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The impact of drenching on the pre-weaning resilience of low birth weight piglets

Dissertation for the degree of Doctor in Veterinary Sciences (PhD) at the University of Antwerp to be defended by Kevin Van Tichelen

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Preface

“Please imagine, gentle reader, that you suddenly find yourself beside a recumbent elephant in a small, locked room. The elephant seems agitated: she periodically jumps to her feet and then crashes to the floor, and may even whirl about and attack you without warning. Unfortunately, you entered the room by being squeezed through a narrow tube and perhaps partly suffocated, so you are none too steady on your feet. You ought to keep as far from the elephant as the limited space permits, but you cannot afford this luxury because you are cold, wet, unclothed, and desperately short of food, and the only source of food is the elephant's milk. Competing for this resource, however, are ten or more individuals like yourself, some twice your body weight, and all murderously aggressive and armed with sharp teeth.

This Tolkienesque torture is, of course, an allegory for the trials facing a new-born piglet, but the danger has been understated considerably: a mature elephant is only about 50 times the size of an adult human, whereas many sows are 200 to 300 times larger than their most diminutive offspring. In light of these odds, it hardly seems surprising that neonatal death is a significant problem in pig production. Such elementary causes as crushing by the sow and failure of the young to achieve adequate nutrition by suckling should account for many of the losses.”

Fraser (1990) [2]

List of abbreviations

EU	European Union
AI	Artificial insemination
SD	Standard deviation
LBW	Low birth weight
Ig	Immunoglobulin
IUGR	Intrauterine growth restriction
AIAO	All-in-all-out
scFOS	Short-chain fructo-oligosaccharides
MLBW	Mean litter birth weight
NEFA	Non-esterified fatty acids
IGF-1	Insulin-like growth factor 1
EDTA	Ethylene diamine tetra acetic acid
RBC	Red blood cell
HCT	Haematocrit
HGB	Haemoglobin
WBC	White blood cell
ROS	Reactive oxygen species
VLBW	Very low birth weight
DMR	Dense milk replacer
ADG	Average daily growth
CI	Colostrum intake
SL	Skin lesion

Summary

In modern pork production, one of the main goals during the past three decades has been to increase litter sizes, and thus, improve sows' production efficiency. However, the genetic selection for larger litters has led to sows often farrowing more piglets than the number of functional teats (hyperprolific sows). Additionally, the increase in litter size has resulted in many low birth weight (LBW) piglets which are associated with a higher perinatal mortality. Thus, selecting more piglets per sow has resulted in both economic (more losses, longer fattening periods, higher labour costs, additional managemental measures) and animal welfare issues (increased pre-weaning mortality). Recent selection programs have made a shift to a more sustainable pork production, not only targeting a large litter size, but also piglet vitality. Nevertheless, there still is a high proportion of LBW piglets in the current pork production that require assistance.
Several interventions have been examined to tackle these problems at the level of the sow (before, during and after gestation), the general management of the farrowing room and the piglet.

This thesis' scope was to enhance the resilience (the capacity to recover in terms of performance and survival) of LBW piglets during the pre-weaning period. Other dissertations have already studied interventions at the piglet level, such as the application of nurse sows, artificial rearing, split suckling, etc. These interventions all require a basic level of vigour from the piglets to be successful which is often lacking in LBW piglets. One intervention that does not require a basic level of vitality is drenching, the oral administration of substances.

During the past years, different studies have focused on drenching bioactive substances. However, these studies only focused on the supplement and neglected any potential effect of the drenching technique itself. Given that LBW piglets are often very weak, drenching might provoke additional stress, and nullify the supplement's effect or even negatively affect the piglet's health. In this thesis' first study, LBW piglets were sham drenched to evaluate the effect of drenching on their body weight, health, and mortality. No harmful or positive effect of drenching was observed, and thus, it was concluded that drenching is a safe tool that can be implemented in a good pre-weaning management.

In a second field-trial, LBW piglets were drenched with bovine colostrum, short-chain fructo-oligosaccharides (scFOS) or quercetin (each dissolved in a plain milk replacer) during the first seven days after birth. The animals' body weight, mortality, skin lesions, and different blood parameters were evaluated between birth and 2 weeks post-weaning. None of the supplemented compounds had a positive effect on any of the parameters, and thus, on the resilience of LBW piglets. Moreover, a negative effect on survival was observed

in piglets that were drenched with scFOS. These results showed that the evaluated bioactive compounds, in their given dosages, were unable to improve the LBW piglets' survival and emphasised the complex, multifactorial origin of pre-weaning mortality.

A final study aimed to determine whether the performance of LBW piglets could be improved by drenching a dense, concentrated milk replacer (DMR) and whether the frequency of drenching and the severity of the LBW played a role. Secondly, this study compared the supplementation of the same milk replacer at two farms with different perinatal management. No effect of drenching DMR on the survival or performance of (very) low birth weight piglets was observed, regardless of farm and, apparently, of the applied management. However, the survival and performance in very LBW (VLBW) piglets were extremely poor, excluding these animals as a target group for drenching. Additionally, mortality rates were lower at the farm with a higher level of perinatal management, suggesting that high-quality care might have more effect on the survival of small piglets than drenching a dense milk replacer.

In conclusion, the studies within this dissertation validated drenching as a safe technique for LBW piglets, but could not find a positive effect on the resilience of the administered substances. Thus, drenching did not suffice as a single intervention to improve the resilience of LBW piglets.
However, these studies pointed out that researchers and farmers must differentiate between VLBW (or intrauterine growth restricted (IUGR)) and LBW piglets, given that only the latter is a target intervention group. In addition, developing a protocol for humane endpoints of VLBW (IUGR) piglets is necessary.
For future studies, it would be interesting to evaluate the long-term effects of drenching, combined with secondary interventions (split suckling, cross-fostering…) in high-quality perinatal management.

Samenvatting

Gedurende de afgelopen drie decennia lag de focus in de varkenssector vooral op het verhogen van de productie d.m.v. een genetische selectie op een hoger aantal biggen per zeug. Deze selectie heeft echter geleid tot zeer hoogproductieve zeugen die vaak meer biggen werpen dan het aantal beschikbare spenen. Bovendien heeft de verhoogde worpgrootte eveneens geleid tot een groter aandeel aan biggen met een te laag geboortegewicht (LBW biggen). Deze LBW biggen zijn geassocieerd met een verhoogde sterfte gedurende de lactatieperiode (voor het spenen). De toename in worpgrootte is m.a.w. geen overdonderd succes gebleken, maar heeft zowel economische (meer biggensterfte, langere opfokperiodes, meer arbeidskosten, enz.) als welzijnsproblemen (verhoogde sterfte van biggen voor het spenen) met zich meegebracht.
Er werden reeds verschillende ingrepen en strategieën onderzocht om deze problematiek op te lossen op het niveau van het algemeen management, de zeugen (voor, tijdens en na de dracht) en de biggen.

Het doel van deze thesis was om na te gaan of er een verbetering van de veerkracht (de prestaties en de overleving) van LBW biggen mogelijk is tijdens de lactatieperiode door biggen een voedingssupplement toe te dienen. Verschillende studies hebben reeds nagegaan of bepaalde interventies LBW biggen konden ondersteunen, zoals het inzetten van pleegzeugen, vroegtijdig spenen, alternerend zogen, enz. Deze interventies vereisen echter allemaal een zekere basisvitaliteit van de biggen. Een interventie die geen basisactiviteit vereist is drenchen, het oraal toedienen van vloeistoffen.

De afgelopen jaren werden er reeds enkele studies uitgevoerd die keken naar het effect van verschillende supplementen. Er werd echter uitsluitend gekeken naar het effect van het toegediend product en niet naar een mogelijke invloed van de handelingen tijdens het drenchen. Aangezien LBW biggen vaak verzwakt zijn, zouden de manipulaties tijdens drenchen (vangen, vasthouden en product toedienen) kunnen fungeren als een acute stressfactor. Hierdoor zou een eventueel positief effect van het supplement kunnen worden teniet gedaan of, in het ergste geval, de gezondheid van de biggen negatief kunnen worden beïnvloed. Tijdens een eerste studie onderging een groep LBW biggen een “sham drenching” (lege spuit in de mond) gedurende zeven dagen en werden deze dieren vergeleken met LBW biggen die zo weinig mogelijk werden gehanteerd. Er werd geen enkel negatief effect van hanteren op de prestaties en de overleving van de LBW biggen geobserveerd. Drenchen kon bijgevolg beschouwd worden als een veilige interventie voor LBW biggen.

Tijdens een tweede veldproef werden LBW biggen gedurende de eerste zeven dagen na de geboorte gedrencht met bovien colostrum, kortketenige fructo-oligosachariden (scFOS) of

quercetine (telkens opgelost in een onverrijkte melkvervanger). Het effect van deze producten op het lichaamsgewicht, de mortaliteit, de huidletsels, en verschillende bloedparameters werd geëvalueerd vanaf de geboorte tot twee weken na spenen. Er kon geen enkel positief effect van de behandelingen worden waargenomen op één van de gemeten factoren. Bovendien was de mortaliteit hoger bij de LBW biggen die gesupplementeerd werden met scFOS. Deze resultaten toonden aan dat geen van de toegediende bioactieve producten de veerkracht van LBW biggen kon verbeteren tijdens de lactatieperiode.

Tijdens een laatste proef werd het effect van een dense melkvervanger bekeken. Daarnaast werd er ook gekeken of er een verschil zat tussen het aantal toedieningen (drie of slechts één toediening) om een eventuele vermindering in arbeidskosten te kunnen bekomen. Deze proef werd uitgevoerd op twee bedrijven met een verschillend kraamstalmanagement. Er kon geen effect van de dense melkvervanger of van het aantal toedieningen worden gevonden op de prestaties en de overleving van LBW biggen (onafhankelijk van het bedrijf). De biggen in de laagste gewichtscategorie (VLBW biggen) vertoonden een zeer hoge mortaliteit en presteerden ondermaats. Bijgevolg kon worden geconcludeerd dat de focus van drenchen vooral op LBW biggen zou moeten liggen en niet op VLBW biggen. Bovendien lag de mortaliteit van de LBW biggen veel hoger in het bedrijf met een beperkte biggenzorg. Dit toonde aan dat een goed management mogelijks een betere invloed heeft op de veerkracht van LBW biggen dan orale supplementatie.

Samengevat, toonden de studies in deze thesis aan dat drenchen een veilige techniek is om toe te passen bij LBW biggen. Drenchen op zichzelf volstaat echter niet om de veerkracht van LBW biggen te verbeteren tijdens de lactatieperiode.
Het is essentieel voor onderzoekers en varkenshouders om een onderscheid te maken tussen VLBW (of intra-uterien groeivertraagde (IUGR)) en LBW biggen, aangezien enkel LBW biggen een doelgroep vormen voor interventies. Er is echter nood aan een protocol met een duidelijk overzicht van humane eindpunten en een geschikte techniek voor euthanasie voor VLBW (IUGR) biggen.
Ook al werden er geen positieve effecten van drenchen gevonden tijdens deze thesis, toch zijn er nog enkele opportuniteiten voor toekomstige studies. Het zou interessant zijn om na te gaan of drenchen een eerder aanvullende rol kan spelen in combinatie met andere ingrepen, zoals bv. alternerend zogen, en een kwalitatief kraamstalmanagement.

Chapter 1.

General introduction

1.1 Pig farming in Belgium

In Flanders, livestock production mainly consists of cattle (1.3 million), poultry (45.1 million) and pigs (5.7 million) [3]. Thus, pig farming is a substantial component of Belgium's livestock. When only considering the absolute meat production numbers, pork production is the most significant contributor, providing over 50% of the total meat production (Figure 1.1) [4].

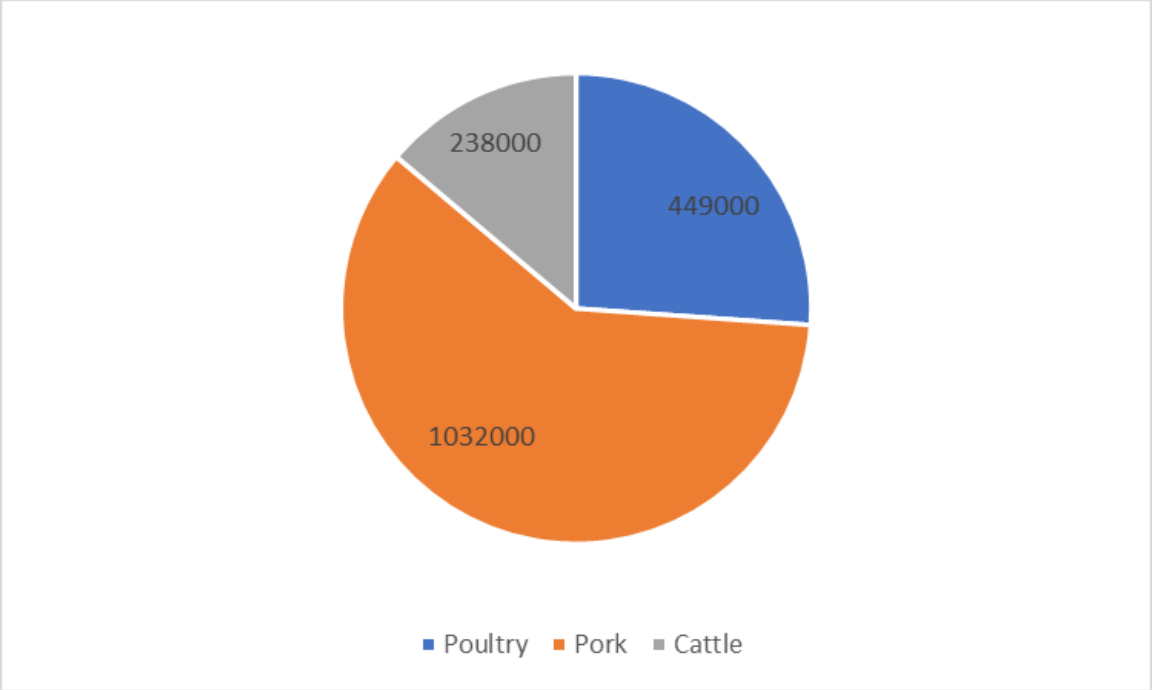

Figure 1.1. Meat production in Belgium (2022). The presented numbers express the total carcass weight in tonnes of poultry (blue), pigs (orange) and cattle (grey) slaughtered in slaughterhouses and on the farm, of which the meat is declared fit for human consumption (adapted from Eurostat [4]).

Pig farming in Belgium has a very regional distribution with over 94% of the farms located in Flanders and only a very small fraction in the Walloon region. This is in contrast with, for example, cattle production which has a more even distribution throughout the country [5]. Even within the Flemish region, pig farms have a very local distribution with most farms located in the west of Flanders (West-Vlaanderen) and the north of Antwerp (Noorderkempen) (Figure 1.2) [3, 5].

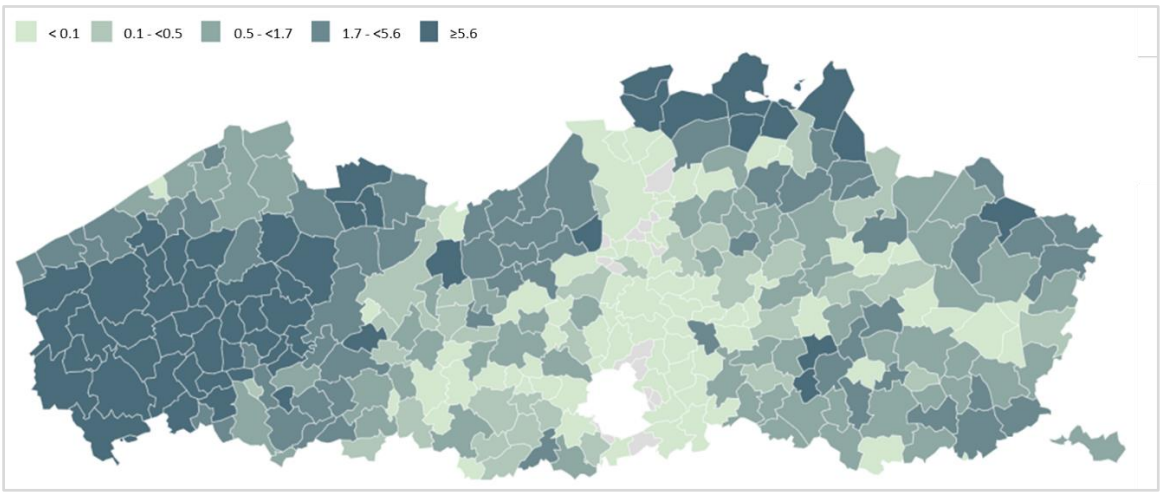

Figure 1.2. Number of pigs per hectare in Flanders' communities in 2022 (adapted from Vlaanderen.be [3]).

The Belgian pig production is not only a significant part of its national livestock, but also an important player in the European Union's (EU) pig market. The biggest pork producers are Spain, Germany and France, but Belgium consistently belongs to the top ten players [4].

The Belgian production generally totals around 250% of the country's internal consumption. Consequently, Belgium has an export-focused production with Germany and Poland being the most important destinations. The main product that is exported is raw, unrefined pig meat for further processing, a rather low-value meat product. On the other hand, Belgium is characterised by high labour costs. Thus, it has a rather low position in the international value chain while having high costs [5].

Like most other EU member states, the number of pigs in Belgium has decreased during the last decade. Since 2010, the pig population has been reduced by 8% (half a million pigs less) [4, 6]. However, the shrinkage in the pig population can be considered as relatively moderate compared to the steep decline in the number of farms. During the past ten years, the number of pig farms has dropped by 32%, and since 1997, even by 66% (Figure 1.3) [6, 7]. This has led to an intense increase in animals per farm. During the past two decades, this upscaling has resulted in farms having more than double the number of pigs than before (from less than 800 per farm in 2000 to over 1600 in 2021) [4, 6, 7]. On average, two-thirds of all pigs in Belgium are kept at farms with 1500 or more pigs [5]. Consequently, farmers are confronted with managemental challenges that are accompanied with higher labour intensity and less opportunities for individual monitoring of the animals.

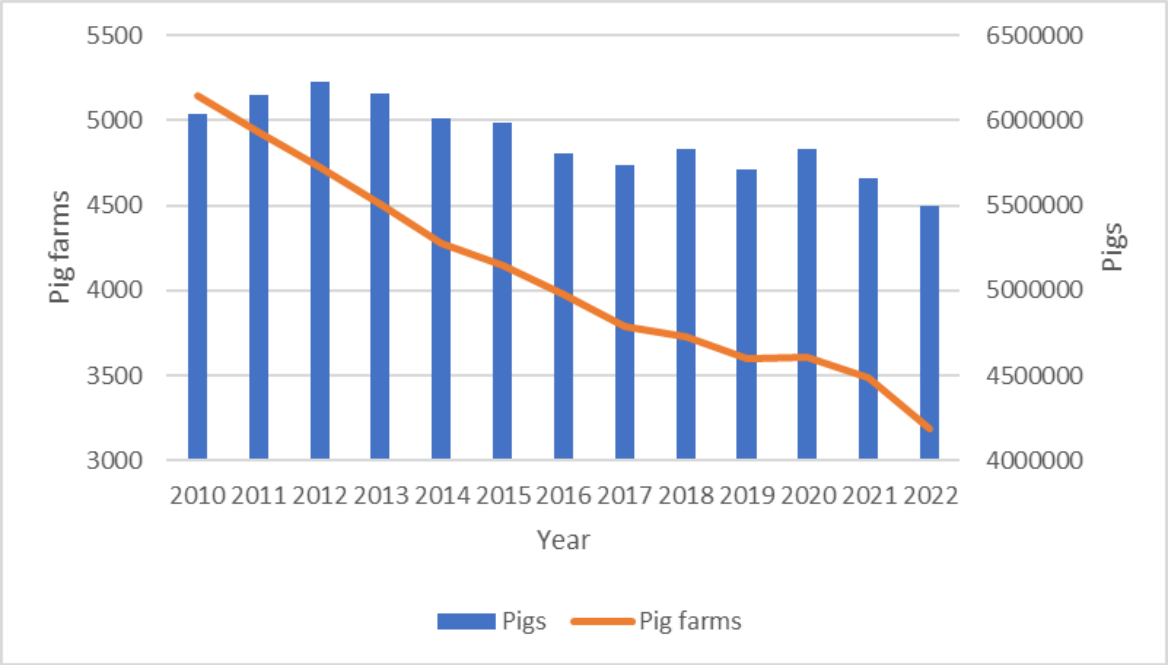

Figure 1.3. Evolution of number of pigs (blue) and pig farms (orange) during the last 13 years in Flanders (adapted from Eurostat [4], Vlaanderen.be [3], StatBel [6] and Landbouwcijfers Vlaanderen [7]).

1.2 Evolution of litter sizes

'Litter size' was defined by Rutherford et al. [1] as all piglets born alive plus all normally developed piglets born dead (i.e. stillborn or malformed piglets that were not viable). This definition excludes partially or fully mummified piglets and only includes animals that participated in the birth process. Nevertheless, the application of this definition often results in higher numbers, as the pig industry generally only counts viable piglets when determining the litter size [8]. The authors also applied a notional categorisation of litter sizes from (abnormally) small to very large, based on thresholds that can have an impact on the perinatal management. Litters of 14 piglets or more were labelled 'large' and litters of more than 20 'very large' (Figure 1.4). One of the most decisive factors for this categorisation is the number of functional teats (teats that are not inverted, blind, too small and produce enough milk to rear a piglet) [9]. When the litter size, following the previously mentioned definition, exceeds the number of functional teats, one or more management interventions are generally required [1, 10]. In most western breeds, the average number of functional teats varies between 12 to 16, but this is also affected by circumstantial factors, such as the position of the sow (lateral position that prevents piglets from suckling at the lower row or restrain the smallest piglets from reaching the upper row), the anatomy of the sow (the sow's hind legs that block the caudal teats) or the construction of the farrowing unit (metal bars from the box that prevent access to the teats) [1, 11].

