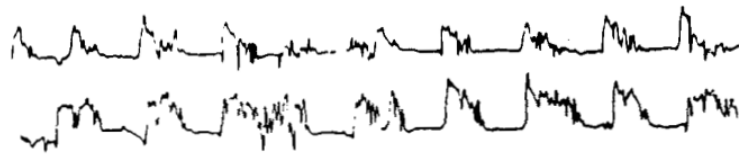
**Piglet expulsion**
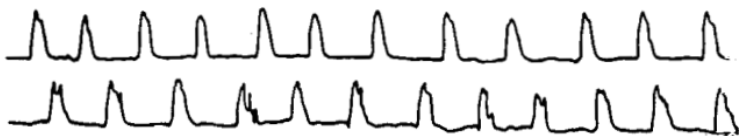(* indicate the birth of a piglet)

**Placentae expulsion**

**2**

**Figure 1.** Myometrial contractions in the sow 12, 5 and 1 hour pre-partum, during parturition and during the placentae expulsion measures by two stain gauges implanted in each uterine horn.

to the proximal end of the horns (Taverne *et al.*, 1979a), but may rebound in the opposite direction when reaching the end of the horn (Taverne, 1982). Empty parts of the horn also contract (Taverne, 1982). Cervico-tubal contractions end when the horn is empty of piglets, indicating that the presence of piglets close to the cervix initiate these contractions (Taverne, 1982). It is estimated that 4 to 5 uterine contractions, with an average duration of 11.5 seconds and an intensity of 9.4 mm Hg are needed to expel one fetus (Mota-Rojas *et al.*, 2005a; Mota-Rojas *et al.*, 2005b; Mota-Rojas *et al.*, 2007). As soon as the horns are completely empty, contractions are only are tubo-cervical and appear very frequent and regular for placentae expulsion (Taverne *et al.*, 1979a; Maffeo *et al.*, 1990). It is unknown whether or not there is synchrony in the timing of contractions between the two horns, but this seems likely, since muscle fibers fuse at the common uterine body (Leibrecht, 1953) and birth order of fetuses from both uterine horns appears to happen fully at random from one horn or the other (Taverne *et al.*, 1977). It is also unclear if crowding of piglets occurs during contractions. It might be that crowding does occur when fetuses are stuck or when a stillborn piglet causes a delay in the birth process. The birth interval after which a stillborn piglets is born is approximately twice as long as that of a liveborn piglet (28 vs. 15 min) (Zaleski and Hacker, 1993; van Dijk *et al.*, 2005). It is unknown if the increase in birth interval is a cause or consequence of the increased birth interval (Vanderhaeghe *et al.*, 2013).

Most studies evaluating the duration of farrowing in sows only consider stage 2 of parturition; the time during which fetuses are expelled (van Rens and van der Lende, 2004; van Dijk *et al.*, 2005; Oliviero *et al.*, 2013; Vallet *et al.*, 2013; Hales *et al.*, 2015; Björkman *et al.*, 2017; Feyera *et al.*, 2018) as this stage determines the level of asphyxiation of piglets and can easily be observed. Asphyxiation mostly occurs due to strong uterine contractions combined with placental space limitation and/or reduced placental-uterine connection, which together reduce or obstruct placental blood flow (Senger, 2003). As an initial response to reduced blood oxygen levels, fetal heartrate drops (Langendijk and Plush, 2019) and fetal movements increase, which in turn promotes myometrial contractions, making it a positive feedback system to reduce the duration of farrowing (Senger, 2003). In fetuses with a prolonged inadequate oxygen supply, blood $CO_2$ concentrations will

rise and hypoxia starts to occur. To reduce fetal oxygen consumption, fetal limb and body movements will reduce (Randall, 1992), fetal heartrate falls (bradycardia) (Randall, 1979; Singer, 1999), and metabolic rate reduces (Singer, 1999). When fetal blood $O_2$ concentration drops below a certain threshold level, ATP production shifts to anaerobic glycolysis and fetal lactate levels increase. This anaerobe metabolism is faster than aerobe metabolism, but can only provide energy for a short period of time (up to 2 min) (Pigozzi *et al.*, 2007). Lactate also reduces blood pH, which can affect functioning of the central nervous or cardiovascular system (Omo-Aghoja, 2014). Lactate levels at birth have been related with chances of dying during lactation. For example, English and Wilkinson (1982) showed that piglets that died pre-weaning had higher blood lactate concentrations at birth than survivors (383 vs. 303 μg lactate/ml blood; P<0.01, respectively). Also Langendijk *et al.* (2018a) found an increase in pre-weaning mortality when blood lactate concentrations in umbilical cord blood was increased (8.5% and 10.9% for 4.45 – 6.40 mmol/L and >6.40 mmol/L, respectively). Thus, the level of asphyxia at birth appears to be related to the chances for pre-weaning survival.

It is not known whether intensity, duration and number of contractions, or the duration of stage 1 of parturition is related to litter size. It is also not known whether the duration of stage 1 and 2 of parturition are related. It is known that the duration of stage 2 of parturition is related to litter size; it indeed takes more time to deliver more piglets (van Rens and van der Lende, 2004). Combining data of 15 studies that measured the duration of stage 2 of parturition in the last 18 years (van Rens and van der Lende, 2004; van Dijk *et al.*, 2005; Oliviero *et al.*, 2009; Hales *et al.*, 2015; Theil, 2015; Björkman *et al.*, 2017; Thorsen *et al.*, 2017; Feyera *et al.*, 2018; Langendijk *et al.*, 2018b; Udomchanya *et al.*, 2019; van den Bosch *et al.*, 2019; Gourley *et al.*, 2020; Nam and Sukon, 2020; Thongkhuy *et al.*, 2020; Nielsen *et al.*, 2021) shows an estimated increase of 27 minutes in duration of stage 2 of parturition per extra piglet (Figure 2, averages per study). The deviation from the predicted line for farrowing duration based on litter size is sometimes quite large, which may be caused by differences in e.g. breed, housing or management (i.e. use of birth assistance and exogenous hormones).
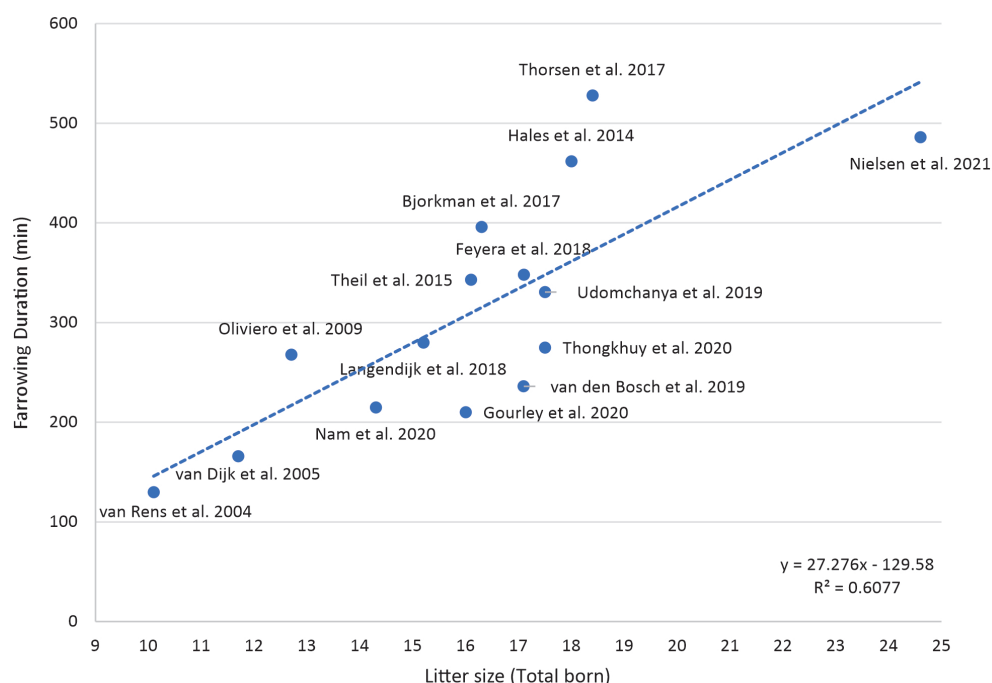
**Figure 2.** Relationship between average litter size and average duration of parturition (stage 2, expulsion of piglets) based on averages of 15 studies conducted over the last 18 years (van Rens and van der Lende, 2004; van Dijk *et al.*, 2005; Oliviero *et al.*, 2009; Hales *et al.*, 2015; Theil, 2015; Björkman *et al.*, 2017; Thorsen *et al.*, 2017; Feyera *et al.*, 2018; Langendijk *et al.*, 2018b; Udomchanya *et al.*, 2019; van den Bosch *et al.*, 2019; Gourley *et al.*, 2020; Nam and Sukon, 2020; Thongkhuy *et al.*, 2020; Nielsen *et al.*, 2021)

Summarizing, the total duration of parturition (stage 1, 2 and 3), in which a sow experiences frequent and powerful uterine contractions, can take up to 24 hours in the hyper prolific sow (Purohit, 2010). Research on duration of farrowing mainly focuses on phase 2 of parturition since this phase is more easy to observe. It is unknow what the impact is of phase 1 on the sow, her piglets and how related phase 1 and 2 of parturition are with each other. Most of the studies studying the intensity, number and duration of uterine contractions for the different phases of parturition in the sow are done three to four decades ago (Taverne *et al.*, 1979a; Taverne, 1982; Maffeo *et al.*, 1990). It is unknown

if the intensity, number and duration of contractions relate to the current litter sizes and other aspects of the modern sow.

## Placental and Umbilical Cord Functionality

The placenta is responsible for nutrient and oxygen exchange between the sow and her fetus. The fetus has a diffuse placenta in which many closely spaced chorionic villi are distributed over the entire outer surface of the chorion (Senger, 2003), which ensures transport and diffusion of nutrients from the maternal to the foetal blood. Additionally, specific structures, so called areolae, on the placenta absorb the products secreted by the endometrial glands (e.g. growth hormones, hormones, transport proteins lymphokines, cytokines) (Senger, 2003). The surface area of the chorio-allantoic membrane mainly increases in size between day 35 to 70 of gestation, with little change between day 70 to 100 of gestation (Knight *et al.*, 1977). Vascularisation of the allantoic membrane starts at approximately day 15 post mating, i.e. 2 days after contact between the trophoblast and maternal epithelium (Dantzer and Leiser, 1994), and increases until mid-gestation, after which vascularity remain relatively constant (Biensen *et al.*, 1998; Wilson *et al.*, 1998). By that time, blood vessels occupy about 3-4% of the chorio-allantoic membrane, but with large variation among individual fetuses, among litters and between breeds (Biensen *et al.*, 1998; Wilson *et al.*, 1998). Blood capillaries from the chorionic villi merge and eventually form larger vessels that enter the umbilical cord (Senger, 2003). In addition to vascularisation, nutrient supply to the fetus is also affected by uterine blood flow, which increases as gestation progresses (Pere and Etienne, 2000). Although it seems likely, it is not known whether or not vascularisation of the placenta and placental blood flow are related. Blood flow (Reynolds *et al.*, 1985) and placental area (Rootwelt *et al.*, 2013) per piglet both seem negatively correlated with litter size, which likely explains why average piglet birth weight decreases as litter size increases (Boulot *et al.*, 2008; Beaulieu *et al.*, 2010; Rootwelt *et al.*, 2012). No studies were found showing a clear relationship between litter size and placental vascularisation. Wilson *et al.* (1998) found differences between breeds in placental vascularisation at the fetal-maternal interface. Vascular density was higher in Meishan placentas compared to Yorkshire placentas, although placental size was

larger in Yorkshire sows. Whether or not placental blood flow differs between breeds has not been evaluated.

Placental characteristics and the incidence pre-weaning mortality appear to be related, although these relationships might be confounded with piglet birth weight. Both placental surface (-20.4%) and placental weight (-14.8%) were lower in piglets that died before weaning compared to surviving conspecifics, which was most likely caused by a lower birth weight of piglets that died before weaning (Rootwelt *et al.*, 2013). Baxter *et al.* (2008) found no difference in vascularization score of placentas of piglets that survived or died before weaning.

The umbilical cord connects the fetus to the placenta. The umbilical cord contains one vein that carries oxygen and nutrient-rich blood to the fetus and two smaller arteries that transport deoxygenated blood from the fetus back to the placenta (Smith and Schenk, 2011). These vessels are surrounded by a so called Wharton's jelly, a gelatinous connective tissue, consisting mainly of hyaluronic acid, in which collagenous and reticular fibres form a loose meshwork (Tantius *et al.*, 2014). An intact and functional umbilical cord is of crucial importance for fetal oxygen and nutrient supply. Umbilical cord length of piglets was found to be 35 cm on average (ranging between 17 to 50 cm) and was positively correlated with piglet weight (Rootwelt *et al.*, 2012). Umbilical cord length is not correlated to the position of a piglet in the uterus, but its elasticity (up to 37.5% of its length) allows it to stretch as a piglet is transported through the uterine horn at parturition. Tension required to break an umbilical cord varies from 545 to 2,000 g (Randall, 1989; Leenhouwers *et al.*, 2002). The percentage of piglets born with a broken umbilical cord lies between 21 to 71% (Rootwelt *et al.*, 2012; Langendijk *et al.*, 2018a) and Rootwelt *et al.* (2012) showed that broken umbilical cords occur most in the second and last third of piglets born (2.3 times more often compared to the first third of piglets born). When or where an umbilical cord breaks has, to our knowledge, not been studied in pigs. It can be hypothesized that the umbilical cord breaks at a weak spot or occurring randomly over the full length of the umbilical cord potentially caused by a weak spot in the Wharton's jelly or the first place where umbilical cord blood flow has stopped. It is also unclear which placental or other sow and/or piglet characteristics might be related with length, thickness, strength or

breaking point of the umbilical cord. It seems likely that larger piglets, which have a larger placenta (Rootwelt *et al.*, 2012, 2013), also have a thicker umbilical cord which may also be less prone to breaking. Curtis *et al.* (1974) suggested that stillborn piglets (that weighed less than live born litter mates) have a smaller umbilical cord that is more likely to break, suggesting a relationship with piglet birth weight and umbilical cord thickness/strength. However, Langendijk and Plush (2019) found a similar weight distribution in live and stillborn piglets indicating the suggestion of Curtis *et al.* (1974) might not be true. Piglets born alive, but with a broken umbilical cord (as observed at the moment of birth) showed a lower vitality score and had an higher risk for post-partum death compared to piglets born with an intact umbilical cord (Rootwelt *et al.*, 2013). A recent review estimated the association between incidence of stillbirth and a broken umbilical cord before expulsion to be 50% or more (Langendijk and Plush, 2019). In addition, even when the cord does not break, extensive stretching leads to vasoconstriction and limited blood flow, increasing the risk for stillbirth (Randall, 1972).

In summary, in larger litters, placental blood flow per piglet is reduced (Reynolds *et al.*, 1985) and placental area per piglet is smaller (Rootwelt *et al.*, 2013), which likely explains why average piglet birth weight is lower (Boulot *et al.*, 2008; Beaulieu *et al.*, 2010; Rootwelt *et al.*, 2012) and partially explains why incidence of pre-weaning mortality increases. An intact and functional umbilical cord is of key for fetal oxygen and nutrient supply and therefore survival. Studies on how, where or when an umbilical cord breaks and which sow and/or piglet characteristics are related to its breaking are limited. A better understanding of the complex interactions between placental/umbilical cord blood flow, contractions, breaking of the umbilical cord and other characteristics of the modern sow might provide insights in how perinatal piglet losses can be reduced. In conclusion, placental and/or umbilical cord blood flow might be under pressure in large litters, which might be related to stillbirth and pre-weaning mortality.

# The Potential of Maternal Nutrition to Reduce Farrowing Duration

Relationships between placental characteristics, uterine contractions, placental and umbilical cord blood flow and piglet losses are summarized in Figure 3. Additionally, in this figure potential effects of maternal nutrients on these events are included. Providing the right nutrients to the sow and her fetuses may not only enhance placental development and fetal growth, but could also affect uterine blood flow in the perinatal period and/or affect the duration of farrowing. Studies evaluating nutritional solutions aiming to reduce farrowing duration by enhancing uterine contractions or affect placental characteristics will be discussed in this paragraph.
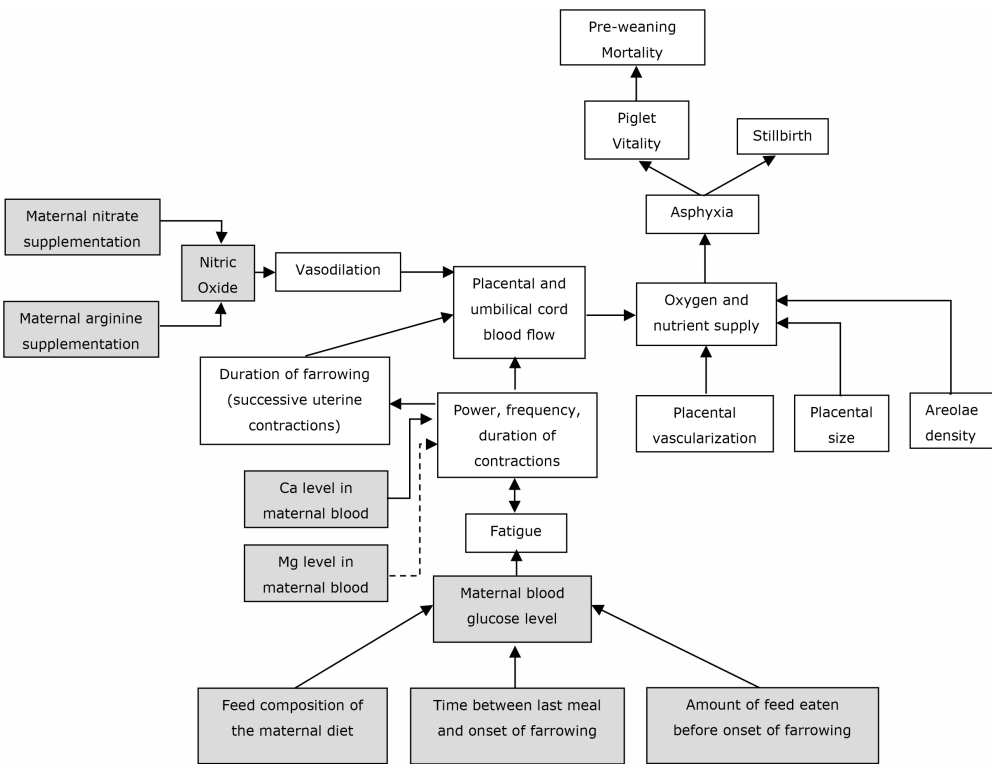


**Figure 3.** Relationship between farrowing and placental characteristics on piglet vitality, incidence of stillbirth and pre-weaning mortality and potential roles of maternal nutrients on these relationships. Dotted lines indicate a negative effect. Grey boxes will be discussed in current paragraph

Nutritional interventions in the perinatal period aiming to decrease stillbirth and to increase piglet vitality right after birth should stimulate uterine contractions (frequency or intensity), increase placental nutrient and/or oxygen supply to the fetus and/or provide the sow with the energy to prevent fatigue. To prevent constipation (Tabeling *et al.*, 2003) and metritis, mastitis and agalactia (MMA) (Cerisuelo *et al.*, 2010) feed allowance in some European countries is lowered to 2.0-3.0 kg/sow/day, beginning 2-3 days before the expected farrowing date. It can be questioned whether or not this lower amount of energy and nutrient intake and the type of nutrients supplied, sufficiently facilitates energy and nutrient requirements during parturition. Concequently, feeding strategies in the perinatal period might need to be reconsidered.

## Energy

The energy requirement for the farrowing process are expected to be comparable to moderate to heavy exercise (van den Bosch *et al.*, 2019). However, recent estimates of energy requirements estimated during the transition period (10 days pre-farrowing to 10 days post-farrowing) included maintenance, heat loss, mammary growth, fetal growth and colostrum/milk production, but not requirements for the farrowing process itself, resulting in the lowest estimated energy requirements at the day of farrowing (Theil, 2015). Also recent evaluations of amino acid and energy requirements did not include parturition (van der Peet-Schwering and Bikker, 2019). Feyera *et al.* (2021) were the first to give an estimate of energy requirements of farrowing, which was based on an evaluation of different feed amounts and therefore daily energy intake around farrowing, aiming for the shortest farrowing duration and lowest number of interventions during farrowing. The estimated energy requirement for farrowing was 16 MJ ME (approximately 30% of the total energy requirements on that day of farrowing), based on a litter size of 18.9 piglets and a farrowing duration of 4.2 h. In Figure 4, the calculated energy requirements of sows provided by Theil *et al.* and Feyera *et al.* are combined for the last day of gestation, the day of farrowing and day 1 and 2 of lactation. These estimates included energy requirements for maintenance purposes, heat loss due to reproduction costs and diet induced thermogenesis (Noblet *et al.*, 1985; Van Milgen *et al.*, 1997), colostrum/milk

production, fetal growth, mammary growth and growth of uterine tissue, nest building behavior and energy requirements for farrowing on the day of farrowing as done by Feyera *et al.* (2021) are shown.
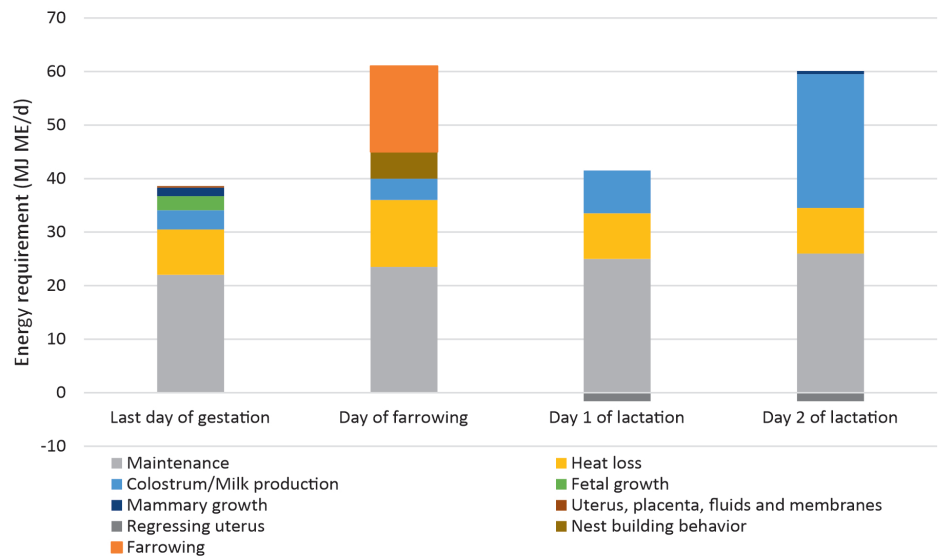


**Figure 4.** Energy requirements of sows on the last day of gestation, the day of farrowing and day 1 and 2 of lactation, adapted from (Theil, 2015), including estimated energy requirements for additional heat loss, nest building behaviour and farrowing (Feyera *et al.*, 2021). ME = Metabolizable energy.

Van Kempen *et al.* (2007) suggested that sow exhaustion during farrowing caused by energy depletion could impair the number and intensity of uterine contractions, thereby increasing the duration of farrowing and consequently, increasing stillbirth rate. That sow exhaustion occurs, was also suggested by Mosnier *et al.* (2010), who found an increased sow plasma lactate concentration at day 1 post-partum (approximately 1.4 mmol/L) compared to day 4 (approximately 0.9 mmol/L). The increased concentration of lactate in sow blood is likely due to increased metabolic activity and uterine contractions of sows during farrowing (also seen by an increase in body temperature (Gourdine *et al.*, 2007)). A recent study, in which lactate levels were determined more frequently around parturition (every 6 h pre-farrowing and every 3 h post-partum), showed that sow blood lactate levels

were indeed increased during parturition, but were already increased at 9 and 3 hours before the expulsion of the first piglet (Nielsen *et al.*, 2021), which is likely related with a higher activity during nest building behavior and by increased uterine contractions during phase 1 of parturition. Three hours after farrowing, lactate levels started to decrease again (Nielsen *et al.*, 2021), indicating sows shifted back to their aerobe metabolism.

## Glucose as a source of energy during farrowing

ATP (adenosine triphosphate), derived primarily from glucose by glucogenesis is the main energy source for uterine contractions (Challis *et al.*, 2000). Blood glucose levels rise during farrowing, which can be explained by the increased glucogenesis under the influence of adrenalin and cortisol (Le Cozler *et al.*, 1999; Rizzo *et al.*, 2011). Sow blood glucose levels originate from carbohydrates in the diet and/or glycogen reserves. Feyera *et al.* (2018) showed that farrowing duration linearly increased with time when the last meal was more than 3.13 ± 0.34 h before the onset of farrowing (defined as the birth of the first piglet, Figure 5A).
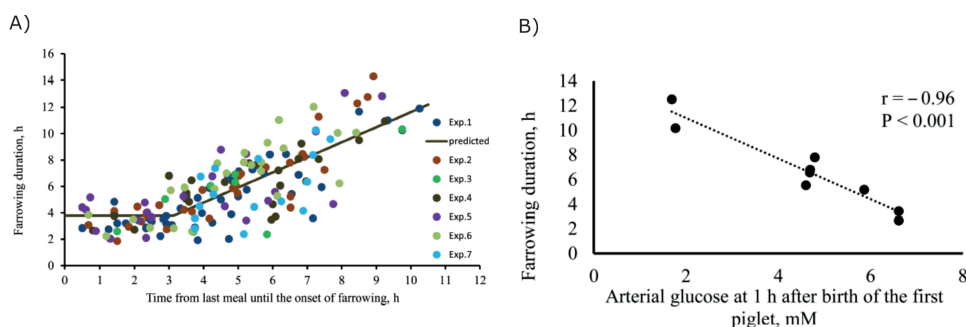


**Figure 5.** Relationship between time from last meal until the onset of farrowing in relation to farrowing duration measured in 7 different experiments (A) and the correlation between arterial glucose concentration 1 hour after the birth of the first piglet and farrowing duration (B) (Feyera *et al.*, 2018).

In addition, a negative correlation was found between sow arterial glucose level, measured 1 hour after the birth of the first piglet and farrowing duration (Figure 5B), suggesting that a low energy status of the sow indeed increased farrowing duration. Although duration of

farrowing has been related to the energy status of the sow, other dietary factors (e.g. type of energy, mineral levels, other supplements) might play a role as well. These factors will be discussed below.

## Other carbohydrates

The role of dietary fibers in sow nutrition around farrowing are mostly studied in relation to the prevention of constipation and therefore easy passage of piglets through the birth canal (Guillemet *et al.*, 2007; Oliviero *et al.*, 2009). Effects of dietary fibers on the duration of farrowing have been reviewed extensively (Peltoniemi and Oliviero, 2015) and will thus not be discussed here. No information is available on possible effects of dietary fibers on uterine contractions. However, dietary fibers can be a source of energy from the gastrointestinal tract up to several hours after a meal (Serena *et al.*, 2007). The type of carbohydrates consumed appears to be an important factor for exercise performance in athletes (O'Reilly *et al.*, 2010). The glycemic index (GI) of carbohydrates is a tool to predict blood glucose, insulin and therefore energy supply of diets (Jamurtas *et al.*, 2019) and high GI foods (e.g. sugar and starches) provide a high and relatively short peak in blood glucose. Low GI feed ingredients (e.g. pectins) could provide lower, but longer peaks in blood glucose. Combining different types of carbohydrates, providing fast, medium and slow release glucose might increase the period after feeding in which sufficient glucose is available to supply the energy needed for the farrowing process.

It can be concluded that a better understanding of perinatal energy requirements (and the composition of these energy sources) perinatal is needed to optimize the farrowing process and consequently reduce piglet losses.

## Calcium and magnesium

Calcium is an essential mineral for muscle contractions (Forman *et al.*, 1981; Carsten and Miller, 1987) and therefore also essential for myometrial contractions during farrowing (Figure 3). Studies evaluating calcium requirements in the peri-partum period are limited. Geisenhauser *et al.* (2012) evaluated a single dose calcium supplementation on top of feed

(400 mmol Ca, source Calcium lactate) on the day of farrowing and found a significant reduction (-34% on sow level) in the incidence of dystocia (defined as birth interval > 60 min) and decreased time for placenta expulsion (4-19 min faster). Le Cozler *et al.* (1999) evaluated plasma calcium levels in gilts before, during and after farrowing and observed no change in plasma calcium levels during parturition (measuring 2h before the birth of the first piglet to 7h after). This is in contrast to a recent study of Nielsen *et al.* (2021), who also evaluated plasma calcium profiles around parturition (measuring 33 hours before the birth of the first piglet until 24 hours after farrowing) and found a drop in calcium levels 9 and 3 hours before the expulsion of the first piglet, but no changes in sow blood calcium levels during the first 24h post farrowing. These ambiguous results in perinatal plasma calcium profiles might be related with differences in litter size (12.2 vs. 24.6 total born for Le Cozler *et al.* (2002) and Nielsen *et al.* (2021), respectively) and/or farrowing duration (175 vs. 486 min for Le Cozler *et al.* (2002) and Nielsen *et al.* (2021), respectively), suggesting that hyper prolific sows have higher calcium requirements in the perinatal period. The benefits of calcium supplementation to sows before farrowing on incidence of stillbirth and piglet vitality remain unclear, but might be related to the plane of feeding in this period and the calcium source and concentration in the diet.

Magnesium promotes the relaxation of smooth muscle cells and inhibits contractions of the uterine myometrium. Magnesium sulphate is used in human medicine to prevent pre-term labor and pre-term birth (Mercer *et al.*, 2009), which suggest that magnesium supplementation hours before farrowing might have a negative effect on myometrial contractions. However, Le Cozler *et al.* (1999) observed a drop in magnesium levels in sow blood 1 h after the first piglet was born, which was likely due to the role of magnesium in dephosphorylation of ATP to provide energy for muscle contractions as ATP must be bound to a magnesium ion to be biologically active. The synergistic and antagonistic role of magnesium with calcium might explain why calcium levels were constant and magnesium levels dropped during parturition (Le Cozler *et al.*, 2002). In pig husbandry, magnesium is used in sow diets as an effective laxative to prevent constipation (Plush *et al.*, 2017). However, as with calcium, research on magnesium supplementation for sows in the perinatal period is limited. Plush *et al.* (2017) showed an increase in stillbirth incidence

(+0.3 stillborn piglets/litter, $P$ = 0.01) when sows were supplemented with magnesium sulphate (2.85 kg/mton feed, receiving 2.5 kg of feed/sow/day) from 5 days pre-farrowing until 3 days post-farrowing. It can be speculated that magnesium induced relaxation of the myometrium increased the duration of farrowing, but this was not evaluated.

It can be concluded that although both calcium and magnesium play an important role in myometrial contractions, research about supplementing sows with one or both of these minerals in the perinatal period is very limited. Consequently, calcium and magnesium requirements of the sow in the perinatal period and potential factors influencing these requirements (e.g. litter size) are currently unknown.

