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Glucose and glycogen levels in piglets that differ in birth weight and vitality

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ABSTRACT

In the pig, intrauterine crowding can greatly affect postnatal characteristics, among which birth weight and locomotion. In a previous study, we discovered that piglets with a low birth weight/low vitality (L piglets) have a reduced motor performance compared to piglets with a normal birth weight/normal vitality (N piglets). A possible explanation is that L piglets lack the energy to increase their motor performance to the level of that of N piglets. Blood glucose levels (GLU) and glycogen concentrations in skeletal muscle of the front (GLY_{FRONT}) and hind leg (GLY_{HIND}) and the liver (GLY_{LIVER}) at birth and during the first 96 h postpartum were compared between L and N piglets. GLU at birth was the same for both groups. After birth, GLU immediately increased in N piglets, whereas it only increased after 8 h in L piglets. L piglets showed a lower GLY_{HIND} at birth and did not use this glycogen during the first 8 h postpartum, while N piglets showed a gradual depletion. GLY_{LIVER} at birth was 50% lower for L piglets and was unused during the studied period while N piglets consumed half of their GLY_{LIVER} during the first 8 h. Based on these results, it is possible that lower glycogen concentrations at birth, the delayed increase in GLU and the lower use of glycogen during the first 8 h after birth negatively affect motor performance in L piglets. However, based on this study, it is unclear whether the low mobilization of glycogen by L piglets is a consequence, rather than a cause of their lower motor performance.

1. Introduction

Due to their large litter size, intrauterine crowding (IUC) is a common phenomenon in pigs (Dziuk, 1985; Fraser, 1990; Perry and Rowell, 1969). This naturally occurring IUC is aggravated in a modern breeding setting, where sows are genetically selected for producing litters of fourteen piglets and more (Rutherford et al., 2011). In these litters, there is an increased heterogeneity of birth weights and an increased number of small piglets (Wise et al., 1997), with an impact on vitality and morbidity.

In a previous paper we discussed how IUC leads to a lower motor performance (indicative of a force deficit) in piglets with a low birth weight (lower than the mean litter birth weight minus the standard deviation (SD))/low vitality (L piglets), compared to piglets with a normal birth weight (within the limits of the mean litter birth weight plus or minus the SD)/normal vitality (N piglets) (Vanden Hole et al., 2018a). In a subsequent study, we learned that the muscles of L piglets are morphologically sufficiently developed and do not contain a lower number of fast-contracting (type II) fibers (Vanden Hole et al., 2018b). Thus we must look beyond the musculoskeletal system for finding an explanation for the reduced motor performance in L piglets. Several reasons for a low viability and vitality in newborn pigs (mainly piglets with a low birth weight) have been proposed, such as weaker thermoregulation mechanisms (Bate, 1993), neonatal asphyxia at birth (Herpin et al., 1996) or a lower degree of arterial oxygen saturation the first hour after birth (Casellas et al., 2004) and lower energy levels (Theil et al., 2014). In this study, we chose to focus on the latter, because an insufficient energy supply has proven to be the main cause of death before weaning (Pettigrew, 1981). Newborn pigs have a high energy demand for thermoregulation and physical activity (e.g. locomotion, suckling and taking ownership of a functional teat) (Theil et al., 2012). They are largely dependent on glycogen in skeletal muscle and the liver for their initial energy provision (De Vos et al., 2016; Herpin et al., 1993; Theil et al., 2011). However, the glycogen stores at birth are limited and, while they are used extensively, they are not (skeletal muscle) to minimally (liver) restored during the first days after birth (Girard et al., 1992;

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Shelley, 1961). Piglets are born with a negative energy balance (Theil et al., 2014) and depend for their energy supply and the transfer of passive immunity (Salmon et al., 2009) on a rapid intake of colostrum (later on early milk and milk, we will refer to all of these in the rest of the paper generally as 'milk intake') (Mount, 1968). The selection for large litters has exacerbated the 'natural' energy deficiency at birth (Rutherford et al., 2013). In addition, milk production and the number of functional teats have not augmented linearly with litter size (for a review, see Andersen et al. (2011)). As such, a larger litter size does not only increase the competition for milk within a litter (Rutherford et al., 2013), it also puts the piglets with a low birth weight at a disadvantage with regard to milk intake (Amdi et al., 2013; Le Dividich et al., 2005; Quesnel et al., 2012). Furthermore, piglets with a low birth weight have a greater energy requirement per kg of birth weight because their high surface-to-volume ratio makes them more prone to heat loss (Close et al., 1985). As such they have to allocate more energy towards homeothermy (Noblet et al., 1987). In addition, it is possible that, compared to their N littermates, L piglets are born with a lower glycogen concentration (in skeletal muscle and the liver) and that, because of an inadequate milk intake, glycogen will be depleted more rapidly as an alternative energy source (see review by Mellor and Cockburn (1986)).