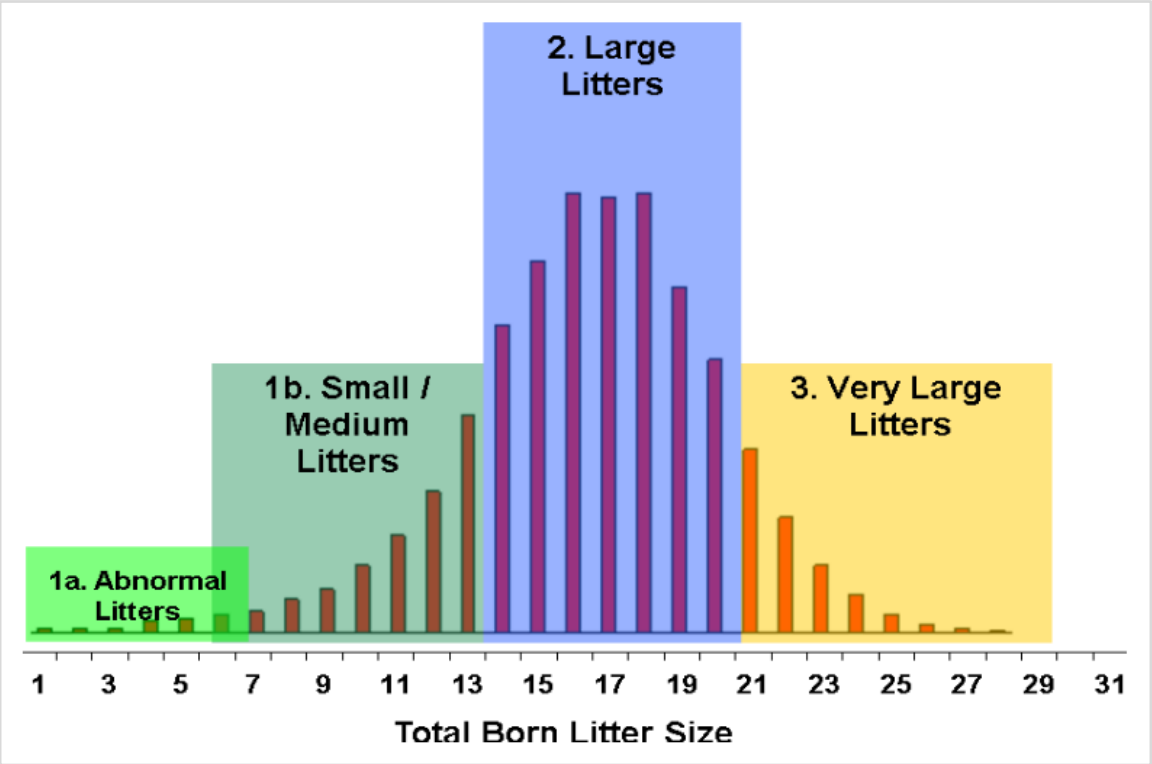

Figure 1.4. Categorisation of litter sizes from abnormally small to very large litters, based on the distribution of litter sizes from Danish data. Piglets that were born alive or normally developed piglets that were born dead (stillborn or non-viable malformed piglets) are included in the litter size calculation (adapted from [1]).

Domestication and production-focused genetic selection have greatly influenced litter sizes. The general domestication of wild boars began around 10,000 years ago, but the selection that led to the currently most common breeds dates back to 200 years ago [12]. During the second half of the 20th century, selection shifted from physical appearance to better production results, such as good carcass traits and a higher growth rate [1]. One of the earliest selection experiments for larger litter sizes started in 1965 [13]. However, the genetic selection for larger litter sizes has mainly been implemented during the last three decades. Since then, the average number of piglets per litter has increased considerably in most countries [14]. The average litter size in Denmark increased from 12.1 in 1996 to 16.1 in 2009 [1]. According to a Dutch review, a similar increase occurred in the Netherlands, where the average litter size has gone up from 11.6 in 1996 to 15.8 in 2016 [15]. In Belgium, a comparable evolution has occurred in litter sizes. Whereas the number of pigs in Flanders has decreased quite steadily, the number of breeding pigs (sows) has dropped more severely during the past years. Since 2010, the number of sows has decreased by 29% (Figure 1.5). In other words, the production-focused selection has led to fewer sows that produce larger litters [4, 6, 7]. These sows often give birth to more piglets than the number of functional teats and are therefore called hyperprolific sows (generally when the litter size exceeds 16 piglets) [16].

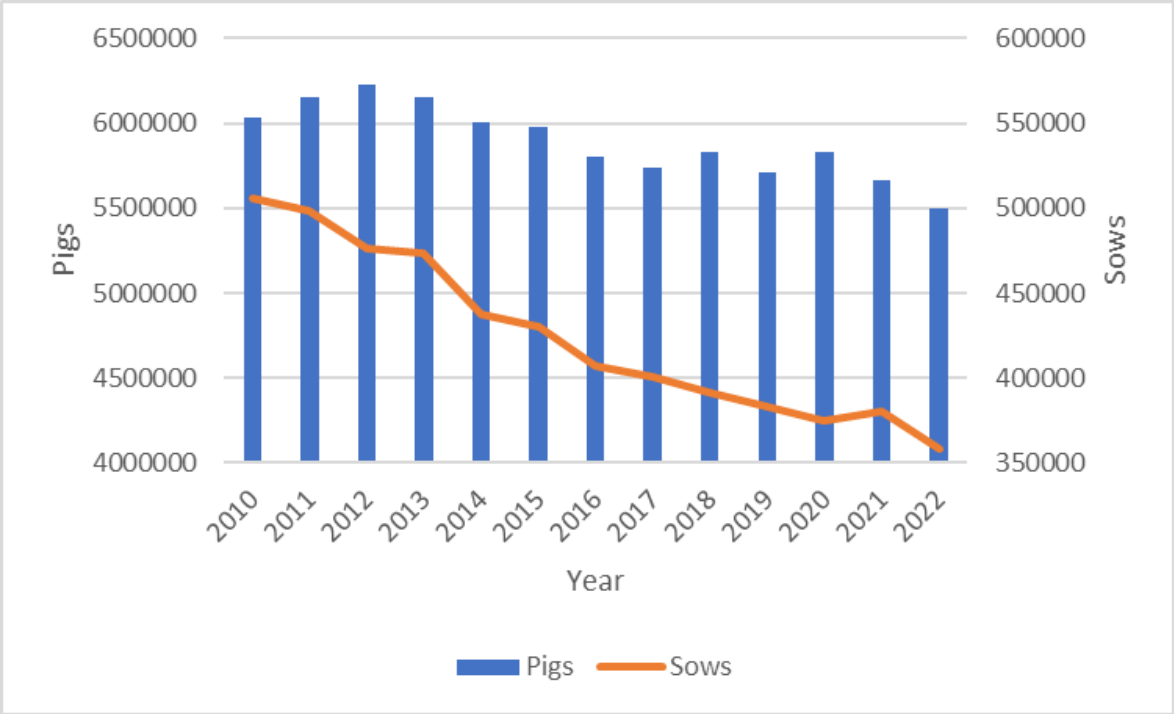

Figure 1.5. Number of pigs (blue) and sows (orange) from 2010 until 2022 in Flanders, Belgium (adapted from Eurostat [4], Vlaanderen.be [3], StatBel [6] and Landbouwcijfers Vlaanderen [7]).

1.3 Impact of increased litter sizes

The introduction of hyperprolific sows has not led to an immaculate increase of production and economic benefits, but has been accompanied by challenges for sows, piglets and farmers (see Figure 1.12 at the end of section 1.4 for schematic overview).

The increase in litter size requires more piglets to be expelled during parturition. Consequently, the farrowing duration has increased significantly over the past decades [17]. This prolongation of farrowing can harm the sows' subsequent performance and health by reducing their fertility (increased repeat breeding rate) [18], impairing the placenta expulsion [19], or increasing the risk of postpartum metritis [20]. Additionally, longer farrowing implies more care and work from nursing personnel [17].

Another negative trait associated with larger litters and prolonged parturition, is an increased stillbirth rate [21]. Since 2010, several studies have reported an average of 5 to 10% stillborns [22]. There are 2 types of stillbirth: type I or ante/pre-partum (death occurs before parturition) stillbirth and type II or intra-partum (death occurs during parturition) stillbirth. Whereas type I stillbirths are usually attributed to an intrauterine infection, type II stillbirths generally have a non-infectious cause, such as asphyxia or dystocia which are more common in larger litters [23]. Type II stillborn piglets are usually normal in size and have a fresh appearance, but are often covered in meconium (hypoxia in utero relaxes the anal sphincter of the foetus, resulting in the expulsion of meconium) [23].

The stillbirth rate can increase significantly when the litter size exceeds 12 piglets. In a study by Lucia et al. [24], the odds of having stillborns were twice as high when sows gave birth to 12 or more piglets. When the farrowing process is prolonged due to the high number of piglets, the risk of hypoxia or other farrowing issues increases [21]. Oliviero and colleagues [25] have observed an average of 1.4 (± 1.5 standard deviation (SD)) stillborn piglets when the farrowing duration was longer than 300 minutes. Contrarily, when farrowing lasted less than 300 minutes, only 0.5 (± 0.9 SD) stillborns were observed. Not only does the duration of farrowing affect the probability of stillbirth, but the birth order also plays an important role. Langendijk et al. [22] noticed an increase in the stillbirth rate with birth order from 2% in the first three piglets to 17% from 13th piglet on (Figure 1.6). Moreover, the duration of farrowing had an amplifying impact on the effect of the birth order on the stillbirth rate. In sows with a farrowing duration which was longer than average, the risk of stillbirth was 23% for piglet 13 and up [22].

Figure 1.6. The effect of birth order on the risk of stillbirth rate, obtained from 37 litters (adapted from [22]).

A rare trait among mammals, is the naturally occurring sibling competition as a part of the reproduction strategy in pigs. Like certain bird species, sows will produce slightly more piglets than the number of offspring that is normally reared. In birds, such as *Casmerodius albus*, the chicks that hatch during the first day will be considerably larger than the chicks that hatch a few days later. The latter chicks will consequently experience much more competition for food, warmth or might even be killed by their older siblings. In other words, the younger chicks will have higher neonatal mortality rates. However, should one of the older chicks die or should there be an abundance of food, then the younger chicks might

thrive as well. Thus, the small investment of a few spare offspring can ensure the parent of a normal number of reared younglings (the energetic cost of producing a new-born is lower than the cost of raising it) [2]. In pigs, this strategy has evolved as well and can be observed in two ways. On the one hand, there is competition for teats between the piglets in which the first-born animals can already suckle before the last piglets are born. On the other hand, there will be a difference in birth weight, creating a competition that favours the piglets with the highest birth weight. Heterogeneity in birth weight is, thus, a naturally occurring phenomenon in pigs [2, 16].
However, the variability in birth weight has increased extensively in hyperprolific sows [21, 26]. When the intrauterine crowding increases (beyond the normal uterine limits), the blood flow in the uterus will increase as well, but not efficiently enough to provide the same amount of blood to all foetuses. Consequently, with every additional foetus, the blood flow per foetus will decrease, resulting in a decreased foetal development [21, 27], and thus, in an elevated proportion of smaller piglets [28]. Quiniou et al. [28] observed that in litters with 11 or fewer piglets, only 7% of the animals could be defined as small (< 1 kg), while 23% of the animals weighed less than 1 kg when the litter size exceeded 15 animals. This is in line with a recent study that showed a reduction in birth weight of 20 g for each additional piglet in the litter, illustrating the negative impact of increased litter sizes on birth weight [26].

1.4 Low birth weight piglets

Piglets that are small for their gestational age are often called low birth weight (LBW) piglets. Definitions or thresholds that determine whether piglets are classified as LBW vary [8]. An early classification defines LBW piglets as those that weigh less than the tenth centile. In other words, piglets that have a birth weight which is lower than that of 90% of the included population are classified as LBW piglets [29]. In a study by Feldpausch et al. [30] a change point or threshold value of 1.1 kg was determined for birth weight, based on pre-weaning mortality. Piglets below this threshold point had a 34.4% pre-weaning mortality rate and were at nearly six times greater risk to die. Therefore, following these authors' findings, LBW piglets could be defined as all piglets that are born below 1.1 kg. Other authors do not classify piglets as LBW, based solemnly on a fixed body mass threshold or percentage, but also take the mean birth weight of a larger population into consideration. For example, Antonides et al. [31] defined LBW piglets as those that weighed 1 SD below the average birth weight of nearly 500 piglets. The abovementioned definitions, however, do not consider the heterogeneity in birth weights within litters. This might have some restrictions, as many problems concerning LBW piglets are the result of their smaller body size compared to their littermates (e.g. less competitive abilities in establishing the teat order [32]).

Larger litters have been associated with higher pre-weaning mortality (between 10 and 20%) in numerous studies. To a large extent, these elevated mortality rates can be attributed to the increased proportion of LBW piglets (reviewed by Rutherford et al. [8] and Muns et al. [32]). A curvilinear relationship between birth weight and pre-weaning mortality has been observed in several studies (Figure 1.7) [30].

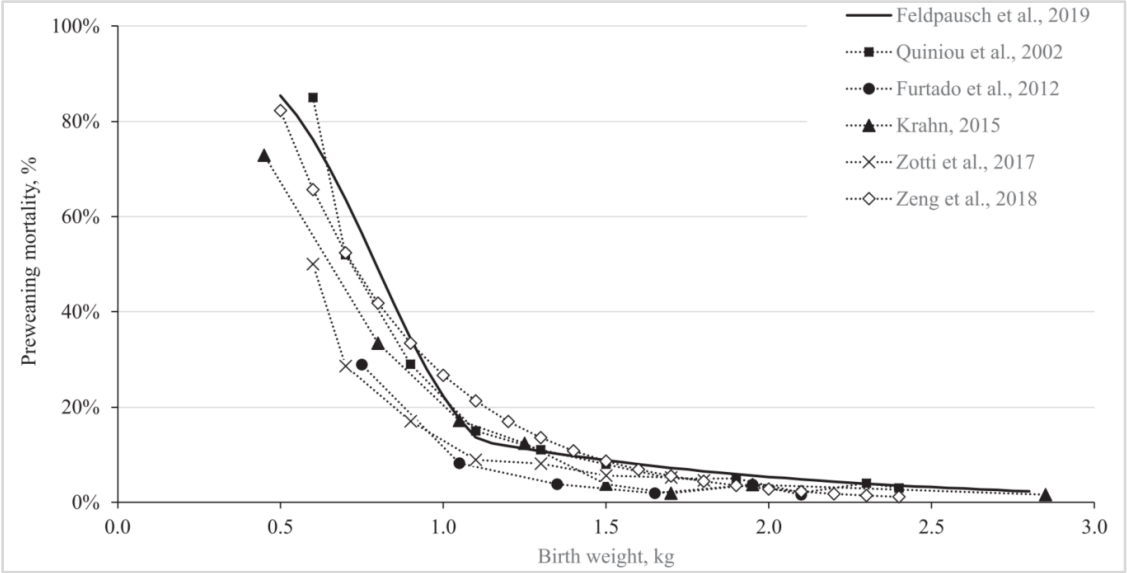

Figure 1.7. Curvilinear relationship between birth weight and pre-weaning mortality across various studies [28, 30, 33-36] (adapted from [30]).

The three leading causes of death in LBW piglets are starvation, hypothermia and crushing, which inevitably interact with one another [8, 21, 32, 37].
In addition to a selection for larger litters, the pig industry has also successfully selected leaner meat at slaughter. As a result, the fat depots have been reduced in sows and suckling piglets alike [38]. Piglets – and LBW piglets in particular – are born with relatively low energy reserves, due to very scarce amounts of adipose tissue (less than 2% body fat in total) and a low liver glycogen supply. Their body reserves are not sufficient for maintenance and heat production, thus, neonatal piglets will quickly enter a negative energy balance (Figure 1.8) [39].

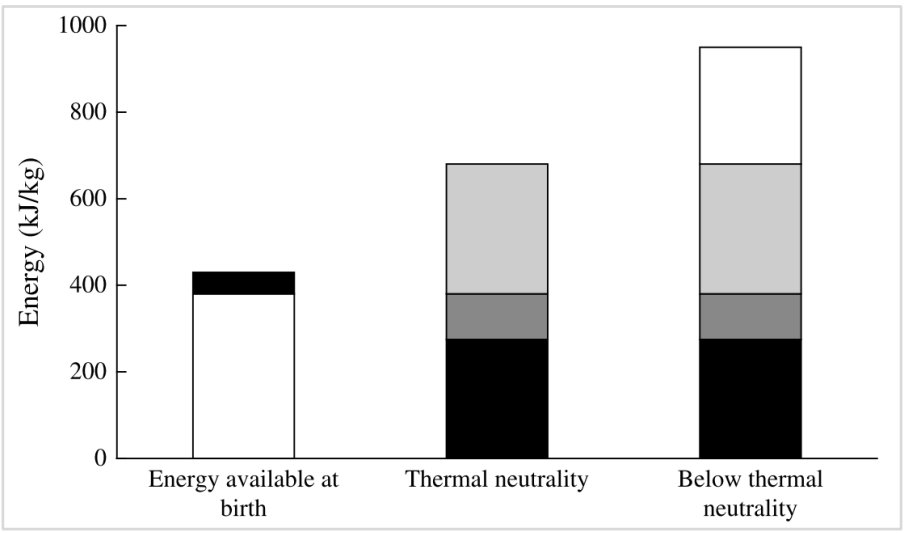

Figure 1.8. Energy reserves at birth (left column; fat (black) and glycogen (white)) and estimated net energy requirements during the first 24 hours of life of piglets surviving to weaning in conditions of thermal neutrality or 5 °C below thermal neutrality (middle and right column, respectively; maintenance (black), physical activity (dark grey), energy retention (light grey) and thermoregulation (white)) (adapted from [39]).

Additionally, piglets are born with a very thin subcutaneous fat layer and a sparse hair covering, requiring them to produce warmth intensely to avoid hypothermia. Piglets mainly rely on shivering for their heat production. As mentioned, piglets' liver glycogen reserves are low and only provide around 15% of the available glycogen. The largest glycogen mobilisation originates from the muscles, illustrating the importance of shivering (and the energy required for immediate postnatal locomotion). Most piglets are born in moderately hypothermal circumstances (18-26°C) and not in a thermoneutral environment (32-38°C), resulting in increased mobilisation of their body reserves. Progressive hypothermia will, therefore, occur in unfed piglets between 8 to 15 hours after birth. Especially in LBW piglets, this heat loss can occur very rapidly, as they have deficient body reserves and a larger surface area to body weight ratio, making them prone to progressive hypothermia ([40] and reviewed by [41]).
Consequently, piglets need an exogenous energy source to survive [40, 42]. This primary source of energy for new-born piglets is colostrum. Colostrum is the first secretion of the mammary glands, secreted shortly before the parturition until approximately 24 hours after the onset of the farrowing process. It is characterised by high levels of bioactive compounds and contains less lactose (2.8% in porcine colostrum), less fat (6.4%), and higher protein levels (16.6%) than milk (4.3%, 10.1%, and 7.5%, respectively) [38, 43, 44]. On average, piglets start suckling around 20-30 minutes after birth and acquire 315-340 g/kg of colostrum [41]. However, the amounts of acquired colostrum are often lower, certainly in LBW piglets [45]. Quesnel and colleagues [43] reviewed that the critical amount of colostrum intake during the first day after birth should be 200 g to benefit the pre-weaning survival. When insufficient volumes of colostrum are ingested, piglets cannot mobilise enough energy to increase their body temperature and improve their viability

(Figure 1.9) [9]. In a study by Devillers et al. [46], piglets that consumed less than 200 g of colostrum showed a mortality rate of 43.4%. When the colostrum intake was above 200 g, mortality dropped to 7.1%. Birth weight affects colostrum intake, and consequently, the probability of dying. However, it should be taken into account that the underlying reason for a reduced colostrum intake (e.g. low vitality) could be the real primary cause of death and not necessarily the reduced colostrum intake. Ferrari et al. [45] observed that LBW piglets (defined by the authors as weighing between 1.1 and 1.2 kg) needed 250 g of colostrum to reduce their probability of mortality to the same level as piglets that weighed more than 1.3 kg. When the colostrum intake was over 250 g, the probability of dying was the same, independent of the birth weight (Figure 1.10). Additionally, an immediate weight gain of 50 g and a long-term gain (from three weeks until 42 days after birth) were observed when 250 g and 290 g of colostrum was ingested, respectively. Thus, in terms of pre-weaning mortality and growth, a consumption of at least 200 g, and 250 g for LBW piglets, is recommended (reviewed by [43]).

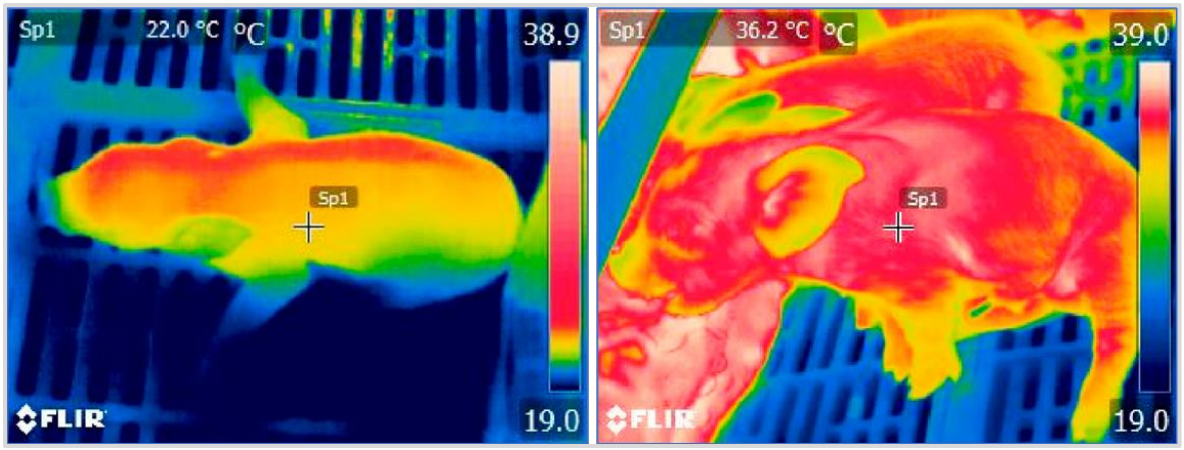

Figure 1.9. Thermal skin image presenting a thermal colour scale (right-hand side of each image). Left: piglet that did not acquire colostrum. Right: piglet receiving colostrum (adapted from [9]).