**Vasoactive components**

Dietary arginine (as recently reviewed by (Palencia *et al.*, 2018; Wu *et al.*, 2018)) and nitrate supplementation (as hypothesized within this thesis) to the sow both aim to influence placental vascularization and/or placental-fetal blood flow. Although converted differently, both arginine (oxidized in a reaction catalyzed by the NO synthase family (Moncada and Higgs, 1993)) and nitrate (non-enzymatically converted via the $NO_3$-$NO_2$-NO pathway (Lundberg *et al.*, 2008)) are precursors for nitric oxide (NO). NO is an endothelium-derived relaxing factor, causing vascular vasodilation (Lundberg and Govoni, 2004; Webb *et al.*, 2008), which plays an important role in regulating placental-fetal blood flow and consequently nutrient and oxygen transfer from mother to fetuses (Bird *et al.*, 2003). This higher blood- and therefore nutrient- and oxygen flow may lead to an increased piglet birth weight and/or oxygenation during parturition, which is hypothesized to lead to a lower incidence of stillbirth, increased vitality and therefore a decreased incidence of pre-weaning mortality. Arginine is mostly supplemented in the first stage of gestation to increase placental angiogenesis (Wu *et al.*, 2004), with several studies showing a beneficial effect on embryo survival, fetal development, placental weight, piglet weight and number born alive (as reviewed by (Palencia *et al.*, 2018; Langendijk, 2021)). Fewer studies have used arginine supplementation up to or close to the moment of parturition. Neither placental weight (when supplementing 1% of L-Arginine from day 22 until day 114 of gestation (Gao *et al.*, 2012)) nor piglet birth

weight and stillbirth rate (when supplementing 25.5 g/d from day 77 of gestation until term (Quesnel *et al.*, 2014)) were affected in these studies. Whether or not a NO precursor can affect placental-fetal blood flow and therefore affect incidence of stillbirth and pre-weaning mortality remains unknown.

## Conclusion

Parturition is not only a stressful, painful and energy demanding event for sows, but also affects perinatal survival of her offspring. With an increase in litter size, farrowing duration increased and uterine blood flow per piglet, placental development and piglet weight have decreased, which increased the challenges to the perinatal piglet. Potential maternal nutritional factors that stimulate uterine contractions and/or increase uterine blood flow (by providing adequate energy and/or minerals or by the use of NO precursors) may reduce the duration of parturition and/or increase perinatal piglet survival. However, knowledge on the exact nutritional requirements before, during and after parturition and the impact of meeting these requirements may have on piglet characteristics and perinatal survival is limited.

2

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# Chapter 3

## Maternal dietary nitrate supplementation lowers incidence of stillbirth in hyper prolific sows under commercial circumstances

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## Simple Summary

Over the last decades, the number of piglets and therefore also the number of stillborn piglets per litter has been increased. Blood and oxygen supply are crucial for piglets to survive the birth process. Blood flow might be increased through vasodilation by dietary nitrate supplementation, which is known in sport nutrition to increase endurance. The current study evaluated effects of nitrate supplementation to sows on the incidence of stillbirth at a commercial farm. In total, 120 sows received either a control diet or a diet containing 0.1% of calcium nitrate from approximately 5 days before until 4 days after farrowing. Number of piglets born alive, stillborn or that died from birth to weaning were recorded. Piglets were weighed at birth, after cross-fostering, 24h after cross-fostering, at 3 days of age and at weaning. Placentas were collected after expulsion and were visually scored on redness. No effect of nitrate supplementation to the sow was found on placental redness, piglet weights and growth or incidence of dead after being born. Dietary nitrate supplementation decreased stillbirth percentage from 9.9 to 7.4 %, making it a potential approach to decrease stillbirth.

## Abstract

The objective of the current experiment was to investigate whether or not maternal dietary nitrate supplementation, a nitric oxide (NO) precursor, could reduce piglet losses under commercial circumstances. In the current experiment, 120 hyper prolific gilts and sows (Landrace x Yorkshire: Danbred) on a commercial farm in Denmark, received either a control lactation diet or a lactation diet containing 0.1% of calcium nitrate (containing 63.1% of nitrate) from approximately 5 days pre-farrowing until day 4 of lactation. Number of piglets born total, alive and stillborn, as well as birth weights, weights after cross fostering (approximately 1 day of age), 24h after cross fostering, day 3 of age and at weaning was recorded. Placentas of sows were collected after expulsion and scored on redness. No effect of nitrate supplementation was found on piglet weight, piglet growth, placental redness score and pre-weaning mortality during lactation. Maternal dietary nitrate supplementation decreased stillbirth percentage with 2.5% (9.9 vs 7.4%; $P = 0.05$). It can be concluded that maternal dietary nitrate supplementation shows potential to decrease incidence of stillbirth in hyper prolific sows.

## Introduction

Stillborn piglets are a great loss and represent a welfare and societal issue for the pig industry (Vanderhaeghe *et al.*, 2013). In hyper prolific herds, like in Denmark, stillbirth percentage went up to on average 10.2% in 2019 (Hansen, 2021). Stillbirths is typically associated with intra-uterine asphyxia or dystocia (Sprecher *et al.*, 1974), in which the placenta and umbilical cord play a crucial role. Fetal asphyxia during birth can partially be explained by; 1) Compression of the umbilical cord and placenta due to successive uterine contractions or when fetuses enter the pelvis (Curtis, 1974), 2) Loss of umbilical cord functionality (e.g. breaking, knots, wrapping around limps, stretching, etc.) (Randall, 1971; Christianson, 1992) or 3) Premature detachment of the placenta. Additionally, stillbirth is related to farrowing duration (Borges *et al.*, 2005; van Dijk *et al.*, 2005; Canario *et al.*, 2006). For example, Langendijk *et al.* (2018) showed an increase in stillborn percentage from 2.7 to 10.7% and 27.3% when farrowing duration increased from less than 2 hours to 4-6 hours and over 8 hours, respectively.

Recently, effects of maternal dietary interventions have been studied on farrowing duration and the risk of stillbirth (Feyera *et al.*, 2018; van den Bosch *et al.*, 2019a; van den Bosch *et al.*, 2019b; Gourley *et al.*, 2020; Oliveira *et al.*, 2020; Feyera *et al.*, 2021). Human sport supplements have been shown to induce vasodilatation, which increases blood flow and consequently oxygen flow in the body (Lundberg and Weitzberg, 2005a; Lundberg *et al.*, 2011; Bailey *et al.*, 2012). Consequently, performance can be enhanced by increasing stamina. It can be hypothesized that comparable effects can be obtained in sows around farrowing. By ensuring a larger blood flow and, consequently oxygen and nutrient flow in the placenta and the umbilical cord during farrowing, the risk for asphyxiation and stillbirth can be reduced.

A potential candidate nutrient that might affect blood supply to target tissue is nitrate (van den Bosch *et al.*, 2019a; van den Bosch *et al.*, 2019b). Dietary nitrate has shown to improve endurance exercise performance in human athletes (Lansley *et al.*, 2011; Cermak *et al.*, 2012; Wylie *et al.*, 2013). Nitrate ($NO_3^-$) in itself is inert, but after conversion to nitrite ($NO_2^-$), mainly facilitated by bacteria in the mouth (Govoni *et al.*, 2008), it is further

reduced to nitric oxide (NO, by denitrifying anaerobic bacteria or periodontal acidity (Lundberg and Weitzberg, 2005b; Gilchrist *et al.*, 2010)), which is a vasoactive component. The $NO_3$-$NO_2$-NO pathway is suggested to be very important in regulation of blood flow (Cosby *et al.*, 2003) as shown by Larsen *et al.* (2006). They found a reduction in blood pressure in healthy volunteers when nitrate was supplemented in the diet for three days. In pigs, dietary nitrate supplementation has to our knowledge only been studied by van den Bosch *et al.* (van den Bosch *et al.*, 2019a; van den Bosch *et al.*, 2019b) in a dose response study, feeding up to 0.24% CaNO3 from 7 days pre-farrowing to 4 days post-farrowing. Piglet vitality, placental size and piglet birth weight were linearly increased with increasing dose of maternal nitrate supplementation (van den Bosch *et al.*, 2019a; van den Bosch *et al.*, 2019b).

It can be hypothesized that maternal nitrate supplementation could lead an increased stamina of the sow and an adequate *in utero* blood flow during farrowing, due to the vasoactive properties of NO. Either one or both of these modes of action might reduce the duration of farrowing and decrease the incidence of stillbirth. The aim of this study was to evaluate effects of maternal dietary nitrate supplementation around farrowing on incidence of stillbirth, piglet performance and pre-weaning mortality on a commercial farm with hyper prolific sows with a high incidence of stillbirth.

## Materials and Methods

All experimental procedures were approved by the institutional animal use and care committee of Wageningen University and Research (Wageningen, the Netherlands) on November 27th, 2014.

### Animals and Diets

The experiment was performed at a commercial farm in Holstebro, Denmark in 2015. In three consecutive batches, 134 hyper prolific crossbred sows (Landrace x Yorkshire: Danbred) were allocated based on parity (range 1 to 9) to one of two treatments containing 0.0% (control) or 0.1% of calcium nitrate ($5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$; containing 63.1%

of nitrate; commercial name Bolifor CNF (Yara Phosphates Oy, Helsingborg, Sweden)) in the final diet. Two concentrates (10% of the final diets) for the control and calcium nitrate group were produced by Cargill Animal Nutrition (Rotterdam, the Netherlands). Calcium levels in the two concentrates were kept constant by exchanging limestone and calcium nitrate. Concentrate compositions are shown in Table 1. On farm, these concentrates were mixed with other raw materials as shown in Table 2 to obtain a final diet fed in a dry mash form. Consequently, sows in the treatment group received a maximum amount of nitrate of 32 mg/kg BW per day, which is considerably lower than the no observed adverse effect level (NOAEL) of 410 mg nitrate/kg BW per day as indicated by the EFSA (EFSA Panel on Contaminants in the Food Chain (CONTAM) *et al.*, 2020).

**Table 1.** Composition of the experimental concentrates (10% inclusion in the final diet), as formulated

| Ingredients (%) | Control | 0.1% CaNO$_3$ | Nutrient levels | Control | 0.1% CaNO$_3$ |
|---|---|---|---|---|---|
| Provisoy | 50.000 | 50.000 | Dry Matter (%) | 94.640 | 94.500 |
| Chicory Pulp | 10.000 | 10.000 | NE (MJ/kg) | 7.011 | 7.095 |
| Calcium Carbonate | 7.351 | 6.856 | Crude protein (%) | 28.500 | 29.950 |
| Monocalcium Phosphate | 6.888 | 6.884 | AID Lys (%) | 1.933 | 1.933 |
| Wheat | 5.797 | 6.602 | AID Met + Cys (%) | 1.141 | 1.146 |
| Salt | 3.757 | 3.454 | Calcium (%) | 4.61 | 4.61 |
| Potassium Chloride | 3.358 | 3.449 | Phosphorus (%) | 1.90 | 1.90 |
| Choline Chloride 60% | - | 2.500 | Sodium (%) | 2.20 | 1.68 |
| Choline Chloride 70% | 2.143 | - | Potassium (%) | 2.89 | 2.89 |
| Sodium Bicarbonate | 2.479 | 1.056 | Magnesium (%) | 1.26 | 1.26 |
| Bolifor CNF[1] | - | 1.000 | | | |
| Soya Oil | 2.000 | 2.000 | | | |
| L-Lysine HCL | 0.439 | 0.417 | | | |
| DL-Methionine | 0.396 | 0.391 | | | |
| Commercial Premix[2] | 5.393 | 5.393 | | | |

[1] Bolifor CNF, available from Yara Phosphates Oy, consists of calcium nitrate ($5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$); containing 63.1% of nitrate.
[2] Commercial sow premix from Cargill Animal Nutrition.

Experimental diets were fed twice a day (7.00 h and 15.00 h) from day 112 of gestation until 4 days after farrowing (based on the individual farrowing date of the sow). Diets were fed restrictedly at 3.8 kg/sow/d on day 112, 2.9 kg/sow/d on day 113 and 114, 2.4 kg/sow/d on day 115 until the day of farrowing. After farrowing, diets were provided at 3.1, 3.7, 4.0 and 4.7 kg/ sow/d at d 1, 2, 3, and 4 after farrowing, respectively. Starting at day 5 of lactation to weaning (day 23.6 ± 2.1) a commercial available lactation diet (15.2%

CP, 9.3 MJ NE/kg) was provided 3 times a day (7.00 h, 12.00 h and 15.00 h) to all sows in a liquid form. Sows had ad libitum access to drinking water. Piglets received potato starch until day 10 of age via floor feeding. From 10 days of age until weaning, a commercial available pre-starter (17.5% CP, 11.9 MJ NE/kg) was provided in a feeding bowl once per day.

**Table 2.** Calculated composition of the experimental diets as mixed on farm (as fed).

|  | Control | 0.1% $CaNO_3$ |
|---|---|---|
| **Ingredients (%)** |  |  |
| Barley | 48.50 | 48.50 |
| Wheat | 25.00 | 25.00 |
| Beet pulp, sugar 5.9% | 8.80 | 8.80 |
| Soybean meal, dehulled | 7.00 | 7.00 |
| Soybean oil | 0.70 | 0.70 |
| Concentrate Control | 10.00 | - |
| Concentrate $CaNO_3$[1] | - | 10.00 |

[1]Bolifor CNF, available from Yara Phosphates Oy, consists of calcium nitrate ($5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$); containing 63.1% of nitrate.

## Animal Housing and Management

Approximately 10 days before the expected farrowing date, pregnant sows were moved to individual farrowing pens with farrowing crates in 1 out of 5 farrowing rooms. Room 1 had 36 pens (used in round 1), room 2 had 24 pens (used in round 1), room 3 had 36 pens (used in round 2), room 4 had 34 pens (used in round 2 and 3) and room 5 also had 34 pens (used in round 3). All rooms had the same type of pens, farrowing crates and flooring. All rooms had unblinded windows with natural light coming in. Pens contained concrete flooring with steel slats under the farrowing crate, located over a manure pit. Each pen contained a piglet nest with heated flooring, a heating lamp set at 30°C and saw dust. Piglets had ad libitum access to drinking water. Cross fostering, all done by the same person, was allowed within treatment groups until 3 days of age. Number and body weight of piglets that were cross fostered, and date and time of cross-fostering was recorded. Litters were standardized to 14-15 piglets per sow, based on her mothering ability and number of functional teats. Left over piglets were placed at foster sows, which were one

week in lactation (sows not in experiment). To prevent errors in feeding or cross fostering, treatments were allocated to the left or right side of the central corridor in each farrowing room. Per farrowing room, allocation of treatment per side was done randomly. Sows were allocated to treatments based on parity.

## Measurements

Sow P2 backfat thickness (on the last rib, 6 cm down the dorsal middle line) was measured by the same person when sows were on average at day 112 of gestation and at weaning. Farrowing induction, medicine administration around or during farrowing and use of birth assistance was recorded. If farrowing was completed at 6.00 AM, gestation length, total number of piglets born (TB), total number of piglets born alive (TBA), and total number of stillborn piglets (TSB, visually determined) were recorded. When sows finished farrowing during the time staff was present (between 5.30 AM and 4.00 PM), weighing took place on that day. Mummified and degenerated piglets were excluded from the total number born. Piglet weights were determined within 24h after birth (daily at 6.00 AM, before cross fostering took place), after cross fostering (between 7.30 AM and 9.00 AM), 24 h later, at 3 days of age and at weaning. Time of weighing was recorded.

Number of dead piglets, reason for mortality (e.g. crushing, weak, starvation, diarrhea and unknown) and weight of dead piglets were registered on a daily basis.

## Placenta Analysis

Placentas of sows were collected during and after farrowing and stored at -20°C. After thawing, each placenta was cut open over the whole length on the lateral side, using the umbilical cord as a reference. Open placentas were spread out on a white triplex board with the umbilical cord facing upwards. Individual placentas were photographed in a room with standardized conditions (no natural light), using a Nikon D80 camera with a Nikon dx swm ed if aspherical 67 lens with fixed settings on height, zoom, color saturation and ISO sensitivity settings. Color of the placenta was scored, using a scoring system of 0 to 4 adapted from Baxter *et al.* (2008). Scores were:

0 = No score possible or placenta was brown, because of deteriorating tissue.

1 = Placenta color is pale pink.

2 = Placenta color is light red or bright pink.

3 = Placenta color is bright red.

4 = Placenta color is deep red.

Placentas with a color score of 0 were removed from the analysis (19 placentas in total).

## Statistical Analysis

One sow aborted after being allocated in the current experiment. Data from sows that received birth assistance (n = 8), refused to eat (n = 2), farrowed too early (one day on feed) (n = 1) or had 8 or more stillborn piglets (n = 2, one in each treatment group) was removed from the dataset. The final dataset contained 120 sows. Parity was classified as class 1: parity 1; class 2: parity 2, 3 and 4; class 3:parity >4. 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 data transformation and was expressed as a percentage of TB. Placental color scores were analyzed as ordinal data. 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:

$$Y_{ijkl} = \mu + \alpha_i + b_j + c_k + \varepsilon_{ijkl}$$

where: $Y_{ijkl}$ = dependent variable, $\mu$ = overall mean, $\alpha_i$ = fixed treatment effect ($i$ = 0.0 or 0.1% of calcium nitrate), $b_j$ = random effect of farrowing room ($j$ = 1, 2, ...,5), $c_k$ = random parity class effect ($k$ = 1, 2 or 3), and $\varepsilon_{ijkl}$ = residual error term. Since the effect of batch and room were confounded, only room was added as a random effect to the model. For gestation length, stillborn rate, birth weights and pre-weaning mortality rate the random effect of days on the experimental diet before farrowing (m = 2, 3, ..., 10) was added to the model. Backfat measurement at weaning were corrected for the number of days between measurements (covariable). Piglet birth weights were corrected for litter size (covariable) and piglet weaning weights were corrected for weaning age (covariable) and number

of piglets weaned (covariable). Sow with litter was considered as the experimental unit. For analysis of placenta scores, the effect of Sow(placenta) was added to the model as a random factor.

Preliminary analyses demonstrated a lack of effects of two potential interactions: 1) between litter size (TB) and treatment (control vs calcium nitrate) and 2) between days on feed before farrowing and treatment. Consequently, results will be expressed per main effect of treatment.

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

## Results

Average gestation length was 117.6 ± 1.2 days (taking the first day of insemination as day 1 of gestation), which led to sows being 5.2 ± 1.3 days on the experimental diets before the moment of farrowing. Mean TB was 18.2 ± 3.5 piglets per litter, with 16.6 ± 3.2 live born and 1.6 ± 1.5 (8.8%) stillborn (all mean ± SD). Piglets were weaned at 23.6 ± 2.1 days of age.

### Sow performance

A significant longer gestation length was found for sows receiving the dietary nitrate (+0.4 days, $P = 0.05$, Table 3). This resulted in sows receiving the experimental diet significantly longer (+0.5 days, $P = 0.03$) than sows in the control group. No difference was found between treatments in backfat thickness of sows at approximately day 112 of gestation, at weaning and backfat loss during lactation (Table 3).

### Piglet weights and average daily gain (ADG)

No effect of maternal dietary nitrate supplementation was found on birth weight of live or stillborn piglets. In addition, no effect of maternal dietary nitrate supplementation

was found on piglet ADG between cross fostering and 24h after cross fostering as well as between 24h after cross fostering and 3 days of age. Lastly, no effect of maternal dietary nitrate supplementation was found on weaning weight.

**Table 3.** Effects of calcium nitrate (0.1% Bolifor CNF) in the maternal diet of sows, fed from approximately 5 days pre-partum until 4 days post-partum on sow backfat thickness, reproductive performance and piglet weight

| | Control | 0.1% Calcium nitrate[1] | *p* Value |
|---|---|---|---|
| *N* **(number of sows/ litters)** | 63 | 57 | |
| Parity before farrowing | 3.3 ± 0.2 | 3.5 ± 0.3 | 0.70 |
| Gestation length (days) | 117.0[b] ± 0.5 | 117.4[a] ± 0.5 | 0.05 |
| Number of days on feed before farrowing (days) | 4.5[b] ± 0.5 | 5.0[a] ± 0.5 | 0.03 |
| **Sow backfat thickness** | | | |
| At approximately day 112 of gestation (mm) | 15.0 ± 0.8 | 15.5 ± 0.8 | 0.37 |
| At weaning (mm)[2] | 12.6 ± 0.6 | 12.7 ± 0.6 | 0.88 |
| Backfat loss during lactation (mm)[2] | 2.8 ± 0.5 | 3.2 ± 0.5 | 0.14 |
| **Reproductive performance** | | | |
| Total born | 18.7 ± 0.5 | 17.9 ± 0.5 | 0.25 |
| Number of piglets after cross fostering | 14.7 ± 0.6 | 15.0 ± 0.6 | 0.59 |
| Number of piglets weaned | 12.7 ± 0.2 | 12.6 ± 0.2 | 0.38 |
| **Piglet weights and ADG** | | | |
| Birth weight live born piglets (kg)[3] | 1.34 ± 0.09 | 1.33 ± 0.09 | 0.76 |
| Birth weight still born piglets (kg)[3] | 1.08 ± 0.07 | 1.08 ± 0.07 | 0.97 |
| Average weight after cross fostering (kg) | 1.23 ± 0.09 | 1.25 ± 0.10 | 0.65 |
| Average weaning weight (kg)[4] | 6.06 ± 0.62 | 6.21 ± 0.63 | 0.48 |
| ADG cross fostering until 24h after cross fostering (g/piglet/day) | 87 ± 13 | 92 ± 13 | 0.65 |
| ADG from 24h after cross fostering until 3 days of age (g/piglet/day) | 119 ± 19 | 121 ± 19 | 0.79 |

[1] Calcium nitrate ($5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$); containing 63.1% of nitrate: commercial name Bolifor CNF, available from Yara Phosphates Oy.
[2] Corrected for number of days between BF measurements.
[3] Corrected for total number born.
[4] Corrected for total number weaned per litter and weaning age.
[ab] Different superscripts indicate a significant difference.

## Piglet survival

A significant lower percentage of stillborn piglets was found when sows received 0.1% of calcium nitrate from approximately 5 days before farrowing onward compared to the control treatment (7.4 vs. 9.9%, respectively $P = 0.05$, Figure 1a). Mortality was significantly lower on day 2 post farrowing for piglets of sows receiving the dietary nitrate

compared to the control (0.9% vs. 2.7% respectively, $P < 0.01$, Figure 1b). However, on the day of birth, day 1 and day 3 of age, piglet mortality was non significantly ($P > 0.05$) higher for litters of which sows received the calcium nitrate compared to the control treatment. This resulted in a lack of effect of nitrate addition on total pre-weaning mortality compared to the control treatment (15.3% vs. 14.3% for control and calcium nitrate, respectively, $P = 0.55$).
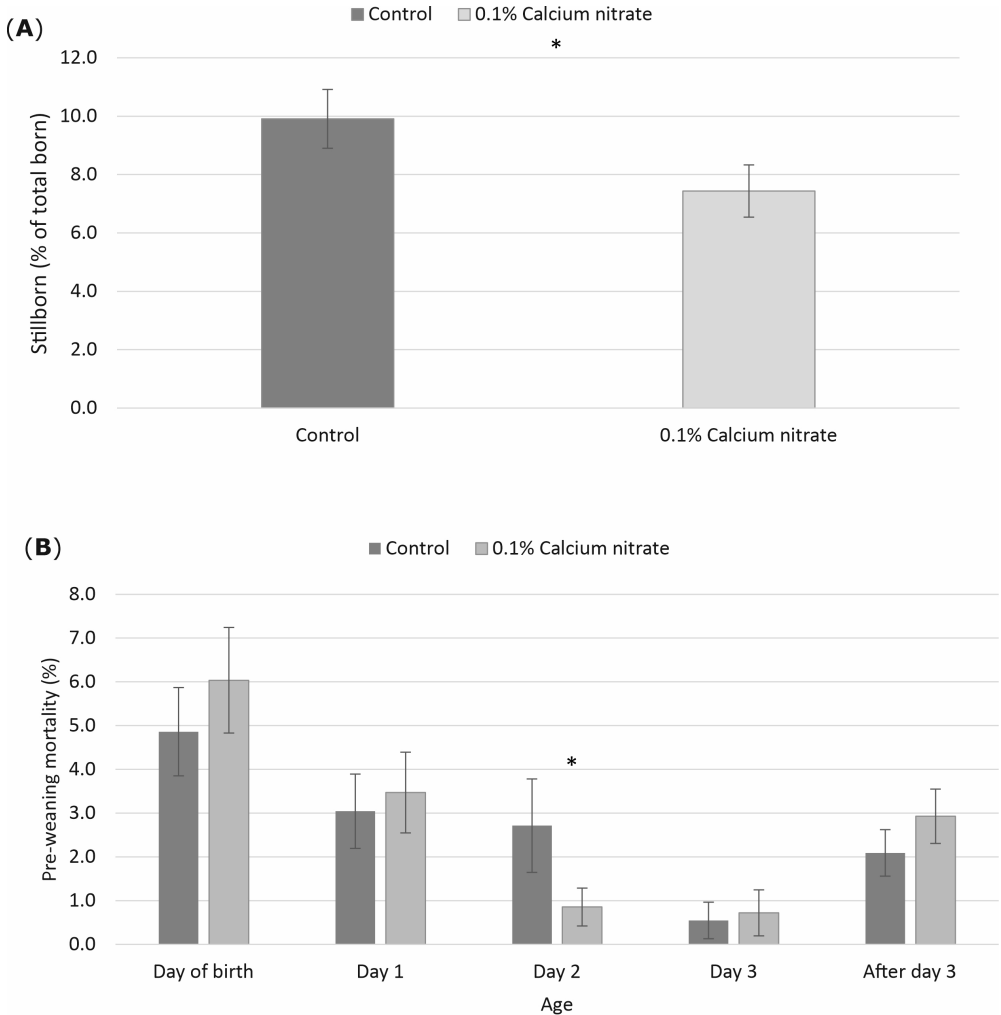


**Figure 1.** Stillborn percentage (**A**) and pre-weaning mortality percentage during the first three days of life (**B**) for piglets born out of sows receiving the control diet or the 0.1% calcium nitrate diet. * Indicates a significant difference ($P < 0.05$).

**Placental color score**

No effect of maternal dietary nitrate supplementation was found on placental color score as shown in Table 4.

**Table 4.** Frequencies of placental color score for the control and 0.1% of calcium nitrate group.

| Score[1] | Control | | 0.1% Calcium nitrate | | Pooled SEM | *p* Value |
|---|---|---|---|---|---|---|
| | n[2] | % | n | % | | |
| | | | | | 3.2 | 0.19 |
| 1 | 27 | 7.8 | 41 | 12.1 | | |
| 2 | 145 | 41.9 | 135 | 39.8 | | |
| 3 | 146 | 42.2 | 144 | 42.5 | | |
| 4 | 28 | 8.1 | 19 | 5.6 | | |
| Total | 346 | 100.0 | 339 | 100.0 | | |

[1]Color of the placenta was scored on a 0 to 4 scale adapted from Baxter *et al.* (2008). 1 = Placental color was pale pink. 2 = Placental color was light red or bright pink. 3 = Placental color was bright red. 4 = Placental color was deep red.
[2]Represents the number of placentas, but data analyzed on sow basis

## Discussion

Dietary nitrate supplementation to sows from approximately 5 days before farrowing until 4 days after farrowing resulted in a small, but significant longer gestation length, a lower stillbirth percentage and a lower pre-weaning mortality rate in piglets at 2 days of age. Overall pre-weaning mortality was not affected by treatment. Placental redness score was not affected when dietary nitrate was supplemented to the maternal diet.

Average litter size and stillborn percentage were high in the current study (18.2 and 1.6, respectively), but comparable to the average production levels in Denmark in 2015 (e.g. 17.6 total born and 1.6 stillborn) (Hansen, 2021). The nitrate dosage in the current study corresponded with the 0.06% dosage, used in previous studies of Van den Bosch *et al.* (van den Bosch *et al.*, 2019a; van den Bosch *et al.*, 2019b). In these studies, no significant effect of maternal nitrate supplementation was found on the incidence of stillbirth, which is in contrast to results found in the current study. It might be that the higher incidence of stillbirth in the current study (8.8%) provided more room for improvement compared to the study of Van den Bosch *et al.* (2019a) (5.8%). Sows in the current experiment

were two days shorter on feed before farrowing compared to the study of Van den Bosch *et al.* (van den Bosch *et al.*, 2019a; van den Bosch *et al.*, 2019b). Based on studies in humans, it is hypothesized that the effects of nitrate supplementation quickly follow after ingestion. In humans, a rapid increase in plasma nitrate levels within the first 30 minutes after ingestion was found, with a peak occurring around 1.5 - 2 hours after ingestion. Plasma nitrate levels stayed elevated for 6 to >11 hours after ingestion (Lundberg and Govoni, 2004; Webb *et al.*, 2008; Miller *et al.*, 2012) and may increase after each meal (resulting in increased saliva production, which contains nitrate), because nitrate is not only ingested when supplemented to the diet, but previous supplied nitrate is taken up by the salivary glands as well. It was estimated that about 25% of all plasma nitrate is taken up by the salivary glands and continuously secreted in saliva, where it is reduced to nitrite by commensal bacteria in the mouth and is then being swallowed (Cosby *et al.*, 2003; Lundberg and Govoni, 2004). In human blood, nitrate has a half-life of 5 to 8 hours (Lundberg and Weitzberg, 2005a; Bryan and Grisham, 2007) and nitrite ($NO_2^-$) of 1 to 5 minutes (Lundberg and Weitzberg, 2005a). NO is a highly reactive free radical with a half-life of only a few seconds. NO (or one of the reaction products) is quickly oxidized to arrange higher nitrogen oxides concentrations, like nitrate and nitrite (Lundberg *et al.*, 2008). On commercial farms, most sows will receive either 2 or 3 meals per day or are fed ad libitum. Supplementation of a source of nitrate to the lactation diet of sows will most likely cause sufficient increase in nitrate levels and therefore continuous synthesize nitrite and NO at the moment of farrowing to ensure the potential effects on endurance and vasodilatation, which may result in a reduction in stillbirth and an increase in piglet vitality. Consequently, it can be hypothesized that, although nitrate was supplemented shortly before the onset of farrowing, the timeframe of supplementation is sufficient to see effects.

Another major source of NO production is via the endogenous L-Arginine-NO synthase pathway, in which L-Arginine is oxidized via the nitric oxide synthase (NOS) family (Moncada and Higgs, 1993). Only a few studies investigated effects of maternal dietary arginine supplementation until right before the moment of farrowing. Che *et al.* (2013) evaluated the supplementation of 1% of L-Arginine to sows from day 30 of gestation to

day 114 of gestation and showed a significant lower number of stillborn piglets (-0.6 pigs, $P < 0.05$) compared to the control. Mateo *et al.* (2007) supplemented 1.0% of L-arginine to gilts from day 30 to 114 of gestation (TB was 11.6 piglets/gilt) and showed a significant lower stillbirth (-1.2 pigs, $P < 0.05$). Gao *et al.* (2012) found no significant difference in the absolute number of stillborn piglets (1.21 vs 1.42, for the control and L-Arginine treatment, respectively), but this lack of effect might be related to the difference in total born piglets (12.46 vs 13.77, for the control and L-Arginine treatment, respectively). Che *et al.* (2013) hypothesized that the reduction in stillbirth can be due to increased utero-placental blood flow and maternal nutrient transfer, which supports a more efficient uterine capacity for fetal growth and development. When this physiological mechanism indeed occurs, it can be speculated that NO production via the maternal diet is particularly of interest in larger litters, because of the average smaller placenta (Baxter *et al.*, 2008). A one to one comparison between studies, using maternal L-arginine supplementation or maternal nitrate supplementation as a NO precursor is not possible, since it is unknown how much NO is produced in the body by both supplements.

Although research on crosslinks between the $NO_3$-$NO_2$-NO pathway and the L-Arginine NO synthase pathway is limited, there are some indications that cross-talk exist between the two pathways in vascular NO homeostasis (Carlström *et al.*, 2015). Lundberg *et al.* (2004) suggested that NO generation from nitrite could be a back-up system for situations in which conditions for inducible NOS (iNOS) production is unfavorable (low oxygenation and acidification). Long-term (8-10 weeks) nitrate supplementation in rats showed a reversible dose dependent reduction in phosphorylated endothelial NOS (eNOS) in the aorta and a lower eNOS-dependent vascular response in vessels from nitrate treated mice (Carlström *et al.*, 2015), suggesting that indeed NOS activity is lower when nitrate is supplemented. Carlstorm *et al.* (2015) suggested that mainly individuals (e.g. elderly) with a compromised eNOS activity might show an increased response to nitrate supplementation (Carlström *et al.*, 2015). Since in the current study dietary nitrate was only supplemented for a total of on average 9 days, it is not expected a reduced eNOS activity occurs.