A significant part of an animal's energy budget is allocated towards locomotor activity (Biewener, 2003). An increase in performance (or speed) requires an animal's muscles to generate larger forces and contract more rapidly (Biewener, 2003; Ørtenblad et al., 2013). In skeletal muscle, glycogen is the main energy source, though the contribution of glucose increases with prolonged activity or exercise (Westerblad et al., 2010). As such, a lower glycogen concentration in the muscles and/or the liver of L piglets at birth and/or a quicker depletion during early development combined with a lower milk (and hence glucose) intake seems a plausible explanation for the observed difference in motor performance between L and N piglets.

The goal of this paper is not to give a complete overview of the energy balance in the newborn pig, but to specifically link energetics to locomotion. As such, we aimed to answer the following questions:

- We hypothesize a lower blood glucose level (GLU) at birth for L piglets, compared to N piglets, indicating a lower allocation of glucose from the sow towards L piglets *in utero*. In addition, we hypothesize a lower GLU for L piglets during early development, indicating they are less apt in retrieving milk from the sow, compared to their N littermates. Alternatively, a lower GLU in L piglets might also indicate lower glycogen stores in the liver or a less efficient use of this liver glycogen. These reasons for a lower GLU in L piglets are not mutually exclusive.
- We hypothesize that L piglets have a lower concentration of glycogen in the skeletal muscles of both the front (GLY_{FRONT}) and hind leg (GLY_{HIND}) at birth and during early development. The muscles of the front and the hind leg are investigated separately because of the functional specialization of legs in quadrupeds. We also hypothesize that, because of their lower ability to get milk from the sow, L piglets will need to use their glycogen reserves more quickly than N piglets and will hence show an early drop in both GLY_{HIND} and GLY_{FRONT}.
- We hypothesize that L piglets have a lower glycogen concentration in the liver (GLY_{LIVER}) than N piglets at birth and during the first 96 h after birth. In addition, we hypothesize that L piglets will use of their glycogen reserves more quickly than N piglets, because of their lower ability to take in milk during the first hours after birth and the need to achieve blood glucose homeostasis.

2. Materials and methods

2.1. Selection of piglets

Institutional and national guidelines for the care and use of animals were followed and all experimental procedures involving animals were approved by the Ethical Committee of Animal Experimentation, University of Antwerp, Belgium (approval number 2015-26).

Thirty-two piglets (Topigs x German Piètrain) were selected from 10 litters in a local farm in October 2016. The same selection procedure was followed as in Vanden Hole et al. (2018a; 2018b). The piglets selected for Vanden Hole et al. (2018b) were used for this study.

Two to 6 healthy piglets were selected per litter (mean number of piglets born per litter was 18.2 (±4.2) (mean ± SD, here and throughout)) in pairs of L and N piglets and ear-notched upon selection. Pairs were sex-matched, with both piglets being male or both being female. An overview of the selected piglets can be found in Supplementary Table S1. Immediately after birth, all piglets from the abovementioned 10 litters were weighed, in order to calculate the mean body mass (BM) at birth per litter. Each piglet was given a vitality score, based on respiration (0–2; 0 = no respiration, 1 = labored respiration, 2 = regular respiration) and locomotion (0–2; 0 = no movement, 1 = piglet can stand up, 2 = piglet is able to take a few steps). Piglets scoring 1 or 2 (out of 4) were considered to be low in vitality, while piglets that scored 3 or 4 were considered to have a normal vitality.

Based on BM and vitality at birth, piglets were classified into either L (n = 15) and N piglets (n = 17). N piglets had a BM at birth that was within the limits of the mean BM at birth of the entire litter ±SD, combined with a normal vitality score. Piglets with a low vitality score and a BM at birth that was lower than the mean BM at birth of the litter – 1 SD, were considered L piglets. Piglets with a normal birth weight and a low vitality or piglets with a low birth weight and normal vitality were not included in the study. This was done purposefully, in order to compare two extremes, caused by IUC (Vanden Hole et al., 2018a, 2018b).

The mean BM at birth of N piglets was 1.37 kg (±0.29), while that of L piglets was 0.79 kg (±0.26).

This study includes 4 time points during early development: 0, 4, 8 and 96 h after birth. The first 3 time points are key in the locomotor development of the young piglet, while 96 h serves as a good control age during the time frame of early development (for a more elaborate explanation see (Vanden Hole et al., 2018a; Vanden Hole et al., 2017)).