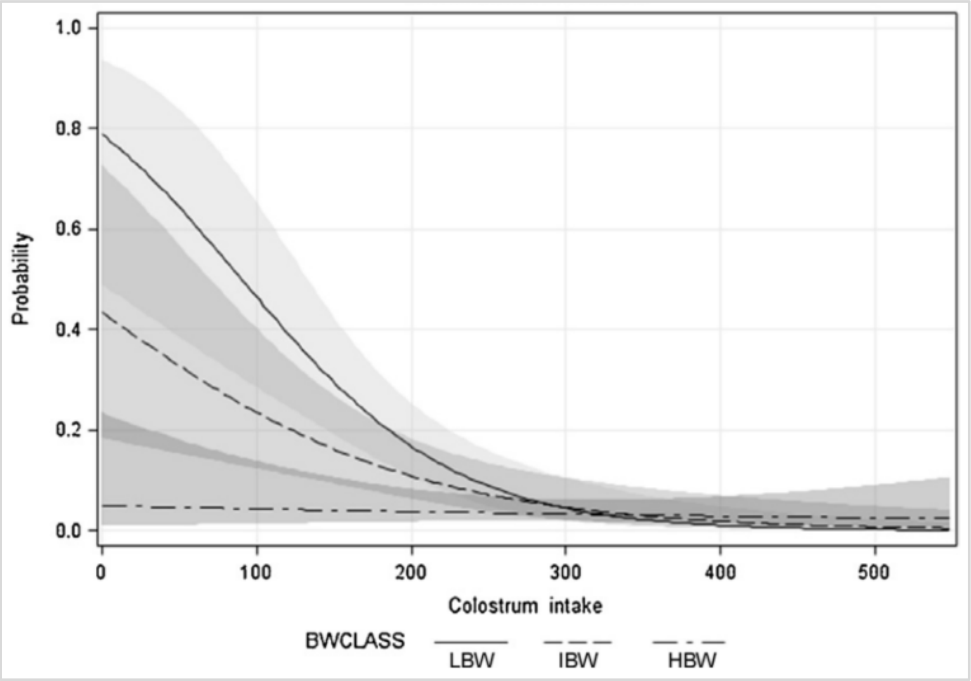

Figure 1.10. Probability of death (with 95% confidence limits), resulting from an interaction between birth weight and colostrum intake (grams) in low birth weight (LBW; 1.1-1.2 kg), intermediate birth weight (IBW; >1.2-1.3 kg) and high birth weight (HBW; >1.3-1.7 kg) piglets (adapted from [45]).

An additional challenge for new-born piglets is to obtain enough antibodies from colostrum. Due to the epitheliochorial placentation in pigs, the maternal blood and nutrients must pass six cell layers [47]. This strongly limits the transport of macromolecules, such as immunoglobulins (Ig), from mother to foetus [48]. Consequently, piglets are born immunologically naïve and rely on an adequate colostrum intake to acquire passive immunity. The predominant antibody in colostrum is Ig G, followed by Ig A and Ig M. The Ig G level in colostrum is very high around parturition, but will start to decrease sharply during the first 12 hours. This emphasises the importance of acquiring enough colostrum, immediately after birth, as the (immunological) quality of colostrum will decrease rapidly (Figure 1.11) (reviewed by [9, 39]). Moreover, piglets only enjoy a time window of 24 hours to absorb intact immunoglobulins. After the gut closure, Ig G will no longer be transferred from the enterocyte into circulation. This process enables neonatal animals – under the assumption that they ingest enough colostrum within the first hours after birth – to acquire sufficient amounts of Ig G while minimizing the chances of invasion by pathogens (reviewed by [39]).

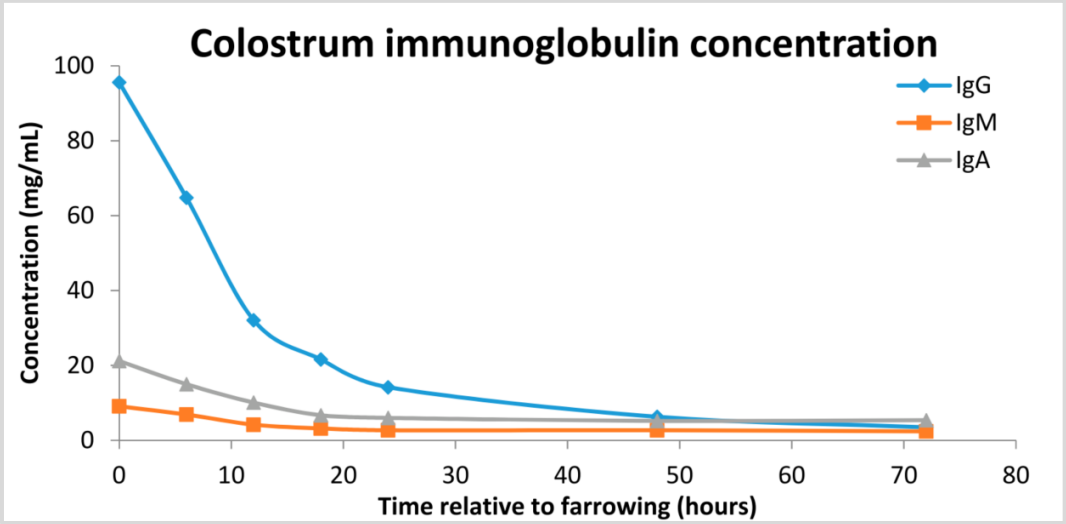

Figure 1.11. Evolution of immunoglobulin (Ig) levels in sow colostrum during the first 72 hours after parturition. A sharp decrease of antibodies (mainly IgG) during the first 12 hours will result in later-born piglets consuming colostrum of lower (immunological) quality (adapted from [9]).

Earlier studies observed no positive correlation between colostrum yield and litter size. In other words, there is a reduction in colostrum intake when the litter size increases [49, 50]. In a recent study, however, the colostrum yield increased by 93.6 g for each additional live-born piglet [51]. On the other hand, this study also noted a reduction in colostrum production of 2.2 g for each additional minute of farrowing, and this study was characterised by larger litters and longer farrowing durations, compared to the earlier studies. Whereas the direct effect of litter size on colostrum yield is still arguable, the colostrum production of hyperprolific sows is generally insufficient to meet the requirements of all piglets [43]. Consequently, the competition for teat access will increase in larger litters which will, in turn, result in a lesser chance for LBW piglets – that are less vigorous and easily outcompeted by larger littermates [32] – to acquire enough colostrum to survive (reviewed by [21]). Additionally, LBW piglets will often not be able to compete for the anterior teats. The anterior teats produce higher volumes of colostrum and have a higher protein synthesis, resulting in an upregulation of immunoglobulins and lactoferrin, in comparison with the more posterior teats. Thus, LBW piglets will often not only consume insufficient amounts of colostrum, but colostrum of a lower quality as well [52, 53].

A third leading cause of death in LBW piglets – next to starvation and hypothermia – is crushing by the sow [37, 54]. As mentioned before, the leading causes of pre-weaning death in LBW piglets are associated with each other, e.g., low energy intake will quickly result in starvation, hypothermia and weakness, stimulating piglets to huddle up against the sow for direct body heat or spend more time near the sow in search of teats, while simultaneously making them less vigorous to jump away when the sow suddenly lies down. Starvation and hypothermia can, hence, make LBW piglets more susceptible to

crushing [54, 55]. This was illustrated by Andersen et al. [56] who observed that, with increasing litter size, crushing only increased for piglets that were not able to ingest milk.

The abovementioned causes of death are, of course, applicable to all piglets, regardless of their birth weight. Moreover, other factors, such as genetics, environment, sex, parity, body conformation etc. all attribute to its multifactorial character (reviewed by [32]). Additionally, not only birth weight will determine the incidence of pre-weaning mortality, but body conformation has been shown to be an important indicator of the survival chances as well. Piglets with a higher body mass index (body weight/(crown to rump length)^2) [57] and piglets that are disproportionately small [58] have a higher likelihood to survive during the pre-weaning period. Nevertheless, LBW piglets are generally at much higher risk to die during the pre-weaning period (reviewed by [21, 59]). In a study by Zeng et al. [36], piglets with a birth weight below 1 kg accounted for 54.1% of the total pre-weaning losses. Moreover, it has been reported that up to 80% of all piglet deaths occur prior to weaning [30, 32], with the most critical time taking place during the first 72 hours after farrowing [32, 59]. Therefore, the primary focus to reduce piglet mortality should be on LBW piglets during the first days after birth.

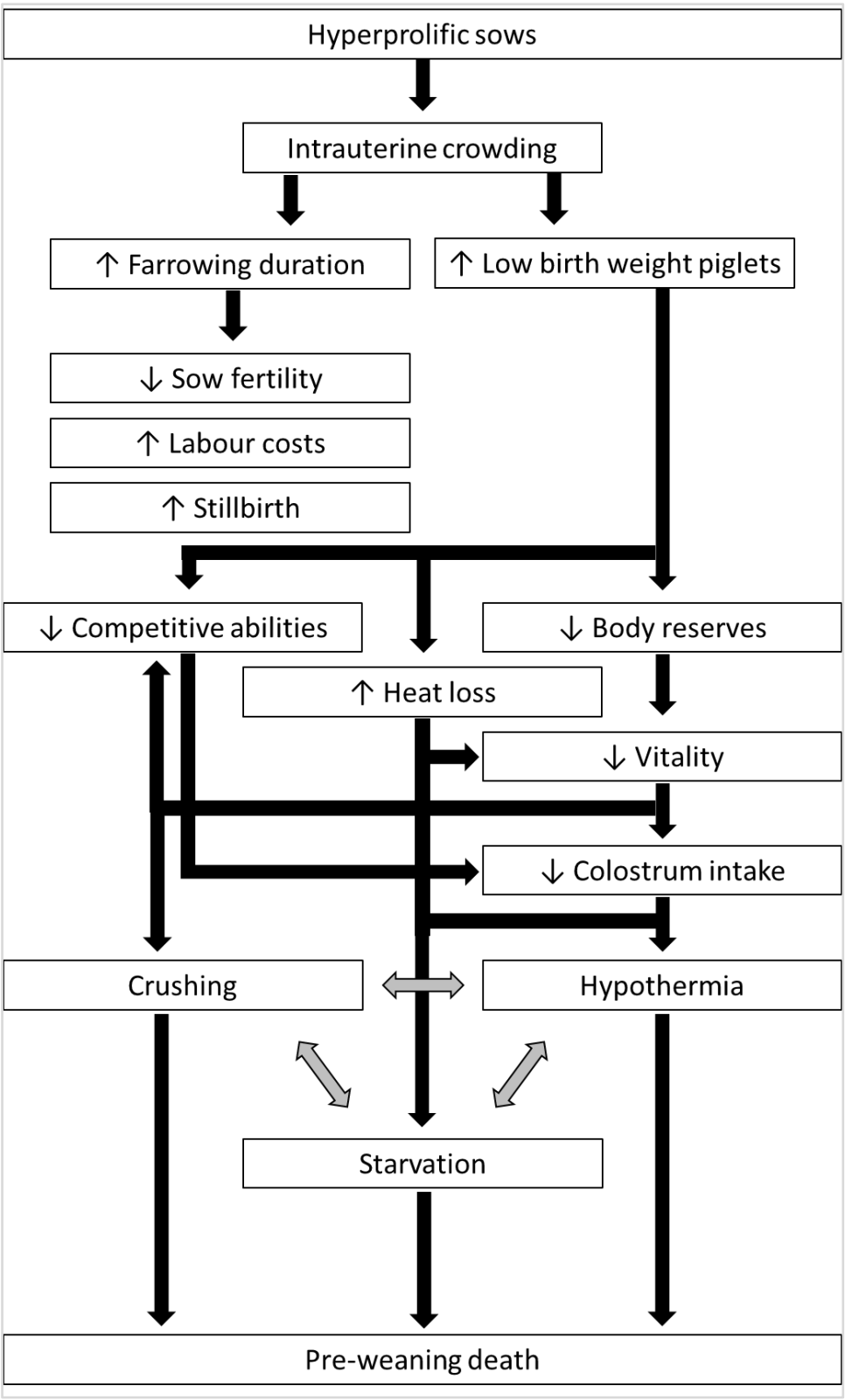

Figure 1.12. Schematic overview of how hyperprolific sows have led to increased pre-weaning mortality rates through higher birth weight heterogeneity. A higher proportion of low birth weight piglets has resulted in a hypothermia-starvation-crushing complex.

1.5 Intrauterine growth restriction

Within the group of LBW piglets, there are animals that are not only small for their gestational age, but have also suffered from an aberrant, impaired development during gestation. This phenomenon is often referred to as intrauterine growth restriction (IUGR). Several studies found an average IUGR prevalence between 18% and 25% [26, 60, 61], but much variation was observed between different litters (0-76.5%) [26]. Nevertheless, the negative impact of IUGR on the offspring's survival and performance challenges all pig farmers, as it is detrimental to both production and animal welfare goals [62, 63].

The causes of IUGR can be divided into three categories: maternal malnutrition (mainly during late gestation), placental dysfunction (due to a crowded gravid uterus) and foetal malfunctioning (often caused by toxins or (viral) infections) (reviewed by [64]). As a result, IUGR piglets cannot express their full genetic growth potential as an embryo and/or foetus. Unlike 'non-IUGR' LBW piglets, these animals will, consequently, not only be born with a low body mass, but will also have smaller organs (reviewed by [26, 47, 64]). This developmental difference allows a differentiation between LBW and IUGR piglets. Traditionally, IUGR piglets are defined based on their foetal or birth weight relative to their gestational age [62]. Additionally, other factors, such as the birth weight in relation to the litter's mean birth weight (between the 5^{th} and 10^{th} centile or below 1.5 SD), are sometimes included as well to determine whether piglets suffer from IUGR [29, 65, 66]. However, the most distinctive differentiation from LBW piglets can be made by including morphological characteristics in the detection of IUGR piglets. Hales et al. [57] defined and scored the severity of IUGR, based on the head morphology. When the piglets had a dolphin-shaped head, bulging eyes and wrinkles perpendicular to the mouth, they were considered IUGR piglets (Figure 1.13). When only one or two of the criteria were observed, the animals were classified as light IUGR piglets. These head morphometrics are the result of a 'brain-sparing effect'. To protect the central nervous system in the presence of IUGR, the blood perfusion of the brain is preferred over that of other organs. Consequently, brain development will be less impaired than other organs, and the brain, thus, has a relatively larger size, and a coherent dolphin-shaped head [67]. The redistribution of the blood not only favours the brain, but also other vital organs, resulting in relatively larger liver, lungs and adrenal glands [68]. Other authors have combined birth weight and head shape and have added a narrow hind part as an additional parameter to define and score the severity of IUGR (Figure 1.13) [26, 69].

The discrimination between LBW piglets and IUGR piglets can be useful in both scientific and farm protocols, since IUGR piglets experience the same disadvantages as LBW piglets due to their small size, but additionally, suffer from an impaired organ development. This makes them even more prone to perinatal mortality and lower performance. Even beyond weaning, IUGR piglets' performance remains lower compared to their larger littermates and they exhibit altered body composition (reviewed by [64]).

Under practical circumstances, it remains arguable how to manage IUGR piglets best. Whereas some authors plea to intervene and support IUGR piglets immediately after

farrowing [70, 71], others suggest that only non-IUGR LBW piglets should be prioritised [21] or that – depending on the severity – IUGR piglets should be humanely killed [72]. Regardless, it remains challenging to implement strategies to improve IUGR piglets' survival efficiently, since they often are not vigorous enough to benefit from the applied intervention (reviewed by [64]).

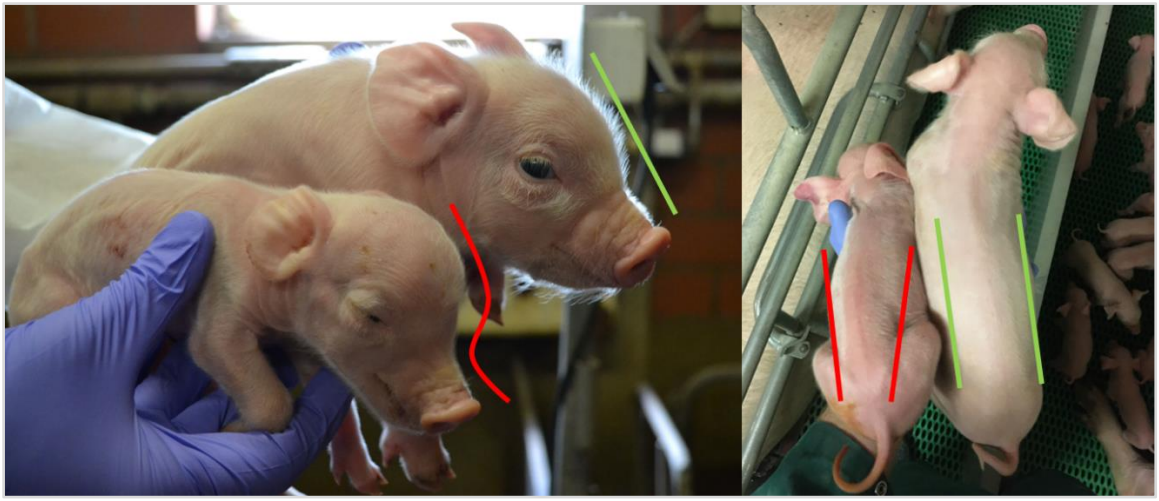

Figure 1.13. Left picture: Normal (back) and intrauterine growth restricted (IUGR) piglet (front); Right picture: normal (right) and IUGR (left) piglet. The IUGR piglets are characterised by a dolphin-shaped head, bulging eyes and a narrowing hind part (adapted from [64]).

1.6 Interventions

Many studies have already focused on interventions that could potentially reduce the negative effects of using hyperprolific sows. These interventions can be performed on the level of breeding (e.g. more balanced selection programs between large litter sizes and the sows' limitations), the sow (e.g. achieving an optimal farrowing duration, acquiring an ideal energy status, preventing maternal fatigue, reducing stillbirth, applying nutritional strategies to improve embryo quality, etc.), the general farm management (e.g. providing an optimal thermal environment) or on the piglet level (reviewed by [72]).
A recent survey [73] looked into the different management strategies of 170 Flemish pig farms. Nearly 70% of the farmers admitted encountering challenges and problems during the pre-weaning and nursery phase. The supernumerary piglets were described as the biggest challenge (48.9%), followed by crushing (17.4%) and diseases (15.2%). Remarkably, the proportion of farmers who apply humane killing as an intervention has decreased over the years (from over 50% in 2014-2015 and 2017-2018 to 30% in 2021-2022), while 69.4% claims to implement more than three management interventions. This suggests a growing awareness and implementation of strategies amongst farmers. Figure 1.14 illustrates the percentages of different interventions that are applied at the enquired farms.

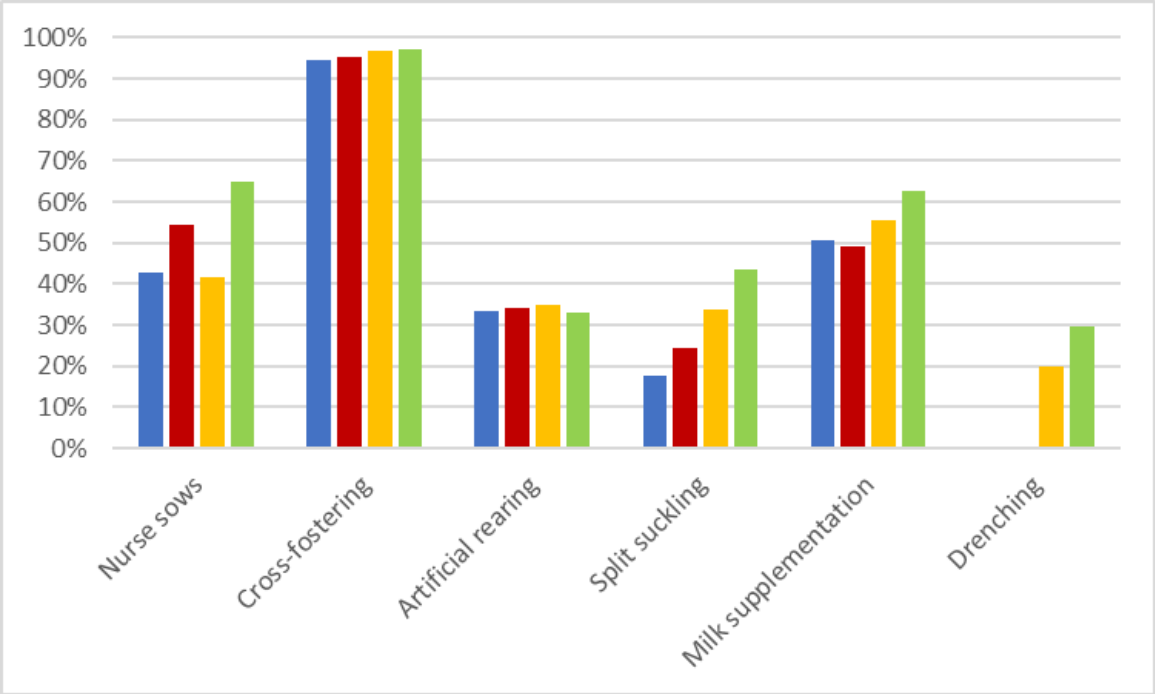

Figure 1.14. Interventions that are applied during the neonatal period at the piglet level, according to surveys in Flanders from 2011-2012 (blue), 2014-2015 (red), 2017-2018 (yellow) and 2021-2022 (green). Each column represents the percentage of farmers who have indicated that the given intervention was applied at their facility (adapted from [73]).

In the following sections, the emphasis will be on strategies that are often implemented on the piglet level during the neonatal period.

1.6.1 Nurse sows

The utilisation of nurse sows implies relocating new-born piglets from one sow (maternal sow) to another (nurse sow) that will rear these piglets until weaning. The transfer should not occur prior to 12 hours after farrowing to allow the piglets to obtain colostrum from their mother (reviewed by [10]).