Gestation length was significantly longer when sows received dietary nitrate supplementation (+0.4 days, $P < 0.05$). It is difficult to say whether or not this is truly caused by treatment

or a results of how gestation length was registered. Day of farrowing was noted when employees were present. Sows which farrowed in the evening when employees left, were registered to have farrowed on the day after. In addition, a non-significant difference in litter size was observed between the control and nitrate supplementation treatment (18.7 vs 17.9 piglets, respectively $P$ = 0.25). A negative correlation exists between litter size and gestation length (Hanenberg *et al.*, 2001; Sasaki and Koketsu, 2007), which is likely caused by an earlier occurrence of fetal stress caused by space limitation due to fetal mass in the uterus, which induces the onset of parturition (Senger, 2003).

An absolute 2.5% reduction in stillbirth percentage ($P$ = 0.05) was found when nitrate was supplemented to sows compared to the control treatment. The reduction in stillbirth percentage might be a result of a shorter duration of farrowing caused by an increased stamina. Farrowing duration is directly linked to the incidence of stillbirth (Borges *et al.*, 2005; van Dijk *et al.*, 2005; Canario *et al.*, 2006). However, farrowing duration could not be registered in this experiment. Van den Bosch *et al.* (2019b) did not find an effect maternal dietary nitrate supplementation on duration of farrowing in a dose response study, which was hypothesized to be due to the short duration of farrowing observed in that study and therefore little room for improvement. Since a significant effect of maternal nitrate supplementation was found on gestation length, it can be hypothesized that the lower stillbirth percentage was caused by this increase in gestation length. Literature mainly describes an effect of gestation length on stillbirth when gestation length is short (< 114 days) (Sasaki and Koketsu, 2007; Rydhmer *et al.*, 2008; Vanderhaeghe *et al.*, 2011). Rydhmer *et al.* (2008) showed a linear decrease of number of stillborn piglets from day 111 until 120 of gestation with a non-significant difference between day 117 and 118 of gestation. It therefore seems unlikely that an increased gestation length of 0.4 days is the driver for 2.5% reduction in stillbirth percentage. In addition, number of days on feed, which is confounded with gestation length, was added to the statistical models to correct for potential effects. No effect of treatment on incidence of pre-weaning mortality during the whole lactation period was found. Van den Bosch *et al.* (2019a) found a trend for a quadratic effect of nitrate dosage on pre-weaning mortality percentage, with the lowest percentage seen at approximately 0.09-0.12% of nitrate. NO is capable of

relaxing the vascular endothelium, causing vasodilatation (Bird *et al.*, 2003; Bailey *et al.*, 2012), which may have led to a larger blood flow, and consequently oxygen flow to the fetuses *in utero*. This larger oxygen flow might have reduced the level of asphyxiation in piglets, causing piglets to be born more vital and therefore reduce the risk for mortality. Van den Bosch *et al.* (2019b) showed a trend ($P = 0.10$) for an increased partial oxygen pressure ($pO_2$) in umbilical cord blood of newly born piglets when an increasing dose of maternal dietary nitrate was fed. In addition, piglet vitality score increased linearly with increasing dosage of maternal nitrate supplementation, which might have been caused by the increased placenta size observed (van den Bosch *et al.*, 2019b) and/or the increased birth weight of piglets (van den Bosch *et al.*, 2019a). Why no clear effect on pre-weaning mortality was found in the current experiment might be related to the use of cross-fostering. Cross-fostering is a common technique, used to match the litter size of a sow to her mothering ability (Alexopoulos *et al.*, 2018). Cross-fostering piglets to another sow has an impact on the environment of the fostered piglet, as well as on the litter this piglet is fostered onto.

No effect of maternal nitrate supplementation was found on placental redness score, which is in line with the study of Van den Bosch *et al.* (2019b). As mentioned before, NO is an endothelial derived relaxing factor, which regulates blood flow across tissues (including the uterus and placenta) and consequently the nutrient and oxygen flow from mother to fetuses (Bird *et al.*, 2003; Bailey *et al.*, 2012). NO also enhances placental vascular growth by placental angiogenesis. Uterine and umbilical cord blood flow increases exponentially throughout gestation to keep up with increasing fetal growth (Reynolds and Redmer, 1995), which means the uterine and placental vascular wall keep remodeling to provide this essential blood flow (Pallares *et al.*, 2008). It was therefore expected that a higher placental redness score could be observed in placentas of sows receiving maternal dietary nitrate supplementation. It might be that due to the short time of nitrate supplementation (e.g. approximately 5 days pre-farrowing), the time frame to adapt placental vascular system was too short and only vasodilation might occur. Widening of the blood vessels could potentially not be visible anymore after placenta expulsion. In addition, placentas were collected after expulsion deprived from maternal circulation for some time. The

dead placenta tissue may not have given us the required information on vasodilatation. Vascularization of the placentas, due to maternal nitrate supplementation has not been studied in the current experiment and could therefore be a topic for future research.

## Ethics Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Wageningen University and Research.

## Conflict of Interest

## Acknowledgements

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# Chapter 4

**Effect 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 piglets 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 pre-weaning mortality. Three hundred and five gilts and sows were allocated to one of 6 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 d0, 72 h of age and weaning. Pre-weaning mortality was determined throughout lactation. BW d0 ($P = 0.04$), as well as BW at 72h 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 ADG 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 pre-weaning mortality of piglets with the lowest level of mortality found at 0.09 - 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 pre-weaning piglet vitality. Exact mode of action and optimal dose of nitrate still need to be elucidated.

**Key words:** birth weight, farrowing, litter uniformity, nitrate, pre-weaning mortality, sow

## 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 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 (Pere 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/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 towards 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 8 consecutive batches, 305 crossbred (Yorkshire x Dutch Landrace; Topigs 20) sows were allocated to one of six diets containing 0, 0.03, 0.06, 0.09, 0.12 or 0.15% of nitrate. Dosages were based off 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 1-9). The source of nitrate used was calcium nitrate ($5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$; containing 63.1% of nitrate; commercial name Bolifor CNF (Yara Phosphatas 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, which contained 90% of ingredients, which was split in 6 homogenous batches. Varying ingredients per experimental diet were added by mixing them into one of the 6 batches of the basal diet. Diets were produced as 4-mm pellets at 70-80ºC. 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 onwards, 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 22-32) a commercially available pelleted lactation diet was fed (14.9% CP, 9.5 MJ 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 onwards, respectively. Feed refusals of sows were removed and weighed before the next feeding. Wet feed refusals were oven dried at 100ºC until weight of the sample did not decrease anymore to determine DM 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).

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

| Item | Experimental lactation diet, % | | | | | |
|---|---|---|---|---|---|---|
| | 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 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[2] | | | 1.00 | | | |
| Sodium chloride | | | 0.36 | | | |
| L-Lysine HCL (78.0%) | | | 0.31 | | | |
| Sodium bicarbonate | | | 0.16 | | | |
| Monocalciumphosphate | | | 0.12 | | | |
| L-Threonine (98.5%) | | | 0.10 | | | |
| L-Tryptophane (98.0%) | | | 0.01 | | | |
| Phyzyme XP 10000 TPT | | | 0.01 | | | |
| Barley | 11.71 | 11.70 | 11.66 | 11.63 | 11.60 | 11.57 |
| Soybean oil | 2.10 | 2.09 | 2.10 | 2.10 | 2.11 | 2.12 |
| Limestone[4] | 2.00 | 1.98 | 1.95 | 1.93 | 1.91 | 1.88 |
| Bolifor CNF[3] | 0.00 | 0.05 | 0.10 | 0.14 | 0.19 | 0.24 |
| **Chemical composition (calculated)[5]** | | | | | | |
| 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 Try, % | 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, %[4] | 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 |

[1] Lactational diets were provided in 2 meals (7.30 AM and 4.30 PM) at 3.25 kg/sow/d from d 108 to 112 of gestation, 2.7 kg/sow/d from d 113 of gestation onwards, 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.

[2] 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 sulphate; 65 mg of Zn as zinc sulphate; 15 mg of Cu as copper sulphate; 4 mg of I as potassium iodide; 0.4 mg of Se as sodium selenite; 10,000 IU vitamin A as Vitamine 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 eq. 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.

[3] The source of nitrate used in this experiment was calcium nitrate ($5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$; containing 63.1% of nitrate; Commercial name Bolifor CNF® available from Yara Phosphatas Oy of Helsingborg, Sweden).

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

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

## Animal housing and management

Approximately one week (d 7.2 ± 1.8) before the expected date of farrowing, the pregnant sows were transported to individual pens (180 x 240 cm) with farrowing crates (55 x 185 cm) in one 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 30ºC. Farrowing was never induced, no 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 only allowed between sows receiving the same treatment, having the same farrowing date and between 24 and 48 h hours after birth (d1). 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, 0.03, 0.06, 0.09, 0.12 or 0.15% of nitrate, respectively). After 48h 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 BW 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 pre-weaning 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 24h after birth

(BW d0). Individual piglet weight was determined again at 72 h of age (BW 72h), i.e. 48 h after the first weighing and at weaning. Uniformity of the litter was expressed by the standard deviation (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. Pre-weaning mortality was calculated by:

*Pre-weaning mortality =*

$$\left( \frac{\textit{Number of piglets lost at the sow between the moment of farrowing and weaning}}{\textit{number of live born piglets + number of piglets added − number of piglets removed at cross fostering}} \right) \times 100\%$$

Livability was calculated by:

$$\textit{Livability} = 1 - \left( \frac{\textit{(number of TSB + number of pre weaning deaths)}}{\textit{TNB + number of piglets added − number of piglets removed at cross fostering}} \right) \times 100\%$$

## 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 behaviour toward the piglets (biting them to death) and was treated with Stressnil (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 analysed as a percentage of TNB.

Variables were analysed 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, $\mu$ = overall mean, $\alpha_i$ = fixed treatment effect ($i$ = 0, 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 $\varepsilon_{ijklmn}$ = residual error term. Sow was considered as the experimental unit. TNB was included as a covariate for BW d0 and litter uniformity d0. Number of piglets after cross-fostering was included as a covariable for BW 72h 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 versus nitrate (0% 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 days, meaning sows received the experimental diets for 7.2 ± 1.8 days 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, 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, 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 ADFI of sows pre-farrowing (2.79, 2.76, 2.74, 2.77, 2.70 and 2.70 kg/sow/d for 0, 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, 2.97 kg/sow/d for 0, 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, 0.03, 0.06, 0.09, 0.12 and 0.15% nitrate, respectively; *P* > 0.05).

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

| Item | Level of nitrate (%) 0 | 0.03 | 0.06 | 0.09 | 0.12 | 0.15 | SEM | P-value | Linear | Quadratic | Contrasts 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[1] | 16.8 | 16.3 | 17.5 | 16.4 | 17.0 | 17.0 | 0.08 | 0.55 | 0.49 | 0.82 | 0.76 |
| Mean BW d0, kg[2] | 1.272 | 1.323 | 1.313 | 1.321 | 1.339 | 1.345 | 0.033 | 0.31 | 0.04 | 0.61 | 0.03 |
| SD BW d0, kg[2] | 0.293 | 0.277 | 0.271 | 0.293 | 0.267 | 0.28 | 0.011 | 0.36 | 0.39 | 0.47 | 0.16 |
| **TBA** | | | | | | | | | | | |
| TBA, n | 15.7 | 15.3 | 16.4 | 15.4 | 15.9 | 15.9 | 1.0 | 0.98 | 0.73 | 0.60 | 0.98 |
| (% of TNB) | -94.2 | -94.1 | -94.6 | -94.4 | -93.9 | -93.9 | | | | | |
| Mean BW d0, kg[2] | 1.288 | 1.338 | 1.323 | 1.336 | 1.35 | 1.364 | 0.033 | 0.29 | 0.03 | 0.78 | 0.04 |
| SD BW d0, kg[2] | 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 | 1.1 | 1.1 | 1.0 | 1.0 | 1.1 | 1.1 | 1.0 | 0.98 | 0.73 | 0.60 | 0.98 |
| (% of TNB) | -5.8 | -5.9 | -5.4 | -5.6 | -6.1 | -6.1 | | | | | |
| **Litter characteristics** | | | | | | | | | | | |
| Number of piglets after cross fostering, n | 14.7 | 14.7 | 15.2 | 14.4 | 14.7 | 14.7 | 1.9 | 0.51 | 0.46 | 0.27 | 0.33 |
| Number of piglets weaned, n | 12.2 | 12.4 | 12.5 | 12.4 | 12.7 | 12.2 | | 0.06 | 0.00 | 0.96 | 0.01 |
| BW 72h, kg[2] | 1.557 | 1.618 | 1.594 | 1.626 | 1.639 | 1.668 | 0.035 | 0.42 | 0.18 | 0.34 | 0.07 |
| SD BW 72h, kg[2] | 0.318 | 0.301 | 0.284 | 0.304 | 0.292 | 0.292 | 0.013 | 0.85 | 0.28 | 0.97 | 0.26 |
| ADG between d0 and 72 h of age, g/pig/d[2] | 96.6 | 103.2 | 100.8 | 101.1 | 102.6 | 105.8 | 7.16 | 0.90 | 0.40 | 0.87 | 0.30 |
| ADG between 72 h of age and weaning, g/pig/d[3] | 258.4 | 258 | 249.4 | 260.1 | 261.2 | 255.9 | 6.38 | 0.45 | 0.42 | 0.88 | 0.78 |
| BW at weaning, kg[3] | 7.967 | 8.003 | 7.798 | 8.132 | 8.056 | 8.021 | 0.271 | 0.38 | 0.05 | 0.85 | 0.30 |
| SD BW at weaning, kg[3] | 1.455 | 1.477 | 1.366 | 1.413 | 1.377 | 1.318 | 0.064 | | | | |

[1] Excluding mummified piglets.
[2] Included TNB as covariate in the model.
[3] Included number of piglets after cross fostering in the model.

**4**

Results on sow reproduction and litter performance are presented in Table 2. Mean BW d0 (for both TNB and TBA) and BW 72h 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 $P < 0.05$ for TNB, TBA and BW 72h, respectively). SD of weight at d0 (for both TNB and TBA) was not affected by treatment. SD of BW 72h 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, while 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 pre-weaning mortality (% of TBA) tended to be lower in the nitrate supplemented groups compared to the control (2.8%, $P = 0.06$; Fig. 1a). For both pre-weaning mortality and livability (Fig. 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 pre-weaning mortality and higher livability at intermediate levels (0.09 - 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 d0, BW 72h and litter uniformity at weaning, all Linear $P < 0.05$) in the current 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 pre-weaning 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 days before farrowing, which seems a relatively short time span to influence fetal gain. The current study is, to 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 oxidised in a reaction catalysed by the nitric oxide synthase (NOS) family (Moncada and Higgs, 1993), and not via the $NO_3$-$NO_2$-NO pathway, like dietary nitrate (Lundberg
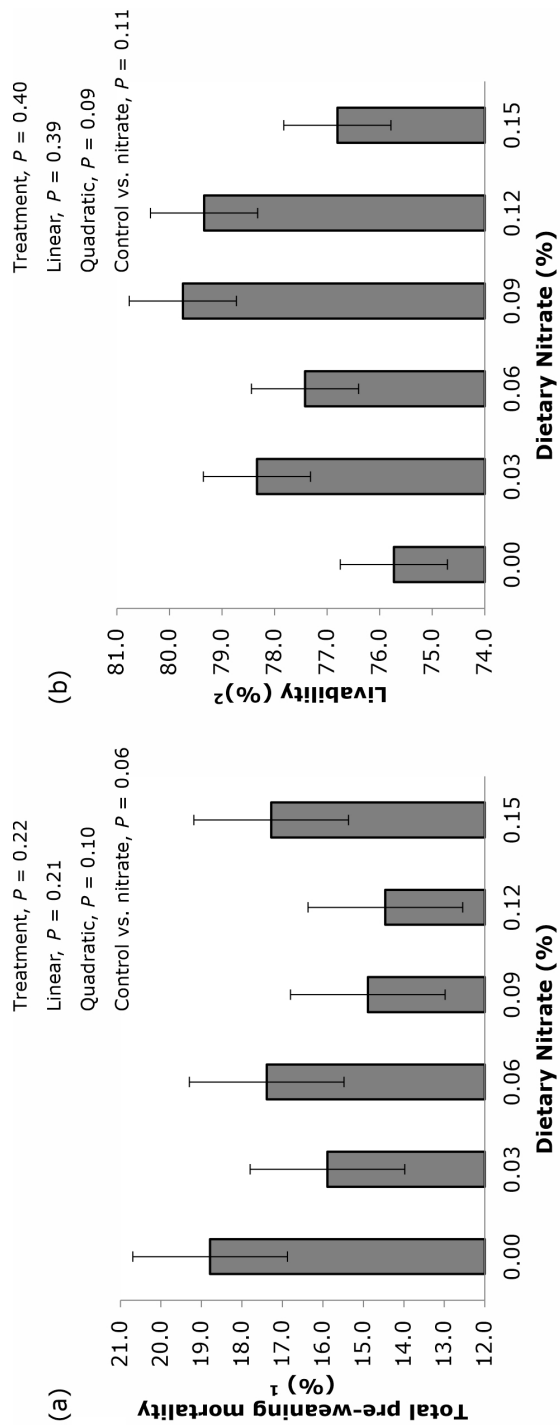
**Figure 1a and b.** LSMeans ± SEM of total pre-weaning mortality (a) and livability (b) per maternal dietary nitrate level.

[1] Pre-weaning mortality is calculated as: $\left( \dfrac{\text{the number of piglets lost at the sow between farrowing and weaning}}{(\text{number of live born piglets} + \text{number of piglets added} - \text{number of piglets removed at cross fostering})} \right) \times 100\%$

[2] Calculated as: $1 - \left( \dfrac{(\text{number of stillborn piglets} + \text{number of pre-weaning deaths})}{(\text{number of total born piglets} + \text{number of piglets added} - \text{number of piglets removed at cross fostering})} \right) \times 100\%$

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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 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 gram/ liveborn piglet, *P* < 0.05) after L-Arginine supplementation. Exact NO production resulting from arginine versus 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 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 foetuses and higher piglet birth weights. In order to get an estimation of the additional blood flow needed for the additional piglet gain seen in our experiment, the study of McPherson *et al.* (2004) and Pere 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 timespan 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 pre-weaning mortality (Leenhouwers *et al.*, 2002; van Rens *et al.*, 2005; Baxter *et al.*, 2008; Rootwelt *et al.*, 2013), and between placental surface area and pre-weaning mortality (Baxter *et al.*, 2008; Rootwelt *et al.*, 2013). Van den Bosch *et al.* (2019) showed a linear increase in placental width as maternal nitrate supplementation increased from 0 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 pre-weaning mortality (excluding stillborn) was higher than Dutch average mortality rates in the Netherlands (15.6% vs. 13.9%, respectively (Agrovision, 2016)), which is likely due to the fact that researchers and other staff were not allowed to interfere with survival. Nevertheless, pre-weaning mortality tended to be higher in the control vs. nitrate supplemented groups ($\Delta$= 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 ($\Delta$= 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, since an estimated two-thirds of the embryonic death losses occur before d 30 of gestation (Bazer *et al.*, 2009). After d 30 of gestation, many factors may contribute to fetal losses (e.g. virus infections, non-uniform 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 days 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.

## Conflict of Interest

M. van den Bosch, I. B. van de Linde, A. A. M. van Wesel and D. Melchior are employed at the Cargill Innovation Centre Velddriel, the Netherlands. A related patent application (PCT/US2015/ 064293) was filed on December 7, 2015, and accepted as WO/ 2016/090366 on April 9, 2021. 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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# Chapter 5

## Effects of maternal dietary nitrate supplementation on farrowing and placental characteristics, level of asphyxiation at birth and piglet vitality

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## Abstract

We investigated whether maternal dietary nitrate supplementation, leading to nitric oxide (NO) formation, would affect duration of farrowing, levels of asphyxiation, vitality of piglets at birth and/or loss of potential viable piglets in the form of stillbirth and pre-weaning mortality. Data were collected from 190 crossbred (Yorkshire x Dutch Landrace) sows, which were allocated, balanced for parity, to six dietary nitrate levels (0, 0.03, 0.06, 0.09, 0.12 or 0.15% of nitrate). Sow received the lactational diet containing nitrate from approximately 7 days before farrowing until 5 days after farrowing. Blood acid-base parameters (pH, $pO_2$, $pCO_2$, $BE_{ecf}$, $HCO_3$, $sO_2$ and lactate) and nitrate concentration were determined in umbilical cord blood. The farrowing process was video recorded and later analyzed for total duration of farrowing, piglet birth interval, piglet vitality was scored and piglet latency to stand right after birth. Placentas were collected after expulsion during and after farrowing. Placenta length and width were measured and placental color scores were assessed based on redness of the placenta. The probability of a higher vitality score of piglets (being more vital) linearly increased with increasing levels of maternal dietary nitrate. This higher vitality score however, was not reflected by changes in the blood acid-base parameters in umbilical cord blood, except for a tendency for a higher $pO_2$ with increasing levels of nitrate, which could have been caused by a quicker onset of respiration or an increased blood flow to the piglets during birth. Placenta width increased with increasing levels of maternal dietary nitrate, but no effect on placenta length and redness was found. Neither duration of farrowing nor birth interval were affected by maternal dietary nitrate level. In conclusion, maternal nitrate supplementation may affect piglet vitality via vasodilatation (placental characteristics) rather than an increase in exercise efficiency (duration of farrowing).

**Key words:** nitrate, piglet vitality, placenta, farrowing, asphyxiation

## Introduction

The loss of potential viable piglets in the form of stillborn piglets or piglets dying before weaning represents an economic and emotional loss for the producer as well as an ethical problem for society (Vanderhaeghe *et al.*, 2013). Stillborn piglets account in global swine production for approximately 3-8% of the total born piglets (Borges *et al.*, 2005; Vanderhaeghe *et al.*, 2010; Vanderhaeghe *et al.*, 2013). Most piglets that die during farrowing (intra-partum) die due to asphyxiation (van Dijk *et al.*, 2008), caused by a broken umbilical cord (approximately 71% of the cases (Rootwelt *et al.*, 2012)) and/or a prolonged duration of farrowing (van Dijk *et al.*, 2005; Oliviero *et al.*, 2010). Intra-partum asphyxiation, when not severe enough to result in death, can reduce vitality of piglets right after birth, which also increases the risk for early pre-weaning mortality (English and Wilkinson, 1982; Baxter *et al.*, 2008). Pre-weaning mortality ranges between 10-20% of live born piglets (Muns *et al.*, 2013; Muns *et al.*, 2016) of which approximately 80% occurs during the first 3 days after birth (Rootwelt *et al.*, 2013). Given the link between farrowing duration, asphyxia and piglet survival, nutritional interventions that aim to shorten farrowing duration by increasing the endurance of sows could decrease stillbirth and increase piglet vitality directly after birth. During the process of contractions and expulsion of the piglets, however, a sow has limited or no feed intake, which suggest that nutritional interventions aiming at improving farrowing should be done before the onset of parturition.

Dietary supplementation with nitrates or nitrites, leading to nitric oxide (NO) production, has been intensively investigated in relation to human health (as reviewed by (Omar *et al.*, 2016)) and exercise performance (as reviewed by (Jones, 2014)). Nitric oxide (NO) can be synthesized from arginine in a reaction catalyzed by the NO synthase (NOS) or via a NOS-independent pathway by a stepwise reduction of nitrate and nitrite into NO (Lundberg *et al.*, 2009). Under hypoxic situations, NO formation via nitrate and nitrite reduction shows a much greater efficacy than the NOS- pathway, because oxygen is not a required substrate of this reaction, whereas it is in the NOS synthase pathway. The NOS-independent pathway appears thus more effective during exercise (Gladwin *et al.*,

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2005) and potentially the process of parturition. In addition to its effect on exercise performance, NO, a major endothelium-derived relaxing factor (Bird *et al.*, 2003; Bailey *et al.*, 2012), has a vasodilative effect and influences vasculogenesis and angiogenesis in placental tissues (Wu *et al.*, 2004). Adequate vasculogenesis and angiogenesis of the placenta are not only important during gestation to ensure the required blood supply for fetal development (Liu *et al.*, 2012), but may also be of importance during the process of farrowing. It might be that, by ensuring a larger blood, and, therefore, oxygen and nutrient flow in the placenta and the umbilical cord during farrowing, the risk for asphyxiation can be reduced. In addition, dietary nitrate supplementation has been found to improve exercise efficiency in athletes by reducing the $O_2$ cost of exercise without increasing lactate production (Larsen *et al.*, 2007), suggesting a lower muscular demand for $O_2$ (Jones, 2014). Improved exercise efficiency of skeletal muscles can lead to an increased output or speed that should result in a shorter time to complete a certain exercise (Jones, 2014). We hypothesize that this concept can be extrapolated to the process of farrowing, but experimental results supporting this hypothesis are not available yet.

In light of the potential beneficial effects of nitrate supplementation on vasodilation and exercise efficiency in humans, maternal dietary nitrate supplementation, leading to NO formation, might represent an efficient strategy to increase 'exercise efficiency' during farrowing, via increased uterine blood flow, decreased farrowing duration or both. These beneficial effects of nitrate supplementation on the farrowing process may increase piglet vitality and reduce risk for still birth in the offspring. To the authors knowledge, no studies have investigated the potential use of maternal dietary nitrate supplementation before and/or during farrowing to decrease the level of asphyxia in new-born piglets or other species. The objective of this study was to determine the effects of different levels of dietary nitrate, supplemented to the sow during the perinatal period, on the farrowing process, the level of asphyxiation of piglets at birth, piglet vitality and placental characteristics.

## Materials and Methods

All procedures in this study were approved by the Animal Use and Care Committee of Wageningen University, The Netherlands, in accordance with EU Directive 2010/63/EU for animal experiments.

### Experimental design

The experiment was performed at the Swine Innovation Centre Sterksel (VIC) of Wageningen University & Research, The Netherlands, and was carried out in 8 successive batches. In total, 190 crossbred (Yorkshire x Dutch Landrace) sows were allocated to one of six dietary treatments in a dose response study. All sow received a commercial available gestation diet upon entering the farrowing room. Allocation to the treatments was based on parity (3.5 ± 2.0; range 1-9), and treatments were distributed among batches, and within and between farrowing units. Average parity was 3.6 (n=33), 3.6 (n=33), 3.5 (n=30), 3.5 (n=29), 3.4 (n=28) and 3.6 (n=31) for diets contained 0, 0.03, 0.06, 0.09, 0.12 or 0.15% of nitrate, respectively. Average parity was 3.5 (n=24), 3.5 (n=24), 4.6 (n=23), 5.3 (n=23), 3.2 (n=23), 2.6 (n=23), 1.7 (n=23) and 4.0 (n=21) for batch 1 to 8, respectively. Diets contained 0, 0.03, 0.06, 0.09, 0.12 or 0.15% of nitrate. The source of nitrate used in this experiment was calcium nitrate ($5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$; containing 63.1% of nitrate; Commercial name Bolifor CNF® available from Yara Phosphatas Oy of Helsingborg, Sweden). Calcium levels in the diets were kept constant by using limestone. Approximately 7 days before the expected date of farrowing, pregnant sows were transported to individual farrowing crates (pen size 180 x 240 cm, crate size of 55 x 185 cm) in four farrowing units. The lactation feed supplemented with nitrate (12.6% CP, 12.3 MJ NE kg-1) was fed restrictedly at 3.25 kg/sow/day between day 108 until 112 of gestation, 2.7 kg/sow/day from day from day 113 of gestation onwards and 2.0 kg/sow/day on the day of farrowing. After farrowing diets were provided at 2.5, 3.0, 3.0 and 3.5 kg/sow/day at day 1, 2, 3 and 4 after the day of farrowing, respectively. Feed was provided twice per day (7.30 AM and 4.30 PM) from the moment sows entered the farrowing units (day 108 ± 1 of gestation) until 4 days after the day of farrowing. Sows

had *ad libitum* access to water. Piglets received a commercial available prestarter from 3 days of age until weaning.

## Data collection

During the whole experiment, farrowing was not induced and no medicine was administered during farrowing. To prevent any effect of human intervention on our data, sows that did receive forms of birth assistance in case of emergency were excluded from the dataset. Since farrowing occurred spontaneously, researchers were present 24 hours per day for 3 days around the expected farrowing date (i.e. 115 days after insemination) to take samples and collect placentas. A mixed blood sample (i.e. from the vein and/or artery) from the umbilical cord was collected within three minutes after birth and analyzed within four minutes after birth, from two random live born piglets out of every four subsequent piglets born, using a 2.5 mL syringe with a 16 mm 21 G needle and sodium oxalate as an anticoagulant. Blood samples were taken without handling piglets to avoid the influence of stress on blood parameters. Blood samples were taken regardless of the condition of the umbilical cord (intact or ruptured).

## Analysis of blood samples

Blood acid-base parameters were analysed within one minute after collection by using the iStat® portable Clinical Analyser (iStat Europe, Birmingham, United Kingdom) and CG8+ cartridges. The iStat® has proven to be a reliable, valid blood gas analyser in new-born piglets (van Dijk *et al.*, 2006). Remaining blood was collected in a 4 mL BD vacutainer® (fluor heparine tube) and stored on ice before being centrifuged at 3000 rpm at 4°C for 10 minutes. Blood plasma was decanted and stored at -20°C for further analysis. Lactate concentration in plasma was determined by using an enzymatic UV test with lactate dehydrogenase with reagents of DiaSys Diagnostic Systems GmbH (Holzheim, Germany). Additionally, plasma nitrate concentration was determined by using the nitrate assay by R&D Systems™ (Biotechne, Minneapolis, United States of America).

## Video analysis

A digital video recorder (Samsung SRD470DP) connected to cameras (Velleman – CCD colour cameras) was used to record the farrowing process of individual sows. Video recordings were analysed by using the Observer XT 10 software package (Noldus Information Technology B.V., Wageningen, the Netherlands). Video material of 6 sows could not be analysed due to limited visibility (control; n=1, 0.03%; n=2, 0.09%; n=1, 0.12%; n=1 and 0.15%; n=1). The total duration of farrowing was calculated as the time in minutes between the birth of the first and last piglet according to the video footage. Birth interval was calculated as the time in minutes between the births (i.e. when is piglet is completely expulsed) of two subsequent piglets. Birth intervals and total farrowing duration was done blinded by three observers. Individual vitality of new-born piglets was scored by using the scoring method as described by Baxter *et al.* (Baxter *et al.*, 2008), but with a scoring time of 30 seconds:

0 = No movement, no breathing within 30 sec after birth.

1 = No movement, but piglet is breathing or trying to breathe within 30 sec after birth (coughing and spluttering).

2 = Piglet is moving, breathing or trying to breathe within 30 sec after birth.

3 = Piglet is moving and breathing well and makes a first attempt to stand within 30 sec after birth.

Time to first attempt to stand, defined as the time between the moment of birth and the first time the sternum is lift from the ground, was scored separately for each individual piglet. Vitality scores were done by blinded by one observer. Time to first attempt to stand were done blinded by three observers.