For each time point we aimed at including 8 piglets with an equal distribution of L piglets/N piglets and females/males. However, in the 0 h group an extra N/L couple had to be selected, because for one N piglet that was euthanized at 0 h, there was no L counterpart present in that litter. This leads to a total of 5 N and 4 L piglets (3 males and 6 females) being included in this 0 h group. In addition, only 7 piglets were included in the 96 h group, because one male L piglet died prior to sampling.

For the GLU measurements, additional samples were available from another study, involving low and normal birth weight piglets of similar ages. These were added to the existing dataset (Supplementary Table S1, numbers between brackets). The piglets in this additional study were selected in the same way and at the same farm as described above, but in October 2017. As such, GLU data from 24 piglets (Topigs x PIC) were added, leading to a total dataset for GLU of n = 56. Of these 24 piglets, 11 were classified as L piglets, 13 as N piglets. These piglets were euthanized at 0, 8 and 96 h (8 piglets per group), with an equal distribution of L and N piglets in the 0 and 8 h group. In the 96 h group, 3 L and 5 N piglets were present. All 24 piglets were female.

2.2. Sampling

Before euthanasia, the selected piglets were deeply anesthetized with a combination of Zoletil 100® (Tiletamine 50 mg/ml, Zolazepam 50 mg/ml; Virbac, Carros, France) and Sedaxyl® (Xylazine hydrochloride 20 mg/ml; Kela, Hoogstraten, Belgium), in a dosage of 0.22 ml/kg BM (administered intramuscularly). Euthanasia itself was performed by transecting the jugular veins and carotid arteries. Immediately after euthanasia GLU was measured with a glucose meter (Onetouch Ultra, Johnson & Johnson, New Brunswick, NJ, USA).

Following the GLU measurement, tissue samples were taken in a standardized way (see below) from 3 muscles from the left front limb (*m.*

brachiocephalicus, *m. triceps caput longum*, *m. extensor carpi radialis*) and 3 muscles from the left hind limb (*m. vastus lateralis*, *m. semitendinosus*, *m. gastrocnemius*) and the liver. From *m. triceps caput longum*, *m. brachiocephalicus*, *m. semitendinosus* and *m. vastus lateralis* the distal part of the muscle was sampled, while from *m. gastrocnemius* the middle part was used. *M. extensor carpi radialis* was split in two through a longitudinal section, of which the most caudal half was sampled. The liver sample was a combined sample of pieces from each of the six lobes (lobus sinister lateralis and medialis, lobus dexter lateralis and medialis, lobus quadratus and lobus caudatus). Immediately after dissection, the samples were snap frozen and stored at -80 °C.

2.3. Glycogen concentration

The protocol for the determination of the glycogen concentration in both muscle and liver tissue is based on the protocol by Theil et al. (2011). One important modification was that in our protocol we took 3 different pieces of tissue per sample which resulted in one average value per sample. This was done because the distribution of glycogen throughout the tissue is heterogeneous (Marchand et al., 2002, 2007; Nielsen and Ørtenblad, 2012; Nielsen et al., 2010; Wanson and Drochmans, 1968).

As such, 3 pieces of tissue of 25 mg (±2 mg) each were taken from each muscle and liver sample. The exact masses of the tissue pieces were documented for later calculations. To break down the tissue and convert the glycogen to glucose, each 25 mg piece of tissue was heated for 2 h at 100 °C with 1 mL of 1 M HCl in a sealed glass tube. Halfway through (after 1 h), the suspensions were sonicated for 5 s at an amplification of 100%. By doing this, the muscle fibers or liver cells were broken down more efficiently. After 30 min of cooling at room temperature, the glass tubes were centrifuged at 2500 g for 20 min at 4 °C. The supernatant was filtered with a prepleated cellulose filter (Whatman plc, Maidstone, Kent, UK) to remove any remaining (not broken down) tissue. Peroxidase-Glucose Oxidase (PGO) enzymes (Sigma-Aldrich, St. Louis, MO, USA) were used for determining the concentration of glucose spectrophotometrically at 450 nm (Tecan Infinite 200 Pro, Männedorf, Switserland). Muscle glycogen was expressed as % of wet tissue weight.