There are three types of nurse sow procedures: one-step, two-step or three-step. In the one-step strategy, the piglets from a selected nurse sow are weaned (after at least 21 days of age) and piglets from sows that have recently farrowed are transferred to the nurse sow. The latter sow will then rear these piglets until they reach the weaning age. The two-step procedure – or cascade fostering – includes an additional 'interim' sow (nurse sow 2). The selected nurse sow's (nurse sow 1) piglets are weaned when they are at least 21 days old. Next, piglets from nurse sow 2 – which has farrowed 4-7 days ago – are transferred to the nurse sow 1 that will rear these animals until weaning age. Supernumerary piglets from recently-farrowed sows can then be transferred to nurse sow 2 to be fostered until weaning. In a three-step nurse sow system, a second interim sow (nurse sow 3) is used that receives newly born LBW piglets (12 hours after birth) from different litters. Consequently,

the LBW piglets will receive a major part of their colostrum intake from this interim sow. At the end of the first day, the LBW piglets are moved to nurse sow 2 that had her own piglets removed between 4-7 days after farrowing (similar to the two-step protocol). This system allows an optimisation of colostrum intake for LBW piglets through a decrease in weight variation between the litters. The second interim sow (nurse sow 3) will have a normal lactation period of at least 21 days (Figure 1.15) (reviewed by [10, 74, 75]).

By implementing a nurse sow strategy, excess piglets from large litters (i.e., piglets that outnumber the number of functional teats) can be placed with sows that have an artificially created capacity to rear piglets, thus, creating a more homogeneous distribution of piglets in the farrowing unit. Consequently, there will be less competition between the piglets, resulting in a higher milk intake and less teat fighting (reviewed by [10]). On the other hand, this strategy requires nurse sows to endure prolonged lactation and an extended stay in the farrowing crate. In the one-step strategy, the nurse sow must remain in the farrowing unit for at least six weeks (if the piglets are weaned at 21 days). The two-step procedure shifts this burden from the interim sow (nurse sow 2) to nurse sow 1, as the total prolonged lactation comprises four and six weeks, respectively. The prolonged lactation can harm the sow's welfare, such as swollen bursae on the legs and more udder injuries [76]. However, it does not appear to negatively impact the sow's body condition score [74, 76]. The higher incidence of udder injuries creates a paradox, given that one of the goals of implementing nurse sows is to reduce sibling competition, and thus, the associated fighting. In the two-step strategy, re-establishing a teat order could lead to more fighting, since the piglets have already had a suckling period of 4-7 days before being transferred to nurse sow 1. In the one-step procedure, less fighting is expected, since the piglets are usually less than a day old (reviewed by [10]). Another complication that might occur when nurse sows are applied, is chilling and starvation due to a long acceptance period by the sow which can take up to six hours in successful piglets and has an average latency of first milk let-down of 4-5 hours (reviewed by [10, 72]). However, it has been stated in different reviews that the risks of chilling, starvation and dying are the same or worse in large litters when no managemental procedures, such as nurse sows, are applied [8, 10]. A final pitfall to consider when nurse sows are used, is violating the 'all-in-all-out' (AIAO) system. By moving piglets or foster sows between farrowing rooms, the AIAO protocol for a given batch is broken. Thus, the risk of introducing pathogens increases from one batch to another (reviewed by [72]).

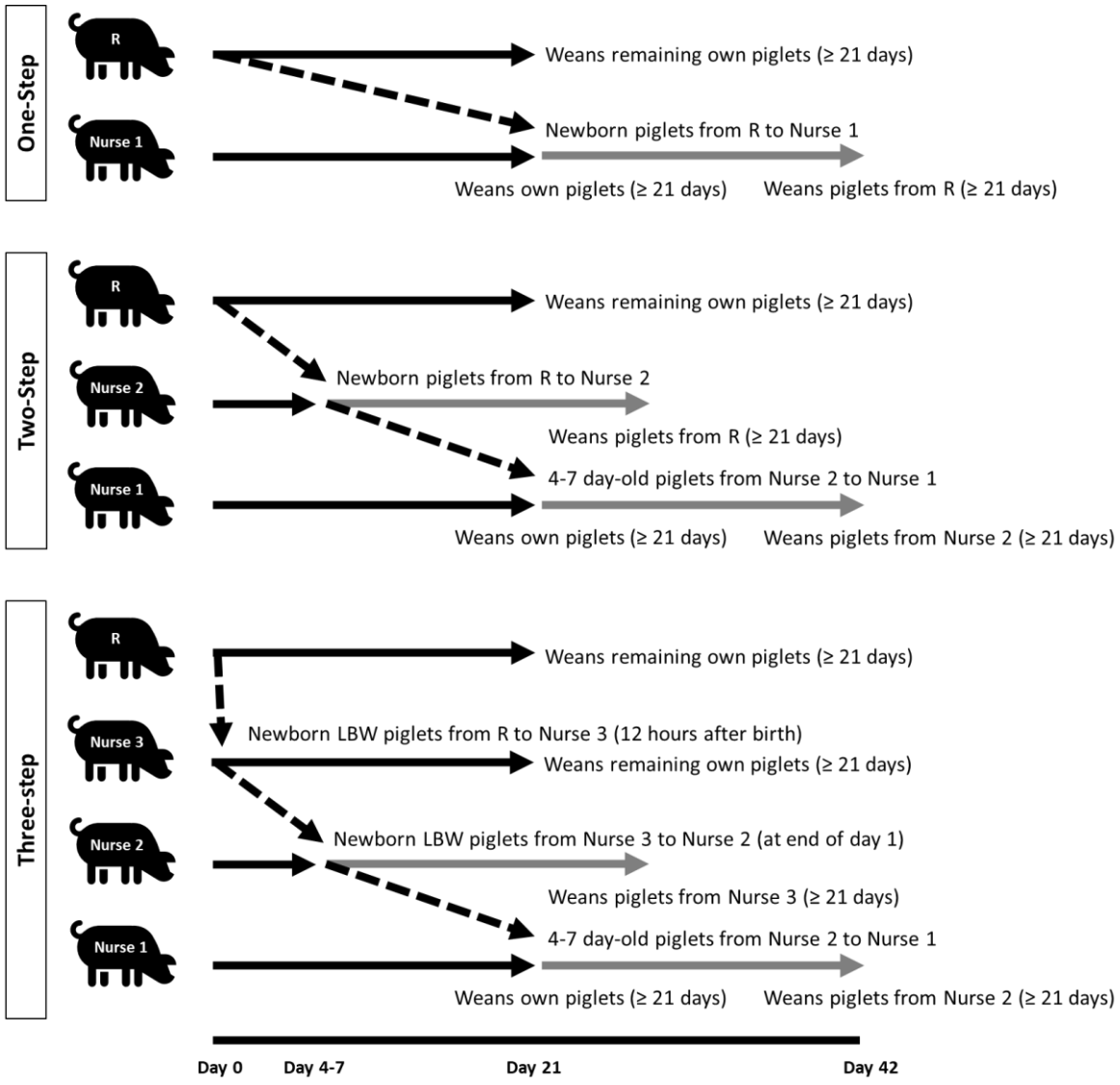

Figure 1.15. One-step, two- step and three-step nurse sow systems when weaning is performed at the age of 21 days. R: mother sow of a large litter from which supernumerary and/or low birth weight (LBW) piglets were removed; Nurse: nurse sow which fosters piglets from a different sow (adapted from [10, 74, 75]).

1.6.2 Cross-fostering

Cross-fostering is a very commonly applied intervention involving the transfer of piglets from their birth sow to another sow. Unlike nurse sows, no artificial udder spaces are created by weaning all piglets from the selected foster sows, but piglets are exchanged between sows from (usually) the same farrowing batch (reviewed by [10, 59, 72, 77]). The advantage of cross-fostering over nurse sows is that the relocation of piglets can be limited to one farrowing room and batch, and thus, has a lower risk of introducing and spreading pathogens (AIAO system is not broken) (reviewed by [9]). On the other hand, cross-fostering is often limited when many sows of the farrowing batch have large litters, while using nurse sows creates additional udder spaces [74].

The types of litters that are created through cross-fostering can vary, but the main goal is to enhance the piglets' survival chances and reduce the need for further interventions, mainly for LBW piglets in large litters (reviewed by [10]).

A first potential aim of cross-fostering is to create litter equalisation. In other words, the intended litters all consist of the same number of piglets or consist of an ideal number of piglets for each individual sow. Consequently, each sow will not have more piglets than her number of functional teats (reviewed by [10]).

Another starting point for cross-fostering can be the formation of litters with piglets that have a similar body weight. This is also referred to as litter standardisation and is often combined with the abovementioned litter equalisation. By creating homogeneous litters in body size, the competition between suckling piglets – at which LBW piglets are strongly disadvantaged – can be reduced (reviewed by [10]). However, Milligan et al. [78] observed an increase in teat disputes when only small piglets were cross-fostered compared to litters that consisted of a combination of small and larger piglets. Simultaneously, this study showed no impact on the growth rate and only a small positive effect on the survival in standardised litters with only small piglets. In a study by Souza et al. [79], however, the survival rate was 100% on day 16 in litters with only small piglets (and small with intermediate piglets), whereas the survival dropped to 83% in cross-fostered litters that had small and heavy piglets in them. Concurringly, Vande Pol et al. [80] observed an improved pre-weaning survival when only small piglets were grouped together. However, by cross-fostering LBW piglets collectively, only heavy piglets remain to be grouped as well. This can harm the latter's growth and survival [80, 81]. Altogether, it can be concluded that LBW piglets are preferably grouped with other small piglets to improve their chances of survival (reviewed by [9]).

From a practical point of view, cross-fostering can also be applied to sort new-born piglets by sex. This prevents farmers from sorting out males from females when the animals are castrated or housed by sex at weaning (reviewed by [10]). Whether cross-fostering by sex is beneficial for the piglets has not yet been investigated. It is mentioned in a review by Alexopoulos et al. [9] that female piglets have better survival chances and grow faster than males during stress, such as the weaning transition. Therefore, it could be interesting to evaluate whether females outperform male piglets when cross-fostered.

Variations that combine cross-fostering and nurse sows are sometimes applied as well. This can include the transfer of unthrifty piglets or split-weaning. When one or more piglets fall behind in their performance, compared to their siblings, they can be transferred to another sow or the larger piglets can be weaned early and be replaced with older, but smaller piglets (collecting unthrifty piglets). When the focus of a farmer is more on the size than the age of the piglets to decide whether the animals should be weaned, small piglets can be grouped and transferred to a nurse sow (recently weaned) that will foster these piglets until they reach the desired body mass (split-weaning). The latter interventions imply that the fostering occurs later in lactation (not during the first day after birth). This can result in more fighting (due to a longer established teat order), an increased risk of rejection by the foster sow and more transfer-related stress (piglets recognise their mother's vocalisations after 36 hours. Thus, the placement with a strange sow could act as an additional stressor) (reviewed by [9, 10]). Contrarily, when piglets are fostered within the first 24 hours after birth, competition and udder disputes appear to be resolved quickly. In litters that are composed of only biological piglets, there will still be fewer teat disputes than in litters with only fostered piglets when fostering is conducted during the first 24 hours, but there will be no lingering effects on behaviour or performance during the subsequent period (reviewed by [9]). Additionally, when piglets are cross-fostered after the age of seven days, it can have a negative impact on the growth rate of both the relocated and remaining piglets (reviewed by [10]).

1.6.3 Artificial rearing

Artificial rearing involves removing piglets from the sow and transferring them to a separate, controlled environment, before the established weaning age. This can be a separate room or an enclosure above the farrowing crate. Within this enclosure, an optimal thermal environment is created (e.g. by providing a heating lamp) and water and milk cups are provided. This strategy is usually applied to rear supernumerary piglets from large litters (after colostrum intake was allowed) or piglets from a nurse sow when the two-step procedure is applied (4-7 days after birth). Additionally, artificial rearing can be used to relocate sick or starving piglets (reviewed by [10, 72]). One example of an artificial rearing system is the Rescue Deck® (S&R Resources LLC, USA). This system is provided with slatted flooring, heating, lighting and artificial feeding systems (milk cups, water cups and creep feed system) (reviewed by [10]).
By providing milk through milk cups, there is no longer a competition for functional teats. As a result, the piglets can acquire higher amounts of milk and obtain a higher growth rate than sow-reared piglets ([82], reviewed by [72]). Schmitt et al. [83], on the other hand, observed impaired growth in artificially reared piglets. Several factors can explain the differences in growth rate between studies, such as the number of piglets in the rearing system, the starting age, the milk composition, the feeding system, spoilage, etc. [83]. Vergauwen et al. [82] observed that artificial rearing resulted in temporary oxidative stress, morphological and permeability changes in the small intestines, similar to conventional weaning. However, these piglets were able to recover within a few days without

compromising their performance. In a study by De Vos et al. [84], artificial rearing resulted in lower body weights after one week, but when the long-term effects (28 days) were evaluated, there was no difference in growth rate. Moreover, this study also made a discrepancy between normal and LBW piglets. Very few differences were observed between these birth weight categories. Thus, artificial rearing can be considered a safe strategy for LBW piglets.
The risk of crushing is eliminated, because artificial rearing does not involve a sow. Consequently, the survival chances of the piglets are increased (mainly of LBW piglets with a lower vitality). Moreover, the absence of a sow allows farmers to implement the system as an alternative or addition to the nurse sow strategy. The (literal) other side of the coin is the high financial investment that is required to create a clean, heated enclosure and that it may not comply with the legislative framework (reviewed by [72]).
An additional concern when artificial rearing is applied, is whether this strategy should be considered as a form of early weaning. The commonly applied definition of weaning is the customisation of young to take nutrition other than by suckling. If this definition is interpreted as the removal from the mother (or another sow) and its milk, artificial rearing can be considered as weaning. However, if weaning is considered the transition from liquid (milk) to solid feeding, the forementioned reasoning does not apply [83]. If artificial rearing were to be treated as a form of early weaning, it would pose welfare questions (deprivation of maternal care). It might even conflict with regulations concerning the weaning age (reviewed by [72]). Schmitt et al. [83] illustrated some welfare issues associated with artificial rearing, such as increased belly-nosing, more tail lesions and a poorer emotional state.

1.6.4 Split suckling

Another alternative to support LBW piglets' colostrum intake, when fostering options are limited, is dividing the litter into different groups. Often, the largest piglets are separated from the smaller and/or less vigorous ones and are kept in a heated creep area or a designated box. This allows the LBW piglets to acquire colostrum without being outcompeted by their larger siblings. Once the first group has been able to suckle, the piglets are marked and swapped with the group of larger piglets. This swapping usually continues throughout the day before all piglets are reunited in the evening. This strategy is called split suckling (reviewed by [9, 10, 72]).
In a study by Morton et al. [85], a higher weight gain was observed after one week when weight-based split suckling was applied (largest piglets were separated once during 1.5 hours). Huser et al. did not find an impact of weight-based split suckling on the growth of small piglets [86], but did observe an increase in the LBW piglets' vigour and survival (increase of 13%) [87].
There appears to be a lack of consensus on how split suckling should be applied (starting time, duration, group size, which piglets should be separated, etc.). Vandaele et al. [88] tried to tackle this knowledge gap by applying split suckling during one or three days and creating piglet groups that were based on both birth order and birth weight. The authors

did not observe an increased growth or survival in small piglets (even though these animals had constant access to the sow, given that only groups of heavy piglets were separated). Performing split suckling during three consecutive days even resulted in higher mortality rates amongst LBW piglets, suggesting that split suckling should be limited to the first day after farrowing.

1.6.5 Supplementation

1.6.5.1 *Supplementary milk*

Milk cups can be installed in the farrowing crate to provide piglets with additional milk as a compensation for the limited, individual milk acquisition in large litters. Following this assumption, a sow should be able to rear more piglets than the number of functional teats, thus, the necessity of using other interventions, such as nurse sows or cross-fostering, will be reduced. Generally, one of two designs is applied: a system that requires activation by the piglet to fill the milk cup (nose against valve) or a system that automatically refills when empty or at regular times (reviewed by [72]). Both designs can be connected to a fully automated system that distributes milk from one central provision point through the entire farrowing room (high in costs) or a low-cost system that needs to be refilled manually (e.g., a small milk tank, connected to the individual milk cups in up to four farrowing pens) [89]. Kobek-Kjeldager et al. [90] found an increased survival in piglets that were provided with valve-activated milk cups. However, this effect was only observed after five days. Given that the most critical period for piglets is during the first three days after birth, and no effect on mortality was seen during this period, it was suggested that the piglets were not able to learn how to activate the milk cups during this critical time. Therefore, a different milk cup design (automatic refill) could potentially result in better survival rates. The authors also observed that the implementation of milk cups was not able to compensate the insufficient milk ingestion of the smaller piglets completely which was illustrated by smaller piglets and more heterogeneity at weaning. The latter confirmed that larger piglets did not only acquire more milk from the sow, but also obtained more milk replacer from the milk cups. Supplementary milk systems could, thus, not eliminate the competition between piglets. This was further supported by Douglas et al. [91] who observed a higher milk replacer intake in LBW piglets when milk cups were only provided to groups with small animals, compared to mixed litters that consisted of LBW and normal sized piglets. Additionally, the hypothesis that milk cups could reduce the risk of crushing and the number of teat disputes were not confirmed in the study by Kobek-Kjeldager et al. [90]. This suggested that piglets still prefer to suckle from the sow, even though an alternative food source is provided.

1.6.5.2 *Drenching*

The abovementioned interventions all have in common that they require a certain vigour from the piglets to reach a teat or a feeding system. However, LBW and IUGR piglets often have reduced vitality and cannot always acquire colostrum or milk without assistance (reviewed by [64]). A strategy to overcome this issue is drenching, the oral administration

of a substance to individual piglets. Before the past decade, explorations on the effects of drenching in neonatal piglets were relatively scarce (reviewed by [92]). In other words, notwithstanding that drenching is a relatively simple and familiar technique, the interest in its potential as a solution to counteract hyperprolific sow-related problems – one of which is the increased proportion of LBW piglets – is rather new. In a recent review by Van Ginneken et al. [64] an overview was provided of several substances that have been supplemented to piglets on the first day of life: porcine and bovine colostrum, energy boosters based on coconut oil, glucose, glycerine, linseed oil, combined preparations, etc. It can be deducted from this overview that drenching LBW piglets has been far from successful to improve the survival or performance (Table 1.1).

As mentioned, piglets are born immunologically naïve and have low energy reserves, especially LBW piglets. Thus, it is critical for their survival to acquire enough colostrum immediately after birth (reviewed by [9, 39]).

By drenching porcine colostrum, an intraspecies source of bioactive compounds and energy can be provided to individual LBW piglets and/or piglets with low vitality. Consequently, the competitive factor – still present when supplementation occurs via milk cups [90, 91] – can be eliminated and a specific dosage can be applied. Porcine colostrum has a gross energy between 5.5 and 8.3 kJ/g (6.7 kJ/g on average). This relatively high energetic content can be attributed to the large amounts of immunoglobulins. However, immunoglobulins are proteins with a high digestive resistance, and thus, do not result in a large amino acid absorption and a subsequent energy supply [44]. The main source of energy in colostrum is fat, mainly composed of long-chain fatty acids (14 or more carbons) and delivers 40-60% of the total energy [39]. Not many studies have been conducted on porcine colostrum supplementation, though. This can be explained by its low availability, given the modest colostrum production by sows and the generally large litters in hyperprolific breeding lines. Amdi et al. [60] supplemented 12 mL/kg sow colostrum to IUGR piglets immediately after birth. The authors observed a rise in rectal temperature of 1°C and an increased blood glucose level after one hour. However, this effect did not last beyond 2 hours after birth, nor was any impact on the body weight found. These results suggested that porcine colostrum should be supplemented in frequent, small portions of a maximum of 20 mL (based on a gastric capacity of 34 mL to avoid overfilling the stomach) to have a more persistent effect. Following these suggestions, Engelsmann et al. [71] supplemented porcine colostrum three times in volumes of 20 mL (in combination with a subcutaneous glucose injection, depending on the treatment group). The authors did not observe an effect on the rectal temperature or performance of IUGR piglets. Only when the colostrum administration was combined with a glucose injection, an effect on body weight was observed. Moreira et al. [93] opted to supplement piglets with larger (total) volumes of colostrum (4 x 20 mL or 4 x 50 mL) and did not notice any effect on body weight. The total supplementation of 200 mL, however, did result in a decreased mortality. Other studies where large volumes were supplemented (2 x 25 mL) showed no effect on body weight or pre-weaning mortality [94, 95]. Muns et al. [96] combined a single colostrum supplementation of 15 mL with cross-fostering but did not see an effect on body weight or mortality. To summarise, these results are often contradictory, but do suggest that porcine

colostrum could be beneficial for LBW piglets when supplemented in portions of 50 mL. However, it remains unclear whether such high dosages are feasible – especially in small piglets – given the maximum gastric volume of 34 mL and the limited availability of porcine colostrum.

A more procurable energy source is bovine colostrum of which the effect on piglets has mainly been examined as a growth promotor in weaned pigs (reviewed by [97]). Unfortunately, studies that focus on neonatal bovine colostrum supplementation are scarce. Viehmann et al. [98] drenched piglets with one dose of bovine colostrum (1 mL) for three consecutive days and saw no effect on body weight or mortality rate. However, they did observe a prolongation of survival – mainly in LBW piglets – when the animals were supplemented. Complementary to these results, slightly higher dosages (2 x 5 mL) did result in a reduced pre-weaning mortality rate (from 18.6% to 6.3% at day 21) [99]. Thus, it appears that drenching LBW piglets with bovine colostrum has the potential to improve their survival. However, Tucker et al. [100] hypothesised that two supplementations of 5 mL might reduce the gastric capacity for subsequent colostrum intake. Given that the mean birth weight of the piglets in the involved study was rather high (LBW piglets were defined as weighing less than 1.35 kg), the supplemented volumes could indeed be too large to benefit LBW piglets with a lower birth weight. Therefore, it would be interesting to evaluate the supplementation of bovine colostrum to LBW piglets in dosages, less than 5 mL (but more than 1 mL).

When the rationale of drenching is purely based on energy supplementation and not on additional elements, such as growth factors or immunoglobulins, other substances can be administered as an alternative for colostrum. Several compounds have been examined, such as coconut oil [101-104], linseed oil, rice bran oil, soybean oil, glycerin [102] and glucose [71]. However, the effect of these supplements on LBW piglets' survival is often absent [71, 101, 102] or contradictory [101-103]. One possible explanation could be the minimal application periods (usually limited to the first day after birth to restrict labour costs) (reviewed by [64]). The general idea of energy supplementation, immediately after birth, is to provide weak, small piglets with an energy boost to improve their vigour to a level that enables them to obtain a first suckle [43]. However, the consulted literature suggests that the limited booster might not suffice to have long-term effects.