## Placenta analyses

Placentas of sows were collected and labelled during and after farrowing and stored at -20°C. Each placenta was cut open over the whole length on the lateral side, using the umbilical cord as a reference. Open placentas were spread out on a white triplex board with the umbilical cord facing upwards with a tape measure across the length and width of the

board. Individual placentas were photographed in a room with standardized conditions (no natural light) using a Nikon D80 camera with a Nikon dx swm ed if aspherical 67 lens with fixed settings on height, zoom, color saturation and ISO sensitivity settings. Length and width of photographed placentas was determined. In addition, color of the placenta was scored using a scoring system of 0 to 4 adapted from Baxter *et al.* (Baxter *et al.*, 2008). Placentas with a color score of 0 were removed from the analysis (0.03%; n=1, 0.06%; n=1 and 0.09%; n=2). Placental measurements and color scores were done blinded by one observer.

0 = No score possible or placenta was brown, because of deteriorating tissue.

1 = Placenta color is pale pink.

2 = Placenta color is light red or bright pink.

3 = Placenta color is bright red.

4 = Placenta color is deep red.

## Statistical Analyses

Data from sows that received birth assistance (n=3; control, 0.03% and 0.12% of dietary nitrate) and sows that were sick or died around farrowing (n=2; 0.12% and 0.15% of dietary nitrate) were removed from the dataset. One sow was removed from the trial since she expressed aggressive behaviour toward the piglets (biting them to death) and was treated with Stresnil during farrowing (n=1; 0.15% of dietary nitrate). Number of sows were 33, 33, 30, 29, 28 and 31 for the control, 0.03%, 0.06%, 0.09%, 0.12% and 0.15% of dietary nitrate, respectively. Model assumptions, i.e. normality and equal variace of the error terms, were checked by inspection of the residual plots. Total duration of farrowing, average birth interval per sow and average latency to first attempt to stand after birth per sow were found to be non-normally distributed. These parameters were normalized by using a base ten logarithm transformation. Placental weight, length and width (continuous data) and placental color score and piglet vitality score (ordinal data) were subjected to a mixed model analysis, using the PROC GLIMMIX procedure in SAS (Version 9.3, 2011, SAS Institute Inc., Cary, NC, United States) according to the statistical model:

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

where: $Y_{ijklmn}$ = dependent variable, $\mu$ = overall mean, $\alpha_i$ = fixed treatment effect ($i$ = 0, 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 unit effect ($l$ = 1, 2, ..., 4), $f_m$ = covariate of days on feed before farrowing ($m$ = 3, 4, ..., 13) and $\varepsilon_{ijklmn}$ = residual error term. Sow was the experimental unit. Contrasts were used to determine significant relationships for linear and quadratic effects of increasing nitrate contents, and to asses the effect of nitrate versus no nitrate (0% nitrate vs. 0.03, 0.06, 0.09, 0.12 and 0.15% nitrate). For total duration of farrowing, average birth interval and average latency to first attempt to stand after birth, the observer was added as a random effect ($n$ = 1, 2 or 3) and total number of piglets born was added as a covariate to the model.

Blood acid-base parameters of umbilical cord blood (continuous data) were subjected to a mixed model analysis, using the PROC GLIMMIX according to the following statistical model:

$$Y_{ijklmnop} = \mu + \alpha_i + b_j + c_k + d_l + f_m + g_n + h_o(\alpha_i) + \varepsilon_{ijklmnop}$$

where: $Y_{ijklmnop}$ = dependent variable, $\mu$ = overall mean, $\alpha_i$ = fixed treatment effect ($i$ = 0, 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 unit effect ($l$ = 1, 2, ..., 4), $f_m$ = random effect of days on feed before farrowing ($m$ = 3, 4, ..., 13), $g_n$ = random effect of birth order ($n$ = 1, 2, ...,24), $h_o(_i)$ = random effect of sows nested within treatment, and $\varepsilon_{ijklmnop}$ = residual error term.

Data are expressed as LSmeans and SEM. Differences were assumed to be significant if $P \leq 0.05$.

## Results

Average gestation length was 115.1 ± 1.6 days on average (taking the first day of insemination as day 1 of gestation), which led to sows being 7.2 ± 1.8 days on feed before the moment of farrowing. Mean litter size was 17.1 ± 3.4 piglets per litter, with 16.1 ± 3.1 live born and 1.0 ± 1.4 stillborn. Out of all sows that farrowed, 818 piglets from 109

litters were sampled. In total, 529 samplings harvested enough blood to do the blood gas analyses and plasma collection, 172 samplings harvested sufficient blood for blood gas analyses only, and 117 samplings were only used to harvest plasma. Mean values for acid-base variables from umbilical cord blood are shown in Table 1.

**Table 1.** Acid-base balance parameters from blood analysed within 4 minutes after birth from the umbilical cord of live born piglets.

| Variable | N | Mean | SD | Range Min | Max |
|---|---|---|---|---|---|
| pH | 701 | 7.52 | 0.12 | 7.03 | 7.93 |
| $pO_2$ (mmHg) | 695 | 33.65 | 9.65 | 11.00 | 74.00 |
| $pCO_2$ (mmHg) | 700 | 34.36 | 8.75 | 12.30 | 66.40 |
| $BE_{ecf}$ (mmol/L) | 674 | 4.80 | 5.21 | -16.00 | 22.00 |
| $HCO_3$ (mmol/L) | 701 | 27.47 | 4.30 | 11.40 | 43.20 |
| $sO_2$ (%) | 701 | 68.03 | 18.69 | 6.00 | 100.00 |
| Lactate (mmol/L) | 635 | 4.04 | 1.80 | 1.46 | 10.64 |
| Nitrate (µmol/L) | 600 | 883.12 | 293.03 | 19.93 | 2056.67 |

## Farrowing characteristics

It took on average 236 ± 121 min (range 65 - 758 min) to complete farrowing. Average birth interval was 15 ± 7 min. Maternal dietary nitrate level did not significantly influence total duration of farrowing and birth interval of piglets (Table 2).

## Umbilical cord blood parameters

pH, $pCO_2$, $BE_{ecf}$, $HCO_3$ and $sO_2$ in umbilical cord blood samples of piglets were not significantly affected by level of maternal dietary nitrate (Table 2). $pO_2$ tended to linearly increase as maternal dietary nitrate levels increased ($P$ = 0.10). Maternal dietary nitrate level significantly affected lactate level in umbilical cord blood of newly born piglets ($P$ = 0.03), but no linear or quadratic relationship was found between treatment and blood lactate levels. Nitrate level in the umbilical cord blood was not significantly influenced by maternal dietary nitrate levels.

**Table 2.** Effects of different levels of nitrate in the diet of sows on farrowing characteristics, acid-base balance parameters of umbilical cord blood of live born piglets and latency to first attempt to stand. Sows received diets containing dietary nitrate from 7 days before expected farrowing date to 5 days after. Data were expressed as average per litter.

| Item | N litters/ treatment (range) | Level of nitrate (%) 0.00 | 0.03 | 0.06 | 0.09 | 0.12 | 0.15 | Pooled SEM | Model P-value | Contrasts Linear | Quadratic | Control vs. all levels of nitrate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Farrowing characteristics** | | | | | | | | | | | | |
| Duration of farrowing (min)[1,2] | 27-32 | 197 | 224 | 214 | 208 | 202 | 228 | 0.050 | 0.82 | 0.59 | 0.95 | 0.36 |
| Average birth interval (min)[2] | 27-32 | 13 | 15 | 13 | 14 | 12 | 15 | 0.047 | 0.36 | 0.76 | 0.65 | 0.47 |
| **Umbilical cord blood parameters** | | | | | | | | | | | | |
| pH | 17-19 | 7.54 | 7.55 | 7.53 | 7.57 | 7.53 | 7.56 | 0.02 | 0.41 | 0.71 | 0.71 | 0.86 |
| $pCO_2$ (mmHg) | 17-19 | 33.56 | 34.65 | 34.68 | 33.86 | 34.95 | 33.83 | 1.49 | 0.91 | 0.86 | 0.47 | 0.45 |
| $pO_2$ (mmHg) | 17-19 | 31.13 | 32.68 | 31.41 | 32.78 | 33.01 | 33.57 | 1.35 | 0.55 | 0.10 | 0.91 | 0.19 |
| $BE_{ecf}$ (mmol/L) | 17-19 | 6.16 | 6.32 | 6.40 | 7.97 | 6.04 | 7.36 | 1.19 | 0.37 | 0.25 | 0.75 | 0.40 |
| $HCO_3$ (mmol/L) | 17-19 | 28.55 | 28.73 | 28.52 | 29.56 | 28.56 | 28.87 | 1.01 | 0.79 | 0.65 | 0.64 | 0.64 |
| $sO_2$ (%) | 17-19 | 65.50 | 68.58 | 66.27 | 68.63 | 67.82 | 70.41 | 2.13 | 0.54 | 0.14 | 0.85 | 0.20 |
| Lactate (mmol/L)[2] | 15-18 | 3.81 | 3.26 | 3.98 | 3.57 | 3.22 | 3.63 | 0.03 | **0.03** | 0.37 | 0.72 | 0.17 |
| Nitrate (µmol/L) | 15-18 | 938.41 | 903.38 | 859.47 | 934.24 | 1018.67 | 930.96 | 97.97 | 0.35 | 0.35 | 0.59 | 0.87 |
| **Post-partum piglet vitality** | | | | | | | | | | | | |
| Latency to first attempt to stand (sec)[2] | 27-32 | 74 | 70 | 76 | 70 | 73 | 77 | 0.03 | 0.83 | 0.68 | 0.53 | 0.85 |

[1] Defined as the time between birth of first and last piglets. Birth is defined as the complete expulsion of a piglet.
[2] Parameters were normalized by using a base ten logarithm transformation. LSMeans were back transformed to orginal scale. Pooled SEM is still reported as transformed value.

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## Piglet vitality

Average vitality score was 2.14 ± 0.84 for all piglets born. Probability of a higher vitality score (being born more vital) linearly increased with increasing levels of maternal dietary nitrate ($P$ = 0.03, Table 3). The latency to first attempt to stand after birth was not influenced by maternal dietary nitrate level (Table 2).

**Table 3.** Effects of different levels of nitrate in the diet of sows on cumulative probability (percentage) of a higher post-partum vitality score (being more vital). n = 25-30 litters per treatment.

| | Cumulative percentage (%) | | |
| --- | --- | --- | --- |
| **Level of nitrate (%)** | **Score 1 or higher** | **Score 2 or higher** | **Score 3** |
| 0.00 | 96.6 | 85.1 | 41.0 |
| 0.03 | 96.5 | 84.7 | 40.3 |
| 0.06 | 96.3 | 84.1 | 39.2 |
| 0.09 | 97.5 | 88.7 | 48.8 |
| 0.12 | 97.1 | 87.3 | 45.7 |
| 0.15 | 97.3 | 88.0 | 47.2 |
| Pooled SEM | 1.4 | 5.1 | 9.4 |
| Model P-value | | 0.14 | |
| Linear contrast | | **0.03** | |
| Quadratic contrast | | 0.91 | |
| Control vs. all levels of nitrate contrast | | 0.34 | |

## Placental characteristics

Average placental length was 65.8 ± 6.3 cm and average placental width was 36.6 ± 2.9 cm. Placental length was not significantly influenced by maternal dietary nitrate level (Figure 1b), but placental width increased linearly with increasing maternal dietary nitrate levels ($P$ = 0.02; Figure 1a). Average placental color score was 2.48 ± 0.76. Probability of a higher placental color score (having a deeper red color) was not affected (linearly, quadratically or no vs. added nitrate; Table 4) by maternal dietary nitrate level.

**Table 4.** Effects of different levels of nitrate in the diet of sows on cumulative probability (percentage) of a higher placental color score (having a deeper red color). n = 25-30 litters per treatment.

| Level of nitrate (%) | Cumulative percentage (%) | | |
| --- | --- | --- | --- |
| | Score 2 or higher | Score 3 or higher | Score 4 |
| 0 | 95.7 | 55.7 | 7.4 |
| 0.03 | 95.7 | 56.2 | 7.5 |
| 0.06 | 95.0 | 51.8 | 6.4 |
| 0.09 | 95.4 | 54.1 | 7.0 |
| 0.12 | 93.9 | 46.9 | 5.3 |
| 0.15 | 95.8 | 56.4 | 7.6 |
| Pooled SEM | 3.5 | 15.6 | 4.4 |
| Model P-value | | 0.81 | |
| Linear contrast | | 0.61 | |
| Quadratic contrast | | 0.48 | |
| Control vs. all levels of nitrate contrast | | 0.64 | |

## Discussion

The aim of our study was to evaluate effects of maternal dietary nitrate supplementation on farrowing characteristics and the level of asphyxiation of piglets right after birth, piglet vitality right after birth, and placental characteristics. Our study shows that increasing levels of nitrate in the diet of the sows during the perinatal period had no significant effect on duration of farrowing or birth interval. The probability of a higher vitality score, as well as placental width increased linearly with increasing levels of maternal dietary nitrate supplementation. Furthermore, $pO_2$ tended to increase linearly as maternal dietary nitrate levels increased. Farrowing is hypothesized to be comparable to moderate to heavy endurance exercise and the level of exercise is believed to increase with increasing litter sizes. Whether the effect of dietary nitrate seen on athlete exercise efficiency may be extrapolated to the process of farrowing is unknown. Although, both exercise as well as farrowing require consistent and prolonged muscle contractions, differences between the two processes exist. Muscle contraction of the uterine walls (mostly smooth muscle) are autonomous and hormonally driven, while during exercise muscle contractions are
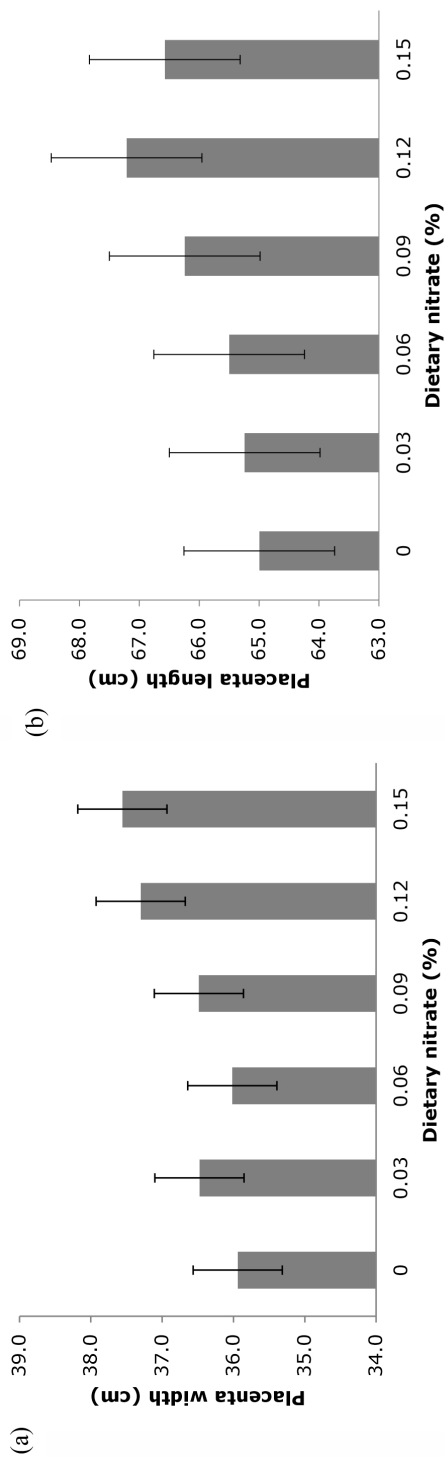
**Figure 1a and b.** LSMeans (± SEM) of placental width (a) and length (b) measured post-partum per maternal dietary nitrate level (n litter per treatment range = 25-30). P values for linear contrast testing are 0.02 and 0.12 for placental width and length, respectively. Note; standard error bars contain all sources of variation in the experiment, including that of random effects (since mixed models were used). However, only residual variance is used to conduct significance test.

driven by the somatic nervous system. However, in the expulsion phase of farrowing a sow is likely, similar to humans, actively pushing out piglets, which is likely a combination of autonomous (e.g. uterus) and somatic muscle use (e.g. abdominal wall and potentially other muscle groups) (as also suggested by (Fereya, 2018)). Whether NO is as effective in autonomous and somatic muscle contractions remains unclear. Hormones like oxytocin, present at high levels during farrowing, may compromise the hypothesized vasodilative effect NO since strong muscle contractions could compromise higher blood flows in the uterus, placenta or umbilical cord (Mota-Rojas *et al.*, 2002).

To our best knowledge, no studies evaluated the effect of maternal dietary supplementation with either nitrate or L-Arginine (both leading to NO formation) on farrowing characteristics. A limited number of studies evaluated effects of nutritional interventions on duration of farrowing and asphyxiation in piglets (Quiniou, 2005; Guillemet *et al.*, 2007; Tydlitát *et al.*, 2008; Vallet *et al.*, 2013), which made it rather difficult to determine *a priori* the expected effect size in our study. In athletes, effects of dietary nitrate supplementation were found to be larger (~12-25% improvement) when looking at time to exhaustion at a fixed work rate (Bailey *et al.*, 2009; Lansley *et al.*, 2011) than when looking at improvements in time to complete a certain exercise (~0.5-2.0% improvement in time-trial performances). Since stamina of sows is believed to be critical for successful farrowing (Van Kempen, 2007), we expected that the effects of dietary nitrate supplementation on farrowing process would be comparable to the improvements seen in the increased time to exhaustion when nitrate was supplemented in athletes. The lack of effect of nitrate supplementation of farrowing duration might be due to the short total duration of farrowing on average compared to other studies also using hyper-prolific, confined sows, meaning there might have been little room for improvement. For instance, Hales *et al.* (2015) found a total duration of farrowing (i.e. time between the first and last born piglet, with 18.0 ± 0.47 total born piglets) of 462 min (95% CI: 381; 552), while the average duration of farrowing in the current study was only 236 ± 121 min (17.1 ± 3.4 total born piglets). It might also be that nutritional interventions aiming to shorten duration of farrowing only benefit sows of certain breeds, parities or under certain housing conditions. For example, Vallet *et al.* (2013) found an interaction between parity

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(gilt vs. sows) and the effect of maternal creatine supplementation on birth interval, when provided to the sows from day 110 of gestation until the moment of farrowing. Creatine, which has also received increasing attention to improve athlete performance in humans, is hypothesized to maintain ATP supplied during powerful anaerobic muscle activity, like farrowing (Persky and Brazeau, 2001; Brosnan and Brosnan, 2007). Another postulate to explain the lack of effects of maternal nitrate on birth interval might also be that only a certain proportion of the litter benefits from the effect of nutritional interventions. Although the hypothesized mode of action of creatine is different from that of nitrate, creatine reduced the birth interval of the last piglet born in the litter in sows, but increased birth interval of the last piglet in the litter for gilts (Vallet *et al.*, 2013). These findings suggest that maternal nutritional interventions during the perinatal period could be used to alter farrowing process in sows, but that the effects are dependent on other parameters, such as sow parity, piglet rank in the litter and hormonal levels.

The level of asphyxiation in new-born piglets was evaluated by collecting and analyzing blood from the umbilical cord of piglets within four minutes after birth. While overall $pO_2$ values were comparable, $pCO_2$ and lactate values were lower, and pH and $BE_{ecf}$ were higher in our study than in the study of Rootwelt *et al.* (2012) who sampled blood from the umbilical vein of piglets from hyper-prolific sows right after birth (14.9 ± 0.6 live born). Differences between studies might be caused by the inclusion of stillborn piglets (6.5%) in the study of Rootwelt *et al.* (2012)*,* whereas only life born piglets were sampled in our study. Although pH, $pCO_2$, $BE_{ecf}$ and $HCO_3$ levels in umbilical cord blood were not influenced by nitrate treatment, $pO_2$ tended ($P = 0.10$) to increase linearly with increasing level of maternal dietary nitrate. $pO_2$ is believed to increase by sevenfold in the umbilical cord artery after the onset of respiration , while $pCO_2$, pH and lactate values are believed to be more stable during the first four minutes after birth (Herpin *et al.*, 1996). It can be speculated that $pO_2$ levels were increased in piglets born from sows that received increasing levels of dietary nitrate, because of a shorter onset of respiration. This would be in line with the significant linear effect of maternal dietary nitrate supplementation on probability for a higher vitality score in piglets, since more vital piglets are more likely to start breathing sooner. Since duration of farrowing was not influenced by the

level of nitrate in the sow diets, it might be that $pO_2$ values in umbilical cord blood of new-born piglets tended to increase with increasing levels of maternal dietary nitrate supplementation, because either maternal blood $pO_2$ levels or placental blood flow to the piglets right before and during birth tended to increase. Nitric oxide, as a vasodilator of endothelial cells, plays an important role in placental blood flow and can, therefore, support an increase nutrient and oxygen transfer to the fetus (Bird *et al.*, 2003; Bailey *et al.*, 2012). This hypothesis can, however, not be confirmed or rejected in our study since maternal $pO_2$ levels during farrowing and umbilical cord blood flow were not determined. No relation was found between maternal dietary nitrate content and nitrate concentration in umbilical cord blood of piglets. Lack of this relation might be due to differences in time between the last meal of the mother and moment of farrowing. In addition, the potential variation in intensity of farrowing could influence the level of hypoxia or the intensity of "exercise", which might affect the conversion of nitrate into NO.

While placental length was not affected by treatment, placental width increased linearly with increasing levels of maternal dietary nitrate, the number of total born piglets was not affected by the dietary treatments. Since in general the number of ovulations is larger than the maximum number of fetuses in the uterus, it is assumed that the maximum uterine capacity is used for fetal development. During late gestation the bilayer in the placenta forms secondary folds increasing the interacting surface area between mother and fetus, which is hypothesized to be an alternative way to increase placental efficiency when lengthily surface expansion is limited due to crowding (Friess *et al.*, 1980; Vallet *et al.*, 2014). This might explain why only difference in placental width and not in placental length was observed in our study. Furthermore, it might also be that nitric oxide (NO), formed after degradation of nitrate, up regulated vascular endothelial growth factor (VEGF), which may have increased placental angiogenesis, thus leading to a wider placenta (Kimura and Esumi, 2003). It is striking that placental width was influenced by the dietary treatment after being on the diet for only 7 days. This is a relatively short time compared to studies investigating the effect of dietary supplementation with arginine to enhance reproductive performance of sows via placental development, in which sows were fed the dietary treatment for 11 to 24 days in early gestation, or for 38 to 92 days in mid-late gestation up

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to the moment of farrowing (as reviewed by Wu *et al.* (2013), (Che *et al.*, 2013; Quesnel *et al.*, 2014)). To our best knowledge, only one study investigated the effect of dietary arginine supplementation on placental weight of sows fed the diet during gestation up to the moment of farrowing (Gao *et al.*, 2012). In their study, Gao *et al.* (2012) found a significant 16% increase in total placental weight for all liveborn piglets when sows were fed 1% of L-arginine-HCl from day 22 until day 114 of gestation compared to an isonitrogenous control diet. The increase in total placental weight was most likely driven by the increase in the number of piglets born alive, since no difference was found on placental weight per live born piglet. Only placental length and width were measured in our study, and further research is needed to evaluate whether an increased placental size also increases placental blood flow and whether that correlates with an higher piglet vitality at birth. Baxter *et al.* (2008) found no significant difference ($P > 0.05$) in placental surface area between piglets that died and survived until weaning. Nevertheless, vitality score right after birth of piglets that survived was significantly higher than that of piglets that died (2.28 vs. 1.77; $P < 0.05$, for surviving and dying piglets respectively), indicating that placental surface area might not be a clear predictor for vitality at birth.

## Conclusions

Our study showed positive effects of maternal dietary nitrate supplementation in the perinatal period on piglet vitality right after birth, but these effects were not driven by a decrease in duration of farrowing. Our results suggest that maternal nitrate supplementation affects placental characteristics, which may lead to an increased oxygen and nutrient flow to the fetuses. However, since vasodilation was not measured in our study, the hypothesis that nitrate supplementation leads to vasodilation of blood vessels in the placenta via NO formation cannot be accepted nor rejected. Our findings, together with the limited amount of research done on maternal nutritional interventions during the perinatal period, support the need for further studies to explore the potential for maternal dietary nitrate to increase piglet vitality at birth and, eventually, reduce stillbirth and mortality in new-born piglets.

## Conflict of Interest

## Acknowledgments

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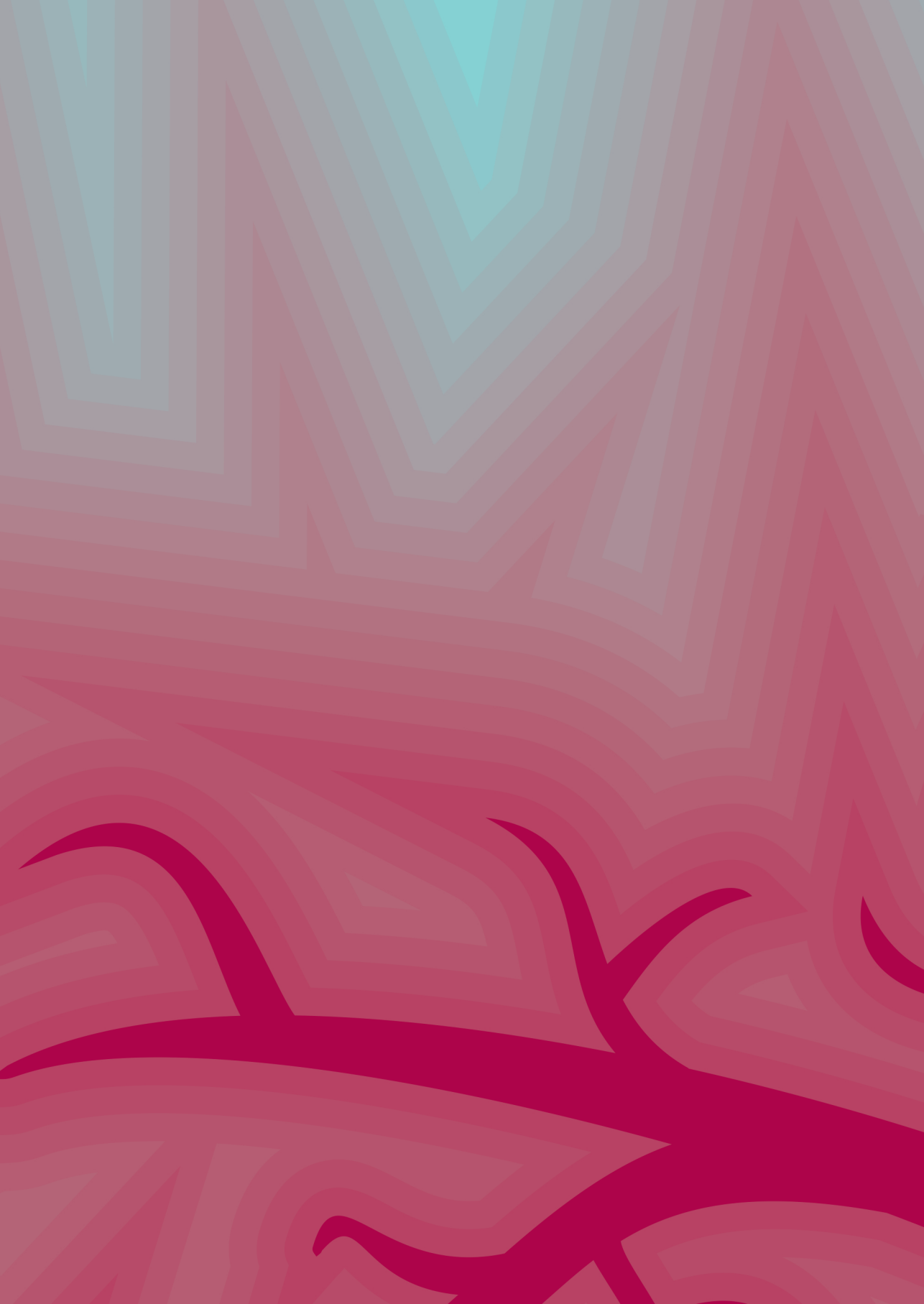
# Chapter 6

## Disentangling litter size and farrowing duration effects on piglet stillbirth, acid-base blood parameters and pre-weaning mortality

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## Abstract

The current study evaluated interactions between farrowing duration and litter size on the level of asphyxia, vitality, percentage of stillbirth and pre-weaning mortality of piglets. Farrowing duration was measured in 159 crossbred gilts and sows (Yorkshire x Dutch Landrace). Litter size ranged between 12 and 21 piglets. Blood acid-base parameters in umbilical cord blood and vitality scores of piglets were determined immediately after birth. Number of piglets born alive and stillborn as well as individual piglet weights at birth were recorded. Pre-weaning mortality (excl. stillbirth) was determined throughout lactation. Litter size as well as farrowing duration were categorized to evaluate the interaction between the two. There tended to be an interaction between litter size and farrowing duration for pre-weaning mortality ($P = 0.10$). In small litters (12-15 piglets), a prolonged farrowing duration (> 250 min) tended to increase pre-weaning mortality compared to a short (< 150 min) and medium farrowing duration (150-250 min), while for large litters (19-21 piglets) a medium to long farrowing duration tended to decrease pre-weaning mortality. No other interactions between litter size and farrowing duration were found. Piglets within large litters showed a higher umbilical cord lactate level ($P < 0.01$), lower average vitality score ($P = 0.01$) and a higher stillborn percentage ($P < 0.01$) compared to piglets within medium size (16-18 piglets) and small litters. Each additional piglet born to a litter linearly decreased average piglet birth weight (17.6 g, $P < 0.01$), increased farrowing duration (11 min, $P < 0.01$) and increased stillbirth (0.5%, $P = 0.04$). A medium farrowing duration resulted in a lower stillborn percentage compared to a short or prolonged farrowing duration, suggesting that farrowing duration might have an optimum. When analyzed linearly, stillborn percentage increased with 1.85% per every 100 min ($P < 0.01$) of farrowing duration. It can be concluded that both litter size and farrowing duration affect stillborn percentage, but independent from each other. However, these two factors tended to interact regarding pre-weaning mortality, suggesting that setting a certain threshold for maximal farrowing duration should be taken with care, because this appears to depend on litter size.

**Key words:** farrowing duration, litter size, stillbirth, pre-weaning mortality, piglets, acid-base blood parameters.

## Introduction

Larger litter sizes in pigs are often accompanied by a higher incidence of stillbirth (Hanenberg *et al.*, 2001; Canario *et al.*, 2006; Distl, 2007; Rosendo *et al.*, 2007) with prolonged farrowing duration being a key driver for this (van Rens and van der Lende, 2004). A longer farrowing duration has been associated with a higher risk of hypoxia in piglets (Herpin *et al.*, 2001), due to succesive uterine contractions, reduction of utero-placental blood flow (Curtis, 1974) or loss of the umbilical cord functionality (Randall, 1971; Christianson, 1992). Depending on the severity, hypoxia may lead to stillbirth or a lower piglet vitality, potentially leading to pre-weaning mortality (Rootwelt *et al.*, 2013). Several studies have suggested that farrowing duration should not exceed a certain treshold level. For example, Oliviero *et al.* (2010) suggested that farrowing duration should not take longer than 300 min, since incidence of stillbirth increased from 0.4 to 1.5 stillborn piglets in litters with a farrowing duration below or above the threshold level of 300 min. Langendijk *et al*. (2018b) showed that stillbirth incidence increased exponentially when farrowing duration took more than 240 min. Stillbith incidence was 2.7%, 6.9%, 10.7%, 13.4% and 27.3% when farrowing duration was less then 120 min, 120-240 min, 240-360 min, 360-480 min or more than 480 min, respectively. They suggested to intervene in the farrowing process from a farrowing duration of 240 min onwards. Interestingly, the average litter size in the study of Oliviero *et al.* (2010) and Langendijk *et al.* (2018b) differed considerably (12.7±3.0 and 15.3±0.5 piglets born total, respectively), whereas the suggested threshold level showed the opposite effect. This suggests that other factors (e.g. breed, sow body condition, average parity of the herd, stress, but also environmental factors like supervised farrowing, the use hormones, climate etc.) can affect the threshold level above which farrowing duration might have negative effects on piglet suvival rates. One of the interfering factors might be litter size. Farrowing duration is positively related to litter size (van Dijk *et al.*, 2005), which might suggest that optimal duration of farrowing depends on litter size. Consequently, it can be speculated that for medium (≥16 total born) or large litters (≥19 total born), prolonged farrowing duration is less detrimental than for smaller litters (12-15 piglets total born), since it simply takes more time to farrow more piglets. The aim of this research was to

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disentangle effects of litter size at birth and farrowing duration on level of hypoxia and incidence of stillbirth and pre-weaning mortality.