2.4. Statistics

To evaluate the effect of age (0, 4, 8 and 96 h) and birth weight category (BW category, L or N piglet) on GLU and GLY, linear mixed models were fitted with JMP® Pro 13 (SAS Institute Inc., Cary, NC, USA). Sex was added as a covariate and sow was added as a random factor to account for the dependence between littermates. For GLU the starting model did not include sex as a covariate, because the number of males and females were quite different (due to inclusion of the data from an extra 24 females in the GLU dataset). Instead, the effect of sampling period was tested, because GLU samples were obtained during two different samplings. This starting model was simplified using stepwise backwards modelling (Draper and Smith, 1998). To meet normality and/or homoscedasticity assumptions GLY_{FRONT}, GLY_{HIND} and GLY_{LIVER} were square transformed and GLU was square root transformed. Effects were considered statistically significant if $p \leq 0.05$. *Post-hoc* analysis with Tukey's correction was used to compare different age groups, except in the case of GLY_{LIVER} where a Kruskal-Wallis test was used due to the SD's being too different. All values are indicated as mean ± SD.

3. Results

Detailed information on means (±SD) across the different groups can be found in the Supplementary Table S1.

For GLU there was a significant interaction between BW category and age, indicating GLU changes differently with age for L and N piglets. For L piglets, GLU was significantly lower at 0, 4 and 8 h, compared to 96 h ($P = 0.0078$, 0.0006 and 0.0197, respectively) (Fig. 1). In N piglets, we noted that GLU at 0 h was significantly lower compared to 4, 8 and 96 h ($P = 0.0175$, 0.0015 (4 and 8 h, respectively) and $P < 0.0001$ (96 h). In other words, we note an earlier increase of GLU in N piglets, compared to L piglets. There was no significant effect of the sampling period.

For GLY_{FRONT}, no significant differences were observed between L and N piglets (Fig. 2A). However, age did have a significant effect on GLY_{FRONT} ($P < 0.0001$). For both L and N piglets GLY_{FRONT} was significantly lower at 96 h than at 0, 4 and 8 h ($P < 0.0001$ (0 h), $P = 0.0007$ and 0.0147 (4, 8 h)). In addition, GLY_{FRONT} was significantly lower at 4 and 8 h, compared to 0 h ($P = 0.0293$ and 0.0008, respectively). As such,

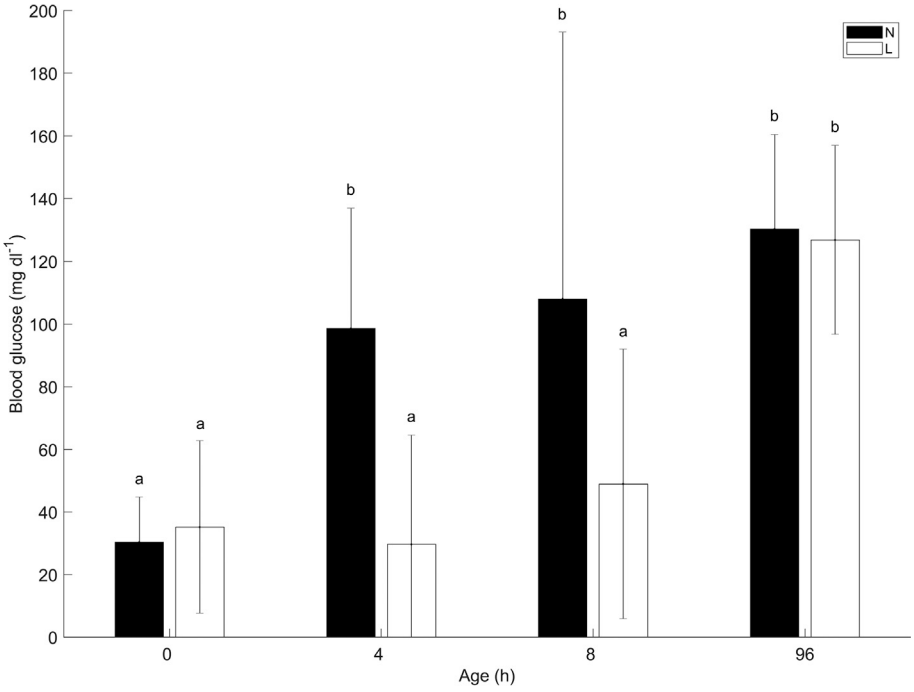

Fig. 1. Blood glucose levels. n = 56. N = piglets with a normal birth weight/normal vitality; L = piglets with a low birth weight/low vitality. A normal birth weight is defined as a birth weight within the limits of the mean birth weight of the litter ±SD, a normal vitality score as 3 or 4 out of 4 (based on activity and respiration). A low birth weight is defined as a birth weight < the mean birth weight of the litter - SD, a low vitality score as 1 or 2 out of 4 (based on activity and respiration). All values are mean ± SD. Significant differences (linear mixed models, $p \leq 0.05$) are indicated by different letters.