Another approach – apart from colostrum or energy supplementation – to enhance the survival chances of LBW piglets, could be to support other compromised traits, such as their impaired gut health [21]. Several studies have focused on the effect of prebiotics – in the form of short-chain fructo-oligosaccharides (scFOS) – on the microbiota of piglets [105-109]. Positive results have been found regarding the immune system and body weight. However, these studies are limited to preterm, weaned or piglets with a birth weight above 1 kg. Thus, the effect of scFOS on LBW piglets is still unknown. Next to a compromised gut health, LBW piglets also suffer from intestinal oxidative stress. Quercetin – a flavonoid antioxidant – has already proven to protect intestinal cells from oxidative damage under in vitro circumstances [110-112]. However, a successful translation of these results to on-farm conditions has yet to be accomplished.

Additionally, drenching requires intensive handling of already weakened piglets (chasing, fixating, supplementing) which raises the question whether these actions might act as an additional stressor, and consequently, nullify a potentially beneficial effect of the administered supplement. Brajon et al. [113] observed that handling young piglets modulates the animals' response and behaviour towards humans. Negative experiences can act as a stressor and have long-term effects up to five weeks. Thus, to avoid additional stress during the pre-weaning period, a positive attitude towards animal handling should be adopted.

Table 1.1. Supplementation of piglets on the first day of life (adapted from [64]).

Supplement		*Dosage*	*Follow-up*	*Body temperature*	*Body weight*	*Pre-weaning mortality*	*Reference*
Porcine colostrum		12 mL/kg BW	8 h pp	Increase 1°C, 1 h after bolus	NT	NT	Amdi et al. (2017)[60]
		2 x 25 mL	20 days pp	NT	No effect	No effect	Viott et al. (2018)[94]
		3x 20 mL	21 days pp	No effect	No effect	NT	Engelsmann et al. (2019)[71]
Bovine colostrum		2 x 5 mL	21 days pp	No effect	No effect	6.3% (vs. 18.6%) mortality at Day 21	Muns et al. (2017)[99]
Energy	***Coconut oil***	2 mL (74 kJ)	21 days pp	No effect	No effect	No effect	Schmitt et al. (2019)[101]
		2 x 1.68 mL (91.69 kJ)	Weaning	NT	No effect	No effect	Manzke et al. (2018)[102]
	Coconut and soybean oil	3 g (81.54 kJ)	21 days pp	NT	No effect	28% (vs. 58%) mortality at Day 21	Declerck et al. (2016)[103]
	Glucose	3 × 6 ml (50 mg/L) SC	21 days pp	No effect	±0.56kg at 21 days pp	No effect	Engelsmann et al. (2019)[71]
	Glycerin	2 × 2.33 ml (91.69 kJ)	Weaning	NT	No effect	No effect	Manzke et al. (2018)[102]
	Linseed oil	2 × 1.4 ml (91.69 kJ)	Weaning	NT	No effect	No effect	
	Rice bran oil	2 × 2 ml (91.69 kJ)	Weaning	NT	No effect	No effect	
	Soybean oil	2 × 1.3 ml (91.69 kJ)	Weaning	NT	No effect	No effect	
Combination	***Milk whey protein and coconut oil***	8ml (180.8 kJ)	20 days pp	NT	No effect	No effect	Viott et al. (2018)[94]
	Porcine colostrum and glucose	3 × 20ml porcine colostrum + 3 × 6ml glucose (50 mg/L) SC	21 days pp	No effect	±0.49 kg at 21 days pp	No effect	Engelsmann et al. (2019)[71]

Abbreviations: BW, Body weight; NT, Not tested; pp, postpartum; SC, subcutaneous.

Chapter 2.

Objectives and thesis outline

The main objective of this PhD-thesis is to evaluate the **effect of drenching on LBW piglets**. More specifically, to investigate if drenching can improve the resilience – the ability to recover from a deprived situation – of small piglets under practical, farm conditions.
Pig farmers face many challenges regarding economic, animal health and animal welfare issues. The strongly incorporated genetic selection for hyperprolific sows has resulted in larger litters, often outnumbering the rearing capacity of sows, resulting in supernumerary offspring and an increased proportion of LBW piglets. During this dissertation, different approaches will be studied to evaluate the potential of drenching as an innovative tool to help farmers tackle their obstacles regarding LBW piglets (Figure 2.1).

Aim 1:
As mentioned in the introduction, the results of studies that examine the effect of substances, given via drenching, are very often inconclusive or contradictory. Most studies only focus on the supplement and neglect any potential effect of drenching itself. Given that LBW piglets are often very weak, drenching might provoke additional stress, and nullify the effect of the supplement or even negatively affect the piglet's health. To test this hypothesis, a first field study evaluated the **safety of drenching** – in terms of its impact on pre-weaning survival, body weight, skin lesions, and biochemical and haematological blood parameters – by comparing sham drenched piglets with piglets that were not handled.
Hypothesis:
The act of drenching will provoke additional stress and affect the LBW piglets negatively.
(Chapter 3)

Aim 2:
Earlier studies have found a positive effect of bioactive substances, such as bovine colostrum, short-chain fructo-oligosaccharides (scFOS, a prebiotic compound) and quercetin (an antioxidant) in pigs. The few studies that have been conducted at farms were conducted on piglets above one kilogram or had inconclusive results. Therefore, the effects on the piglets' performance of the **oral supplementation of bovine colostrum, scFOS or quercetin** for seven days to LBW piglets was examined during a field experiment.
Hypothesis:
Drenching bovine colostrum, scFOS or quercetin will improve the resilience of LBW piglets.
(Chapter 4)

Aim 3:

Following the results from the abovementioned studies, a third field experiment was conducted to assess the **effect of a dense, concentrated milk replacer** that acted as an energy booster. Additionally, the **number of applications** (once or three times) was tested to potentially reduce labour costs and a **lower weight limit** was evaluated up to which drenching would have an effect. Finally, the field trial was repeated at a second farm to estimate the **confounding effect of the perinatal management**.

Hypotheses:

- Drenching a dense, concentrated milk replacer will improve the resilience of LBW piglets.
- Drenching LBW piglets three times with a dense milk replacer will improve the resilience of LBW piglets more than only supplementing the animals once.
- Piglets with a birth weight below 750 g are too weak to experience any benefits from drenching a milk replacer.
- In the case of a higher level of perinatal care, drenching a dense milk replacer will improve the performance of LBW piglets more than in the case of lower perinatal care.

(Chapter 5)

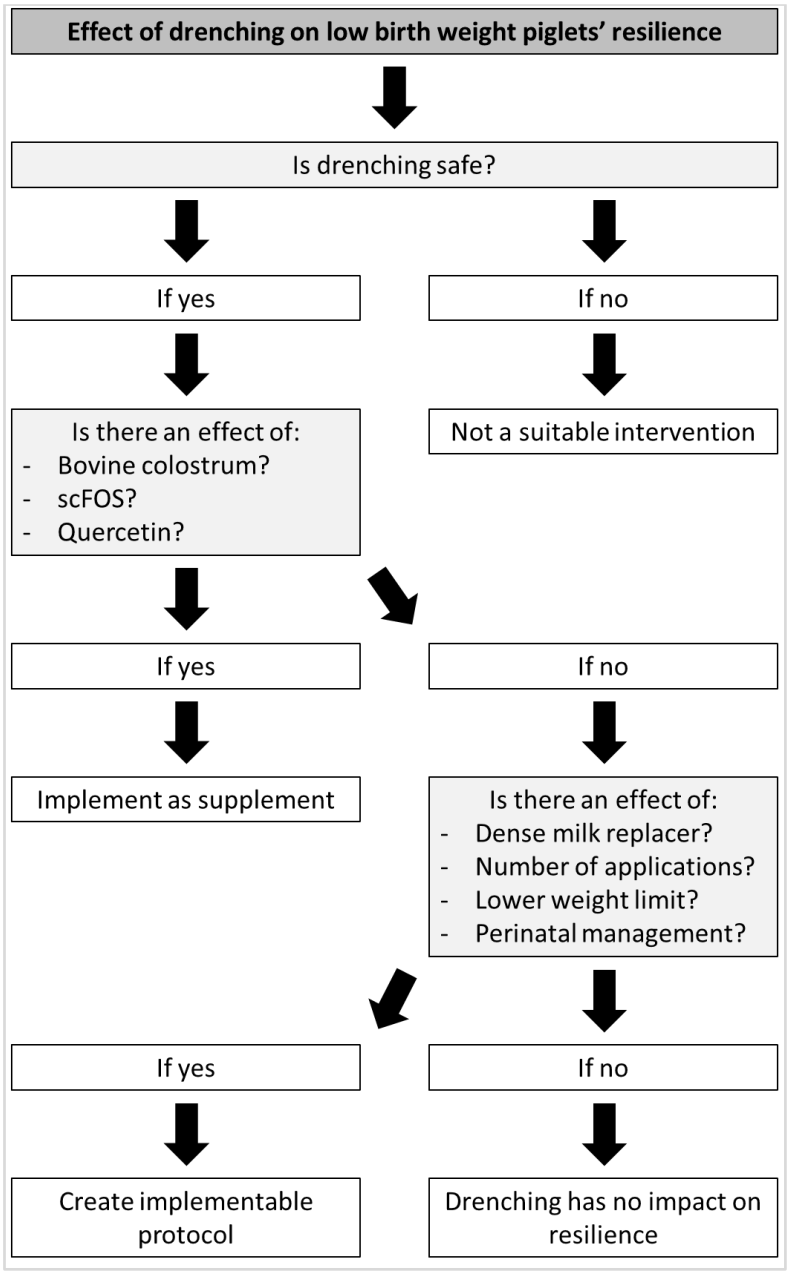

Figure 2.1. Overview of objectives

Chapter 3.

Handling associated with drenching does not impact survival and general health of low birth weight piglets

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Abstract

*The increase in litter sizes in recent years has resulted in more low birth weight (LBW) piglets, accompanied by a higher mortality. A potential intervention to overcome this is drenching bioactive substances. However, if the act of drenching provokes additional stress in LBW piglets, it might counteract the supplement's effect and be detrimental for the piglet's survival. To study the effect of the drenching act, piglets from 67 sows were weighed within 4 hours after birth. The mean litter birth weight (MLBW) and SD were calculated. LBW piglets (n = 76) were defined as weighing between (MLBW-1*SD) and (MLBW-2.5*SD). They were randomly allocated to two treatments: "sham" (conducting the act of drenching by inserting an empty 2.5 mL syringe in the mouth during 20 s, once a day, day 1 till day 7; n = 37) or "no treatment" (no handling; n = 39). On day 1, 3, 9, 24 and 38, piglets were weighed and scored for skin lesions. Blood samples were collected on day 9 and 38 and analysed to determine glucose, non-esterified fatty acids (NEFA), urea, immunoglobulin G (IgG), insulin-like growth factor 1 (IGF-1) and a standard blood panel test. There was no difference between sham drenched and untreated piglets regarding any of the parameters. In conclusion, this study showed that drenching does not impose a significant risk to LBW piglets and can be applied safely during the first 7 days after birth.*

3.1 Introduction

A frequently used strategy to supplement individual (LBW) piglets with a given amount of milk or substance in a certain dosage is drenching. However, most studies only focus on the effect of the supplemented product, whereas the effect of drenching itself is often neglected [20,21,23,30]. Drenching usually involves catching and fixating the animals, thus, potentially acts as a stressor. Given the LBW piglets' vulnerable condition, this potential stressor might further aggravate their chance of survival and negatively influence their health and survival, rather than provide the animals with additional energy or bioactive compounds. Consequently, it could be expected that sham drenching LBW piglets daily during their first week of life might result in higher mortality rates when these weakened animals are confronted with normal husbandry procedures that act as acute stressors, such as castration or teeth clipping. Therefore, it was hypothesised in this study that the act of drenching would provoke additional stress and affect LBW piglets negatively, resulting in a worsened performance (body weight, general (blood) health parameters) and a higher pre-weaning mortality.

3.2 Materials and methods

3.2.1 Ethical approval

This study was reviewed and approved by the Ethical Committee for Animal Experimentation of the University of Antwerp (ECD 11/2018) and was compliant with national legislation and European guidelines (2010/63/EC).

3.2.2 Animals

The experiments were conducted on a commercial farm in Meer (Hoogstraten, Belgium). All sows (Topigs20 (n = 58), Norwegian Landrace (n = 9)) were kept in individual farrowing crates of 2.25 × 0.60 m that were located in pens of 2.50 × 1.75 m. The parity of the sows varied from 1 to 10, with a mean parity of 4.35 ± 2.11 standard deviation (SD). The sows were fed with a commercial gestation diet up to farrowing. After farrowing, all sows were switched to a commercial lactation diet. Declared nutrient and chemical composition can be found in Table 3.1. Piglets included in the study, as well as their littermates, were subjected to the standard handling procedures in the farm: before the age of one week, all piglets were ear tagged, tail docked, received a 200 mg iron dextran injection and all male piglets were castrated using meloxicam analgesics. Piglets were weaned at the age of 3 weeks.

Table 3.1. Nutrient and chemical composition of sows' gestation and lactation diets.

Composition	*Gestation diet*	*Lactation diet*
Crude protein (%)	13.9	15.0
Crude fat (%)	3.8	3.8
Crude fibre (%)	8.3	7.4
Crude ash (%)	5.1	5.4
Total sugars and starch (%)	40.0	41.2
Lysine (%)	0.7	0.8
Methionine (%)	0.3	0.3
Phosphor (%)	0.5	0.6
Calcium (%)	0.7	0.8
Vitamin E (mg/kg)	150.0	150.0
Vitamin A (IU/kg)	10,000	10,000
Vitamin D3 (IU/kg)	2,000	2,000
Iron (mg/kg)	53.0	53.0
Iodine (mg/kg)	2.0	2.0
Copper (mg/kg)	5.0	5.0
Manganese (mg/kg)	43.0	43.0
Zinc (mg/kg)	15.0	15.0
Selenium (mg/kg)	0.4	0.4

3.2.3 Piglet selection

All piglets were weighed within 4 hours after parturition. For each litter, the mean birth weight (MLBW) and SD were calculated. Piglets with a birth weight of MLBW-2.5*SD < piglet birth weight < MLBW-1*SD were characterised as LBW piglets. In each litter, a maximum of two LBW piglets was selected and ear tagged. A sample size calculation was performed, using G*Power [114]. In total, 76 LBW piglets were selected, spread over 6 farrowing rounds and 67 sows. The body weight, skin lesion score and mortality of the piglets were recorded on the day of birth (d1), on day 3 (d3), day 9 (d9), 2 days after weaning (d24) and 2 weeks after weaning (d38).

3.2.4 Experimental treatments

To minimise the influence of sow effects and due to the large number of LBW piglets needed to observe a potential effect of drenching, treatments were allocated on piglet level rather than litter level. The LBW piglets were randomly allocated to a treatment: "sham" or "no treatment (none)". The "sham" intervention implied a fake drenching by inserting an empty 2.5 mL syringe into the piglet's mouth. This was repeated once a day during the first week after birth (d1 till d7). Based on preliminary testing with volumes of 2 mL of milk, the average catching and drenching time was 29.6 sec ± 8.1 SD per piglet (average catching time by 1 person: 10.5 sec ± 5.9; average drenching time: 19.0 sec ± 5.7).

Therefore, every piglet was sham drenched during 20 s. Animals that belonged to the “no treatment” group were left in the pen and were not picked up nor drenched.

3.2.5 Data collection

3.2.5.1 *Skin lesion scoring*

Skin lesion scoring has been validated as an indicator for aggressive behaviour [115]. It is often applied in studies that focus on supplementation or diets [116, 117], following the hypothesis that the supplement might provide the piglet with additional energy, allowing it to demonstrate more competitive, aggressive behaviour. To determine whether handling (drenching) LBW piglets had an effect on their competitive behaviour, and consequently, to ensure that any observed difference in skin lesions during future supplementation studies can be attributed to the supplement and not the act of drenching, each LBW piglet was scored for skin lesions on the snout and skin.
A skin lesion score was given using the following scoring system according to Rundgren and Löfquist [118], Pluske and Williams [119] and Parrat et al. [120]:

0: no lesions
1: <5 superficial lesions (skin unbroken)
2: 5–10 superficial lesions or <5 deep lesions (skin broken and evidence of haemorrhage)
3: >10 superficial lesions or >5 deep lesions

The skin lesion scoring was performed on d1, d3, d9, d24 and d38.

3.2.5.2 *Blood sampling*

At the end of the drenching period (d9) and 2 weeks after weaning (d38), an 8 mL blood sample was taken from the cranial vena cava. For ethical reasons, no more than 2 attempts to draw blood were allowed. The blood sample was divided into 3 tubes: 1 serum tube, 1 ethylene diamine tetra acetic acid (EDTA) tube and 1 heparin tube. The serum and EDTA tubes were sent to Animal Health Care (Torhout, Belgium) for routine biochemical and haematological analysis. The following biochemical parameters were determined: glucose, non-esterified fatty acids (NEFA) and urea. The haematological analysis determined the levels of red blood cells (RBCs), the haematocrit (HCT), the haemoglobin (HGB) levels, the lymphocytes, monocytes, neutrophils, eosinophils, basophils, the total white blood cell (WBC) count and the platelet (thrombocyte) levels.
The heparin tube was centrifuged at 1500 g (3500 rpm) for 10 min at 4 °C. Next, the supernatant or plasma was collected and kept at −80 °C until further analysis.

3.2.5.3 *IgG and IGF-1 analysis*

The immunoglobulin G (IgG) and insulin-like growth factor 1 (IGF-1) levels were measured using a porcine competitive inhibition and a sandwich enzyme immunoassay, respectively (IgG: Cloud-Clone Corp., (Katy) Texas, USA, CEA544Po; IGF-1: Cloud-Clone Corp., (Katy)

Texas, USA, SEA050Po). The collected plasma was diluted (1/2500 and 1/50, respectively) and IgG and IGF-1 levels were determined according to the manufacturer's instructions. All samples were loaded in triplicate.

3.2.6 Statistical analysis

To evaluate the potential effect of drenching on all outcome variables, linear mixed models were fitted in JMP Pro 14 (SAS Institute Inc., Cary, NC, USA). Treatment and age were added as fixed effects and sex was considered a covariate (with the exception of the IgG and IGF-1 analysis, due to a limited number of male animals (n = 3)). In addition, all 2-way interactions between treatment, age and sex were included. Interactions in third degree were not added, as these would have made the model too complex. Given the fact that the piglets were selected over a period of 10 months (6 selection rounds), the farrowing round was added as a random effect. To account for the dependence between littermates and the multiple measurements that were performed on the same piglets, the sow (nested in the farrowing round) and the piglet (nested in sow which was nested in the farrowing round) were included, respectively, as random effects as well. Sows that had been used for piglet selection during previous farrowing rounds were neglected, thus, each sow was only included once. This starting model was simplified using stepwise backwards modelling, during which all non-significant effects were removed from the starting model. To meet normality and/or homoscedasticity, body weight, NEFA, urea, IgG, IGF-1 and neutrophil levels were log transformed, while the other outcome variables required no transformations. Effects were considered statistically significant if $p \leq 0.05$. Post-hoc analysis with Tukey's correction was used to compare different groups. All values are presented as median ± SD. To evaluate the probability of more severe skin lesions occurring in certain treatment or age groups, an ordinal logistic regression model was used in which treatment, age and their interaction were added as model effects. Next, this model was simplified using stepwise backwards modelling by removing all non-significant ($p > 0.05$) effects. The probability of a higher mortality between the different groups was evaluated by a Cox's proportional hazard model. Treatment, age, sex and their interactions were added as fixed factors. A post-hoc analysis was performed using risk ratios. Additionally, mortality was visualized using Kaplan–Meier curves.

3.3 Results

3.3.1 Body weight

There were no significant interactions present. No significant difference in body weight was observed between piglets that were sham drenched and piglets that received no treatment (p = 0.203) or between males and females (p = 0.441). As expected, the body weight increased over time (day 1: 0.84 ± 0.20 kg, day 3: 0.97 ± 0.28 kg, day 9: 1.76 ± 0.58 kg, day 24: 3.45 ± 1.17 kg, day 38: 5.30 ± 1.74 kg); $p < 0.001$) (Figure 3.1).

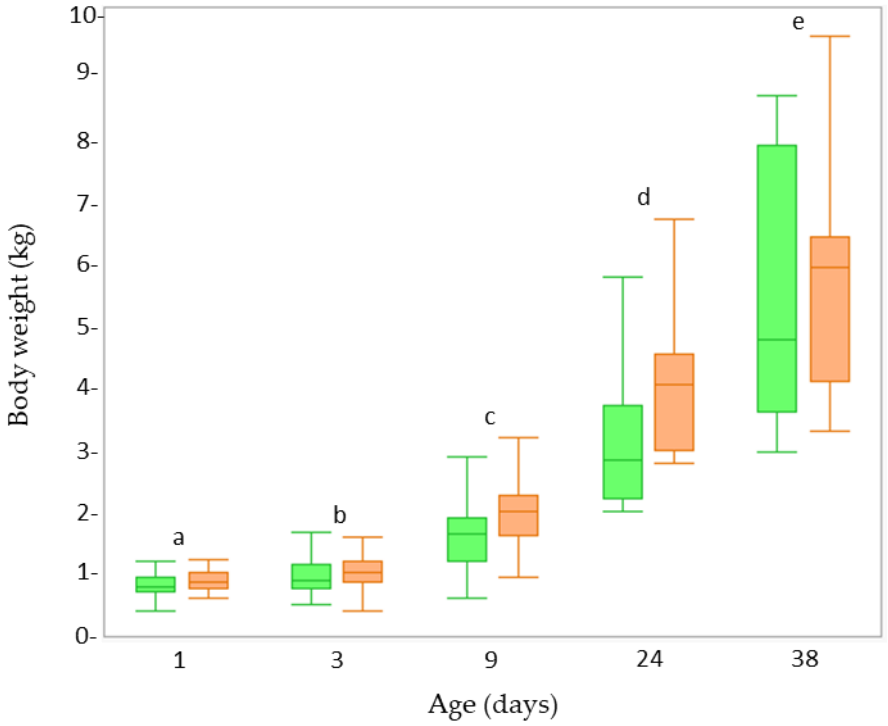

Figure 3.1. Boxplot of body weight (n = 76) from low birth weight piglets that received no treatment (green box; n = 39) or were sham drenched (orange box; n = 37) at different time points (day 1 (n = 76), day 3 (n = 50), day 9 (n = 40), day 24 (n = 35) and day 38 (n = 28)). Significant age differences (linear mixed models, Tukey post-hoc analysis, $p \leq 0.05$) are indicated by a different letter.