## Material and Methods

### Animals

In total, 190 gilts and sows (Yorkshire x Dutch Landrace, Topigs Norsvin) of parity 1 to 9, in 8 consecutive batches were used in this study. The study was performed at the Swine Innovation Centre Sterksel of Wageningen University & Research, The Netherlands. Per batch, 4 farrowing units were used, each containing 12 farrowing pens. Animals entered the farrowing room approximately 7 days before the expected farrowing date (i.e. d 115 after insemination) and were placed in individual farrowing crates (pen size 180 x 240 cm, crate size of 55 x 185 cm). No nesting material was provided. Data of sows used in the current study were obtained during a feeding experiment, evaluating effects of supplementing 0,00%, 0.03%, 0.06%, 0.09%, 0.12% and 0.15% of nitrate in the perinatal period on piglet survival as described by Van den Bosch *et al.* (van den Bosch *et al.*, 2019a; van den Bosch *et al.*, 2019b). Lactation diets were provided twice daily (7.30h and 16.30h) from the moment sows entered the farrowing room (d 108 ± 1 of gestation) until weaning (d 27.2 ± 1.7 post-partum). Sows had ad libitum access to water. Each pen had a piglet nest with a heating lamp set at 30ºC. During farrowing, supervision was present for 24 h a day, but it was not allowed to use any intervention during farrowing or interfere with piglet survival after birth by e.g. saving them from crushing or placing them at the udder or in the piglet nest. Farrowing was not induced and sows which received birth assistance or medication during farrowing were excluded from the experiment. Cross-fostering took place between sows that farrowed on the same day and only between 24 and 48 h after birth. Litters were standardized aiming for 15 piglets per sow. Piglets that were fostered on or off the sow were selected randomly. Number of dead piglets, reason for death (e.g., crushing, splay legs, starvation, lameness, weak, low birth weight, and unknown as scored by farm staff), and weight of dead piglets were registered on a daily basis. Pre-weaning

mortality excluded stillborn piglets. Pre-weaning mortality was calculated by the following equation:

$Pre\text{-}weaning\ mortality =$

$$\left( \frac{Number\ of\ pre\text{-}weaning\ deaths\ (excl.TSB)}{TBA + number\ of\ piglets\ added - number\ of\ piglets\ removed\ at\ cross\ fostering} \right) \times 100\%$$

Piglets received a commercially available pre-starter from 3 days of age until weaning (17.4% CP, 11.6 MJ NE/kg; Top Wean, Agrifirm, Apeldoorn, the Netherlands).

## Measurements

Within three minutes after birth, a mixed blood sample (i.e. from the vein and/or artery) was taken from the umbilical cord (intact or ruptured) from two randomly chosen live born piglets out of every four subsequent piglets born. A 2.5 mL syringe with a 16 mm 21 G needle and sodium oxalate as an anticoagulant was used. Blood acid-base parameters were analysed within one minute after collection, using the iStat® portable Clinical Analyser (iStat Europe, Birmingham, United Kingdom) and CG8+ cartridges. Piglets were not handled during sampling to prevent stress. After blood sampling, piglets were tagged to record their birth order. Remaining blood was collected in a 4 mL BD vacutainer® (fluor heparin tube) and stored on ice before being centrifuged at 3000 rpm at 4°C for 10 minutes. Blood plasma was decanted and stored at -20°C for further analysis. Lactate concentration in plasma was determined, using an enzymatic UV test with lactate dehydrogenase with reagents of DiaSys Diagnostic Systems GmbH (Holzheim, Germany).

A digital video recorder (Samsung SRD470DP) connected to cameras (Velleman – CCD colour cameras, Gavere, Belgium) was used to record the farrowing process of individual sows. Video recordings were analysed, using the Observer XT 10 software package (Noldus Information Technology B.V., Wageningen, the Netherlands). Video material of 6 sows could not be analysed due to limited visibility. Individual vitality of new-born piglets was scored from video during the first 30 seconds after birth, using the scoring method as described by Baxter *et al.* (2008):

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0 = No movement, no breathing.

1 = No movement, but piglet is breathing or trying to breathe (coughing and spluttering).

2 = Piglet is moving breathing or trying to breathe.

3 = Piglet is moving and breathing well and makes a first attempt to stand.

Total number of piglets born (excluding mummified and degenerating piglets), number of piglets born alive and number of stillborn piglets were recorded after farrowing was completed. A stillborn piglet was defined as a piglet born without any respiration, but potentially with a heartbeat. A flotation test was performed to determine whether a piglet was a true stillborn. Approximately 2 cm$^2$ of lung tissue was removed after dissection on the day of birth and placed in a bowl of water (Leenhouwers *et al.*, 1999). When lung tissue floated, the piglet was scored as a pre-weaning death instead of a stillborn. Both alive and stillborn piglets were individually weighed and numbered within 24 h after birth. Pre-weaning piglet mortality and weight of dead piglets were registered on a daily basis.

## Statistical analyses

In total, data of 6 sows were removed from the dataset due to receiving birth assistance (n=3), sickness or death of the sow (n=2) or receiving treatment for aggressive behavior during farrowing (n=1). Sows that had a total litter size of less than 12 piglets (n=11) or more than 21 piglets (n=14) were excluded from the analyses due to limited number of sows per litter size. The final dataset contained data of 159 litters. Residual plots were used to check model assumption (e.g. normality and equal variance of the error terms). Total duration of farrowing, which was defined as the time between the birth of the first and the last piglet in a litter, was non-normally distributed and data was transformed by using a base ten logarithm. Total stillborn piglets (TSB) was found to be non-normally distributed even after transformation, and was expressed as a percentage of total number born (TNB). Pre-weaning mortality was analysed as a probability of the total number born alive (TBA).

To disentangle effects of litter size and farrowing duration or to test whether or not litter size and farrowing duration interact on piglet characteristics, litter size and farrowing duration were both categorized into 3 classes. Litter sizes of 12 to 15 piglets were classified as class 1 (n = 41), 16 to 18 piglets as class 2 (n = 71) and 19 to 21 piglets as class 3 (n = 47). Duration of farrowing was classified as less than 150 min (short; n = 59), 150 to 250 min (medium; n = 57) and over 250 min (long; n = 43). The GLIMMIX procedure in SAS (Version 9.3, 2011, SAS Institute Inc., Cary, NC, United States) was used with the following model;

$$Y_{ijklmno} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + c_k + d_l + f_m + g_n + \varepsilon_{ijklmno}$$

Where $Y_{ijklmno}$= dependent variable, $\mu$ = overall mean, $\alpha_i$ = fixed effect of litter size class ($i$ = 1, 2 or 3), $\beta_j$ = fixed effect of farrowing duration class ($j$ = 1, 2 or 3), $\alpha\beta_{ij}$ = the interaction between litter size class and farrowing duration class, $c_k$ = random parity class effect ($k$ = 1, 2 or 3; parity 1: class 1, parity 2, 3 and 4: class 2 and parity >4: class 3), $d_l$ = random farrowing unit effect ($l$ = 1, 2, .., 4), $f_m$ = random feeding treatment effect ($m$ = 1, 2, ..,6), $g_n$ = random batch effect ($n$ = 1, 2, .., 8) and $\varepsilon_{ijklmno}$ = residual error term. Sow was considered as the experimental unit.

Besides the cathegorized effect, also the fixed (reported as P$_{LS}$) and linear effects of litter size (reported as P$_{LS\ Lin}$) were assesed. Variables were subjected to the following statistical model, using a PROC GLIMMIX:

$$Y_{ijklmn} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + c_k + d_l + f_m + \varepsilon_{ijklmn}$$

Where $Y_{ijklmn}$ = dependent variable, $\mu$ = overall mean, $\alpha_i$ = fixed effect of litter size ($i$ = 12, 13, .., 21), $\beta_j$ = fixed effect of farrowing duration, $\alpha\beta_{ij}$ = interaction between litter size and farrowing duration, $c_k$ = random batch effect ($k$ = 1, 2, .., 8), $d_l$ = random parity class effect ($l$ = 1, 2 or 3; parity 1: class 1, parity 2, 3 and 4: class 2 and parity >4: class 3), $f_m$ = random feeding treatment effect ($m$ = 1, 2, .., 6) and $\varepsilon_{ijklmn}$ = residual error term. Sow was considered as the experimental unit. Additionally, the linear effect of litter size with other variables were assed by using contrasts. For vitality score, the observer was added to the model as a random effect ($n$ = 1, 2 or 3).

To access linear effects of farrowing duration (reported as P$_{FD}$), variables were subjected to the following statistical model, using a PROC GLIMMIX:

$$Y_{ijklmn} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + c_k + d_l + f_m + \varepsilon_{ijklmn}$$

Where $Y_{ijklmn}$ = dependent variable, $\mu$ = overall mean, $\alpha_i$ = linear effect of farrowing duration, $\beta_j$ = fixed effect of litter size ($j$ = 12, 13, .., 21), $\alpha\beta_{ij}$ = interaction between farrowing duration and litter size, $c_k$ = random batch effect ($k$ = 1, 2, .., 8), $d_l$ = random parity class effect ($l$ = 1, 2 or 3; parity 1: class 1, parity 2, 3 and 4: class 2 and parity >4: class 3), $f_m$ = random feeding treatment effect ($m$ = 1, 2, .., 6) and $\varepsilon_{ijklmn}$ = residual error term. Sow was considered as the experimental unit. For vitality score, the observer was added to the model as a random effect ($n$ = 1, 2 or 3). Preliminary analysis in model 2 and 3 demonstrated a lack of effect of the interaction between litter size and farrowing duration. Concequently, results will be expressed per main effect.

For all models and analyses, differences were considered to be significant at $P \leq 0.05$ and $0.05 < P \leq 0.10$ as a tendency. Data is expressed as LSMeans ± SEM or as regression coefficients ($\beta$).

## Results

The average TNB was 17.1 ± 3.4 (mean ± SD) piglets per litter, with 16.1 ± 3.1 live born and 1.0 ± 1.4 stillborn (5.8%). It took sows on average 236 ± 121 min (range 65 - 515 min) to complete farrowing. In total, 818 piglets from 109 litters were blood sampled via the umbilical cord, from which 529 samplings harvested enough blood to do both blood gas analyses and lactate analyses, 172 samplings harvested sufficient blood for blood gas analyses only, and 117 samplings were only used to harvest plasma for lactate analyses.

### Litter size class and farrowing duration class interaction

There was no interaction between litter size class (LSC; small: 12-15 piglets, medium: 16-18 piglets and large litters: 19-21 piglets) and farrowing duration class (FDC; short: <150 min, medium: 150-250 min and long: >250 min) on umbilical cord blood gasses,
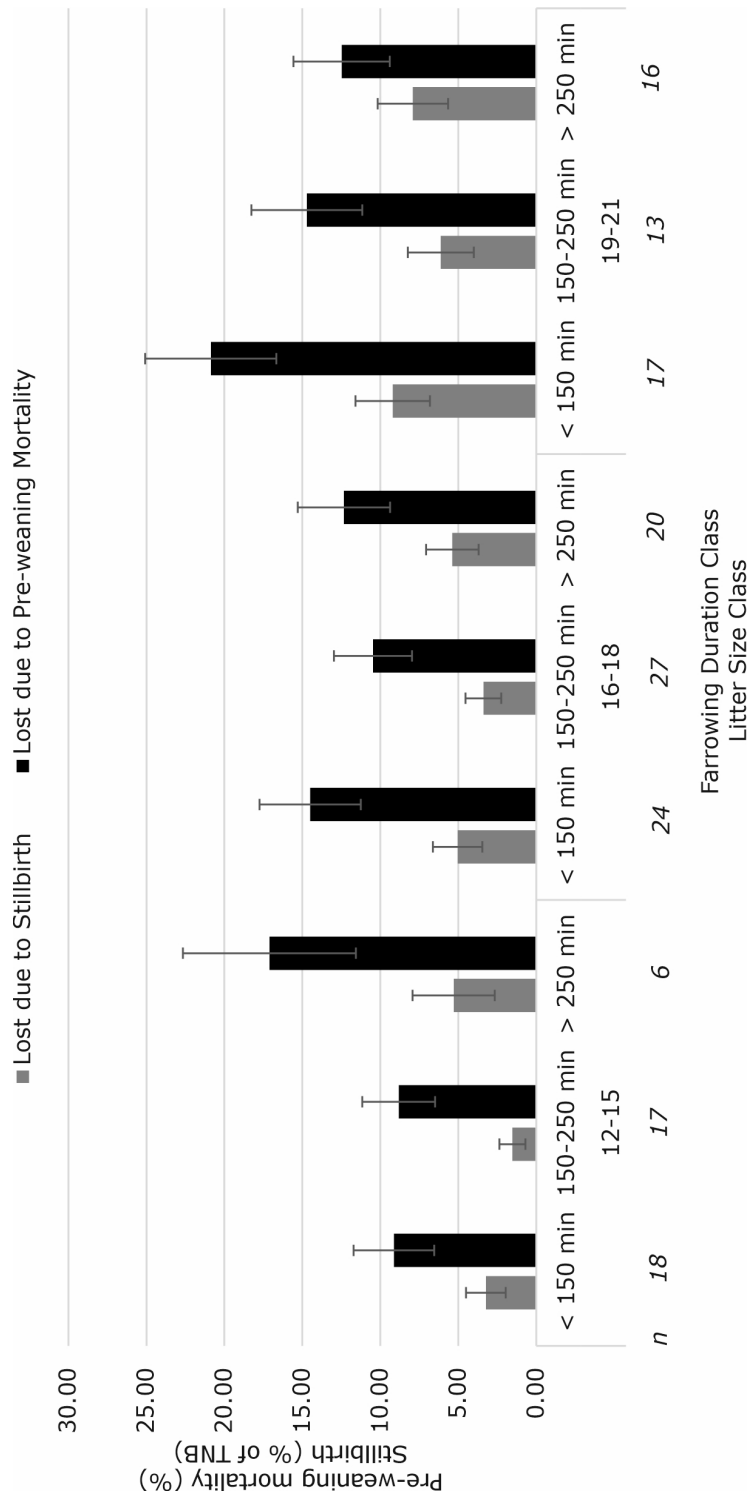
**Figure 1.** Interaction between farrowing duration (classified) and litter size (classified) on percentage of stillbirth ($P = 0.77$) and pre-weaning mortality ($P = 0.10$) (LSMeans±SEM). n = number of litters per category.

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vitality score (data not shown) and percentage of stillborn piglets (Figure 1). A tendency ($P$ = 0.10, Figure 1) for an interaction between LSC and FDC was found on percentage of pre-weaning mortality. For small litters, a long duration of farrowing tended to increase pre-weaning mortality compared to a short and medium farrowing duration, while for large litters, a medium to long duration of farrowing tended to decrease pre-weaning mortality. For medium litter sizes, duration of farrowing did not affect incidence of pre-weaning mortality.

Because no other interactions were found between LSC and FDC, main effects are presented separately (Table 1 and 2). Table 1 shows the main effects of level of hypoxia and piglet characteristics per LSC. Large litters showed a significantly higher lactate level ($P$ < 0.01), lower average vitality score ($P$ = 0.01) and a higher percentage of stillborn piglets ($P$ < 0.01) compared to medium and small litters. Other umbilical cord blood gasses were not different between litter size classes.

**Table 1.** Effects of litter size class (total born) on umbilical cord blood parameters immediately after birth, vitality of piglets, incidence of stillbirth and pre-weaning mortality (LSMeans±SEM).

| | Litter size class | | | SEM | P-value |
|---|---|---|---|---|---|
| | 12-15 | 16-18 | 19-21 | | |
| N (litters) | 41 | 71 | 47 | | |
| Average parity | 2.8 | 3.3 | 4.4 | | |
| **Umbilical cord blood parameters** | | | | | |
| pH | 7.52 | 7.51 | 7.51 | 0.02 | 0.79 |
| $pCO_2$ (mmHg) | 34.9 | 35.3 | 34.5 | 1.32 | 0.75 |
| $pO_2$ (mmHg) | 35.1 | 35.6 | 35 | 1.18 | 0.9 |
| $BE_{ecf}$ (mmol/L) | 4.73 | 4.43 | 3.78 | 0.69 | 0.49 |
| $HCO_3$ (mmol/L) | 27.5 | 27.1 | 26.7 | 0.61 | 0.58 |
| $sO_2$ (%) | 67.8 | 68.8 | 66.8 | 2.09 | 0.58 |
| Lactate (mmol/L)[1] | 3.87[b] | 4.12[b] | 4.89[a] | 0.32 | < 0.01 |
| Average vitality score[2] | 2.29[a] | 2.16[ab] | 2.09[b] | 0.07 | 0.01 |
| Stillbirth (% of total born) | 2.98[b] | 4.52[b] | 7.65[a] | 1.83 | <0.01 |
| Pre-weaning mortality (%) | 11.2 | 12.3 | 15.7 | 3.1 | 0.07 |

[1] Parameter were normalized by using a base ten logarithm transformation. LSMeans were back transformed to original scale. Pooled SEM is still reported as transformed value.
[2] Individual vitality of new-born piglets was scored during the first 30 seconds after birth by using the scoring method as described by Baxter *et al.* (2008)
[ab] Different superscripts represent a significant difference between litter size classes.

Main effects of FDC on level of hypoxia as well as piglet vitality, stillbirth and pre-weaning mortality are shown in Table 2. Piglets born from sows with a short farrowing duration showed a similar partial oxygen pressure in umbilical cord blood compared to piglets born from sows with a long duration of farrowing, but a higher level than piglets born from sows with a medium duration of farrowing. In addition, piglets born from sows with a short duration of farrowing tended to show or showed the lowest acid-base balance ($BE_{ecf}$, $P$ = 0.06) and bicarbonate concentration ($HCO_3$, $P$ = 0.02) in umbilical cord blood compared to piglets born when farrowing duration was medium or long. Oxygen saturation level ($sO_2$) tended to be higher ($P$ = 0.07) in piglets born from sows with a short duration of farrowing compared to a medium or long duration of farrowing. Vitality score was not different between farrowing duration classes, but percentage of stillborn piglets tended to be higher ($P$ = 0.06) in piglets born from sows with a medium duration of farrowing than in piglets born from sows in both other farrowing duration classes.

**Table 2.** Effects of farrowing duration class on umbilical cord blood parameters immediately after birth, vitality of piglets, incidence of stillbirth and pre-weaning mortality (LSMeans±SEM).

| | Farrowing duration class (min) | | | SEM | P-value |
| | <150 | 150-250 | >250 | | |
|---|---|---|---|---|---|
| N (litters) | 59 | 57 | 43 | | |
| Average parity | 3.3 | 3.2 | 4.0 | | |
| **Umbilical cord blood parameters** | | | | | |
| pH | 7.51 | 7.52 | 7.51 | 0.02 | 0.57 |
| $pCO_2$ (mmHg) | 34.40 | 35.40 | 34.90 | 1.38 | 0.63 |
| $pO_2$ (mmHg) | 37.2a | 33.8b | 34.7ab | 1.23 | 0.05 |
| $BE_{ecf}$ (mmol/L) | 3.23 | 4.67 | 5.04 | 0.71 | 0.06 |
| $HCO_3$ (mmol/L) | 26.1b | 27.6a | 27.7a | 0.65 | 0.02 |
| $sO_2$ (%) | 70.20 | 65.8 | 67.4 | 2.23 | 0.07 |
| Lactate (mmol/L)[1] | 4.18 | 4.33 | 4.37 | 0.32 | 0.74 |
| Average vitality score | 2.14 | 2.16 | 2.23 | 0.06 | 0.37 |
| Stillbirth (% of total born) | 5.35 | 3.19 | 6.10 | 1.66 | 0.06 |
| Pre-weaning mortality (%) | 14.2 | 11.1 | 13.8 | 2.94 | 0.14 |

[1] Parameter were normalized by using a base ten logarithm transformation. LSMeans were back transformed to original scale. Pooled SEM is still reported as transformed value.
[2] Individual vitality of new-born piglets was scored during the first 30 seconds after birth by using the scoring method as described by Baxter *et al.* (2008).
[ab] Different superscripts represent a difference ($P \leq 0.05$) between farrowing duration classes.

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## Linear effects of litter size

Figure 2A shows the absolute number of piglets born total, born alive, stillborn, died before weaning and number of piglets weaned when litter size increased from 12 to 21 piglets. For each additional piglet born to the litter the percentage of stillbirth increased linearly by 0.5% ($P_{Lin}$ = 0.04, Figure 2B). Pre-weaning mortality increased by 1.1% for each additional piglet born to the litter ($P_{Lin}$ < 0.01, Figure 1B). Average birth weight of piglets decreased by 17.6 g per piglet with each additional piglet born to the litter ($P_{Lin}$ < 0.01). The variation in birth weight within a litter, expressed as SD was not related to litter size. Farrowing duration increased linearly with litter size (10.7 min per extra piglet between 12 and 21 piglets, Figure 3, $P_{Lin}$ < 0.01). Average vitality of piglets within a litter decreased linearly (Figure 4, $P_{Lin}$ = 0.03) by 0.02 per piglet when litter size increased from 12 to 21 piglets.
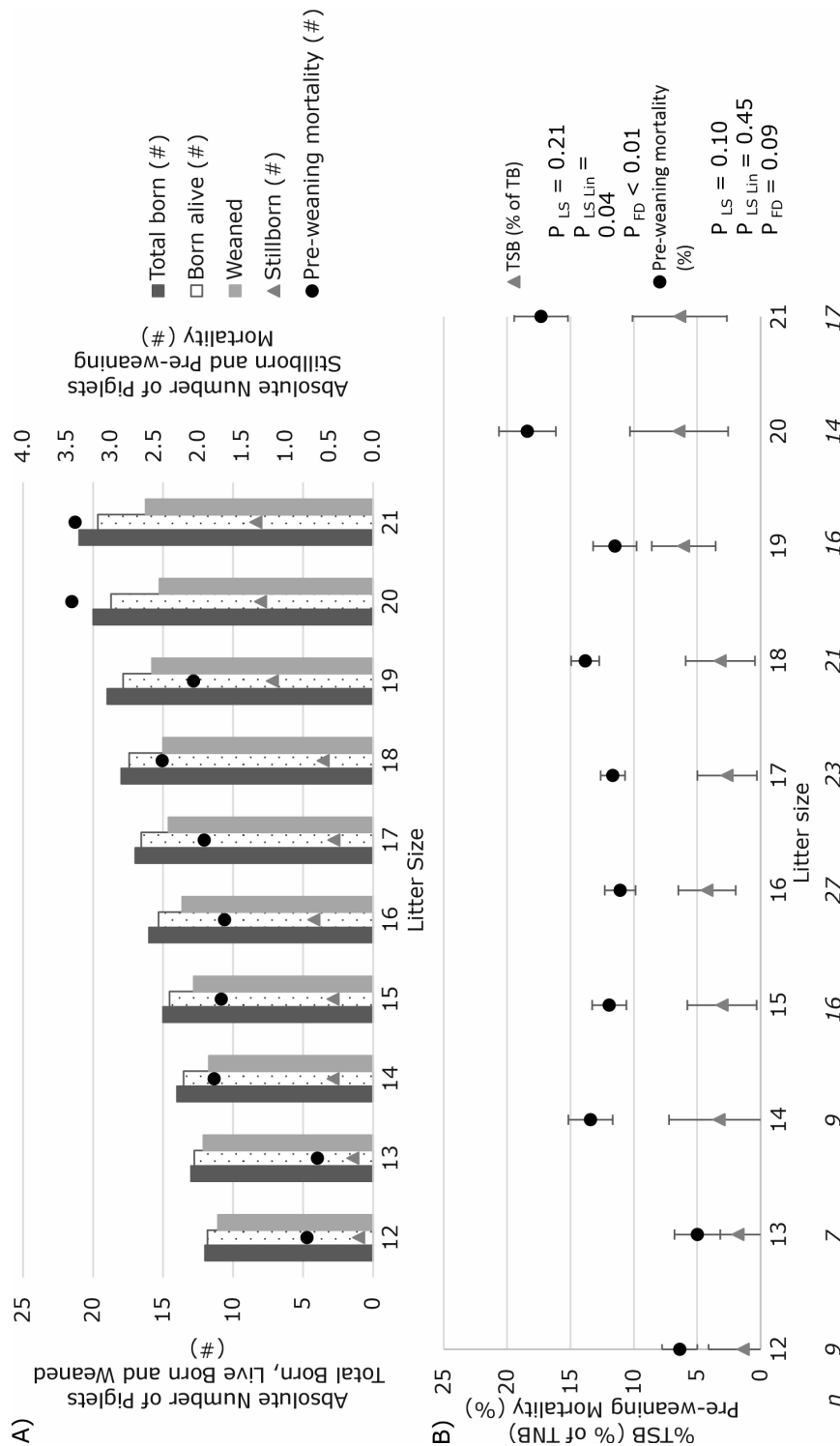
**Figure 2.** Absolute number of piglets born total, born alive, stillborn, lost due to pre-weaning mortality and weaned per litter size (A) and incidence of stillbirth (▲) and pre-weaning mortality (●) per litter size (LSMeans ± SEM) (B). Analyses performed on individual litters. $P_{LS}$ is the P value for the fixed effect of litter size. $P_{LS\ Lin}$ is the P value for the linear effect of litter size and $P_{FD}$ is the effect of farrowing duration class. $n$ = number of litters per litter size.
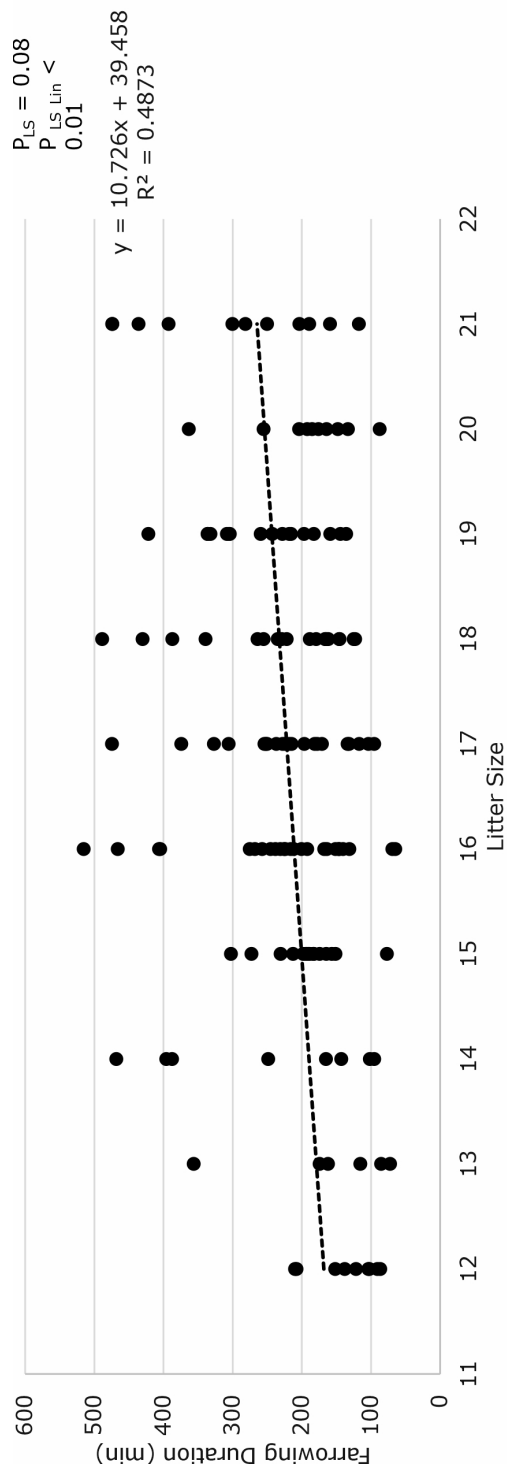
**Figure 3.** Relationship between litter size and farrowing duration. Total duration of farrowing was defined as the time between the birth of the first and the last piglet in a litter. $P_{LS}$ is the P value for the fixed effect of litter size. $P_{LS\ Lin}$ is the P value for the linear effect of litter size.
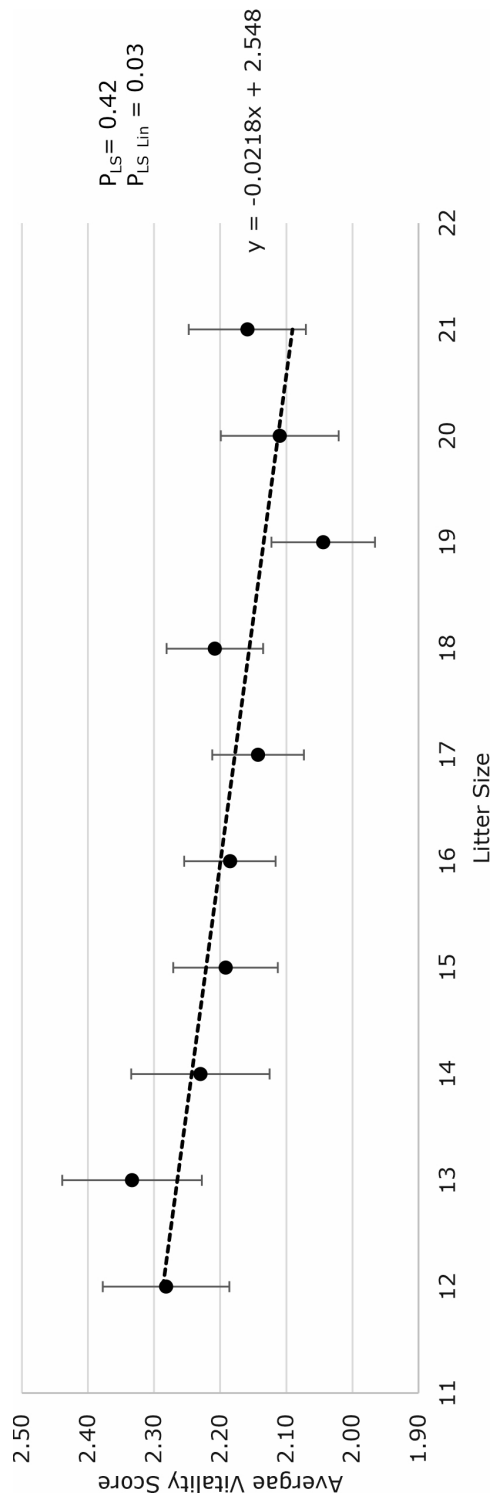
**Figure 4.** Relationship between litter size and average piglet vitality score conducted within 30 seconds after birth, using the scoring method as described by Baxter *et al.* (2008) (LSMeans±SEM). Analysis was performed on individual litters. $P_{LS}$ is the P value for the fixed effect of litter size. $P_{LS\,Lin}$ is the P value for the linear effect of litter size.