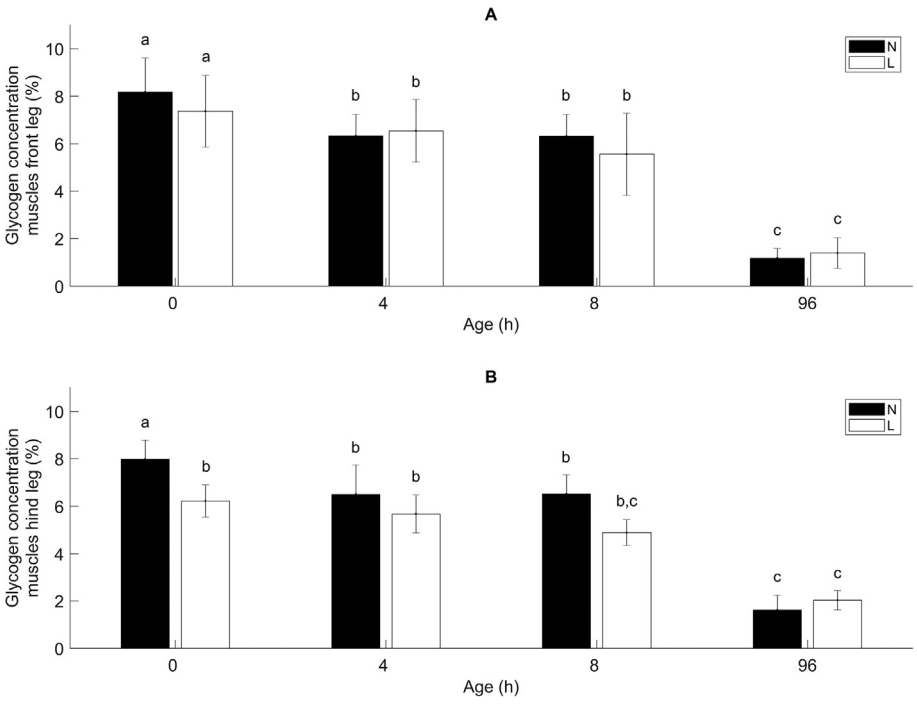

Fig. 2. Glycogen concentration in skeletal muscle. A. Glycogen concentration front leg (n = 32). B. Glycogen concentration hind leg (n = 32). N = piglets with a normal birth weight/normal vitality; L = piglets with a low birth weight/low vitality. A normal birth weight is defined as a birth weight within the limits of the mean birth weight of the litter ±SD, a normal vitality score as 3 or 4 out of 4 (based on activity and respiration). A low birth weight is defined as a birth weight < the mean birth weight of the litter - SD, a low vitality score as 1 or 2 out of 4 (based on activity and respiration). All values are mean ± SD. Significant differences (linear mixed models, $p \leq 0.05$) are indicated by different letters.

GLY_{FRONT} decreased between 0 and 4 h, and between 8 and 96 h. We also found that for both L and N piglets males had a higher GLY_{FRONT} than females ($P = 0.0231$).

For GLY_{HIND} we observed a significant interaction between age and BW category, indicating GLY_{HIND} changes differently with age for L and N piglets (Fig. 2B). In L piglets, GLY_{HIND} was significantly lower at 96 h, compared to the GLY_{HIND} at 0 and 4 h ($P = 0.0048$, and 0.0129, respectively). In N piglets GLY_{HIND} was significantly lower at 96 h, compared to 0, 4 and 8 h ($P < 0.0001$ (0 h), $P = 0.0020$ and 0.0046 (4 and 8 h). In addition, GLY_{HIND} was significantly higher at 0 h, compared to 4 and 8 h ($P = 0.0488$ and 0.0113). In other words, GLY_{HIND} drops significantly between 0 and 4 h, and then again between 8 and 96 h (but remains constant between 4 and 8 h). The GLY_{HIND} at 0 h was significantly different between L and N piglets ($P = 0.0128$).

For GLY_{LIVER} we observed an interaction between age and BW category, indicating GLY_{LIVER} changes differently with age for L and N piglets (Fig. 3). For L piglets, there was no significant age-effect, while there was for N piglets ($P = 0.0305$). For N piglets, GLY_{LIVER} at 0 h was significantly higher than at 8 h ($P = 0.0238$) and 96 h ($P = 0.457$). Comparing L and N piglets, we saw a significantly different GLY_{LIVER} at 0 h ($P = 0.0208$).