3.3.2 Glucose levels

There were no significant interactions. There was no effect of age or sex. Glucose levels did not differ between piglets that were sham drenched and piglets that received no treatment (Table 3.2).

3.3.3 NEFA levels

There were no significant interactions. Sham drenching had no effect on blood total NEFA levels, nor did the levels differ between male and female pigs. The NEFA levels were significantly lower at the age of 38 days when compared to the age of 9 days (Table 3.2).

3.3.4 Urea levels

There were no significant interactions. Urea levels did not differ between piglets that were sham drenched and piglets that received no treatment. Urea levels did not differ significantly between males and females. Urea levels were significantly lower on day 38 compared to day 9 (Table 3.2).

3.3.5 IgG levels

There was no significant interaction between treatment and age. Age did not have an effect on IgG levels. IgG levels did not differ between piglets that were sham drenched and piglets that received no treatment (Table 3.2).

3.3.6 IGF-1 levels

There was no significant interaction between treatment and age. IGF-1 level was significantly higher on day 38 compared to day 9. IGF-1 levels did not differ between piglets that were sham drenched and piglets that received no treatment (Table 3.2).

3.3.7 Haematological analysis

There were no significant interactions. The sex of the animals had no effect on the RBC level, the HCT, the HGB level, the lymphocyte concentration, the monocyte level, the basophil level and the thrombocyte level. However, the total amount of WBCs was significantly higher in males than in females. This increased WBC count in males was due to a higher neutrophil and eosinophil concentration. There was a significant age effect present for the RBCs, the HCT, the HGB, the total WBC count, the lymphocytes, the monocytes and the thrombocytes. There was no age effect on the neutrophil, eosinophil and basophil concentration. No difference was seen between animals that were sham drenched or received no treatment for the RBC level, the HCT, the HGB level, the total WBC count, the lymphocytes, the monocytes, the neutrophils, the eosinophils, the basophils and the thrombocytes (Table 3.2; Table S1 (Supplementary material)).

Table 3.2. Blood values (median ± SD) of glucose, non-esterified fatty acids (NEFA), urea, immunoglobulin G (IgG), insulin-like growth factor 1 (IGF-1), red blood cells (RBCs), haematocrit (HCT), haemoglobin (HGB), white blood cells (WBCs), lymphocytes, monocytes, neutrophils, eosinophils, basophils and thrombocytes, presented by age, sex and treatment from selected low birth weight piglets (linear mixed models, Tukey post-hoc analysis, $p \leq 0.05$).

	Age			**Sex**			**Treatment**		
	Day 9	**Day 38**		**Female**	**Male**		**No treatment**	**Sham**	
	Median ± SD	**Median ± SD**	***p*-value**	**Median ± SD**	**Median ± SD**	***p*-value**	**Median ± SD**	**Median ± SD**	***p*-value**
Glucose (mmol/L)	6.87 ± 1.64	6.40 ± 1.70	0.300	6.40 ± 1.92	6.75 ± 1.03	0.960	6.55 ± 2.02	6.56 ± 1.32	0.341
NEFA (mmol/L)	0.58 ± 0.79	0.17 ± 0.18	<0.001	0.46 ± 0.47	0.45 ± 1.05	0.925	0.47 ± 0.52	0.33 ± 0.82	0.125
Urea (mmol/L)	3.66 ± 0.88	1.66 ± 0.69	<0.001	2.84 ± 1.41	2.32 ± 1.13	0.479	3.24 ± 1.13	2.32 ± 1.40	0.081
Ig G (mg/mL)	3.31 ± 4.93	2.97 ± 1.02	0.344	3.24 ± 4.27	2.55 ± 1.13	0.773	3.39 ± 5.50	2.55 ± 1.79	0.560
IGF-1 (ng/mL)	8.95 ± 15.99	38.61 ± 21.02	0.008	23.48 ± 25.05	15.03 ± 14.48	0.298	23.48 ± 27.05	20.17 ± 17.62	0.175
RBC (10^{12}/L)	4.27 ± 0.42	5.69 ± 0.56	<0.001	5.23 ± 0.96	5.33 ± 0.89	0.687	5.11 ± 1.04	5.46 ± 0.88	0.769
HCT (%)	30.45 ± 2.50	35.9 ± 3.51	0.005	33.5 ± 3.58	32.30 ± 4.96	0.600	32.60 ± 3.88	33.20 ± 4.15	0.759
HGB (g/dL)	8.40 ± 0.71	10.40 ± 1.12	0.001	9.20 ± 1.43	9.35 ± 1.26	0.814	9.20 ± 1.70	9.65 ± 1.17	0.418
WBC (10^3/µL)	10.73 ± 5.10	17.95 ± 4.20	0.021	13.18 ± 5.61	18.24 ± 4.11	0.039	12.66 ± 6.58	17.81 ± 4.39	0.908
Lymphocytes (10^3/µL)	4.18 ± 1.75	7.96 ± 1.62	0.001	6.15 ± 2.42	7.06 ± 1.87	0.616	4.94 ± 2.82	6.93 ± 1.90	0.950
Monocytes (10^3/µL)	0.89 ± 0.37	1.55 ± 0.57	<0.001	0.94 ± 0.52	1.47 ± 0.70	0.086	1.08 ± 0.51	1.12 ± 0.64	0.952
Neutrophils (10^3/µL)	5.83 ± 3.15	8.33 ± 2.90	0.172	6.14 ± 3.22	9.70 ± 2.24	0.005	5.49 ± 3.77	8.40 ± 2.52	0.612
Eosinophils (10^3/µL)	0.10 ± 0.20	0.19 ± 0.13	0.817	0.13 ± 0.11	0.27 ± 0.20	0.038	0.12 ± 0.12	0.19 ± 0.17	0.913
Basophils (10^3/µL)	0.02 ± 0.06	0.01 ± 0.02	0.177	0.01 ± 0.05	0.02 ± 0.01	0.552	0.01 ± 0.06	0.02 ± 0.01	0.440
Thrombocytes (10^3/µL)	1087.50 ± 374.48	444.00 ± 173.61	<0.001	646.00 ± 458.02	560.00 ± 283.45	0.461	764.00 ± 535.69	596.00 ± 334.41	0.689

3.3.8 Skin lesion scores

There were no significant interactions. No influence of sex was observed (p = 0.394). Age did have an effect on the severity of skin injuries (p = 0.001). The highest probability of piglets having skin lesions was at the age of 38 days, followed by 24 days, 9 days, 1 day and 3 days. Thus, the post-weaning period posed the highest risk. Piglets that were sham drenched showed no higher risk of having skin lesions compared to piglets that were not treated (p = 0.247).

3.3.9 Mortality

The 2-way interactions of treatment*age, sex*age and treatment*sex were not significant (p = 0.959, p = 0.970 and p = 0.696, respectively). There was no effect of sex (p = 0.320) or treatment (p = 0.619). However, there was a significant effect of age on mortality (p < 0.001). All considered piglets had the highest risk of dying on the first day, with this risk decreasing over the following time points. Consequently, mortality was highest during the first 9 days (47.37%), with the most critical moment being the first 3 days (mortality of 34.21%) (Figure 3.2).

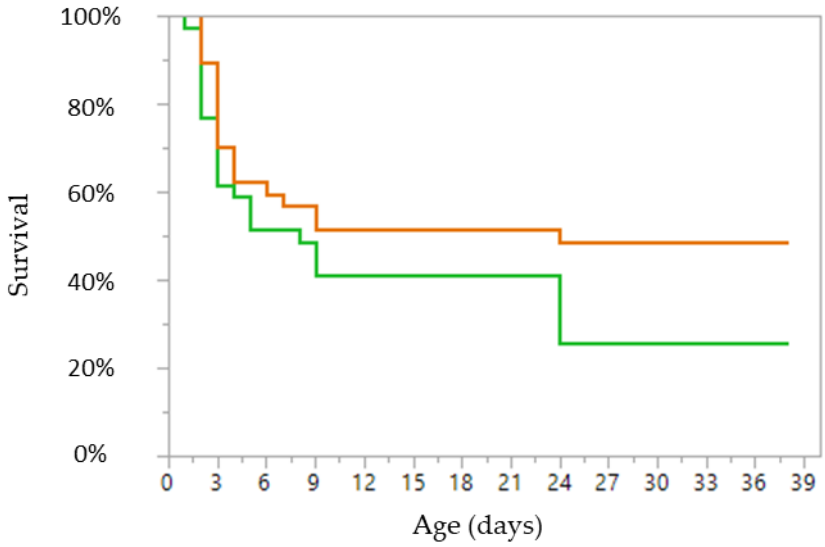

Figure 3.2. Cumulative mortality of low birth weight piglets that were sham drenched (orange line, n = 37) or received no treatment (green line; n = 39) over time. Cox's proportional hazard model showed that the animals had the greatest risk of dying during the first day after birth, with the risk decreasing over the following time points (Kaplan–Meier survival plot, p ≤ 0.05).

3.4 Discussion

In order for LBW piglets to acquire an adequate amount of energy and nutrients, farmers are often suggested to drench them with milk replacer, colostrum or enriched formula [21,23,24,37,38]. However, supplementing piglets via drenching implies chasing, picking up, restraining and drenching the animals while they are often agitated or scared. It was hypothesised in this study that the act of drenching piglets with a low birth weight, and consequently, a lower energy reserve and chance of survival [103], might aggravate their already weakened situation. If drenching LBW piglets causes an additional burden, it might counteract any potentially positive effect of the supplemented substance. In this respect, the literature data are conflicting, as Declerck and colleagues [103] observed a reduction in mortality when they supplemented LBW piglets (<1.00 kg) with a coconut oil containing booster on the day of birth, whereas an earlier study by Santos et al. [104] did not report an effect on mortality, even though coconut oil was supplemented at a higher total energetic dosage to piglets of a similar birth weight (0.60–0.90 kg) on both the first and second day of life. A later study, in which LBW piglets were supplemented with coconut oil on the day of birth and the second day, also did not show a reduced mortality when comparing supplemented and non-supplemented piglets. It must be mentioned, though, that during the latter study, piglets with a higher birth weight (<1.20 kg) were classified as LBW and the total supplemented energy was lower than the previously mentioned studies [102]. The results of these last two studies support the hypothesis that drenching could nullify a potentially positive effect of the supplemented product. The present study examined the effect of drenching for seven days after birth vs. non-drenching on the body weight, skin lesion score and survival of exclusively LBW pigs at days 1, 3, 9, 24 (weaning) and 38 (post-weaning).

No difference in body weight was found between LBW piglets that were sham drenched and LBW piglets that were not drenched across the experimental period. Similar results were found in a previous study by de Oliveira et al. [121]. In the latter study, there was no difference in body weight between handled (enforced stroking) and non-handled piglets. Strangely, in this study [121] piglets that were not handled, but exposed to humans, were heavier than their handled littermates. These findings suggest that the performance of piglets, in terms of body weight, is sensitive to the level of exposure or induced stress. This was confirmed in another study showing that the body weight of piglets which experienced human handling as too stressful was affected negatively [122]. What is more, previous exposures to humans that were experienced as negative have been shown to persist in piglets' memories for up to five weeks. Thus, any interaction with humans could potentially have a detrimental effect on the pig's performance, even beyond weaning [113, 122, 123]. The results on the body weight of LBW piglets in our study demonstrate that the level of stress caused by drenching does not impact piglet's growth. Given that the pigs in this study were considered less resilient because of their low body weight and younger age, this lower resilience did not seem to apply to a more profound stressor as drenching. Due to the LBW piglets' low energy reserves [32, 103] and smaller size, they are generally unable to

compete against heavier littermates for the better, anterior teats, resulting in an insufficient colostrum intake [2,29,46]. This inadequate consumption of colostrum further depletes the LBW piglets' energy reserves, which can explain why the animals were not able to struggle much during this experiment's drenching moments and did not experience this as a significant stressor. Moreover, since LBW piglets are easily outcompeted by their heavier litter mates, it can be assumed that they did not experience previous stressful experiences due to fighting. This can be an additional explanation as to why the LBW piglets did not experience drenching as very stressful, but instead showed rather meek behaviour. In a second part, the present study looked into different biochemical (glucose, NEFA, urea, IgG and IGF-1) and haematological (RBCs, HCT, HGB, WBCs, lymphocytes, monocytes, neutrophils, eosinophils, basophils and thrombocytes) blood parameters after the drenching period (day 9) and two weeks after weaning (day 38) to asses a potential effect of drenching as a chronic stressor. By comparing these blood values between sham drenched and non-drenched LBW piglets, any difference could be attributed to the act of drenching, allowing future supplementation studies to distinguish differences in blood values, due to the act of drenching, from those that are caused by the supplement. Repetitive, unpleasant handling of pigs induces chronic stress and results in elevated corticosteroid, adrenalin and noradrenalin levels [23, 124, 125]. Corticosteroids, such as the glucocorticoid cortisol, stimulate gluconeogenesis, resulting in higher glucose levels, and inhibit the protein metabolism, resulting in higher protein levels in the blood [126]. Additionally, cortisol has a stimulating role in the urinary excretion of urea [127], and stress increases the circulating levels of NEFAs [128]. If drenching LBW piglets for seven days were experienced as an unpleasant handling, higher glucose, lower urea and higher NEFA levels would have been expected. However, no differences were seen between the treatment groups. As mentioned before, it should be taken into account that LBW piglets often have low body energy reserves, a reduced ability to mobilize these reserves [32, 42, 103, 129, 130] and less energy uptake [46, 131]. Thus, it is plausible that the relatively mild stressor (i.e. drenching) was not sufficient enough to mobilise the piglets' already low glycogen reserves (for a longer period). Moreover, any additional stressor related procedures (i.e. ear tagging, tail docking and castration) had to be considered as well. These interventions have been known to induce pain and cause acute stress, thus increasing the cortisol levels and potentially masking the more subtle effect of drenching-induced stress [132]. However, the blood samplings were performed at least two days after these interventions and cortisol levels are normally only elevated up to 4 h, 2–7 h and 0–1 h after ear tagging, castration and tail docking, respectively [132-135]. Thus, when considering the effect of these procedures on cortisol and, consequently, glucose, NEFA and urea, no interference was expected at the blood sampling moment at day 9 of life. NEFA and urea levels were both lower at two weeks after weaning compared to immediately after the drenching period. Plausible explanations for these findings could be that neonatal piglets have a higher demand for fatty acid mobilization and gluconeogenesis in response to an increased energy demand, as suggested by Madsen et al. [136], and the high amount of fats that can be found in the sow's milk [137]. Since this study showed no effect of drenching on the protein or lipid metabolism, nor any increase (or decrease) in glucose levels, and high (or

low) blood glucose concentrations are correlated to the chance of survival [125], it can be assumed that drenching did not impose a significant threat on the pigs' survival chances.
No differences in IgG and IGF-1 levels were found between drenched and non-drenched piglets. These findings suggested that handling LBW piglets did not interfere (negatively) with their suckling behaviour. Since colostrum is very rich in immunoglobulins (with IgG being the most abundant) [42] and growth factors [99, 138], a reduction in plasma IgG and IGF-1 levels would be expected if the induced stress of drenching had affected the LBW piglets' suckling. Similar to the biochemical blood analysis, no difference in haematology was seen between sham drenched and non-drenched LBW piglets. These results further confirmed that drenching did not have a negative impact on LBW piglet's metabolism. There was a higher leucocyte – mainly neutrophil – count in male piglets than in female piglets, regardless of their age or treatment. This increase in males was not due to castration, as this normally results in leucocyte trafficking, during which white blood cells are redistributed to other organs, such as the skin [139]. A plausible explanation would be that the male animals suffered more from infections, which might be represented by the higher, albeit not significant, mortality in male piglets. However, no monitoring of infections was conducted, so no conclusions regarding the reason for the increased leucocyte number could be given. Similar to our results, higher neutrophil and eosinophil levels were found in male piglets in comparison with female piglets by Olufunke et al. [140].
Skin lesions were scored to determine whether drenching affects the aggression that was experienced by the LBW piglets. Apart from aggressive behaviour that is driven by the formation of social or dominance hierarchy, pigs are prone to show more aggression when exposed to stressful circumstances such as low environmental enrichment or mixing with unfamiliar pigs [141]. This was reflected in the present study by the higher presence of skin lesions during the post-weaning period, when unfamiliar piglets were put together in the same pen. It was hypothesised that the additional stress from drenching piglets could potentially increase their aggression as well. On the other hand, de Oliveira et al. [121] suggested that forced human handling could reduce the piglets' fear. This could increase their ability to cope with stress and reduce intraspecific aggression. In the present study, skin lesions were not affected – positively nor negatively – by drenching. The act of sham drenching might have been more stressful to the piglets than the enforced stroking that was performed in the study by de Oliveira et al. [121], thus it induced more stress and eliminated any positive effect on their behaviour. Contrarily, drenching might not have been such a big stressor for the piglets that they responded with more aggression towards their conspecifics. More behavioural and stress research, such as fear and aggression testing (e.g., novel object test, open-field test) and measurements of biomarkers such as salivary cortisol or alpha-amylase, would be required to determine any other effects of drenching on the behaviour and stress response of LBW pigs.
In agreement with previous studies, mortality was highest during the first 7 days, with the most critical period being the first three days [28, 46] for both drenched and non-drenched animals. After this critical first week, mortality declined in the sham drenched group, with only 8% of the animals dying after the drenching period and less than 3% dying after weaning. The non-drenched piglets suffered nearly three times as many losses after the

first week (23%), with 15% of the piglets dropping out post-weaning. Given the multifactorial aetiology and complexity of mortality in piglets [32], this observation could be important, but requires further research as no statistically significant difference was found in the present study. The sham drenched piglets possibly benefitted from having been picked up and returned to the farrowing crate. During this study, the piglets were often asleep when they were picked up, and thus, awoken and agitated. Putting an empty syringe in their mouth could have activated their suckling reflex and stimulated the piglets to suckle after being returned to the sow. The untreated animals were left alone and remained asleep and therefore missed out on this extra suckling bout, which could explain the difference between these two treatment groups. However, since no difference was seen between drenched and non-drenched animals for the other tested parameters (e.g., body weight), the activation of the piglets by picking them up was probably not enough for them to increase their suckling performance in a matter to gain weight or improve their immunity. During the present study, piglets were not returned in a standardised way (e.g., always at a teat), so confounding was possible. Although there was no significant interaction between sex and treatment, male animals did seem to have lower survival chances when not being handled. In both male and female animals, mortality remained lower than 60% when they were sham drenched. In the untreated group, however, less than 10% of the male piglets survived, while female piglets had survival rates similar to the sham drenched animals. It was already reviewed by Muns et al. [32] that male piglets were more likely to die before weaning even if they were heavier. Even though male piglets are often born heavier than females, a male-biased susceptibility to mortality seems to exist. The higher energy demands in male piglets could be attributed to secondary sexual characteristics, such as larger body size and tusks, resulting in poorer thermoregulatory abilities and decreased immunocompetency [142]. The results of the present study suggested that male LBW animals could benefit from being handled during the neonatal period, while female animals might not experience any positive effect from early human handling. Further research to determine this potentially sex-biased effect is required before applying different management strategies for LBW piglets, depending on their sex.

3.5 Conclusions

Sham drenching did not affect LBW piglets' performance or mortality during the drenching period, the suckling period and after weaning. Thus, drenching can be applied safely in underprivileged piglets as an intervention to enhance their survival chances. For studies examining the effects of supplements, it is advised to always incorporate a non-drenched group into the experimental set-up. Consequently, it will be possible to attribute any observed effect to either the supplemented product or the act of drenching.

Chapter 4.

Drenching bovine colostrum, quercetin or fructo-oligosaccharides has no positive effect on health or survival of low birth weight piglets

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Abstract

*The introduction of hyperprolific sows has resulted in more low birth weight (LBW) piglets, accompanied by higher mortality. A possible strategy to enhance the resilience and survival of LBW piglets is oral supplementation (drenching) of bioactive substances. This study evaluated the supplementation of bovine colostrum, short-chain fructo-oligosaccharides (scFOS) or quercetin that were dissolved separately in a milk replacer. The study was divided into two sub-experiments. First, the milk replacer was compared with a sham drenched group. Secondly, each dissolved compound was compared with the milk replacer. The LBW piglets, defined as weighing between (mean litter birth weight -1*SD) and (mean litter birth weight -2.5*SD), were randomly allocated to the different treatments and drenched once a day for seven days. On day 1, 3, 9, 24 and 38, piglets were weighed and scored for skin lesions. Blood samples were collected on day 9 and 38 and analyzed to determine glucose, non-esterified fatty acids, urea, immunoglobulin G, insulin-like growth factor 1, and a standard blood panel test. There was no difference between sham drenched piglets and piglets that were drenched with milk replacer regarding any of the parameters. No effect was observed between the milk replacer group and any of the bioactive compounds either, except a higher mortality within the scFOS group. In conclusion, this study showed that drenching the evaluated bioactive compounds, in the used dosages, did not improve LBW piglets' resilience or survival and more research is required to determine the effect of scFOS on small piglets.*

4.1 Introduction

A potential strategy to enhance the resilience and survival of LBW piglets is through supplementation of energy or bioactive substances [92, 99, 101-104, 136, 143]. Energy supplementation mainly attempts to prevent energy depletion, and consequently, starvation and hypothermia [99, 101, 102, 104]. However, piglets are not only born with low energy reserves but are also immunologically naïve [144]. They require an exogeneous source of both energy and immunoglobulins (i.e. colostrum) [21, 145]. LBW piglets often fail to retrieve an adequate colostrum intake due to their low viability or their inability to compete with bigger littermates for functional teats [9]. To tackle the reduced colostrum consumption, the supplementation of bovine colostrum has been evaluated in different studies [93, 99, 146-148]. In most of these studies, an improved immunological response was observed after colostrum supplementation [99, 146, 147]. However, under practical conditions, where small dosages and simple handling are required, the supplementation of colostrum does not always result in better survival. Some authors have found higher survival rates with small dosages of (bovine) colostrum [99], whereas others could only observe an improved survival when (porcine) colostrum was supplemented in high volumes [93].