## Linear effect of farrowing duration

Figures 5 and 6 show the relationship between farrowing duration and incidence of stillbirth and pre-weaning mortality. Incidence of stillbirth increased by 1.85% per 100 min of farrowing duration ($P_{FD}$ < 0.01) and pre-weaning mortality increased by 0.46% per 100 min of farrowing duration ($P_{FD}$ < 0.01).
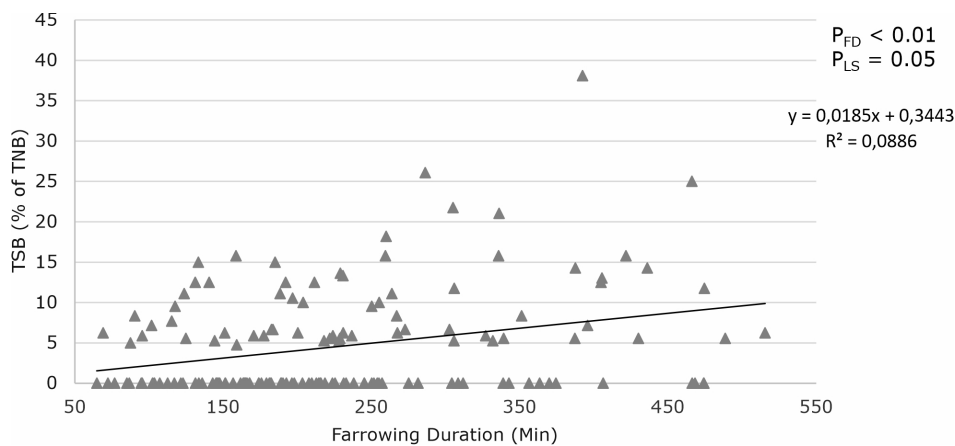


**Figure 5.** Relationship between farrowing duration and incidence of stillbirth (total still born as percentage of total born). $P_{FD}$ is the P value for the effect of farrowing duration. $P_{LS}$ is the P value for the fixed effect of litter size.
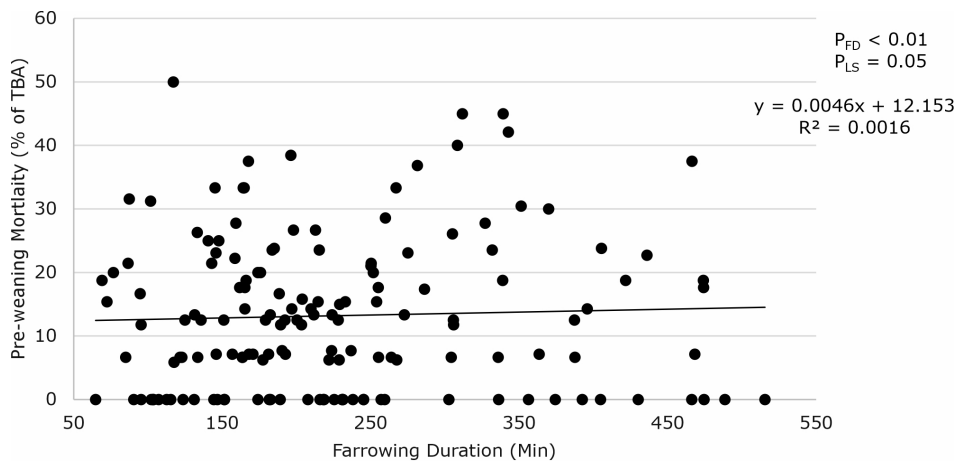


**Figure 6.** Relationship between farrowing duration and pre-weaning mortality (piglets lost as percentage of total born alive). $P_{FD}$ is the P value for the effect of farrowing duration. $P_{LS}$ is the P value for the effect of litter size.

## Discussion

Larger litter sizes are associated with a higher percentage of stillborn piglets (Zaleski and Hacker, 1993; van Dijk *et al.*, 2005) and pre-weaning mortality rate (Roehe and Kalm, 2000; Lund *et al.*, 2002; Rutherford *et al.*, 2013). Larger litter sizes are also associated with longer farrowing duration (van Rens and van der Lende, 2004). Because of this entangling of litter size and farrowing duration, it is unclear what the impact of litter size as such is on stillbirth or pre-weaning mortality or what the impact of a prolonged farrowing duration for larger litters is for these losses. This study therefore evaluated effects of litter size, farrowing duration and their interaction on the level of asphyxia, piglet vitality and percentage of stillbirth and pre-weaning mortality (excl. stillbirth) within a litter. Litter size class had a strong significant effect ($P \leq 0.01$) on lactate levels in umbilical cord blood, average vitality score and stillbirth percentage, all increasing as litter size class got larger. For farrowing duration class effects were more moderate ($p \geq 0.01$ or trends) and most favorable levels were mostly observed in piglets born from sows with a medium farrowing duration. This suggests that the effect of litter size on umbilical cord blood gasses, vitality and stillborn percentage is stronger than the effect of farrowing duration.

Data leveraged in the current study originate from a study evaluating a dose response of maternal nitrate supplementation (as described in (van den Bosch *et al.*, 2019a; van den Bosch *et al.*, 2019b)) in which no effect of treatment was found on duration of farrowing (van den Bosch *et al.*, 2019b), litter size and incidence of stillbirth (van den Bosch *et al.*, 2019a). There tended to be a quadratic effect of dosage of nitrate supplementation on pre-weaning mortality, however, all dietary treatments were distributed approximately equally across all farrowing durations.

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### Litter size class and farrowing duration class interaction

No interaction was found between LSC and FDC on umbilical cord blood parameters, piglet vitality score and percentage of stillborn piglets. A tendency for an interaction between LSC and FDC was found on percentage of pre-weaning mortality. Litters of 12 to 15 piglets showed the lowest incidence of pre-weaning mortality when farrowing duration did not

exceed 250 min. In litters of 16 to 18 piglets, no effect of farrowing duration was found on percentage of pre-weaning mortality, but in litters of 19 to 21 piglets, it appeared to be beneficial to have a longer farrowing duration, since pre-weaning mortality rate tended to decrease as farrowing duration increased. These results suggest that, looking at pre-weaning mortality, the optimal farrowing duration might differ per LSC. Strikingly, the same interaction was not seen in relation to stillbirth incidence, which was expected to be more directly linked to farrowing duration than pre-weaning mortality. However, it has to be mentioned that cross-fostering was applied and therefore pre-weaning mortality numbers include piglets from the birth litter as well as piglets fostered onto a sow. Piglets were not cross fostered solely to other sows within the same farrowing duration group. English and Wilkinson (1982) showed that piglets that died before three weeks of age had higher blood lactate concentrations at birth than piglets that survived (383.3 vs. 303.0 µg lactate/ml blood for piglets that died and survivors, respectively; $P < 0.01$), showing that level of asphyxiation at birth appears to be related to pre-weaning mortality rate. Asphyxia at birth impacts the time to reach the udder and the quantity of colostrum ingested (Langendijk *et al.*, 2018a), but also, likely linked to that, asphyxia is linked to lower body temperatures and consequently higher risks of death due to hypothermia (Herpin *et al.*, 1999; Orozco-Gregorio *et al.*, 2008; Alexopoulos *et al.*, 2018). However, in the current study, vitality scores and umbilical cord acid base blood parameters did not reflect the effect found on pre-weaning mortality. Although both piglet vitality score (Baxter *et al.*, 2008) as well as umbilical cord acid base blood parameters at birth (English and Wilkinson, 1982) have been linked pre-weaning mortality percentage other indicators, like time to reach the udder, colostrum intake or body temperatures (which were not evaluated in this study), may have reflected the effect found on pre-weaning mortality.

Average duration of farrowing of sows in the current study was short (average 236±121 min with an average litter size of 17.1±3.4 piglets) compared to other studies. The highest FDC contained sows exceeding 250 min of farrowing time, which is below the formerly indicated threshold level of 300 min, after which the risk for stillbirth increased (in a herd with an average litter size of 12.7 piglets) (Oliviero *et al.*, 2010). Level of asphyxia and incidence of stillbirth increases with birth order (English and Wilkinson, 1982; Herpin

*et al.*, 1996) and average blood $pCO_2$ increases with litter size (Herpin *et al.*, 1996), which might explain why larger litters show a higher incidence of asphyxia and stillbirth. However, Langendijk *et al.* (2018a) reported the risk of being a stillborn for piglets with birth order 13 or up was 9% when farrowing duration took less than 280 min and 23% when farrowing duration exceeded 280 min. This suggests that total farrowing duration seems to surpass the effect of birth order. It can be speculated that that the main driver for piglet losses is the power of and frequency of uterine contractions rather than just the duration of farrowing. Uterine contractions decrease blood flow of the uterus and gaseous exchange through the placenta (Tucker and Hauth, 1990). A short duration of farrowing with powerful contractions will likely increase intrapartum death, which is also demonstrated in studies evaluating the effect of oxytocin treatment (Mota-Rojas *et al.*, 2002; Alonso-Spilsbury *et al.*, 2004). Although it is likely that polytocous species experience an increased number of uterine contractions during parturition compared to monotocous species (Senger, 2003), it is unclear whether or not power and frequency of contractions during parturition is different between sows with different litter sizes. However, it is known that uterine blood flow per fetus decreases when litter size increases (Pere and Etienne, 2000), likely caused by a smaller placenta (Baxter *et al.*, 2008; Rootwelt *et al.*, 2013). Consequently, it can be speculated that the effect of powerful contractions (as hypothesized to be the case during short duration of farrowing and high oxytocin levels) might be more detrimental in larger litters when piglets are already subjected to a lower blood flow compared to smaller litters. This could explain the trend for a higher incidence of pre-weaning mortality in larger litters with a short duration of farrowing.

**Litter size**

LSC significantly influenced incidence of stillbirth, umbilical cord lactate levels and average vitality score of the litter. Successive uterine contractions can lead to a repetitive obstructed blood flow to the fetus, causing a more anaerobic metabolism, which is represented in umbilical cord lactate levels (van Dijk *et al.*, 2008; Langendijk and Plush, 2019). Perinatal asphyxia has been related to a lower postnatal vitality and higher postnatal mortality until 10 days of age (Herpin *et al.*, 1996), which might be caused by altered expression

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patterns of stress related proteins in the brain, heart and intestines (Trujillo-Ortega *et al.*, 2007). The significant decrease in average vitality score and the trend for a higher pre-weaning mortality percentage in the higher LSC found in the current study are aligned with this hypothesis. In addition, studies have shown an increase in umbilical cord blood pH as birth order increases (Herpin *et al.*, 1996; van Dijk *et al.*, 2008), which is related to a higher average umbilical cord lactate levels of litters in higher litter size classes as measured in the current study. When analyzed linearly, the increase in litter size was, in our study, related to an increase in farrowing duration. Farrowing duration increased by 10.7 min per piglet additionally born to the litter. Combining data on average litter size and farrowing duration of several studies published between 2004 and 2018 (van Rens and van der Lende, 2004; van Dijk *et al.*, 2005; Oliviero *et al.*, 2010; Hales *et al.*, 2015; Theil, 2015; Björkman *et al.*, 2017; Thorsen *et al.*, 2017; Langendijk *et al.*, 2018b), resulted in an estimation of 44 min extra farrowing time per piglet added to a litter. This is considerably higher than the 11 min in the current study, which might be related to the relatively short duration of farrowing seen in the current study as will be further discussed below.

**Farrowing duration**

As mentioned before, farrowing duration of sows in the current study was relatively short compared to other studies despite of no interventions being used. Sows in our study farrowed on average 17.1±3.4 piglets in 236±121 min (13.8 min per piglet). Feyera *et al.* (2018) found a duration of farrowing of 348±162 min for sows with an average litter size of 17.5±3.8 (19.9 min per piglet) and Björkman *et al.* (2017) found a farrowing duration of 396±234 min for 16.3±3.6 piglets (24.3 min per piglet). The difference in farrowing duration among studies might be caused by sow breed, sow body condition, management, parity, piglet birth weight or feeding regime around farrowing (Pejsak, 1984; Le Cozler *et al.*, 2002; van Rens and van der Lende, 2004; Canario *et al.*, 2006; Oliviero *et al.*, 2010; Vanderhaeghe *et al.*, 2010; Hales *et al.*, 2015). Based on this variation among studies, it can be concluded that farrowing duration should always be considered in perspective of the specific farm circumstances. This also suggests that using a fixed threshold for maximum farrowing duration before interventions should take place, as suggested by

Langendijk *et al.* and Oliviero *et al.* (Oliviero *et al.*, 2010; Langendijk *et al.*, 2018b), should be considered with care. Several studies found a relationship between farrowing duration and the incidence of stillbirth (Zaleski and Hacker, 1993; van Dijk *et al.*, 2005; Oliviero *et al.*, 2010; Langendijk *et al.*, 2018a), while the relationship between farrowing duration and pre-weaning mortality is less described (English and Wilkinson, 1982; Baxter *et al.*, 2008), but also less clear. This is in line with findings in our study, which showed that farrowing duration did influence incidence of stillbirth. When classified in a short, medium or long farrowing duration, incidence of stillbirth tended to be lowest when farrowing duration was between 150 to 250 min (medium duration) when comparing a short and long duration of farrowing. This is likely caused by the percentage of sows within each class lacking stillborn piglets, which was considerably higher in sows with a medium duration of farrowing than in both other farrowing duration classes (46.0%, 65.1% and 39.5% for a short, medium or long farrowing duration class, respectively). These results suggest an optimum in farrowing duration in relation to incidence of stillbirth, instead of shorter being better. On the one hand duration of farrowing should not be too short, since intense uterine contractions and abdominal straining may reduce placental blood flow and therefore oxygen exchange between mother and fetus (Curtis, 1974). On the other hand, a prolonged duration of farrowing can result in a higher risk for hypoxia for the piglets, since piglets are longer subjected to successive uterine contractions and potentially impaired oxygen exchange (Herpin *et al.*, 1996; Herpin *et al.*, 2001). Umbilical cord blood parameters seem to give a limited picture on the course of parturition and did not allign well with stillborn rate in the current study. Blood pH, $p\text{CO}_2$ and lactate were not significantly affected by farrowing duration class, whereas $p\text{O}_2$ and $\text{HCO}_3$ did and $\text{BE}_{ecf}$ tended to. Most favorable levels (high $p\text{O}_2$, low $\text{BE}_{ecf}$ and low $\text{HCO}_3$) were observed in piglets born from sows with a short farrowing duration (<150 min) and levels were not significantly different between piglets born from sows with a medium (150-250 min) or long (>250 min) duration of farrowing. Findings are partly in line with van Dijk *et al.* (2006), who divided parturition in three parts and found significantly lower umbilical cord blood pH, $\text{HCO}_3$ and $\text{BE}_{ecf}$ and significantly higher $p\text{CO}_2$ in piglets born in the last third part compared to piglets born in the first and second third part of parturition. Based

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on the current results, it can be hypothesized that optimal farrowing duration is not fixed, but depends on litter size, as discussed above.

## Conclusion

This study provides further evidence to support the influence of high litter sizes and long farrowing durations on the incidence of stillbirth and pre-weaning mortality. Of these two factors studied, litter size is suggested to be a larger driver for stillbirth than farrowing duration, since a clear linear relationship was found between litter size and incidence of stillbirth. For pre-weaning mortality, litter size and farrowing duration tended to interact, suggesting that optimal farrowing duration depends on litter size. Setting a fixed threshold for maximum farrowing duration to intervene in the farrowing process should thus be handled with care.

## Ethics Statement

All procedures in this study were approved by the Animal Use and Care Committee of Wageningen University, The Netherlands, in accordance with EU Directive 2010/63/EU for animal experiments.

## Conflict of Interest

M. van den Bosch and I. B. van de Linde were employed at the Cargill Innovation Centre Velddriel, the Netherlands. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Chapter 7

**General Discussion**

## Introduction

The main aim of this thesis was to investigate whether or not supplementation of nitrate to the sow, leading to NO formation, during the perinatal period is an applicable method to decrease incidence of stillbirth and pre-weaning mortality, in light of the following hypotheses, related to the potential effects of NO; 1) decreasing farrowing duration via an increased stamina of the sow and/or 2) increase piglet vitality due to an increased oxygenation via the vasoactive capabilities of NO. To investigate this, piglets characteristics linked to the level of asphyxia and vitality immediately after birth, birth weight and gain and placental characteristics of litters from sows receiving dietary nitrate were examined.

In the following paragraphs, the results obtained in this thesis will be discussed. First, the potential of maternal dietary nitrate supplementation to increase piglet vitality and decrease piglet losses will be presented. Secondly, it will be discussed, which elements of my hypotheses were proven and which ones need further research. The risks linked to nitrate toxicity in swine will be explained next, after which, limitations of this thesis and ideas for future research, with special emphasis on energy intake for farrowing, will be discussed. Lastly, overall conclusions of this thesis are drawn.

### The potential of maternal nitrate supplementation to increase piglet vitality at birth and decrease piglet losses

*Chapters 3, 4 and 5* describe results of two studies in which calcium nitrate was supplemented to sows in the perinatal period. Calcium nitrate was selected as the source of nitrate to use, since it is a safe (non-explosive) source and a registered feed ingredient within the EU. In *Chapter 3* (Study I) this was done under commercial circumstances on a farm in Denmark with hyper prolific sows (18.2 piglets born total per litter). Sows received a diet with 0.1% calcium nitrate (0.06% of nitrate) or a control diet without supplemented nitrate from day 112 of gestation until 4 days after farrowing. In *Chapters 4 and 5* (Study II) sows received a diet with one of five different levels of nitrate (0.03%, 0.06%, 0.09%, 0.12% and 0.15%) or a control diet without supplemented nitrate from

day 108 of gestation until 4 days after farrowing. In Study I, 0.06% of dietary nitrate supplementation showed a 2.5% (9.9% vs. 7.4%, $P$ = 0.05, for the control and 0.06% nitrate treatment, respectively) decrease in incidence of stillbirth. In Study II, this effect on the incidence of stillbirth was not observed (5.8%, 5.9%, 5.4%, 5.6%, 6.1% and 6.1% for the control, 0.03%, 0.06%, 0.09%, 0.12% and 0.15% of dietary nitrate, respectively). In *Chapter 4* (Study II) a tendency ($P$ = 0.10) for a quadratic effect on incidence of pre-weaning mortality was found with the lowest incidence of pre-weaning mortality found at intermediate levels of nitrate (-3.9% and -4.3% for 0.09% and 0.12% of nitrate, respectively compared to the control). In *Chapter 3* (Study I) no effect of 0.6% nitrate supplementation was found in incidence of pre-weaning mortality. The different results found on incidence of stillbirth and pre-weaning mortality between Study I and II might be explained by either:

1. *Management factors interfering with the experiment.* Study I was conducted under commercial circumstances and although registered and done only within treatment, cross-fostering was applied a lot (no cross-fostering, moving 1 or 2 piglets or moving 3 or more piglets per litter took place in 3%, 4% and 93% of the litters, respectively) and might have blurred the effects of treatment on incidence of pre-weaning mortality. Incidence of pre-weaning mortality during Study I was not higher compared to the on farm average during the 12 months before the onset of Study I which was likely due to continuing of human interference on pre-weaning mortality by saving piglets (e.g., prevent crushing or by placing them under the heating lamp or at the udder). In Study II, in which a tendency for a quadratic effect on incidence of pre-weaning mortality was found, cross fostering was only done to standardize litters (no cross-fostering, moving 1 or 2 piglets and moving 3 or more piglets per litter took place in 24%, 42% and 34% of the litters, respectively) and 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). Incidence of pre-weaning mortality during Study II was approximately 5% higher compared to the average levels at the facility during the 6 months before the onset of Study II when standard management practices were applied. This indicates

that the combination of management strategies as mentioned, have a substantial impact on pre-weaning mortality. In general, it is difficult to decide how to deal with cross-fostering and standardisation of litters in sows studies in which pre-weaning mortality is one of the main parameters of interest. On one hand, not standardising litter sizes can lead to a loss of piglets due a mismatch of number of piglets at the sow and the number of functional teats of the sow, simply due to a lack of milk for all piglets. On the other hand, standardisation of litters through cross-fostering can interfere with the effects of treatment, since piglet relocation and timing of this relocation might not only impact the piglet fostered, but also the litter and the sow a piglet is fostered on or off to in terms of stress and competition (Milligan *et al.*, 2001; Deen and Bilkei, 2004; KilBride *et al.*, 2014; Muns *et al.*, 2014). It is unclear which of both strategies (cross-fostering or interference with piglet survival) has played the largest role, but it can be imaged that particularly the lack of interference with piglet survival might have impacted pre-weaning mortality. The exact impact of cross-fostering on incidence of pre-weaning mortality is more difficult to interpret, since it depends on strategy, timing and farm conditions (Milligan *et al.*, 2001; Deen and Bilkei, 2004; Muns *et al.*, 2014; Muns *et al.*, 2016).

2. *The basal level of stillbirth and pre-weaning mortality within the herd used for the experiment (room for improvement)*. Level of stillbirth was higher in Study I versus Study II (8.8% vs. 5.8%, respectively), which may have led to more room for improvement in the first study. Incidence of pre-weaning mortality was higher in Study II versus Study I (16.4% vs. 14.8%, respectively).

3. *Feeding strategy and daily nitrate intake of the sows during the perinatal period.* In Study I and II, experimental diets were provided for a different number of days before the onset of farrowing (5 vs. 7 days in Study I and II respectively) and different dosages of nitrate and different feeding levels were used. Therefore, an overview of daily nitrate intake in the perinatal period of Study I and II is shown in Figure 1.

The days on feed before the onset of farrowing were selected due to practical reasons, since dietary nitrate was added to the lactation diet, which was provided when sows entered the farrowing room. Number of days sows were on feed was added as a covariable
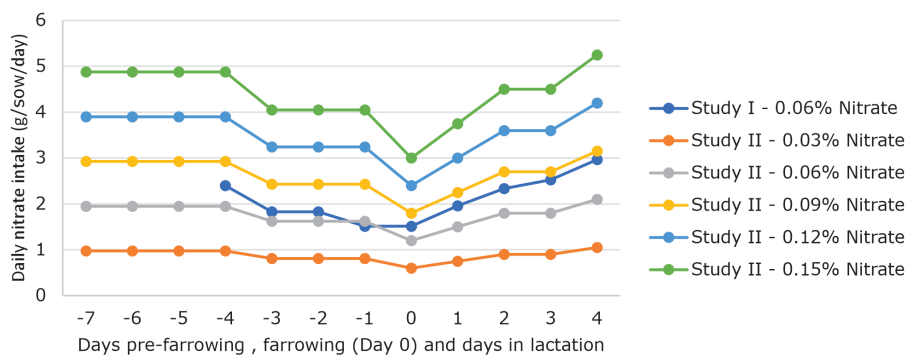
**Figure 1.** Daily nitrate intake during the perinatal period of the different dosages fed in study I and II. In both studies, calcium nitrate ($5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$; containing 63.1% of nitrate; commercial name Bolifor CNF (Yara Phosphates Oy, Helsingborg, Sweden)) was used.

in statistical models in Study I and II and showed no significant effect on incidence of stillbirth, pre-weaning mortality and piglet birth weight. It has to be stated that days on feed before the onset of farrowing is not only confounded with gestation length, but also with the number of days sows received a lactation diet (higher total nutrient intake before the onset of farrowing). These confounding effects make it difficult to appoint effects of days on feed before the onset of farrowing solely to total nitrate intake of sows. Nonetheless, additional analysis showed no interaction between number of days on feed and nitrate supplementation for Study I and II. Concluding, the additional analysis on data of Study I and II do not provide sufficient insights in what the exact impact of number of days nitrate is supplemented before the onset of farrowing is, since this was not a parameter of interest in the experimental design of these studies.

In human blood, nitrate has a half-life time of 5-8 hours (Lundberg and Weitzberg, 2005; Bryan and Grisham, 2007) and effects of nitrate supplementation on exercise performance are seen when either provided 3 days (daily) before (Larsen *et al.*, 2007; Bailey *et al.*, 2009; Bailey *et al.*, 2011; Lansley *et al.*, 2011) or acute (within 3-1 hours) before the exercise performance was tested (Bescós *et al.*, 2011; Kenjale *et al.*, 2011; Jones, 2014), again suggesting that the effect of nitrate is quite acute and that thus the number of days sows are

on feed before onset of farrowing is not that important on exercise performance and sow stamina. However, in *Chapter 4*, a significant effect of maternal nitrate supplementation on piglet birth weight was found (on average 47 grams/piglet when comparing no nitrate supplementation vs. nitrate supplementation, $P < 0.05$), which was not found in Study I. Birth weight is a large determining factor in piglet mortality, but also directly impacts thermoregulation capacity and growth (Muns *et al.*, 2016). The effect of maternal nitrate supplementation found in *Chapter 4* may have been driven by the higher daily nitrate intake and longer time sows received dietary nitrate before the onset of farrowing (Figure 1). It is hypothesized that by supplying maternal nitrate longer before the onset of farrowing, a higher blood and thereby nutrient supply to the piglet in utero (due to the vasoactive properties of NO) is established, which results in higher birth weights. It seems very unlikely that an increase of in total 73 gram/piglet (control vs. 0.15% of nitrate, $P_{Lin}$ = 0.04; *Chapter 4*) in birth weight is caused by acute nitrate supplementation, but by an increased blood and thereby nutrient flow for several days. Average fetal gain of piglets in the last week of gestation ranges between 40.3 (between day 108 and 115 of gestation (McPherson *et al.*, 2004)) and 85.8 gr/piglet/day (between day 110 and 114 of gestation (Wu *et al.*, 1999)). Maternal nitrate supplementation dosed at 0.15% (starting 7 days before farrowing) increased fetal gain with 10 gr/piglet/day, which is approximately a 12-25% increase compared to the average fetal gain in the study of Wu *et al.* (1999) and McPherson *et al.* (2004). In the discussion of *Chapter 4*, calculations were made on the amount of additional blood flow required to obtain the differences in piglet birth weight that was observed. It was calculated that, 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. Average intrauterine fetal blood flow was 564.2 mL/min/fetus, making the expected increase in blood flow for the 0.15% dosage of nitrate 6.26 mL/min/fetus (an increase of 1.1% compared to the average intrauterine fetal blood flow). Calculations on the additional blood flow needed and the calculated increase in fetal gain are speculative, but seem realistic. However, whether or not these are achieved remains to be investigated.

Despite of the previously hypothesized acute effects of nitrate supplementation on sow stamina and the lack of interaction between the number of days on feed and nitrate

supplementation, it is advised to supplement nitrate starting at least 5-7 days before the expected farrowing date to achieve the effect seen on piglet birth weight. Since the effect on birth weight was linear, it might be that a higher nitrate inclusion, higher feeding levels of diets containing dietary nitrate, and/or by supplementing nitrate already in late gestation, could contribute to a larger effect.

Concluding, maternal dietary nitrate supplementation at a concentration of 0.06-0.12% might have the potential to affect incidence of stillbirth and pre-weaning mortality when provided from 5-7 days onward before the onset of farrowing. Likely, effects on incidence of stillbirth and pre-weaning mortality might be more profound when the incidence of stillbirth and pre-weaning mortality on farm is high (~8.8 and ~14.8%, respectively) and human interference with piglet mortality (e.g. saving and/or cross-fostering) is kept to a minimum.

**Maternal nitrate supplementation in the hyper prolific sow**

The title of this thesis is "Supporting the hyper prolific sow and her litter through the perinatal period by dietary nitrate supplementation". In *Chapter 3, 4* or *5* it was not evaluated whether or not maternal nitrate supplementation is more beneficial for sows having larger litter sizes. It can be speculated that nitrate supplementation is more important for larger litter due to uterine crowding (Kemp *et al.*, 2018), the decrease in uterine blood flow per fetus (Pere and Etienne, 2000) and the increased duration of farrowing (van Dijk *et al.*, 2005). Bjorkman *et al.* (2020) defined sows with litter sizes of 16 piglets or more as hyper prolific, meaning most of the sows in Study I were hyper prolific. Data from Study I was leveraged to evaluate the interaction between litter size class (LSC; less than 16, 16-18, 19-21 and more than 21 piglets) and treatment (Control vs. 0.06% of nitrate). Statistical models were the same as described in *Chapter 3*, but LSC and the interaction between LSC and treatment were added to the model as fixed effects. No significant interaction was found on the incidence of stillbirth. A tendency ($P = 0.06$) and a significant ($P = 0.03$) interaction between LSC and treatment was found for pre-weaning mortality and birth weight of piglets, respectively (Figure 2A and B).
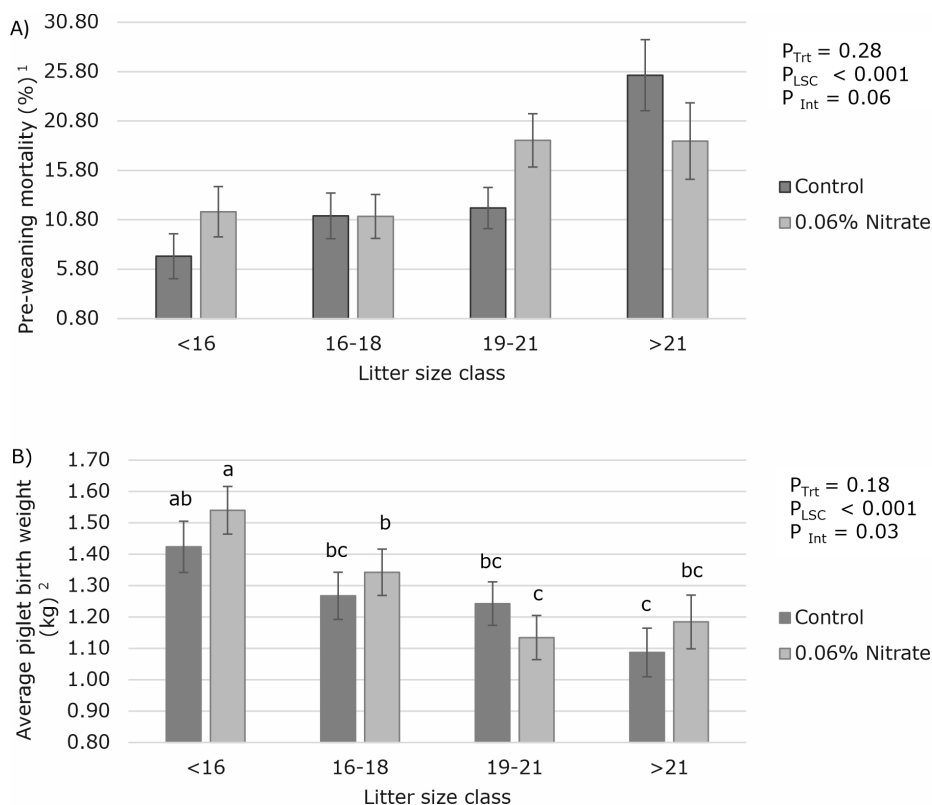
**Figure 2.** Interaction between treatment (Control vs. 0.06% of nitrate) and litter size class (LSC) (< 16, 16-18, 19-21 and >21) for pre-weaning mortality (A) and average piglets birth weight (B) leveraging data of Study I as describe in *Chapter 3*. $N = 8$ to 24 per treatment and per LSC.

[abc]Different superscripts indicate significant differences.

[1]Mortality is calculated as:

$$\left( \frac{\text{the number of deaths (excl. stillbirth)}}{\text{(born alive+number of piglets added−number of piglets removed at cross fostering)}} \right) \times 100\%$$

[2]Birth weight of live born piglets.

In the control treatment, a tendency to a higher piglet mortality was observed for litters larger than 21 compared to litters of 21 piglets or smaller, while when 0.06% nitrate was supplemented to the sow diet, mortality did not differ between the litter size classes (Figure 2A, $P_{int}$ = 0.06). Although a significant interaction between treatment and LSC was found on piglet birth weight (Figure 2B, $P_{int}$ = 0.03), the interaction did not show clear beneficial effects of maternal nitrate supplementation on birth weight for larger

litters. A similar exercise was done for Study II, but since 6 different nitrate levels were used, it was decided to classify litter size in two classes based on the hyper prolificacy definition of Bjorkman *et al.* (2020) (<16 piglets or ≥ 16 piglet born total per litter). Statistical models were the same as described in *Chapter 4*, but litter size class (LSC) and the interaction between LSC and treatment were added to the model as fixed effects. No significant interaction was found on the incidence of stillbirth.