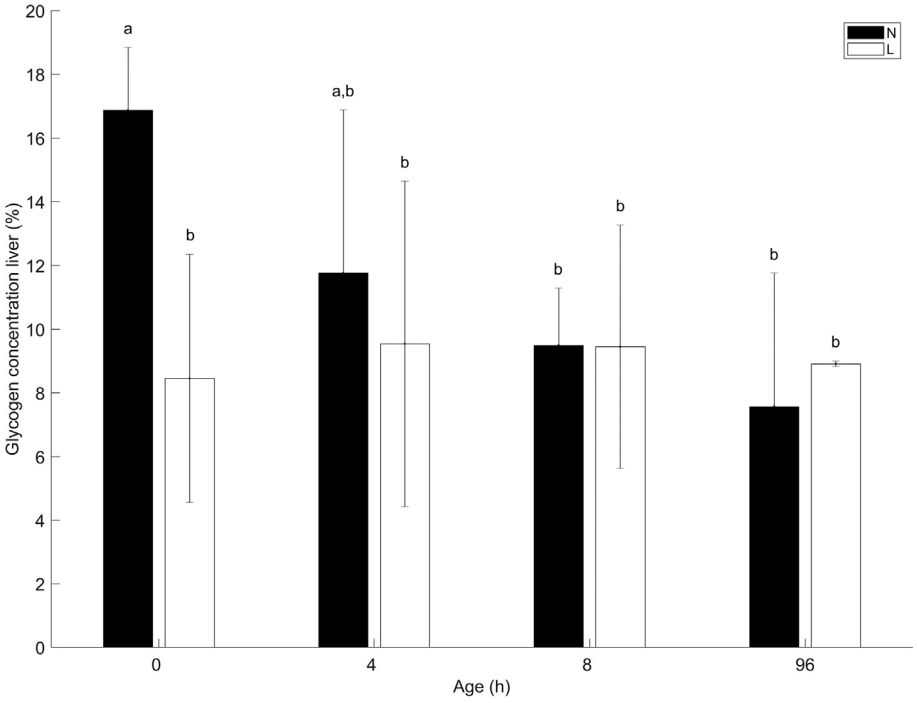

Fig. 3. Glycogen concentration in the liver. n = 32. N = piglets with a normal birth weight/normal vitality; L = piglets with a low birth weight/low vitality. A normal birth weight is defined as a birth weight within the limits of the mean birth weight of the litter ±SD, a normal vitality score as 3 or 4 out of 4 (based on activity and respiration). A low birth weight is defined as a birth weight < the mean birth weight of the litter - SD, a low vitality score as 1 or 2 out of 4 (based on activity and respiration). All values are mean ± SD. Significant differences (linear mixed models, $p \leq 0.05$) are indicated by different letters.

4. Discussion

4.1. Do L piglets have a lower blood glucose level at birth and during the first 96 h after birth, compared to N piglets?

After birth, GLU increased for both L and N piglets and reached similar levels at 96 h. However, we observed a different pattern for L and N piglets, with an earlier increase (between 0 and 4 h) of GLU for N piglets and a later, but similar increase (between 8 and 96 h) in L piglets. Several, non-exclusive reasons for this lower GLU level during the first 8 h after birth for L piglets can be proposed. From our results we see that there is no mobilization of glycogen from the liver in L piglets (see further), which is normally an important factor in maintaining glucose homeostasis during the first 12 h after birth (Ballard and Oliver, 1963; Dawkins, 1963; Pegorier et al., 1982; Rial and Nicholls, 1984; Snell and Walker, 1973). In addition, L piglets might have a lower milk intake than N piglets (as shown by e.g. De Vos et al. (2014) and Declerck et al. (2017)), leading to lower levels of glucose and galactose (the latter used for hepatic gluconeogenesis) (Girard et al., 1992). The first suckling usually occurs about 20–30 min after birth (Le Dividich and Noblet, 1981), but, even then, fierce competition among littermates might prevent L piglets from getting enough milk to increase GLU during these first 8 h. Competitively, L piglets are at a disadvantage, not only because of their smaller size, but also because they are more cold-sensitive than their heavier littermates (Close et al., 1985). They are at greater risk for hypothermia because heat loss per unit of body mass is inversely related to size (for a review, see Herpin and Le Dividich (1995)). Hypothermia reduces the vigor of piglets, because they need to allocate a lot of their energy towards maintaining their body temperature (Herpin et al., 2002a, 2002b; Mount, 1968). As such, it has been shown that there is a depressive effect of cold on milk intake (Le Dividich and Noblet, 1981), but also that it increases glucose turnover rate, accelerates glycogen depletion in the muscles and stimulates glucose uptake by piglet skeletal muscle quickly after birth (Herpin et al., 1992; Lossec et al., 1998a, 1998b). All these glucose-metabolism adaptive changes could also contribute to the lower GLU levels observed in L piglets and can lead to less competitive nursing behavior and consequently milk intake (Le Dividich and Noblet, 1981). Similarly, a reduction in teat-seeking activity has been found in newborn lambs kept in cold conditions (Alexander and Williams, 1966).