It goes without saying that any form of supplementation requires well-balanced gut health, a trait known to be impaired in LBW piglets [21, 149-151]. In that respect, providing LBW piglets with prebiotics can be interesting. Short-chain fructo-oligosaccharides (scFOS), prebiotics that consist of non-digestible chains of one glucose molecule linked to two to four fructose molecules, have proven to be beneficial in piglets. scFOS induce a shift in the gut microbiota, favouring *Bifidobacteria* and *Lactobacillus spp.*, which efficiently ferment scFOS into short-chain fatty acids [105]. Additionally, scFOS supplementation influences the neonatal immune system by stimulating the secretion of immunoglobulin A, increasing activated T-cells and interferon γ [106, 107], and improves performance in terms of body weight at weaning (in piglets with an average birth weight of 1.35 kg) [108]. However, most studies involving scFOS are based on maternal supplementation or supplementation of preterm, weaned, or piglets with a birth weight higher than 1 kg [105-109]. The effect of scFOS on LBW piglets with a birth weight below 1 kg remains unknown.

Another important requirement for a well-functioning digestive tract is the maintenance of an intestinal redox balance. Newborn piglets, and more notably LBW piglets, often suffer from oxidative stress, an imbalance between the production and the ability to eradicate reactive oxygen species (ROS) [152-154]. At the gut level, oxidative stress can result in a disrupted intestinal barrier and an abnormal intestinal development that lead to an increased permeability for pathogens or toxins [155, 156] and insufficient nutrient absorption [153, 157]. In this regard, supplementing LBW piglets with an antioxidant could counterbalance intestinal oxidative stress and its downstream negative effects on gut health. Quercetin, a ubiquitously present flavonoid in plants, is a strong antioxidant [158]. During in vitro studies, quercetin has already proven to have beneficial effects – improved viability, barrier function, and reduced levels of ROS – on H_2O_2 stressed intestinal porcine

epithelial cells from the jejunum of unsuckled neonatal piglets (IPEC-J2) [110-112]. Another study, using IPEC-1 cells, recently showed that quercetin was able to protect the intestinal cells from diquat-induced oxidative damage [159]. However, the translation of quercetin's positive in vitro results into field trials with LBW piglets remains difficult. No positive effect of quercetin supplementation was found in LBW piglets in terms of body weight and intestinal morphology when given 10 or 50 mg/kg during the first week after birth. However, 10 mg/kg quercetin did appear to improve the intestinal barrier function [160]. Other in vivo studies that examine the effect of quercetin in pigs usually focus on weaned piglets [161-166]. Thus, very little is known about the potential effects of quercetin supplementation in neonatal, LBW piglets.
Given the limited knowledge concerning the effects of the previously mentioned compounds on LBW piglets, the current study aimed to examine whether supplementation of bovine colostrum, scFOS, or quercetin during the first week of life, could improve the resilience of LBW piglets. In that respect, each compound was given once daily, during seven days and different zootechnical parameters, i.e. body weight, skin lesions and mortality (on day one (d1), day three (d3), day nine (d9), two days (d24) and two weeks post-weaning (d38)) were examined. Additionally, a blood sample was collected for haematologic and biochemical analysis (on d9 and d38).

4.2 Materials and methods

4.2.1 Ethical approval

This study was reviewed and approved by the Ethical Committee for Animal Experimentation of the University of Antwerp (ECD 11/2018) and was compliant with national legislation and European guidelines (2010/63/EC).

4.2.2 Animals

The study was conducted on a commercial farm (Hoogstraten, Belgium). All sows (Topigs20 (*n* = 98), Norwegian Landrace (*n* = 12)) were kept in individual farrowing crates (2.25 x 0.60 m) that were located in pens (2.50 x 1.75 m) with slatted flooring. A nesting area was provided with an unslatted floor and a top cover. The parity of the sows varied from one to ten, with a mean parity of 4.34 ± 2.13 SD. The sows were fed with a commercial gestation diet up to farrowing. After farrowing, all sows were switched to a commercial lactation diet. Piglets included in the study, as well as their littermates, were subjected to the standard handling procedures in the farm: before the age of one week, all piglets were ear-tagged, tail docked, received 200 mg iron dextran I.M. and male piglets were castrated using meloxicam analgesics (0.4 mg/kg I.M.). Piglets were weaned at the age of 22.2 ± 0.6 days.

4.2.3 Piglet selection

All piglets were weighed within four hours after birth, and subsequently, the mean birth weight of each litter and the SD were calculated. LBW piglets were defined as having a birth weight between (mean birth weight litter – 1 SD) and (mean birth weight litter – 2.5 SD). A maximum of two LBW piglets was selected in each litter to minimize the effect of sow traits and was identified by a coloured ear tag. Following a statistical power analysis (G*Power [114]), a total of 188 LBW piglets was selected, spread over six farrowing rounds and 110 sows.

4.2.4 Experimental treatments

The experimental set-up consisted of two experiments for which the 188 selected piglets were randomly allocated to five treatments: a sham group (*n* = 37), milk replacer (*n* = 38), bovine colostrum (*n* = 38), scFOS (*n* = 39), and quercetin (*n* = 36).
To ensure that the milk replacer, acting as the solvent for the bioactive compounds that were supplemented during the second experiment, did not affect the LBW piglets' survival or health, a pre-experiment was conducted during which any effect of the milk replacer against the effect of drenching was tested. In this experiment two groups of piglets were included: the sham intervention group (*n* = 37) and the group drenched with a plain milk replacer (*n* = 38). The sham intervention implied a fake drenching by inserting an empty 2.5 mL syringe into the piglet's mouth for ± 20 sec. The drenching duration was based on preliminary testing, during which the average catching and drenching time was 29.6 ± 8.1 sec per piglet (average catching time by one person: 10.5 ± 5.9 sec; average drenching time: 19.0 ± 5.7 sec).
This was repeated once a day during the first week after birth (day one till day seven). The other group of piglets was drenched daily during the first week of life with a plain milk replacer (at 25°C) which was not enriched with immunoglobulins, prebiotic fibers, or antioxidants (other than propyl gallate or butylated hydroxyanisol) (Table 4.1). One dose of milk replacer provided 8.95 kJ metabolizable energy to the drenched LBW piglet.
In a second experiment, the daily oral addition of bioactive compounds to the milk replacer was compared with the daily oral supplementation of a milk replacer. The LBW piglets were randomly allocated to one of four treatments: milk replacer (i.e. same group as during preliminary experiment (*n* = 38)), bovine colostrum (*n* = 38), scFOS (*n* = 39) or quercetin (*n* = 36) supplementation. Within every litter, a maximum of two piglets was selected and allocated to different treatments. Consequently, for every treatment, there was only one piglet per sow.
All groups received a daily oral supplementation of 2 mL at ± 25°C from day one until day seven.
The bovine colostrum (Volostrum®, Volac International Ltd., United Kingdom) was dissolved in the aforementioned milk replacer at a concentration of 0.45 g/mL, resulting in supplementation of 0.9 g per piglet. This dosage was chosen, following the results of studies where beneficial aspects of bovine colostrum on growth performance, intestinal

development, immune parameters, and sanitary status of pigs were demonstrated when given during the early post-weaning period (dose of 1 mL) [167, 168] and pre-weaning period (dose of 1 mL) [98] or as commercially available supplements containing bovine colostrum (dose of 1-2 mL) [99].

Based on previous studies by Le Bourgot et al. [106, 169], Apper et al. [109] and Ayuso et al. [108], scFOS (64.8 g/100 mL active product, Profeed L95, Beghin-Meiji, Tereos, Marckolsheim, France) were supplemented to the allocated piglets in a dosage of 1 g scFOS/day (1.54 mL scFOS Profeed L95 + 0.46 mL milk replacer; 2 mL in total).

Quercetin (Sigma-Aldrich, Overijse, Belgium) was supplemented in a volume of 2 mL milk replacer containing 10 mg of quercetin. The dose of 10 mg was based on studies by Cermak et al. [170], Bieger et al. [164], Wein and Wolffram [171], Vergauwen et al. [111], Zou et al. [162] and Van le Thanh et al. [166].

Table 4.1. Nutrient, chemical and energetic composition of the milk replacer in the supplemented concentration (25 g milk powder in 100 mL water).

Analytical constituents		***Nutritional additives***	
Crude protein (%)	5.0	*Vitamin A (IU/100 mL)*	625
Crude fat (%)	4.0	*Vitamin D3 (IU/100 mL)*	125
Crude ash (%)	1.9	*Vitamin E (mg/100 mL)*	2
Crude fibre (%)	0	*Vitamin K (mg/100 mL)*	0.1
Moisture (%)	75.8	*Vitamin C (mg/100 mL)*	4
Lactose (%)	9.6	*Vitamin B1 (mg/100 mL)*	0.2
Lysine (%)	0.4	*Vitamin B2 (mg/100 mL)*	0.2
Methionine (%)	0.2	*Vitamin B6 (mg/100 mL)*	0.1
Cystine+Methionine (%)	0.3	*Vitamin B12 (µg/100 mL)*	1
Calcium (%)	0.1	*Iodine (mg/100 mL)*	0.03
Sodium (%)	0.2	*Manganese (mg/100 mL)*	1.1
Phosphorus (%)	0.2	*Zinc (mg/100 mL)*	2.1
Magnesium (%)	0.03	*Selenium (mg/100 mL)*	0.01
Iron (mg/100 mL)	1.9	*Propyl gallate (mg/100 mL)*	0.1
Copper (mg/100 mL)	3.9	*Butylated hydroxyanisole (mg/100 mL)*	0.1
Energetic value			
Metabolizable energy (kJ/100 mL | kcal/100 mL)		450 | 107	
Net energy (kJ/100 mL | kcal/100 mL)		357 | 86	

4.2.5 Data collection

4.2.5.1 *Skin lesion scoring*

A skin lesion score (for the entire body) was given using the scoring system according to Rundgren and Löfquist [118], Pluske and Williams [119] and Parrat et al. [120]:

0: no lesions
1: < 5 superficial lesions (skin unbroken)
2: 5-10 superficial lesions or < 5 deep lesions (skin broken and evidence of haemorrhage)
3: > 10 superficial lesions or > 5 deep lesions

The skin lesion scoring was performed on d1, d3, d9, d24 and d38.

4.2.5.2 *Blood sampling*

On d9 and d38, an 8 mL blood sample was taken from the cranial vena cava. The blood sample was divided into three tubes: one serum tube, one EDTA tube, and one heparin tube. The serum and EDTA tubes were sent to Animal Health Care (Torhout, Belgium) for routine biochemical and haematological analysis. The following biochemical parameters were determined: glucose, non-esterified fatty acids (NEFA), and urea. The haematological analysis determined the levels of red blood cells (RBC), the haematocrit (HCT), the haemoglobin (HGB) levels, the lymphocytes, monocytes, neutrophils, eosinophils, basophils, the total white blood cell (WBC) count, and the platelet (thrombocyte) levels.
The heparin tube was centrifuged at 1500 x g for 10 min at 4°C. Next, the plasma was collected and kept at -80°C until further analysis.

4.2.5.3 *IgG and IGF-1 analysis*

The immunoglobulin G (IgG) and insulin-like growth factor 1 (IGF-1) levels were measured using a porcine competitive inhibition and a sandwich enzyme immunoassay, respectively (IgG: Cloud-Clone Corp., Katy, Texas, USA, CEA544Po; IGF-1: Cloud-Clone Corp., Katy, Texas, USA, SEA050Po). The collected plasma was diluted (1/2500 and 1/50, respectively) and IgG and IGF-1 levels were determined according to the manufacturer's instructions. All samples were analyzed in triplicate.

4.2.6 Statistical analysis

To evaluate the potential effect of drenching on all outcome variables, linear mixed models were fitted in JMP Pro 15.1 (SAS Institute Inc., Cary, NC, USA). Treatment and age were added as fixed effects, and sex was considered a covariate. In addition, all two-way interactions between treatment, age and sex were included. Given the fact that the piglets were selected over ten months (six selection rounds), the farrowing round was added as a random effect. To account for the dependence between littermates and the multiple measurements that were performed on the same piglets, the sow (nested in the farrowing

round) and the piglet (nested in sow which was nested in the farrowing round) were included, respectively, as random effects as well. Sows that had been used for piglet selection during previous farrowing rounds were neglected, thus each sow was only included once. This starting model was simplified using stepwise backwards modelling, during which all non-significant effects were removed from the starting model. Body weight, NEFA, urea, IgG, and IGF-1 levels were log-transformed. The *p*-threshold for significance was set at 0.05. When required, post-hoc analysis with Dunnett's correction was used to compare different bioactive compounds with the milk replacer (control group). Tukey's correction was used to compare the different age groups during a post-hoc analysis. Given that not all values had a normal distribution and required a logarithmic transformation, all values are presented as median ± SD. To evaluate the probability of more severe skin lesions occurring in certain treatment or age groups, an ordinal logistic regression model was used. The probability of higher mortality between the different groups was evaluated by Cox's proportional hazard model. A post-hoc analysis was performed using risk ratios. Additionally, mortality was visualized using Kaplan-Meier curves.

4.3 Results

4.3.1 Milk replacer compared with sham group

4.3.1.1 *Body weight*

Body weight did not differ between LBW piglets that received milk replacer and piglets that were sham drenched ($p = 0.808$). Sex did not affect body weight ($p = 0.396$). The LBW piglets gained weight during the experimental period ($p < 0.001$) (Figure 4.1).

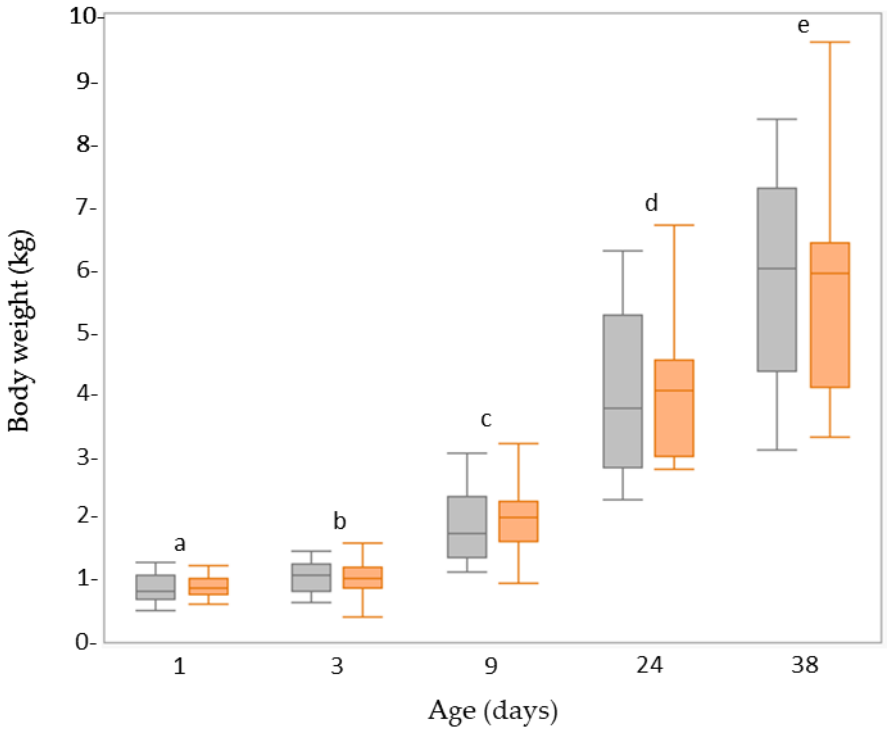

Figure 4.1. Boxplot of body weight from low birth weight piglets at day 1 (n = 75), day 3 (n = 56), day 9 (n = 46), day 24 (n = 41) and day 38 (n = 39) that were drenched with a milk replacer (grey box, n = 38) or sham drenched (orange box, n = 37) once a day from day 1 until day 7. Significant age differences (linear mixed models, Tukey post-hoc analysis, p ≤ 0.05) are indicated by a different letter.

4.3.1.2 *Biochemical analysis*

There was no difference in blood glucose, NEFA, urea, IgG or IGF-1 levels between animals that were sham drenched and animals that received milk replacer. Sex did not affect any of the biochemical parameters. On d9, the NEFA, urea and IgG levels were significantly higher than on day 38 (NEFA: $p < 0.001$; urea: $p < 0.001$; IgG: $p = 0.020$). IGF-1 levels were significantly higher on day 38 compared to day 9 ($p = 0.001$). Regarding glucose, no age effect was observed (Table 4.2; Table S2 (Supplementary material)).

Table 4.2. Blood values (median ± SD) of glucose, non-esterified fatty acids (NEFA), urea, immunoglobulin G (IgG), insulin-like growth factor 1 (IGF-1), red blood cells (RBC), haematocrit (HCT), haemoglobin (HGB), white blood cells (WBCs), lymphocytes, monocytes, neutrophils, eosinophils, basophils and thrombocytes, presented by age, sex and treatment from selected low birth weight piglets (linear mixed models, $p \leq 0.05$).

	Age			***Sex***			***Treatment***		
	Day 9	**Day 38**		**Female**	**Male**		**Milk replacer**	**Sham**	
	Median ± SD	**Median ± SD**	***p*-value**	**Median ± SD**	**Median ± SD**	***p*-value**	**Median ± SD**	**Median ± SD**	***p*-value**
Glucose (mmol/L)	6.77 ± 0.98	6.30 ± 0.93	0.125	6.45 ± 1.00	6.56 ± 0.94	0.479	6.30 ± 0.86	6.56 ± 1.32	0.400
NEFA (mmol/L)	0.52 ± 0.47	0.08 ± 0.28	<0.001	0.33 ± 0.44	0.32 ± 0.51	0.882	0.32 ± 0.56	0.33 ± 0.82	0.562
Urea (mmol/L)	3.21 ± 1.02	1.58 ± 1.29	<0.001	2.91 ± 1.46	2.00 ± 1.18	0.077	2.46 ± 1.37	2.32 ± 1.40	0.495
IgG (mg/mL)	4.77 ± 2.97	2.79 ± 1.75	0.020	3.70 ± 2.92	3.55 ± 2.14	0.957	5.41 ± 2.81	2.55 ± 1.79	0.057
IGF-1 (ng/mL)	14.96 ± 8.70	24.01 ± 25.43	0.001	25.75 ± 19.26	20.13 ± 12.19	0.137	27.46 ± 15.31	20.17 ± 17.62	0.647
RBC (10^{12}/L)	4.24 ± 0.57	5.60 ± 0.55	<0.001	5.37 ± 0.92	5.25 ± 0.96	0.923	5.29 ± 0.99	5.46 ± 0.88	0.151
HCT (%)	29.75 ± 4.10	35.10 ± 3.62	0.001	32.90 ± 3.59	33.50 ± 6.15	0.445	33.20 ± 5.51	33.20 ± 4.15	0.665
HGB (g/dL)	8.10 ± 1.37	10.05 ± 0.87	<0.001	9.65 ± 1.31	9.20 ± 1.63	0.996	9.40 ± 1.67	9.65 ± 1.17	0.858
WBC (10^3/µL)	13.38 ± 4.38	18.24 ± 4.01	<0.001	15.67 ± 5.55	18.20 ± 4.59	0.533	16.30 ± 5.74	17.81 ± 4.39	0.606
Lymphocytes (10^3/µL)	4.95 ± 1.62	7.92 ± 1.34	<0.001	7.12 ± 1.95	7.17 ± 2.06	0.358	7.17 ± 2.11	6.93 ± 1.90	0.960
Monocytes (10^3/µL)	0.90 ± 0.32	1.74 ± 0.70	<0.001	1.13 ± 0.66	1.49 ± 0.78	0.839	1.49 ± 0.78	1.12 ± 0.64	0.600
Neutrophils (10^3/µL)	6.25 ± 2.98	8.89 ± 3.28	0.054	7.03 ± 3.73	9.31 ± 2.91	0.700	7.23 ± 3.93	8.40 ± 2.52	0.984
Eosinophils (10^3/µL)	0.07 ± 0.16	0.21 ± 0.12	0.200	0.15 ± 0.08	0.21 ± 0.18	0.008	0.16 ± 0.12	0.19 ± 0.17	0.361
Basophils (10^3/µL)	0.02 ± 0.01	0.01 ± 0.01	0.537	0.01 ± 0.01	0.02 ± 0.01	0.299	0.01 ± 0.01	0.02 ± 0.01	0.752
Thrombocytes (10^3/µL)	846 ± 421.24	443 ± 168.39	0.002	586 ± 351.91	468 ± 304.67	0.189	404 ± 329.13	596 ± 334.41	0.053

4.3.1.3 *Haematological analysis*

No difference was seen between animals that were sham drenched or received milk replacer. The sex of the animals only affected the eosinophil values with significantly higher levels in male piglets ($p = 0.008$). At day 38, the blood values were higher compared to day 9 for the RBCs ($p < 0.001$), the HCT ($p = 0.001$), the HGB ($p < 0.001$), the total WBC count ($p < 0.001$), the lymphocytes ($p < 0.001$) and the monocytes ($p < 0.001$). However, thrombocyte levels were lower on day 38 ($p = 0.002$). There was no age effect on the neutrophil, eosinophil and basophil concentration (Table 4.2; Table S2 (Supplementary material)).

4.3.1.4 *Skin lesion scores*

Animals that received milk replacer had a higher probability of more severe skin lesions than piglets that were sham drenched ($p = 0.018$). Sex had no effect on the skin lesion score odds ($p = 0.181$). Age affected the odds of observing more skin lesions, with the highest odds of having severe skin injuries on day 24, followed by day 38, 1, 9 and 3, respectively ($p < 0.001$).

4.3.1.5 *Mortality*

Treatment did not affect mortality ($p = 0.614$). There was no difference in mortality between males and females ($p = 0.966$). The LBW piglets were most likely to die on the day of birth, with the odds of dying decreasing with increasing age ($p < 0.001$) (Figure 4.2).