An interaction between LSC and treatment on pre-weaning mortality percentage (Figure 3A) and average piglet birth weight (Figure 3B) was found. The interaction between LSC and treatment found on average birth weight and pre-weaning mortality and is likely driven by the difference in response of non-hyper prolific versus hyper prolific sows receiving 0.03% and 0.15% of nitrate. A closer look showed that 4 out of 24 non-hyper prolific sows receiving 0.03% of nitrate had a small litter (≤9 piglets) and therefore high birth weights. This might have also led to lower incidence in pre-weaning mortality observed in piglets of non-hyper prolific sows receiving 0.03% of nitrate. Based on the analyses done for Study I and II, the hypothesis that maternal nitrate supplementation would be more beneficial for sows with higher litter sizes could not be proven.

### Did nitrate supplementation prove to be vasoactive and/or increase the stamina of the sow?

Two hypotheses were formulated to explain why maternal dietary nitrate supplementation could be beneficial for the sow when supplemented during the perinatal period and more specifically during the process of farrowing;

1.  In humans, dietary $NO_3^-$ supplementation increases not only exercise efficiency as shown by a decrease in $VO_2$ max (a measure for the maximum amount of oxygen a body can utilize for ATP production during exercise) without increasing blood lactate values (Bailey *et al.*, 2012), but also exercise tolerance as shown by an increased time till exhaustion (Bailey *et al.*, 2009; Bailey *et al.*, 2011; Lansley *et al.*, 2011) and can therefore increase exercise performance as shown by a reduced time to run a certain distance (Hopkins *et al.*, 1999). It can be hypothesized that $NO_3^-$ supplementation

may increase stamina of sows during farrowing, thus decreasing the duration of farrowing, which would in turn decrease the levels of asphyxiation and therefore reduce early mortality and stillbirth occurrences in piglets.
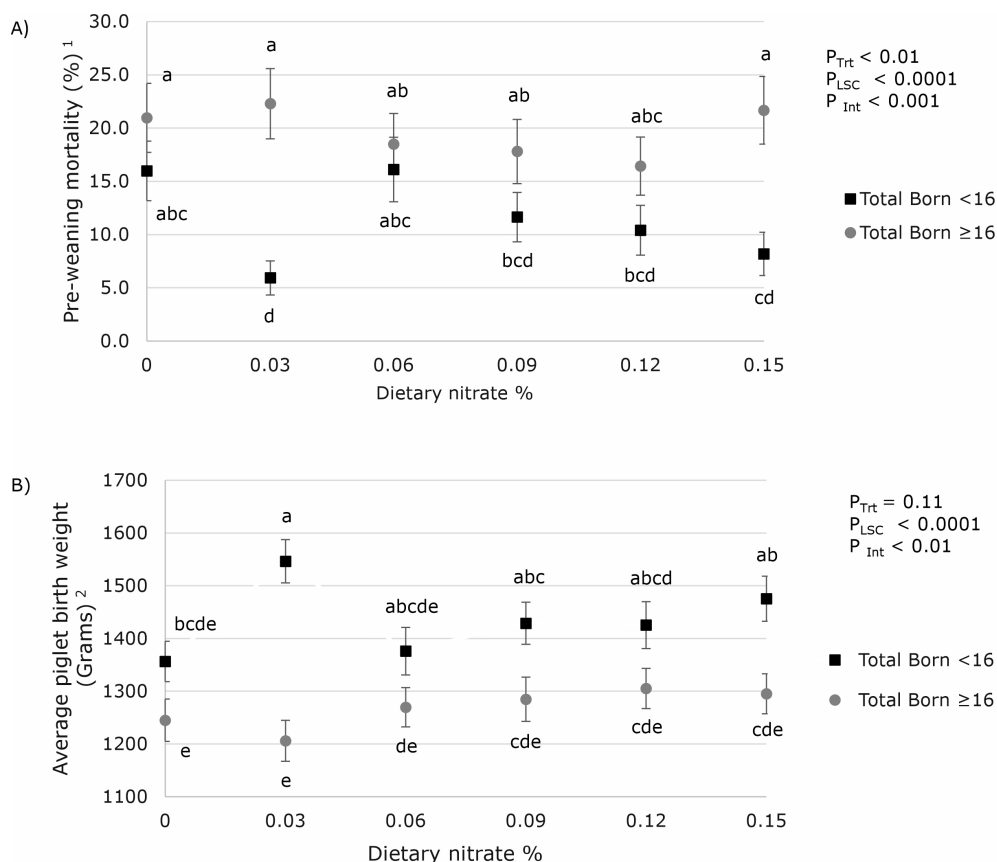


**Figure 3.** Interaction between treatment (Control, 0.03, 0.06, 0.09, 0.12 and 0.15% of nitrate) and litter size class (LSC) (total number born < 16 vs. ≥ 16) for pre-weaning mortality (A) and average piglets birth weight (B) leveraging data of Study II as describe in *Chapter 4*. N = 19-30 per treatment and per LSC. [abcde] Different superscripts indicate significant differences.

[1] Mortality is calculated as:

$$\left( \frac{\text{the number of deaths (excl. stillbirth)}}{\text{(born alive} + \text{number of piglets added} - \text{number of piglets removed at cross fostering)}} \right) \times 100\%$$

[2] Birth weight of live born piglets.

2. Nitric oxide (NO) formation from $NO_3^-$ is an endothelium relaxing factor, inducing vasodilation, which is known to be increased under hypoxic situations and result in lower blood pressure (Webb *et al.*, 2008; Omar *et al.*, 2016). It was hypothesized that, by ensuring a larger blood flow and thereby, oxygen flow in the placenta and the umbilical cord during farrowing, the risk for asphyxiation in piglets can be reduced.

I hypothesized in *Chapter 5* that the linear effect of maternal dietary nitrate seen on piglet vitality right after birth and a tendency for higher $pO_2$ levels in umbilical cord blood of piglets, was likely driven by the vasodilative effect of NO rather than an increased stamina of the sow, since no effect on farrowing duration was found. Placenta width increased linearly with increasing dose of maternal nitrate ($P_{lin}$ = 0.02, *Chapter 5*) and also a linear increase in piglet birth weight was observed ($P_{lin}$ = 0.04, *Chapter 4*). Vascularisation of placenta's was not evaluated, since this was expected to be constant in the last stage of gestation (Biensen *et al.*, 1998; Wilson *et al.*, 1998) and due to the short period of supplementation (approximately 7 days). Consequently, it is not possible to determine whether or not vascularisation, placental blood flow or a combination of both is the main driver for the increased placental size and piglet birth weight and may therefore increase vitality and umbilical cord oxygen levels. Placental blood flow at day 103 of gestation is highly correlated to both placental weight (r = 0.90, *P* < 0.001) and fetal weight (r = 0.83, *P* < 0.01) (Wootton *et al.*, 1977), indicating that it is difficult to appoint the obtained effects to one single factor. Although invasive (as described by for example (Wootton *et al.*, 1977; Gilbert and Leturque, 1982; Pere and Etienne, 2000)), measurements on placental blood flow would be possible and could give insight in the hypothesized mode of action.

Farrowing duration was the only measurement used in this thesis to evaluate whether or not maternal nitrate supplementation affected stamina of the sow and no effect of dosage of nitrate was found (*Chapter 5*). To obtain more insight whether or not dietary nitrate supplementation affects the stamina of the sow during farrowing, it would be interesting to gain more insight in physiological parameters of the sow around farrowing. During an intense activity over a prolonged period of time, like farrowing, muscles should ideally perform aerobic. However, when the demands for oxygen exceeds the arterial blood supply, the animal switch to anaerobic metabolism and lactate is produced by the muscles and

released into the bloodstream (Nielsen *et al.*, 2021). As described in *Chapter 2*, sow blood lactate levels are increased shortly before and during the farrowing process (Mosnier *et al.*, 2010; Nielsen *et al.*, 2021). Especially when farrowing duration is prolonged, it would be interesting to see if there is an interaction with nitrate supplementation on blood lactate levels. However, since Larsen *et al.* (2007) found a lower $O_2$ cost for exercise (approximately -5%) following nitrate ingestion in humans without an increase in blood lactate levels (which suggests an increased muscle efficiency), it would be of interest to also determine the $VO_{2max}$ in sows to gain insight in muscle efficiency and therefore stamina in addition to blood lactate levels. $VO_{2max}$ is a measure for the maximum rate (V) of oxygen ($O_2$) the body can absorb during a minute and is a measure for how well the body can leverage $O_2$ for ATP production. In humans, the $VO_{2max}$ can be measured by measuring $O_2$ in- and exhalation during increased physical exercise. The $VO_{2max}$ is reached when the inhaled $O_2$ levels are not increasing anymore, while exercise intensity is still increasing. Whether or not sows reach their $VO_{2max}$ during farrowing is unknown. $VO_{2max}$ has been determined in miniature pigs (Norton *et al.*, 1990), meaning that this might be possible in sows as well. Although farrowing and exercise are both physical activities, differences between the two processes exist. Sow activity during the expulsion phase is not solely a process driven by the somatic nervous system, but also include autonomous and hormonal driven activity (e.g. uterine contractions). It would therefore be interesting to measure uterine contractions in sows during the farrowing process to determine whether or not maternal dietary nitrate supplementations affects power and potentially also duration and frequency of uterine contractions.

It can be concluded that maternal dietary nitrate supplementation appears to have a larger effect on vasodilation since no effect on sow stamina (farrowing duration) was found.

## Nitrate toxicity and potential risks

Although nitrate supplementation in humans is mainly used to improve health (Omar *et al.*, 2016) and increase athletic performance (Jones, 2014), feeding nitrate to animals might be considered to be controversial. Main reason for that is the risk of methaemoglobinaemia after nitrate ingestion. After dietary nitrate is converted into the more reactive nitrite in

the animal, nitrite can oxidize the ferrous ion ($Fe^{2+}$) of the haem group of the haemoglobin molecule to a ferric ion ($Fe^{3+}$), which is unable to carry oxygen. Methaemoglobinaemia can occur when methaemoglobulin (MethHb; $HbFe^{3+}$) levels in the blood are above approximately 5% (Lundberg *et al.*, 2008). Acute toxicity in humans is expected around 10% of MetHb (Wright *et al.*, 1999) and is expected to be similar in pigs (van de Ligt *et al.*, 2021). Most of the studies that evaluated effects of dietary nitrate or nitrite supplementation on methaemoglobinaemia used a single oral dose instead of a continuous supplementation over a certain period (i.e. when added to the feed). The putative positive or negative effects of continuous or periodical supplementation of low levels of dietary nitrate on health of pigs have not been studied widely yet. EFSA's (European Food and Safety Authority) NOAEL (no-observed-adverse-effect level) recently set the safety benchmark value for nitrate in swine at 410 mg per kg of body weight per day (EFSA Panel on Contaminants in the Food Chain (CONTAM) *et al.*, 2020). After the EFSA review, Doepker *et al.* (2021) conducted a more broad review on swine, evaluating more than 30 relevant studies, including case reports and reviews, examining calcium, potassium, sodium, or unspecified nitrate salts at doses up to 1,800 mg nitrate/kg BW/day for exposures ranging from 1 to 105 d. They concluded with moderate-to-high confidence that the NOAEL for nitrate supplementation in swine is likely between 600 and 800 mg of nitrate/kg BW/day. Van der Ligt *et al.* (2021) supplemented sows 0.12% and 0.60% of nitrate, starting 4 days before farrowing until weaning and evaluated methaemoglobin levels in sows (4 days pre-farrowing, and day 8 and 21 of lactation, Figure 4A) and suckling piglets (at day 7 of age and at weaning, Figure 4B). No signs of nitrate toxicity were observed, indicating that dosing nitrate up to 5 times as high as used in *Chapter 4* and *5* can be considered to be safe to use in sows.
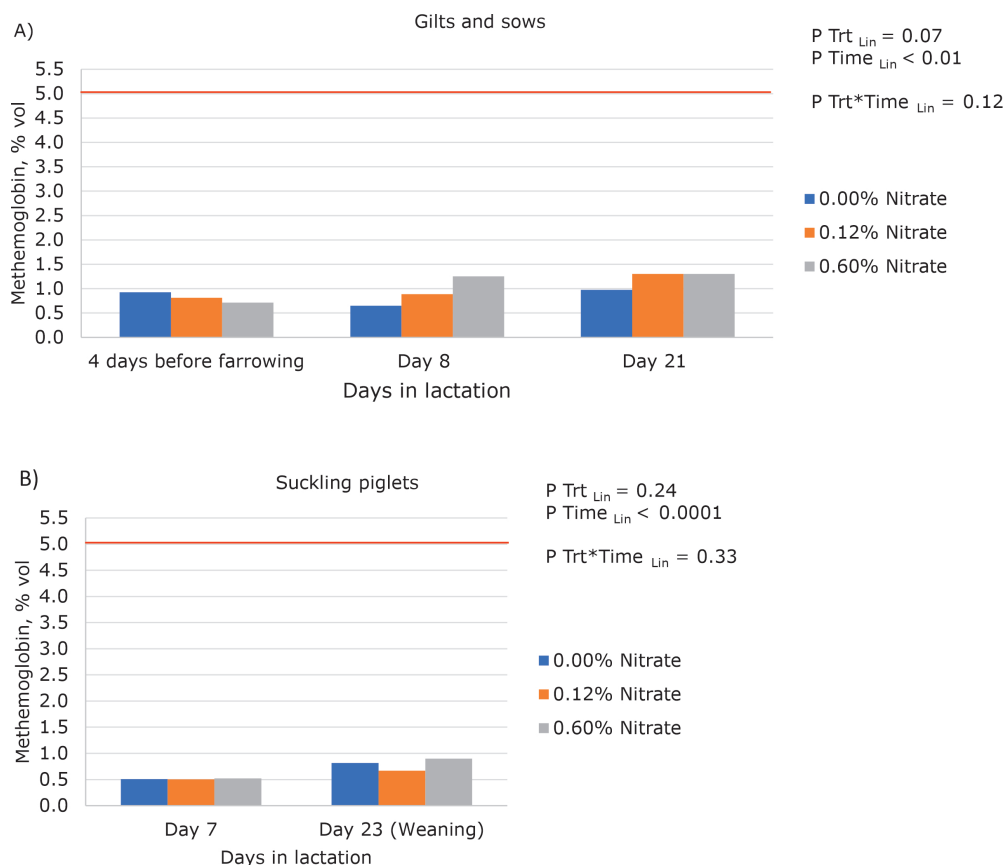
**Figure 4.** Methaemoglobin concentration in blood in response to maternal dietary nitrate supplementation in the perinatal period on 4 days before farrowing and day 8 and 21 of lactation in sows (A) and day 7 and 23 in sucking piglets (B) as adapted from (van de Ligt *et al.*, 2021). The red line indicates the level of methaemoglobin above which methaemoglobinaemia can occur (Lundberg *et al.*, 2008).

In this thesis, sows were restrictedly fed up to the moment of farrowing and 2.7 kg of lactation feed offered per day from day 113 of gestation until farrowing day. Sows weighed 266.0 ± 36.8 kg on average at day 108 of gestation, meaning that nitrate intake in our trial was maximal 15.2 mg/kg of body weight per day in the last 2 days before farrowing in the diet with the highest nitrate concentration. Levels of nitrate intake used in this thesis were low compared to the EFSA's (European Food and Safety Authority) NOAEL levels and the review of Doepker *et al.* (2021).

It can be concluded that the used nitrate concentrations in this thesis are not expected to have a deleterious effect on the sow and piglets.

## Limitations and suggested future research

Since only a limited number of studies has been done evaluating dietary nitrate supplementation to the sow, several research questions remain after the work done within this thesis.

Whether or not NO is indeed causing a vasodilative effect in the placenta and therefore enhances blood flow to the fetusses and how long this vasodilative effect remains after a sow had her meal containing nitrate remains unclear. In *Chapter 5*, nitrate levels of umbilical cord blood of piglets from sows receiving the different levels of dietary nitrate was measured. No linear effect of treatment was found on nitrate levels in umbilical cord blood of piglets. In addition, when comparing the control to all 5 levels of maternal dietary nitrate, no significant difference in umbilical cord nitrate levels was found. That does not necessarily mean the vasoactive properties of NO, the active component aimed to get in the placenta, are not achieved. However, to prove this, utero-placental blood flow would need be measured after nitrate ingestion and even during the farrowing process. When assuming circulating nitrate level in sow blood are related to NO formation, it can be hypothesized that NO formation and therefore the vasoactive effects of NO can be optimized by 1) Daily nitrate intake of sows (e.g. dosage and amount of feed) and 2) Synchronizing the moment of nitrate intake and onset of farrowing (e.g. half-life, timing of meals and number of meals per day). Within this thesis, I did not confirm whether or not the half-life of 5-8 hours for nitrate (Lundberg and Weitzberg, 2005; Bryan and Grisham, 2007) found in humans is also applicable for sows. It can be hypothesized that by knowing the exact half-life of nitrate in sows, a further optimisation could be made in terms of dosage to supplement and feeding strategy (amount of feed, number of meals per day and feeding time before the onset of farrowing). This might, however, be a complex exercise due to the large variation between sows in not only farrowing duration and therefore the level of acidification and oxygenation that affects nitrate conversion (Cosby *et al.*, 2003; Lundberg and Weitzberg, 2005; Shiva *et al.*, 2007; Lundberg *et al.*, 2008; Bailey *et al.*,

2012; Ferguson *et al.*, 2016), but also the variation in the onset of farrowing and the time between which the sow consumed her last meal and the onset of farrowing. Feyera *et al.* (2018) showed that when the time between the last meal consumed by the sow and the onset of farrowing (measured by the expulsion of the first piglet) exceeded 3.13 ± 0.34 h, farrowing duration increased linearly from that point onwards (Figure 5, *Chapter 2*). Study I and II were both conducted in 2014 and 2015, respectively, so I was at that time not aware of this relationship. In hindsight, this would have been an important factor to measure and include as a covariable in the statistical models used. In Study II (in which farrowing duration was measured), feeding times were fixed at 7.30 AM and 4.30 PM and the exact onset of farrowing was recorded. However, whether or not a sow consumed her meal right at or shortly after feeding (which might not always be the case around the onset of farrowing) is unknow and could therefore not be included. When assuming sows did eat their last meal before the onset of farrowing and evaluating the time between provision of the last meal and the onset of farrowing, no clear relationship was found on farrowing duration as shown in Figure 5.
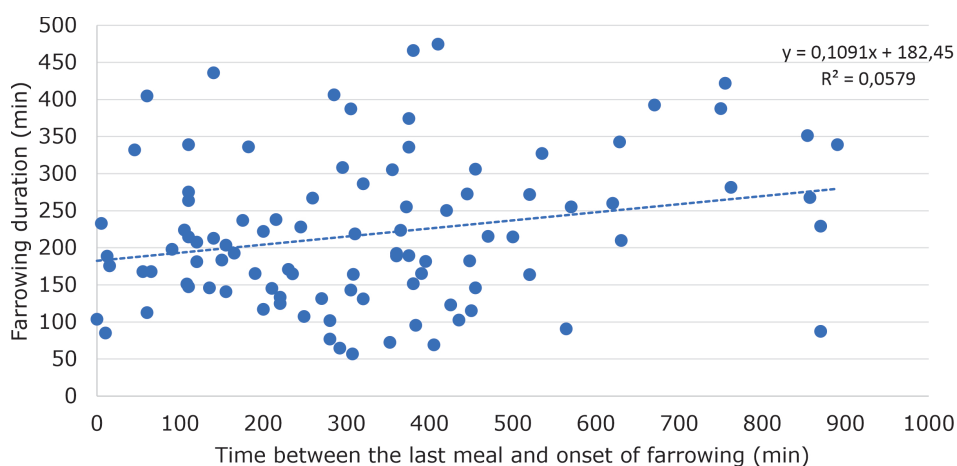


**Figure 5.** Relationship between the time between the last provided meal and the onset of farrowing and farrowing duration in Study II.

Several interactions between the effect of nitrate dosage and other (classified) factors (e.g. litter size class and total number of days on feed) were evaluated. There are several

more which might be of interest. The main aim of Study II, in which most physiological measurements were done on piglets, was to evaluate dosage of nitrate and not to evaluate these interaction. With six treatment groups within Study II, the number of replications per treatment per factor was in some cases too low to draw solid conclusions. It is therefore advised to design a study with a control and a limited number of maternal nitrate supplementation levels and evaluate the interactions of several factors with treatment on placental characteristics, farrowing duration, incidence of stillbirth, piglet vitality, level of asphyxia, birth weight, piglet growth and incidence of pre-weaning mortality, plus several physiological sow parameters, like lactate and $VO_{2\,max}$. In addition, it would be interesting to evaluate whether or not nitrate supplementation is beneficial for specifically the last proportion of the litter born. A limited number of treatments will make insights in these interactions more clear.

It can be speculated that nitrate supplementation in early gestation may have the same beneficial effects on angiogenesis (Ziche and Morbidelli, 2000) as observed with L-arginine supplementation, since both pathways generate NO. However, whether or not the positive effects (e.g. embryo survival, increased placental weight and the number and weight of piglets born alive (Palencia *et al.*, 2018)) found of arginine supplementation in (early) gestation can be observed by supplementing nitrate remains unclear, since arginine is not only a pre-cursor for NO, but also for many other biologically active molecules, like ornithine, polyamines (putrescine, spermine and spermadine), creatine, and agamatine (Wu and Morris Jr, 1998). Exact timing of supplementation of maternal dietary nitrate in early gestation and dosage to be used are unknown. It can be speculated that nitrate supplementation would mainly be beneficial when vascularisation of the placenta takes place (starting approximately at day 15 post mating (Dantzer and Leiser, 1994) until mid-gestation (Biensen *et al.*, 1998; Wilson *et al.*, 1998)) and the placenta size is increasing (mainly between day 35 and 70 of gestation (Knight *et al.*, 1977)). However, also during late gestation when fetal growth is high (Noblet *et al.*, 1985) maternal nitrate supplementation could enhance piglet birth weights, like observed in *Chapter 4*. Long-term nitrate supplementation might, however, impact eNOS (endothelial Nitric Oxide Synthase, primarily responsible for the generation of NO in the vascular

endothelium) activity, since long-term (8-10 weeks) nitrate supplementation in rats showed a reversible dose dependent reduction in phosphorylated endothelial NOS (eNOS) in the aorta and a lower eNOS-dependent vascular response in vessels from nitrate treated mice (Carlström *et al.*, 2015). This suggests that NOS activity is lower when nitrate is supplemented. Carlstorm *et al.* (2015) suggested that mainly individuals with a compromised eNOS activity (e.g. elderly) might show an increased response to nitrate supplementation (Carlström *et al.*, 2015). Whether or not higher parity sows have a compromised eNOS activity and therefore benefit more from maternal nitrate supplementation remains unclear.

## Providing the sow the right amount of energy on the day of farrowing

As mentioned in *Chapter 2*, the total duration of farrowing (stage 1, 2 and 3) can take up to 24 hours in the hyper prolific sow (Purohit, 2010) and will increase with increasing litter sizes (Peltoniemi *et al.*, 2020). When we expect from our sows to give birth to larger litters, we should provide her with the right nutrients to perform this activity. Focus on energy requirements seems a logical first step, since farrowing is likely a high energy demanding activity (van den Bosch *et al.*, 2019; Feyera *et al.*, 2021). Providing the metabolic energy for the sow to go through the process of farrowing could be done in different ways;

1. By setting feeding times of the last meal as close as possible to the onset of farrowing. This can be done by increasing the number of meal and/or provide meals close to the time of day when most sows within the herd give birth. This might mean that feedings are not just done during the day when staff is present, but night feedings should be considered. Feyera *et al.* (2018) showed that farrowing duration linearly increased with time when the last meal was more than 3.13 ± 0.34 h before the onset of farrowing (defined as the birth of the first piglet, Figure 5A in *Chapter 2*). This indicates that to minimize farrowing duration, sows should receive their last meal as close as possible to 3.13 h before the onset of farrowing. In addition, a negative correlation was found between sow arterial glucose level, measured 1 hour after the birth of the first piglet and farrowing duration (Figure 5B in *Chapter 2* (Feyera *et al.*,

2018)), suggesting that a low energy status of the sow indeed increased farrowing duration.

2.  By optimizing the total daily energy intake of the sow based on the average litter size and/or farrowing duration of the herd. Only two studies evaluated different daily energy intakes in sows on the day of farrowing (Che *et al.*, 2019; Feyera *et al.*, 2021). Strategies of how the energy intake was increased differed between these two studies. Che *et al.* (2019) increased energy level of the diet by increasing fat levels (soy oil) and increasing daily feed supply with 0.2 kg/sow/day (from day 90 of gestation until farrowing), while Feyera *et al.* (2021) increased feeding levels (from 1.8 to 5.0 kg/sow/day form day 108 of gestation until 24h after farrowing). Both studies also differed in average litter size and average farrowing duration observed. It is known that farrowing duration increases with increasing litter size (Figure 2, *Chapter 2* and Figure 3 *Chapter 6*), making it assumable that also energy requirements for farrowing increase with litter size. For estimating the energy requirements per piglet born or per 60 min of farrowing, it was assumed that the energy requirements were met when farrowing duration was most short. For these treatments number of piglets born and average farrowing durations are shown in Table 1. Calculations on average energy requirement per piglet and per 60 min for both studies turned out the be strikingly close. Calculations suggest that optimal daily energy intake on the day of farrowing depends on the average litter size of the herd (2.44 MJ ME/ piglet born) and/or farrowing duration (8.66 MJ ME/ hour of farrowing duration). Since only two studies were available, the findings should be confirmed in additional experiments.

Calculations also suggest that sows are typically underfed on the day of farrowing. As an example, sows in Study I, which were fed a fairly typical amount and lactation diet for Dutch standards, received 2.0 kg of feed containing 12.35 MJ ME/kg on the day of farrowing (total daily energy intake of 24.7 MJ ME/day) and gave birth to on average 16.8 piglet. Using the average energy requirement on the day of farrowing as calculated in Table 1 per piglet, sows in the herd of Study I should receive 41.0 MJ ME on the day of farrowing, and thus received 16.3 MJ ME too little.

**Table 1.** Calculation on estimated energy requirements on the day of farrowing per piglet and per 60 min of farrowing based on Che *et al.* (2019) and Feyera *et al.* (2021).

| Study | Che *et al.* (2019) | Feyera *et al.* (2021) | Average |
|---|---|---|---|
| Average litter size | 14.8 | 20.4 | |
| Average farrowing duration (min) | 238.4 | 359.8 | |
| Optimal daily energy intake determined based on the shortest duration of farrowing (MJ ME/ sow/ day) | 33.8 | 53 | |
| **Energy requirement on the day of farrowing per piglet (MJ ME)** | **2.28** | **2.60** | **2.44** |
| **Energy requirement on the day of farrowing per 60 min of farrowing (MJ ME)** | **8.49** | **8.83** | **8.66** |

I hypothesize that farrowing duration on farms can be reduced by;

- Feeding at least 3-4 meals/sow/day around the expected farrowing date of the sow.
- Increase the daily energy intake of sows on the day of farrowing aiming for 2.44 MJ ME per piglet born, using the average litter size on farm to optimize per farm.

**Infobox 1.** Practical advice on management strategies during farrowing that can reduce incidence of stillbirth and pre-weaning mortality

Next to nutrition there are several other factors known that can influence the incidence of stillbirth and pre-weaning mortality. Several will be discussed here.

**Birth assistance**
Birth assistance is the help of piglet delivery that are blocking the birth canal via through vaginal palpations and the use of oxytocin. When to apply birth assistance is a subjective matter with limited scientific research to support a clear guideline. *Chapter 6* indicated that setting a certain threshold level for farrowing duration should be taken with care, since this appears to depend on litter size. Several studies showed a decrease in piglet losses when vaginal palpation was applied in cases of dystocia (Holyoake *et al.*, 1995; White *et al.*, 1996; Le Cozler *et al.*, 2002). Others found a negative correlation between vaginal palpation and the incidence of stillbirth, which might be due to the fact that vaginal palpation was mostly applied when problems occurred during farrowing (Holm *et al.*, 2004;

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Canario *et al.*, 2006; Vanderhaeghe *et al.*, 2010). In practice, it is stated that birth assistance should be applied when the sow is under obvious stress and 1) one or two dry piglets are present and 2) obvious straining and contractions occur with no result. With vaginal palpation comes a risk of injury and infections and should therefore be applied with care. Disinfection of hands, the vulva, the use of a disinfectant lubricant and plastic sleeve is advised. In Study II, no form of birth assistance was applied unless absolutely necessary. Strikingly, only 3 out of 305 sows received birth assistance, while no difference in incidence of stillbirth was seen 6 months before onset of the experiment and during the experiment. This suggest that providing birth assistance in most cases seems to more a precaution than a necessity.

Oxytocin can be used during dystocia to enhance uterine contractions (Straw *et al.*, 2000). Before applying oxytocin, it should be checked whether or not a piglet is stuck in the birth canal or an attempt could be made to release endogenous oxytocin by manual induction of the Ferguson reflex and/or massaging the udder of the sow (Björkman and Grahofer, 2020). Too high dosages (>10 IU) of exogenous oxytocin can lead to damage of the umbilical cord (Randall, 1972) or a decreased placental blood flow (Tucker and Hauth, 1990) and it is therefore recommended, when needed, to administer oxytocin at a level of 5-10 IU one or two times (Peltoniemi *et al.*, 2019).

**Housing**

Aiming for the lowest incidence of stillbirth and pre-weaning mortality, the following aspects in housing of sows during the perinatal period seems to be most optimal; Sows should be housed in a farrowing pen containing a (re)movable farrowing crate and should only be crated after farrowing is completed until 36-72 hours post farrowing (Spicer *et al.*, 1986; Rootwelt *et al.*, 2013; Hales *et al.*, 2015; Glencorse *et al.*, 2019). A recent meta-analysis including 22 studies concluded that loose farrowing reduces the incidence of stillbirth (with approximately 22%) compared to crates (Glencorse *et al.*, 2019). The effect of provision of nesting substrate on incidence of stillbirth is less clear (Yun and Valros, 2015; Bolhuis *et al.*, 2018). Nesting material tended to lower the incidence of crushing during farrowing for sows in both pens and crates (Bolhuis *et al.*, 2018). The relative risk of piglet mortality, mainly due to crushing, increased with 14% when sows are housed in a pen (no-confinement at all) versus continuous confinement in crates throughout lactation (Glencorse *et al.*, 2019). Lastly it is advised to avoid dense bedding (like uncut straw) after farrowing since it can be a risk for movement of low vitality piglets and therefore increase incidence of pre-weaning mortality

(Rutherford *et al.*, 2013). It is therefore advised to not overuse or remove this bedding after farrowing when used as nesting material.

**Sow body condition and constipation**
Fatness of the sow has been linked to an increased farrowing duration (Oliviero *et al.*, 2009; Oliviero *et al.*, 2010), which is either caused by a more narrow birth canal, or by an increased storage of lipid-soluble steroids, like progesterone (Björkman and Grahofer, 2020), which might delay the pre-partum decline in progesterone, affecting oxytocin receptor activation (Oliviero *et al.*, 2008; Peltoniemi *et al.*, 2019). This could lead to weaker uterine contractions. Constipation should be prevented since this is positively correlated to farrowing duration (Oliviero *et al.*, 2010), which might be related to causing a physical blockage near the birth canal (Peltoniemi *et al.*, 2019) or by the pain, stress and discomfort of the constipation that affects the duration of farrowing (Peltoniemi *et al.*, 2019).