Overall, it is hard to conclude whether GLU is the limiting factor for motor performance during the first four days after birth. The similar GLU levels at birth, but a lower motor performance in L piglets, point to other causes for a lowered motor performance. On the other hand, around 4 and 8 h, the lower GLU in L piglets might contribute to their inability to increase their motor performance to the level of that of N piglets.

4.2. Do L piglets have a lower glycogen concentration in the muscles at birth and during the first 96 h after birth than N piglets?

We noted different results for GLY_{FRONT} and GLY_{HIND}, both at birth and during the immediate neonatal period. At birth, we saw no difference between L and N piglets for GLY_{FRONT}, while GLY_{HIND} was lower for L piglets, compared to N piglets. This contrasts with other reports. Widdowson (1974) and De Vos et al. (2016) found no difference in glycogen levels at day 0 and day 3 between piglets with a normal birth weight and growth retarded piglets/low birth weight piglets, respectively.

Immediately after birth GLY_{FRONT} levels reduced at a similar rate in L and N piglets. On the other hand, for GLY_{HIND} the depletion pattern differed between L and N piglets, with the pattern in N piglets being similar to that of GLY_{FRONT}. For L piglets, GLY_{HIND} remained constant during the first 8 h, only after which it decreased. This raises the question whether this gradual pattern of depletion in GLY_{HIND} and GLY_{FRONT} of N piglets, starting immediately after birth, reflects the normal pattern of muscular glycogen depletion. Other studies on the depletion of muscular glycogen report a similar pattern (Elliot and Lodge, 1977; Herpin et al., 2002b). If we consider this gradual depletion the standard pattern of glycogen utilization in skeletal muscle, then L piglets show a deviation by having a lower GLY_{HIND} at birth and not using their available muscular glycogen during the first 8 h after birth.

The different results that were obtained for the front and hind leg might be explained by the functional specialization of the front and hind limbs with regard to quadruped locomotion. The hind limbs are responsible for generating large (accelerating) forces, while the front limbs are mainly responsible for support of the center of mass (Biewener, 2003; Merkens et al., 1993; Payne et al., 2005; Witte et al., 2004). Given that the available glycogen in a myocyte can only be used by that myocyte, a lower GLY_{HIND} for L piglets may greatly affect forward motion and hence motor performance (as measured in Vanden Hole et al. (2018a; 2017) by speed and its components stride length and stride frequency) during the first 8 h of life.

The importance of the early use of glycogen stores has been confirmed in multiple studies (e.g. Elliot and Lodge (1977); Theil et al. (2011)), however, L piglets did not use the available glycogen in the hind limb during the first 8 h. This might be a (partial) explanation for the lower motor performance in L piglets, at least during the first 8 h after birth. However, it is hard to state whether the low mobilization of glycogen by L piglets is preventing them from increasing their motor performance or whether a lower motor performance (lower activity levels, possibly caused by low blood glucose levels during the first 8 h after birth and lower starting levels of glycogen) is causing the piglets not to access their glycogen.

Alternatively, one might consider that L piglets can safeguard their muscular glycogen, because they can get a sufficient amount of energy elsewhere. However, this theory is highly unlikely, given the low GLU and GLY_{LIVER} shortly after birth and their limited fat reserves and ability to oxidize protein (De Vos et al., 2016; Herpin et al., 1993; Le Dividich et al., 1991; Lin et al., 2013; Marion and Le Dividich, 1999), but also given the specific use of muscular glycogen for locomotion and thermoregulation. Herpin et al. (2002a) state that skeletal muscle plays a key role in maintaining homeothermy in newborn pigs, by means of shivering thermogenesis. More likely, they are somehow unable to make use of their available muscular glycogen reserves. We will not go into detail on this hypothesis for two reasons. Firstly, a detailed description of the several (hormonal) pathways and enzymes would go beyond the scope of this paper. Secondly, though the biochemistry of glycogen utilization is well understood, the precise mechanism linking glycogen reserves to muscle function remains elusive (Ørtenblad et al., 2013).

4.3. Do L piglets have a lower glycogen concentration in the liver (GLY_{LIVER}) at birth and during the first 96 h after birth than N piglets?

During the first 8 h after birth, N piglets showed a gradual decrease in GLY_{LIVER}, after which it remained at a constant level. By 96 h after birth, about 55% of GLY_{LIVER} at birth was depleted in N piglets. L piglets had a lower GLY_{LIVER}, compared to N piglets at birth and this level showed no change at all from birth up to and including 96 h. This indicates that L piglets did not use liver glycogen during the first 96 h after birth, which is surprising, because other studies have found a mobilization of liver glycogen to be both common and necessary in newborn pigs, thereby contributing to glucose homeostasis (e.g. Swiatek et al. (1970); Theil et al. (2014); Le Dividich et al. (1994)). The questions we are left with here are: why did L piglets have lower glycogen stores in the liver at birth and why did they not use these reserves during the first 96 h after birth? It is unclear whether these lower levels at birth are due to a lower allocation of glucose (as a building block for glycogen) to L piglets *in utero*. However, a consequence of these low levels at birth might be that L piglets are unable to use their available reserves, perhaps because they are already too low to start with at birth. For several species a critical glycogen level in the liver has been reported, a threshold below which the liver glycogen no longer contributes to blood glucose levels. This critical value can differ greatly between species (0.1% in rabbits,

compared to 1% (of wet weight) in humans) (Shelley and Neligan, 1966). It is possible that for pigs, this threshold value is higher, especially given their higher glycogen reserves (Shelley (1961) reports average values of 8–10% at birth across species). However, looking at our data this critical value seems to be around 8%, which in comparison to other species (see above) and other reports is high (e.g. 4% in Mersmann (1971), 2% in Elliot and Lodge (1977) and Theil et al. (2011)).

Alternatively, the glycogenolysis pathway might be (at least partially) inhibited. As mentioned before we will not elaborate on the latter hypothesis, because this is beyond the scope of this paper.

4.4. Why do L piglets show a reduced motor performance at 96 h after birth, even though the levels of blood glucose and glycogen are not lower, compared to those of N piglets?

At 96 h after birth, none of our investigated variables were different for L and N piglets, while motor performance was still lower in L piglets (see Vanden Hole et al. (2018a)). In their review on energetics in newborn pigs, Mellor and Cockburn (1986) state that the energy requirements increase after the first day after birth. The main cause for this spectacular higher need after a few days is because there is little change in the physiological mechanisms to prevent heat loss during the first week after birth. As such, it is possible that at 96 h of age L piglets (because of their high sensitivity to hypothermia, see before) still have to allocate more of their available energy towards maintaining a constant body temperature, leaving less energy available to boost their motor performance. Also, during the first week after birth, other energy sources become more important, such as lipids and proteins (from milk) (Le Dividich and Seve, 2000). For a review on this topic, see Mellor and Cockburn (1986). As such, an impaired intake or digestion of these lipids and proteins, or any problem with regard to making these substrates available as a viable energy source for locomotion, might come into play during the first week after birth.

5. Conclusion

We found no difference in GLU at birth for L and N piglets. However, after birth we observed an immediate increase in GLU in N piglets, while in L piglets a similar increase occurred only after 8 h. With regard to the glycogen concentration, differences between N and L piglets were noted for GLY_{HIND} and GLY_{LIVER} (not GLY_{FRONT}). Both were lower at birth for L piglets, and showed either a slower depletion pattern (GLY_{HIND}) or were not used at all (GLY_{LIVER}) in this group. Based on these results, it is possible that L piglets' lower glycogen levels at birth (in the hind limb and the liver) negatively affect their motor performance, making them less apt at retrieving early milk. This can in turn cause GLU to rise less quickly, compared to that of their N littermates. However, given the same GLU level for L and N piglets at birth, it is unclear if glucose is actually the limiting factor for motor performance. With regard to the different depletion pattern of glycogen in L and N piglets it is (at this stage) impossible to state whether the low mobilization of glycogen in skeletal muscle by L piglets is a cause or a consequence of a lower motor performance. On the other hand, around 96 h after birth we noted no difference between L and N piglets in blood glucose levels or glycogen concentration, while we know (from our earlier study) that motor performance at this point in time is still lower for L piglets. To further elucidate the effect of energy levels on motor performance a few days after birth, other energy sources should be studied.

Declarations

Author contribution statement

Charlotte Vanden Hole: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Miriam Ayuso: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Peter Aerts: Conceived and designed the experiments; Wrote the paper.

Sara Prims: Performed the experiments; Wrote the paper.

Steven Van Cruchten: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Chris Van Ginneken: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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