## Conclusions and take home messages

Within the current swine industry, most sows are nutritionally not optimally supported to go through the demanding perinatal period, including the parturition process a crucial event related to piglet losses. Within this thesis, it is concluded that maternal nitrate supplementation at a concentration of 0.06-0.12% has the potential to reduce the incidence of stillbirth and pre-weaning mortality when provided from 5-7 days before the onset of farrowing onwards to sows independent of litter size. The effects founds are likely more due to an effect on vasodilation than on stamina, but more research is needed to confirm this hypothesis. Effects on incidence of stillbirth and/or pre-weaning mortality might be more profound when the incidence of stillbirth and pre-weaning mortality on farm is high and human interference with piglet mortality is kept to a minimum. Nitrate levels used within this thesis do not seem to have a deleterious effect on sows and piglets and are well below the recently set NOEAL level of EFSA. The current work and reviewed studies in literature suggest nutritional opportunities aiming to support the sows in the perinatal period and during farrowing may hold part of the solution to reduce piglet losses on farm. Rethinking the current status quo of sow nutrition in the perinatal period

is especially of importance when litter sizes, and with that farrowing duration and piglet losses, keep increasing.

**Take home messages**

1. Supporting the sow in the perinatal period via dietary nitrate supplementation seems a promising method to increase piglet survival independent of litter size.
2. Maternal dietary nitrate supplementation appears to have a larger effect on vasodilation than on stamina.
3. Nitrate concentrations used within this thesis are not expected to have a deleterious effect on the sow and piglets.
4. Optimal duration of farrowing is not a "one size fits all" and is likely dependent of litter size.

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**Summary**

**Nederlandse Samenvatting**

**Acknowledgements**

**About the author**

## Summary

Stillbirth and pre-weaning mortality of piglets do not only represent an economical loss, but also a welfare and ethical problem, which is damaging to the image and potential "licence to produce" of the swine industry. In the Netherlands, approximately 8% of piglets is stillborn and of the live born piglets, on average 12.2% is lost due to pre-weaning mortality. Farrowing is a crucial and challenging event for both the sow and her piglets. For the sow, parturition is an energy demanding, stressful and painful event. The full parturition process (myometrial contraction, dilation of the cervix, piglet and placenta expulsion) can take up to 24h and this duration is increasing as litter size is increasing. Exhaustion of the sow is likely to occur and can impair the number and intensity of uterine contractions, thereby increasing the duration of farrowing. For piglets, farrowing is also a stressful event and the odds of dying are highest during parturition and the first days of life. As farrowing duration increases, the risk for hypoxia due to a limited or complete cut off of the blood flow in the umbilical cord increases, leading either to stillbirth of a reduced vitality right after birth due to asphyxia. In addition, in larger litters, the placental area per piglet is smaller and placental blood flows per piglet is reduced compared to smaller litters. Post-farrowing, piglets are at risk for dying. Piglets are born wet, with a high surface/volume ratio and with a lack of brown adipose tissue (which is used for thermoregulation), meaning that piglets are at high risk for hypothermia, lethargy, and therefore a higher latency to suckle. Consequently, they have a lower ability to move quickly and the risk of being crushed by the sow is increased. It is therefore crucial that piglets are born vital, are able to get to the piglet nest for warmth or get to the udder to consume colostrum and to increase its own heat production.

Maternal nutrition is, next to several other sow and farm management factors, a factor to potentially reduce piglet losses. Sports nutrition in humans is a good source of inspiration for potential nutritional solutions or supplements that could support the sows through the process of farrowing. A new method to enhance competitive performance in athletes, by enhancing athletic performance or to increase endurance, is red beetroot juice. One of the main active compounds in beetroot juice causing these effects is nitrate ($NO_3^-$). Nitrate itself

is not known for specific physiological functions, but *in vivo*, nitrate is non-enzymatically reduced to nitrite ($NO_2^-$) and eventually to nitric oxide (NO), a multivarious messenger molecule with important vascular functions, which particularly occur in environments of hypoxia and acidosis during high physical activity, like farrowing. In addition, in athletes nitrate supplementation showed an increased exercise efficiency and an increased time until exhaustion. Maternal dietary nitrate supplementation is hypothesized to support the sow and her litter in the perinatal period due to 1) the vasodilative effect NO has by being a major endothelium-derived relaxing factor. By ensuring a larger blood, and therefore oxygen, flow in the placenta and the umbilical cord during farrowing, the risk for asphyxiation can be reduced. 2) By reducing the duration of farrowing by improving sow stamina, which would in its turn decrease the incidence of stillbirth and decrease the levels of asphyxiation in new born piglets. The main aim of this thesis was to investigate whether or not supplementation of nitrate to the sow, leading to NO formation, during the perinatal period is an applicable solution to decrease incidence of stillbirth and pre-weaning mortality.

To test if maternal nitrate supplementation can indeed decrease the incidence of stillbirth and pre-weaning mortality, two studies were conducted within this thesis. In Study I, described in *Chapter 3*, initial testing was done on a commercial farm in Denmark with hyper prolific sows (18.2 piglets born total per litter) with a relative high incidence of stillbirth (1.6 piglets per litters) and pre-weaning mortality (14.8% of piglets born alive). In Study I, 120 hyper prolific sows (Landrace x Yorkshire; Danbred) were allocated based on parity and received either a control diet without nitrate or a diet containing 0.06% of nitrate (0.1% of calcium nitrate; $5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$; containing 63.1% of nitrate) from approximately 5 days before the onset of farrowing until day 4 of lactation. Calcium levels in the diets were kept constant by exchanging limestone with calcium nitrate. The number of piglets born alive, stillborn, or that died from birth to weaning were recorded. Piglets were weighed at birth, after cross-fostering, 24 h after cross-fostering, at 3 days of age, and at weaning. Placentas were collected after expulsion and were visually scored on redness. No effect of nitrate supplementation was found on piglet weights, piglet growth, placental redness score, and total pre-weaning mortality during lactation. Maternal dietary

nitrate supplementation decreased stillbirth percentage with 2.5% (9.9 vs. 7.4% for sows receiving the control vs. 0.06% of nitrate, respectively; P = 0.05).

Since Study I was the first time maternal dietary nitrate supplementation in the perinatal period was evaluated, the optimal dosage of nitrate supplementation for both sow and piglets performance was unknown. Therefore in Study II, as described in *Chapter 4* and *Chapter 5*, different dosages of nitrate supplementation from day 108 of gestation until day 5 of lactation were evaluated. In *Chapter 4*, effects of dosage of maternal nitrate supplementation on piglet survival, body weight and litter uniformity are described. In eight consecutive batches, 305 crossbred (Yorkshire × Dutch Landrace; Topigs 20) sows were allocated to one of six diets containing 0.00% (control), 0.03%, 0.06%, 0.09%, 0.12%, or 0.15% of nitrate. Data included number of piglets born alive, being stillborn, and weaned, as well as individual piglet weights at birth, 72 hours of age and weaning. In *Chapter 5*, effects of nitrate supplementation on placental characteristics, levels of asphyxia in piglets and piglet vitality measured immediately after birth were evaluated within a subset of sows (190 out of the 305 sows). Blood acid-base parameters (pH, $pO_2$, $pCO_2$, $BE_{ecf}$, $HCO_3$, $sO_2$ and lactate) and nitrate concentration were determined in umbilical cord blood. The farrowing process was video recorded and later analysed for total duration of farrowing, piglet birth interval, piglet vitality and piglet latency to stand right after birth. Placentas were collected after expulsion during and after farrowing. Placenta length and width were measured and placental color was scored based on redness of the placenta. Maternal nitrate supplementation tended to show a lower incidence of pre-weaning mortality when dosed at 0.09-0.12% (-3.9% and -4.3% compared to the control, respectively, *Chapter 4*). In addition, a linear effect of dosage of maternal nitrate supplementation was found on piglet birth weight (*Chapter 4*), which might be caused by a linear increase in placental width (*Chapter 5*). Litter uniformity (SD) at birth was not affected by maternal nitrate supplementation level (P > 0.10), but SD 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). Piglet weight at weaning and average daily gain of piglets during lactation were not affected by maternal nitrate supplementation (*Chapter 5*). Vitality score of piglets linear increased

with an increasing dosage of nitrate, and also partial oxygen pressure ($pO_2$) in umbilical cord blood measured immediately after birth (*Chapter 5*) tended to be positively related to nitrate dosage. These higher $pO_2$) levels could have been caused by a quicker onset of respiration or an increased blood flow and therefore oxygen flow during birth. However, other blood acid-base parameters in the umbilical cord blood were not affected. No effect of dosage of nitrate supplementation was found on duration of farrowing and placenta redness score (*Chapter 5*). It was concluded that obtained effects of maternal dietary nitrate supplementation appears to have a larger effect on vasodilation (indicated by the effects found on piglet vitality and partial oxygen pressure in umbilical cord blood) than on stamina (since no effect on farrowing duration was found). Additional analyses on data of Study I and II (as described in the *General Discussion*) showed that hyper prolific sows (≥16 piglets) did not benefit more from maternal nitrate supplementation compared to non-hyper prolific sows.

Nitrate concentrations used within the current thesis are not expected to have deleterious effects on the sow or piglets. The highest nitrate intake within this thesis was 15.2 mg per kg of body weight per day, which is well below the set NOAEL (no-observed-adverse-effect level) of 410 mg per kg of body weight per day as determined by EFSA (European Food and Safety Authority).

Data of in total 159 crossbred gilts and sows of Study II was leveraged to evaluate the interactions between farrowing duration and litter size on the level of asphyxia, vitality, percentage of stillbirth and pre-weaning mortality of piglets aiming to disentangle effects of litter size at birth and farrowing duration. Litter size (small: 12-15 piglets, medium: 16-18 piglet and large litter at birth: 19-21 piglets) as well as farrowing duration (short: < 150 min, medium: 150-250 and a long duration of farrowing: >250 min) were categorized to evaluate the interaction between the two. In small litters, a prolonged farrowing duration tended (P = 0.10) to increase pre-weaning mortality compared to a short and medium farrowing duration, while for large litters a medium to long farrowing duration tended to decrease pre-weaning mortality. No other interactions were found. Each additional piglet born to a litter linearly decreased average piglet birth weight with 17.6 grams (P < 0.01), increased farrowing duration with 11 min (P < 0.01) and

increased stillbirth with 0.5% (P = 0.04). A medium farrowing duration resulted in a lower stillborn percentage compared to a short or prolonged farrowing duration, suggesting that farrowing duration might have an optimum. When analysed linearly, stillborn percentage increased with 1.85% per every 100 min (P < 0.01) of farrowing duration. It was concluded that both litter size and farrowing duration affect stillborn percentage, but independent from each other. However, these two factors tended to interact regarding pre-weaning mortality, suggesting that setting a certain threshold for maximal farrowing duration should be taken with care, because this appears to depend on litter size.

## Conclusion

Dietary maternal nitrate supplementation from 5-7 days before the onset of farrowing onwards and dosed at 0.06% - 0.12% decreased incidence of stillbirth and tended to decrease pre-weaning mortality. It appears that the obtained effects of maternal dietary nitrate supplementation likely have a larger effect on vasodilation than on stamina. Additional analyses showed that hyper prolific sows do not benefit more from maternal nitrate supplementation compared to non-hyper prolific sows. Daily nitrate intake achieved within this thesis were well below the recently set NOEAL level of EFSA and did not seem to have harmful effects on sows and piglet.

# Nederlandse Samenvatting

Door optimalisatie van management en fokkerij brengen zeugen steeds meer gezonde biggen voort. Echter, zoals vaak bij dieren met grotere hoeveelheden nakomelingen, sterven een aantal biggen tijdens of vlak na de geboorte. Sterfte van biggen (doodgeboren of voor het spenen) zorgt niet alleen voor economische verliezen, maar is ook een welzijnsprobleem in de varkenshouderij. In de Nederlandse varkenshouderij wordt ongeveer 8% van alle biggen doodgeboren. Vervolgens sterft nog eens 12.2% voor het spenen (het einde van de zoogperiode). Het werpen is een bepalende en uitdagende fase voor zowel de zeug als de biggen. Het totale werpproces (het moment van de eerste weeën tot de geboorte van de placenta's) kan in totaal 24 uur duren en neemt verder toe naarmate een zeug meer biggen krijgt. Het is zeer waarschijnlijk dat de zeug tijdens het werpen uitgeput raakt, wat weer invloed heeft op de kracht en frequentie van de weeën. Voor biggen is het werpen een stressvol en levensbepalend moment. Hoe langer het werpen duurt, hoe groter de kans op zuurstofgebrek doordat de bloedtoevoer door de navelstreng deels wordt afgekneld of doordat de navelstreng breekt. Hierdoor bestaat niet alleen het risico op doodgeboorte, maar ook op een verminderde vitaliteit na geboorte. Grote tomen lopen meer risico doordat de grootte van de placenta en de hoeveelheid bloed die door de placenta en navelstreng naar de big toestroomt per big afneemt. Ook worden biggen nat geboren en hebben weinig vetreserves bij de geboorte. Dit betekent dat het risico op onderkoeling groot is. Het is daardoor van belang dat een big vitaal wordt geboren en snel richting het uier kan gaan om biest te drinken en zo zijn warmteproductie te stimuleren en/of in het warme biggennest te gaan liggen. Onderkoeling kan zorgen voor sloomheid waardoor het risico om te sterven, bijvoorbeeld doordat ze onder de zeug terechtkomt wanneer deze gaat liggen, toeneemt.

Voeding van de zeug is een van de factoren die doodgeboorte en sterfte voor spenen bij biggen zou kunnen verminderen. Sportvoeding bij mensen is een goede bron van inspiratie voor nieuwe ideeën. Het idee wat we in deze thesis getest hebben komt dan ook uit sportvoeding. Een nieuwe methode voor sporters om hun prestaties te verbeteren is door rode bietensap te nemen. Een van de actieve componenten in dit rode bietensap is

nitraat ($NO_3^-$). Nitraat zelf doet fysiologisch niet veel, maar wordt in het lichaam omgezet naar nitriet ($NO_2^-$) en naar uiteindelijk stikstofoxide (NO). Stikstofoxide is een molecuul met vele eigenschappen, waarvan het reguleren van de bloedstoom door middel van bloedvatverwijding (vasodilatatie) er één is. De omzetting van nitraat in het lichaam naar stikstofoxide gebeurt met name wanneer de hoeveelheid zuurstof in het lichaam laag is (hypoxie) en het lichaam verzuurd gedurende lichamelijke inspanning, zoals tijdens de bevalling. Bij sporters heeft het consumeren van nitraat voor een inspanning geleid tot een beter uithoudingsvermogen. Het wordt verondersteld dat het voeren van nitraat aan de zeug in de periode rondom het werpen de zeug en de biggen kan ondersteunen door 1) de bloedstroom regulerende werking van NO wat bloed en daardoor zuurstof naar de biggen kan verhogen en hiermee het risico op zuurstoftekort tijdens de bevalling vermindert. 2) Door het uithoudingsvermogen van de zeug te verbeteren waardoor de totale duur van het werpen wordt verkort en het aantal doodgeboren biggen zou kunnen verminderen en de vitaliteit van pasgeboren biggen kan verhogen. Het hoofddoel van deze thesis was onderzoeken of het verstrekken van nitraat aan de zeug gedurende de periode rondom werpen, een methode is om doodgeboorte en sterfte van biggen voor spenen te verlagen.

Om te onderzoeken of nitraat verstrekking aan de zeug inderdaad de kans op doodgeboorte en sterfte van biggen voor spenen vermindert, zijn twee studies uitgevoerd. In Studie I, beschreven in *Hoofdstuk 3*, werd het verstrekken van nitraat aan de zeug getest op een commercieel varkensbedrijf in Denemarken waar hoogproductieve zeugen werden gehouden (18.2 totaal geboren biggen per toom). Op dit bedrijf was de incidentie van doodgeboorte (1.6 biggen per toom) en sterfte voor spenen (14.8% van alle levend geboren biggen) hoog. In Studie I werden 120 hoogproductieve zeugen (Landras x Yorkshire; Danbred) op basis van hun pariteit (hoe vaak ze biggen hebben gehad) ingedeeld in een controlegroep (welke geen nitraat toegevoegd kreeg aan het voer) of een groep die 0.06% nitraat (0.1% calcium nitraat; $5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$; welke 63.1% nitraat bevat) toegevoegd kreeg aan het voer. De behandelingen startten 5 dagen voor de verwachte dag van werpen en duurden tot en met 4 dagen na het werpen. Aangezien calcium nitraat niet alleen het nitraatgehalte, maar ook het calciumgehalte in het voer verhoogt, is krijt aan

het controlevoer toegevoegd om het totale calciumgehalte in het voer gelijk te houden. Tijdens het experiment zijn het aantal levend- en doodgeboren biggen en de sterfte van biggen tot spenen per zeug geregistreerd. Biggen werden meerdere keren gewogen: op de dag van geboorte, nadat ze waren overgelegd (een methode om het aantal biggen per zeug gelijk te houden), 24 uur na overleggen, bij een leeftijd van 3 dagen en bij het spenen. De placenta's werden verzameld en gescoord op roodheid als maat van doorbloeding. In Studie I werd geen verschil gevonden in geboortegewicht, groei van de biggen, roodheid van de placenta's en sterfte van biggen voor spenen tussen de twee behandelingen. Het verstrekken van nitraat aan de zeug verlaagde het percentage doodgeboren biggen met 2.5% ten opzichte van de controle (9.9% versus 7.4% voor de controle zeugen en de zeugen welke nitraat vertrekt kregen aan hun voer; P = 0.05).

Aangezien Studie I de eerste studie was waarin het verstrekken van nitraat aan zeugen in de periode rondom werpen werd getest, was de optimale dosis om het grootste effect te vinden onbekend. In Studie II, zoals beschreven in *Hoofdstuk 4* en *5*, werden daarom verschillende doseringen van nitraat in het voer getest vanaf dag 108 van de dracht tot en met 5 dagen na het werpen. *Hoofdstuk 4* beschrijft de effecten van de verschillende doseringen van nitraat op bigoverleving, biggewichten en uniformiteit in gewicht besproken. In acht opeenvolgende rondes werden 305 zeugen (Yorkshire x Nederlands Landras; Topigs 20) toegewezen aan een van de zes behandelingen. Dit waren de controle behandeling (welke geen toegevoegd nitraat bevatte) of een voer met 0.03%, 0.06%, 0.09%, 0.12% of 0.15% toegevoegd nitraat. Het aantal levend- en doodgeboren biggen, individuele biggewichten op de dag van geboorte, 72 uur na geboorte en bij spenen werden geregistreerd. In *Hoofdstuk 5* worden de effecten van de verschillende doseringen nitraat op eigenschappen van de placenta, asphyxia (verstikking) en vitaliteit van biggen direct na de geboorte beschreven. Deze intensievere metingen werden bij een deel van het totaal aantal zeugen (190 van de 305 zeugen) uitgevoerd. Verschillende bloedgassen en zuur-base parameters (pH, $pO_2$, $pCO_2$, $BE_{ecf}$, $HCO_3$, $sO_2$ en lactaat) en de nitraat concentratie werden geanalyseerd in een bloedmonster genomen uit de navelstreng van de pasgeboren biggen. Het werpproces werd opgenomen met camera's en later geanalyseerd op totale werpduur, het geboorte interval tussen

opeenvolgende biggen, bigvitaliteit en de duur tussen de geboorte van een big en zijn eerste poging tot staan. Lengte en breedte van placenta's werd gemeten en de roodheid werd gescoord. Nitraat verstrekking liet een tendens voor een lagere sterfte voor spenen zien wanneer deze werd gedoseerd op 0.09-0.12% (respectievelijk -3.9% en -4.3% ten opzichte van de controle behandeling; *Hoofdstuk 4*). Tevens werd een lineair effect van de nitraatdosis op geboortegewicht van de biggen gevonden (hoe hoger de dosis, hoe hoger het geboortegewicht; *Hoofdstuk 4*), wat verklaard kan worden door ook een lineaire toename in placentabreedte (*Hoofdstuk 5*). De uniformiteit in geboortegewicht van biggen werd niet beïnvloed door nitraatdosering, maar een trend (P = 0.07) voor meer uniformiteit (lagere standaarddeviatie) in biggewichten op 72 uur leeftijd werd gevonden wanneer alle nitraatbehandelingen samen werden vergeleken met de controlegroep. De standaarddeviatie in biggewicht nam bij spenen lineair af naarmate de dosis nitraat toenam (toename in uniformiteit). Zowel de groei van de biggen tijdens de zoogperiode als de gewichten van de biggen bij spenen werden niet beïnvloed door de verstrekte dosis nitraat (*Hoofdstuk 5*). Zowel de vitaliteitscore van biggen als de zuurstofdruk in navelstrengbloed namen lineair toe naarmate de dosis nitraat toenam (*Hoofdstuk 5*). Het kan zijn dat de hogere zuurstofdruk in het navelstrengbloed verklaard wordt door een grotere bloedtoevoer en daardoor zuurstofstroom naar de biggen of doordat de big sneller begint te ademen na geboorte door een hogere vitaliteit. Echter, andere bloedwaardes in het navelstrengbloed werden niet beïnvloed door dosering van nitraat. Ook werd geen effect gevonden van de dosis nitraat op de totale werpduur en de roodheid van de placenta (*Hoofdstuk 5*). Uit Studie II werd geconcludeerd dat het waarschijnlijker lijkt dat nitraat een effect heeft op de vasoactiviteit (door het effect gevonden op big vitaliteit en zuurstof druk in het navelstreng bloed) dan op het uithoudingsvermogen van de zeug (aangezien geen effect is gevonden op werpduur). Extra analyses op data van Studie I en II (zoals beschreven in de *Algemene Discussie*) lieten zien dat hoog productieve zeugen (zeugen die 16 biggen of meer krijgen) geen extra voordeel ondervonden van nitraatverstrekking ten opzichte van minder productieve zeugen.

De data van 159 zeugen uit Studie II werd verder gebruikt om meer inzicht te krijgen of werpduur of toomgrootte of beide het meeste effect heeft op mate van asphyxia, vitaliteit,

percentage doodgeboren biggen en sterfte tot spenen. Hiervoor werden zowel toomgrootte bij geboorte (klein: 12-15 biggen, medium: 16-18 biggen en groot: 19-21 biggen) en werpduur (kort: <150 min, gemiddeld 150-250 min en lang: >250 min) geclassificeerd om naar de interactie te kijken van deze twee factoren. Een trend voor een interactie (P = 0.10) werd gevonden voor percentage sterfte voor spenen. Deze liet zien dat in kleine tomen met een lange werpduur de sterfte voor spenen toenam vergeleken met een gemiddelde of korte werpduur, terwijl bij grote tomen sterfte voor spenen juist toenam als het werpen kort duurde in vergelijking met een gemiddelde of lange werpduur. Geen andere interacties werden gevonden. Elke big extra in een toom zorgde voor een afname in het gemiddelde geboortegewicht van 17.6 gram (P < 0.01), voor een toename in werpduur van 11 min (P < 0.01) en een toename in percentage doodgeboren biggen van 0.5% (P = 0.05). Een gemiddelde werpduur zorgde voor het laagste percentage doodgeboren biggen vergeleken met een korte of lange werpduur, wat de suggestie wekt dat werpduur een optimum heeft. Wanneer werpduur lineair werd geanalyseerd, nam bij elke 100 min toename in werpduur, het percentage doodgeboren biggen met 1.85% toe (P < 0.01). Zowel toomgrootte als werpduur hebben dus een effect op het percentage doodgeboren biggen, maar onafhankelijk van elkaar. De trend voor een interactie die gevonden werd tussen werpduur en toomgrootte op sterfte voor spenen suggereert dat we voorzichtig moeten zijn met het stellen wat een maximale werpduur voor alle tomen, omdat deze afhankelijk lijkt te zijn van de toomgrootte.

## Conclusie

Verstrekking van nitraat aan de zeug vanaf 5-7 dagen voor werpen, gedoseerd op 0.06-0.12% verlaagde het percentage doodgeboren biggen en liet een trend in de verlaging van sterfte van biggen voor het spenen zien. Het lijkt erop dat de effecten van nitraatverstrekking aan de zeug meer veroorzaakt worden door een effect op vasodilatatie dan op het uithoudingsvermogen van de zeug. Extra analyses lieten zien dat hoogproductieve zeugen geen extra profijt hebben van nitraatverstrekking vergeleken met minder productieve zeugen.

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De eerste jaren van mijn promotieonderzoek was ik werkzaam bij Cargill en zoals velen van jullie weten was innovatie centrum in Velddriel mijn "thuisbasis". Bij deze wil ik nogmaals al mijn oud-collega's bedanken. Te beginnen bij Patricia: Patrieske, dank je wel voor al je steun en luisterende oor als collega en vriendin. Ook wij hebben heel wat gedeeld in de jaren dat we samen mochten werken en ik heb genoten van elk moment. Je bent nog lang niet van me af. Elke, ook wij kennen elkaar al heel wat jaren. Van bier drinken in de studentenkroeg, naar samen een van de mooiste producten ontwikkelen die Cargill ooit heeft gehad (ben totaal niet bevooroordeeld). Wat een lol hebben we gehad! We stelden elkaar regelmatig de vraag waar we dachten over 5 jaar te staan, en ik kan niet wachten om te zien waar we over de volgende 5 jaar weer zijn. Irene, wat had ik toch zonder jou gemoeten. Je hebt me niet alleen ontzettend geholpen en veel geleerd over statistiek, maar ook wij hebben heel wat afgelachen. Na een aantal jaren samen studeren, werken bij Cargill, ben je inmiddels weer mijn collega bij Agrifirm. John en Edwin, met jullie beiden heb ik een goede band op gebouwd en altijd super fijn samengewerkt! De wetenschap met de praktijk combineren was toch wel onze gemeenschappelijke deler. Maar uiteraard ook de vele slappe klets van jullie! Alcina, you saw me most of the way

through my PhD and we shared a lot of ups and downs in our work and private lives. Our managers changed (a lot), but we always kept going. Thanks for all the fun experiences we shared and that unforgettable wedding of you and Leonel. David, als er iemand is die weet wat ik de afgelopen jaren heb meegemaakt ben jij het wel. Jij zat immers in mijn schuitje. Ook jij hebt me heel wat "motivational speeches" moeten geven. Ik heb je doorzettingsvermogen altijd ontzettend bewonderd. Dank je wel voor je steun, het af en toe even lekker klagen, de vele goeie gesprekken. Anja, vriendin, wat een steunpilaar ben jij geweest. Je wist me altijd weer op te beuren als ik met de moed in mijn schoenen aan je balie stond en ik blijf je daar altijd dankbaar voor! En wat zou Velddriel zijn zonder al die andere fijne (oud)collega's! Nog een laatste speciaal bedankje voor Johan, Corry, Anita, Chantal, Rosalie, Maarten, Rens, Roy, Hans, Kaat, Joost, Kristel, Esther, Maud, Gijs, Marijn, Hester, Evelien, Peter, Elham, Lieske, Hsuan, Qiong, Anton, Jeroen, Alla, Mark, Hink, Henk, Peer en alle anderen. Of course also a very special thank you to all my international colleagues around the globe. Richard, we worked together in de Swine R&D team for several years. Thanks for the fun times! One more special thanks to my former Danish colleagues for helping out with arranging one of the first trials we ever did with nitrate! Tak Jørn and Lars (that is as far as my Danish goes).

Uiteraard waren ook verschillende mensen van de leerstoelgroep ADP betrokken bij mijn onderzoek. Tijdens het uitvoeren van mijn proef in Sterksel heb ik veel kunnen rekenen op Bjorge en Fleur. Jullie behulpzaamheid en ervaring met alles wat met varkens te maken had kwam enorm goed van pas. Uiteraard ook een speciaal woord van dank voor de rest van de medewerkers van de leerstoelgroep die ADP altijd tot een gezellig en warm nest lieten voelen. A special thanks to Caroline! You supervised me for a short time. Thanks for helping me develop my writing skills and your structured feedback. In die 7 jaar zijn er heel wat kamergenoten voorbij gekomen, waarvan ik velen nog steeds af en toe tegenkom in het kleine agrarische wereldje. Merel, Renny, Pieter, Carla, Mariska, Maarten, Jan, Allyson, Anne, Lara en Jesse, dank jullie wel voor de gezelligheid! Lora en Nanette, ook jullie bedankt als eindeloze vraagbakens! Studie 2 is uitgevoerd op het varkens innovatie centrum in Sterksel, waar Nienke, Pim, Carola en vele anderen me hebben geholpen met de vele metingen en wegingen.

Tijdens mijn PhD heb ik ook een aantal studenten mogen begeleiden. Bram, jij had de nobele taak de proef in Denemarken te coördineren wat je goed af ging. Je draaide je hand er niet voor om, om een vrieskist vol placenta's naar Nederland krijgen of honderden biggen te wegen. Na je studie hebben we nog even samengewerkt als collega's. Altijd goed om een "mede-varkensgek" in de buurt te hebben. Chantal, na de proef in Sterksel zijn we inmiddels niet alleen collega's, maar ook vriendinnen geworden. Welkom "on the bright side" van het varkensonderzoek. Op naar een eindeloze hoeveelheid bier, sushi en plantjes! Marloes en Birgit, ook dankzij jullie inzet en gezelligheid is de proef in Sterksel niet alleen een mooie proef, maar ook nog eens een gezellige ervaring geworden.

In de afrondende fase van mijn PhD ben ik aan een nieuwe baan begonnen bij Agrifirm. Wederom stond ik voor de uitdaging werk en het afronden van mijn PhD met elkaar te combineren. Dank aan mijn collega's binnen het R&D monogastrics team voor het begrip, de motiverende woorden en de ruimte die ik kreeg om het af te maken.

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Rob, achter elke sterke vrouw staat een man ;) Je hebt me zien genieten, klagen, worstelen, mijn mijlpalen in mijn PhD zien vieren, me zien vallen en opstaan. Je bleef in me geloven ook al deed ik dat zelf soms niet meer. Dank je wel voor je lieve woorden, het sparren,

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*I have no special talents. I am only passionately curious.* – Albert Einstein

Moniek

## About the author

Moniek van den Bosch was born in Wezep, the Netherlands, on August 28, 1985. She grew up in Wezep and graduated from the Carolus Clusius College in Zwolle in 2004. She moved to Wageningen and obtained her BSc in Animal Sciences at Wageningen University. In 2008 she started her MSc Animal Sciences also at Wageningen University, with a specialization in Adaptation Physiology. Moniek conducted her minor thesis at the Animal Breeding and Genetics group, working on estimating genetic parameters of purebred landrace sows at Hendrix Genetics. During her overseas internship in Palmerston North, New Zealand, she looked into the relation between placentome size and gestational age in dairy cattle. Her major thesis was at the Adaptation Physiology group, studying the second litter syndrome in sows, before receiving her Master degree in Animal Science in September 2010.

After graduation Moniek started as a Research Nutritionist Swine for Provimi. Her main focus was on piglet nutrition, both pre and post-weaning, aiming to maximize feed intake and minimizing gut damage through weaning. After the merger of Provimi and Cargill Animal Nutrition, she continued as a Research Scientist Swine and her focus moved more to sow performance and piglet livability. She was involved in strategic and agility projects in which ideation, testing and analysis lead to applicable solutions for customers. In February 2015, Moniek got the opportunity to start her PhD at the Adaptation Physiology Group of Wageningen University and Research next to her job at Cargill, of which the results are presented in the current thesis. In July 2017, Moniek moved to a Senior Research Scientist position in which she was responsible for ideation and innovation of tomorrow swine

nutrition, mainly specialized in sow nutrition. Moniek is mother of two sons: Cas (October 2015) and Nils (January 2018).

In December 2020 Moniek left Cargill Animal Nutrition and started as a Senior Researcher Swine at the Royal Agrifirm Group, where she currently plays a role in designing the research portfolio for swine. Here she continues to use her curiosity, eagerness to learn, and passion to make difficult things easy, to translate science to on-farm solutions and on-farm problems to the right scientific questions. She has a passion for sow production, the physiological effect of nutrition in the body and reproduction.