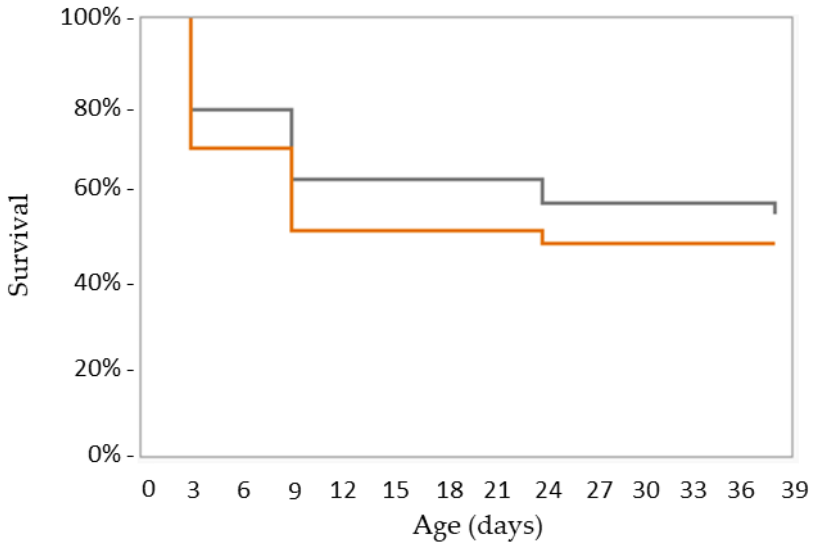

Figure 4.2. Cumulative mortality of low birth weight piglets that were drenched with milk replacer (grey line, n = 38) or sham drenched (orange line; n = 37) over time. Cox's proportional hazard model showed that the animals had the greatest risk of dying during the first day after birth, with the risk decreasing over the following time points (Kaplan–Meier survival plot, p ≤ 0.05).

4.3.2 Bioactive substances compared with milk replacer

4.3.2.1 *Body weight*

Drenching LBW piglets with bovine colostrum, quercetin or scFOS did not result in a different body weight than LBW piglets that were drenched with milk replacer ($p = 0.715$). Males and females did not have significantly different body weights ($p = 0.770$). As expected, the body weight increased during the experimental period ($p < 0.001$) (Figure 4.3).

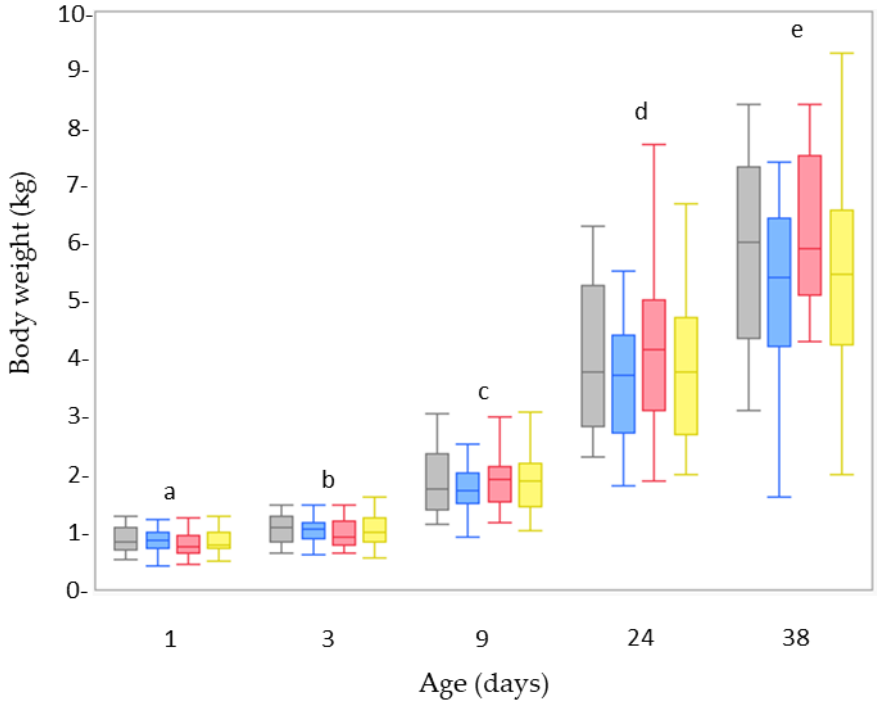

Figure 4.3. Boxplot of body weight from low birth weight piglets that were drenched with milk replacer (grey box: d1 n = 38, d3 n = 30, d9 n = 25, d24 n = 22, d38 n = 21), bovine colostrum (blue box: d1 n = 38, d3 n = 28, d9 n = 22, d24 n = 19, d38 n = 18), short-chain fructo-oligosaccharides (red box: d1 n = 39, d3 n = 23, d9 n = 15, d24 n = 14, d38 n = 21 n = 11) or quercetin (yellow box: d1 n = 36, d3 n = 26, d9 n = 21, d24 n = 19, d38 n = 18) once a day from day 1 until day 7. Body weight is presented at different time points: day 1 (n = 151), day 3 (n = 107), day 9 (n = 83), day 24 (n = 74) and day 38 (n = 68). Significant age differences (linear mixed models, Tukey post-hoc analysis, p ≤ 0.05) are indicated by a different letter.

4.3.2.2 *Biochemical analysis*

No treatment or sex effect was observed (Table 4.3; Table S3 (Supplementary material)). On day 9, glucose, NEFA, urea and IgG levels were significantly higher than on day 38 (glucose: $p = 0.009$; NEFA: $p < 0.001$; urea: $p < 0.001$; IgG: $p = 0.029$). On day 38, the IGF-1 concentrations were significantly higher than on day 9 ($p < 0.001$) (Table 4.4; Table S4 (Supplementary material)).

Table 4.3. Blood values (median ± SD) of glucose, non-esterified fatty acids (NEFA), urea, immunoglobulin G (IgG), insulin-like growth factor 1 (IGF-1), red blood cells (RBC), haematocrit (HCT), haemoglobin (HGB), white blood cells (WBCs), lymphocytes, monocytes, neutrophils, eosinophils, basophils and thrombocytes, presented by treatment from selected low birth weight piglets (linear mixed models, p ≤ 0.05).

	Treatment				
	Milk replacer	**Colostrum**	**Quercetin**	**scFOS**	***p*-value**
	Median ± SD	**Median ± SD**	**Median ± SD**	**Median ± SD**	
Glucose (mmol/L)	6.30 ± 0.86	6.00 ± 1.20	6.17 ± 1.37	6.23 ± 1.07	0.466
NEFA (mmol/L)	0.32 ± 0.56	0.38 ± 0.56	0.45 ± 0.42	0.46 ± 0.72	0.799
Urea (mmol/L)	2.46 ± 1.37	2.73 ± 1.23	2.32 ± 1.09	1.84 ± 1.60	0.121
IgG (mg/mL)	5.41 ± 2.81	3.11 ± 1.17	4.07 ± 3.09	2.62 ± 2.37	0.146
IGF-1 (ng/mL)	27.46 ± 15.31	16.87 ± 15.55	18.59 ± 13.39	13.51 ± 17.73	0.292
RBC (1012/L)	5.29 ± 0.99	5.37 ± 0.96	5.01 ± 1.11	5.28 ± 1.07	0.580
HCT (%)	33.20 ± 5.51	35.50 ± 3.82	33.90 ± 4.59	33.90 ± 4.23	0.096
HGB (g/dL)	9.40 ± 1.67	9.80 ± 1.32	9.05 ± 1.80	9.85 ± 1.25	0.151
WBC (103/µL)	16.30 ± 5.74	19.45 ± 6.39	16.50 ± 5.27	15.50 ± 6.07	0.324
Lymphocytes (103/µL)	7.17 ± 2.11	8.71 ± 2.10	6.84 ± 2.23	6.91 ± 3.31	0.362
Monocytes (103/µL)	1.49 ± 0.78	1.06 ± 0.43	1.39 ± 0.75	1.18 ± 0.64	0.295
Neutrophils (103/µL)	7.23 ± 3.93	8.82 ± 3.30	8.25 ± 3.47	8.42 ± 3.02	0.833
Eosinophils (103/µL)	0.16 ± 0.12	0.14 ± 0.10	0.10 ± 0.10	0.23 ± 0.16	0.158
Basophils (103/µL)	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.823
Thrombocytes (103/µL)	404 ± 329.13	546 ± 316.93	484 ± 335.40	402 ± 346.78	0.590

Table 4.4. Blood values (median ± SD) of glucose, non-esterified fatty acids (NEFA), urea, immunoglobulin G (IgG), insulin-like growth factor 1 (IGF-1), red blood cells (RBC), haematocrit (HCT), haemoglobin (HGB), white blood cells (WBCs), lymphocytes, monocytes, neutrophils, eosinophils, basophils and thrombocytes, presented by age and sex from selected low birth weight piglets (linear mixed models, $p \leq 0.05$).

	Age			***Sex***		
	Day 9	**Day 38**	***p*-value**	**Female**	**Male**	***p*-value**
	Median ± SD	**Median ± SD**		**Median ± SD**	**Median ± SD**	
Glucose (mmol/L)	6.38 ± 1.14	5.90 ± 1.07	0.009	6.17 ± 1.10	6.18 ± 1.16	0.466
NEFA (mmol/L)	0.57 ± 0.58	0.08 ± 0.24	<0.001	0.43 ± 0.59	0.39 ± 0.54	0.974
Urea (mmol/L)	2.65 ± 1.19	1.76 ± 1.29	<0.001	2.47 ± 1.29	2.47 ± 1.34	0.409
IgG (mg/mL)	4.27 ± 2.66	2.63 ± 2.37	0.029	3.91 ± 2.90	3.22 ± 2.42	0.843
IGF-1 (ng/mL)	9.19 ± 12.11	25.35 ± 20.13	<0.001	20.81 ± 23.12	15.66 ± 16.17	0.363
RBC (1012/L)	4.27 ± 0.62	6.09 ± 0.64	<0.001	5.35 ± 1.03	5.19 ± 1.03	0.631
HCT (%)	31.90 ± 4.17	37.50 ± 3.83	<0.001	33.55 ± 4.31	33.90 ± 5.28	0.359
HGB (g/dL)	8.50 ± 1.26	10.40 ± 1.07	<0.001	9.55 ± 1.46	9.35 ± 1.68	0.255
WBC (10^3/µL)	13.51 ± 4.13	20.38 ± 4.95	<0.001	16.21 ± 5.66	17.76 ± 5.98	0.771
Lymphocytes (10^3/µL)	5.52 ± 2.00	8.58 ± 1.70	<0.001	7.58 ± 2.39	7.33 ± 2.31	0.363
Monocytes (10^3/µL)	0.91 ± 0.33	1.56 ± 0.72	<0.001	1.33 ± 0.72	1.20 ± 0.65	0.283
Neutrophils (10^3/µL)	6.85 ± 2.94	9.42 ± 3.41	0.001	8.11 ± 3.48	8.04 ± 3.51	0.942
Eosinophils (10^3/µL)	0.08 ± 0.07	0.21 ± 0.12	<0.001	0.16 ± 0.12	0.10 ± 0.12	0.553
Basophils (10^3/µL)	0.01 ± 0.01	0.01 ± 0.01	0.762	0.02 ± 0.01	0.01 ± 0.01	0.064
Thrombocytes (10^3/µL)	815 ± 367.24	364 ± 204.30	0.001	529 ± 330.26	463 ± 321.85	0.235

4.3.2.3 *Haematological analysis*

There were no significant interactions. There was no effect of treatment on any of the blood parameters (Table 4.3; Table S3 (Supplementary material)). Sex did not affect any examined haematological parameter. On day 9 blood values were significantly lower than on day 38 for RBCs ($p < 0.001$), HCT ($p < 0.001$), HGB ($p < 0.001$), WBC ($p < 0.001$), lymphocytes ($p < 0.001$), monocytes ($p < 0.001$), neutrophils ($p = 0.001$) and eosinophils ($p < 0.001$). There was no age effect on the basophil levels. Thrombocyte level was significantly higher in LBW piglets at the age of 9 days compared to those at 38 days ($p = 0.001$) (Table 4.4; Table S4 (Supplementary material)).

4.3.2.4 *Skin lesion scores*

The odds of having more skin lesions were not affected by treatment ($p = 0.248$). No sex effect was observed. The probability of observing more severe skin lesions was highest during the post-weaning period: highest on day 24, followed by day 38, 1, 9 and 3 ($p < 0.001$).

4.3.2.5 *Mortality*

LBW piglets that were drenched with scFOS were more likely to die than those that were drenched with milk replacer ($p = 0.034$). None of the other treatments affected the mortality rate (Figure 4.4). No sex effect was observed ($p = 0.833$). The LBW piglets were most likely to die on the day of birth, with the odds of dying decreasing with increasing age ($p < 0.001$).

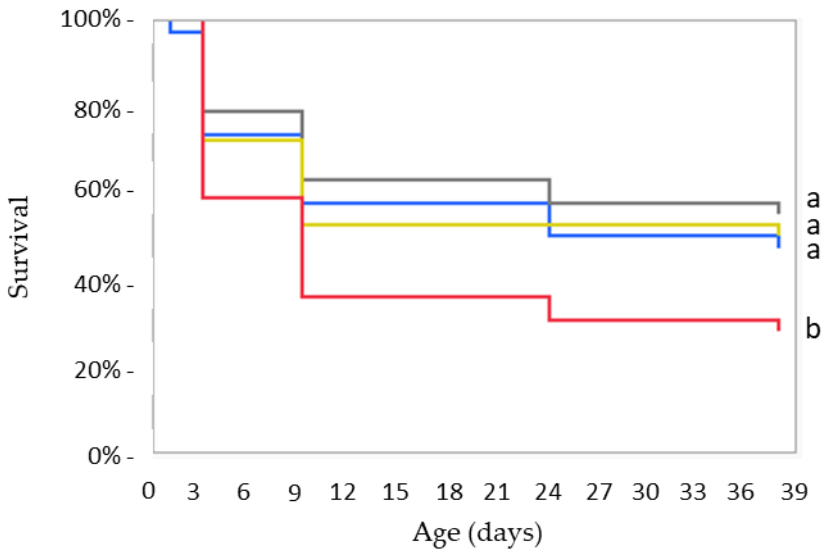

Figure 4.4. Cumulative mortality of low birth weight piglets that were drenched with milk replacer (grey line; n = 38), colostrum (blue line; n = 38), quercetin (yellow line; n = 36) or short-chain fructo-oligosaccharides (scFOS; red line; n = 39) over time. Significant differences between treatments are indicated by a different

letter. The animals had the greatest risk of dying during the first day after birth, with the risk decreasing over the following time points (Cox's proportional hazard model, Kaplan-Meier survival plot, $p \leq 0.05$).

4.4 Discussion

4.4.1 Effect of milk replacer

Given that a plain milk replacer was used as a solvent for all supplemented bioactive substances, a first step consisted of discriminating the possible effect of the milk formula with that of the drenching act on the LBW piglets. Therefore, the supplementation of LBW piglets with the milk replacer during seven days was compared with the sham-drenched group.

Overall, the milk replacer supplementation did not affect the performance or survival of LBW piglets. These results were expected since only a very small dose was given to the animals. Other studies that did find an effect of milk formula supplementation often provided milk in larger dosages (using milk cups or other automated systems) during longer periods or switched to artificially rearing at an older age, as recently reviewed by Huting et al. [172] and Baxter et al. [72].

Similar to the abovementioned performance results, the biochemical and haematological parameters showed no differences between sham drenched LBW piglets and LBW piglets that received the milk replacer.

LBW piglets that were supplemented with the milk replacer were more likely to have severe skin lesions than those that were sham drenched. It was hypothesised in this study that supplementing LBW piglets with health-promoting substances would improve their resilience and vitality and, as a consequence, these piglets could engage more in competitive fights for functional teats. However, given the very small caloric dose of 8.95 kJ per day that was given to the piglets and the lack of any enrichment in the milk formula, the milk replacer was very unlikely to improve their vitality. Moreover, even though skin lesion scoring has been validated to be an indicator of aggressive behaviour, the scoring system without registration of injury locations lacks to differentiate between lesions that are the result of reciprocal fights and bullying. Therefore, the higher odds of skin lesions in milk-fed piglets cannot be explained without additional behavioural testing.

4.4.2 Effect of bioactive substances

One of the objectives of the current study was to evaluate the effect of bovine colostrum supplementation, in a convenient set-up involving a dosage of 2 mL, during seven days. It was hypothesised that the supplemented colostrum would provide the LBW piglets with milk-borne bioactives, such as immunoglobulins and growth factors, and consequently, improve their resilience, performance, and survival. However, no effect was observed on any of the parameters when colostrum-supplemented LBW piglets were compared to the milk replacer group. These results are in line with an earlier field study by Viehmann and colleagues [98] in which neonatal piglets, including LBW piglets, were supplemented with 1 mL of bovine colostrum during three days. This study did not find any improvement on

body weight and cumulative mortality (until ten days after birth) either. However, the authors did observe a prolonged survival time for piglets that were supplemented with colostrum. The animals that died within the first ten days after birth, survived three days longer on average when supplemented with bovine colostrum. In our study, mortality was registered on day 1, 3, 9 and 38, so no exact survival time was calculated. However, at the three different time points (i.e. day 1, 3 and 9) mortality was never significantly different between colostrum and milk replacer supplemented piglets. This indicates that colostrum, supplemented for the first seven days of life, did not enhance the survival time of the LBW piglets during this period. The prolongation of the supplementation period in our study did not improve the results observed by Viehmann et al. [98], possibly because we included piglets with a lower average birth weight than those in their experiment, and consequently, a higher dosage might have been required. Additionally, in our study, and contrarily to Viehmann et al. [98], there was less monitoring during parturition and no heating plates were provided. Perhaps most notably was the absence of any increase in IgG levels in the blood of LBW piglets that were drenched with bovine colostrum. Other studies have observed an increase in IgG or immunological proteins after bovine [98, 99] or porcine [96] colostrum supplementation. In our study, IgG levels were below the critical level for pre-weaning survival of 10 mg/mL [173, 174] and resembled the IgG levels that were found in a group of piglets that were separated from the sow and fed milk replacer during the critical first 12 hours of life [96]. This suggests that the LBW piglets in the present study were unable to ingest enough colostrum. The lower chances to compete against heavier littermates possibly further aggravated the absence of any assistance immediately after birth to achieve their first suckle. Overall, the development of a neonatal colostrum supplementation strategy remains difficult. Some studies find no results after providing piglets with high doses of sow colostrum [95, 96], whereas others find positive results on mortality with only small dosages of bovine colostrum [99]. These, often contradicting, results underline the complex, multifactorial cause of pre-weaning mortality. Furthermore, it appears that providing neonatal LBW piglets with a sufficient amount of energy is more important than ensuring that they acquire adequate IgG levels. This is demonstrated by the present study where a low caloric colostrum supplementation failed to improve LBW piglets' survival, whereas similar studies with high energetic compounds were able to observe a positive effect [72, 99, 103]. Also, Moreira et al. [93] observed similar IgG levels in piglets that received 120 or 200 mL of porcine colostrum, a higher survival rate in the 200 mL fed group, but no differences in antibody levels in piglets that had died. These results indicated that IgG concentration was most likely not the main factor that influenced the piglets' survival.

A second bioactive compound, scFOS, was supplemented to examine its effect on the performance and survival of LBW piglets. No effect of scFOS on the body weight, blood parameters, or skin lesions was observed. A study by Schokker et al. [175] did observe an increased body weight at weaning and an altered mucosal gene expression in the gut after fructo-oligosaccharide supplementation. This different result could be attributed to the different supplementation regimen (5x our dose and supplementation for 13 days (days 2 till 14 of age)) and the higher birth weight of the piglets in their study [175]. However, Ayuso

et al. [108], supplemented scFOS in the same dosage as in the present study and compared supplementation for 7 days with 21 days. The authors found an increased body weight at weaning and during the post-weaning period in normal birth weight piglets, but not in LBW piglets and piglets with a high birth weight, and only in case the animals were supplemented for 21 days. These results suggest that the absent increase of body weight at weaning in our study was most likely due to the LBW and/or limited drenching period of seven days. In the current study, scFOS supplementation did not only fail to improve LBW piglets' performance and health but negatively impacted their survival rate. The cumulative mortality was higher in piglets that belonged to the scFOS group, compared to those in the milk replacer group. These results contravene the study by Ayuso et al. [108] where the post-weaning mortality tended to be lower in LBW piglets that were drenched during seven days, albeit not significantly. However, the average birth weight of LBW piglets in the latter study was higher than in our study (1.02 kg vs. 0.86 kg). Our results suggest that birth weight could play an important role in the efficacy of scFOS. It appears that scFOS supplementation longer than 1 week can have positive effects on piglets with a birth weight above 1 kg, but could have detrimental effects in compromised, LBW piglets below 1 kg.

Given that LBW piglets are known to suffer from oxidative stress [152-154], quercetin was supplemented to test the hypothesis that the beneficial effects on the oxidative status found in vitro [110-112, 159] can be translated into an improved resilience of neonatal LBW piglets. However, no treatment effect was observed on any of the measured parameters. These results are consistent with an earlier study by Vergauwen et al. [160] that did not find any effect of quercetin after supplementation of 50 mg quercetin in LBW piglets. The authors selected LBW piglets with similar birth weight and drenched the same dose of quercetin (10 mg/kg) until day 7 after birth, resembling the experimental set-up of the current study. However, Vergauwen et al. did not drench on the first and second day after birth and artificially reared the LBW piglets to ensure a suboptimal redox status. Contrarily, our study consisted of an uninterrupted supplementation during the first seven days after birth and, no piglets were artificially reared. In contrast, other in vivo research on quercetin supplementation in weaned or growing pigs observed inconsistent effects on body weight or growth, although higher dosages up to 25 mg/kg appeared to have a beneficial impact [161, 176]. On the other hand, Vergauwen et al. [160] did not observe an improvement in the body weight of LBW piglets after a high-dose supplementation of 50 mg/kg. Thus, more research is needed to understand the impact of quercetin on pig health and performance. Additionally, it would be interesting to examine the bioavailability of quercetin when dissolved in milk. If quercetin interacts with certain milk compounds, the absorption and subsequent plasma concentrations could be affected negatively. In this respect, water would be a better solvent than milk replacer. However, orally administered quercetin to pigs has been shown to be conjugated into glucuronides, resulting in a bioavailability up to 17%, even when given simultaneously with the pigs' morning ration [177]. Moreover, Lesser et al. [178] observed a higher absorption of quercetin in the presence of fat due to the incorporation of the flavonoid into the micelles after lipid digestion. In the study by Vergauwen et al. [160] quercetin was dissolved in milk replacer as well and a positive effect on the intestinal permeability was observed, suggesting that the bioavailability of quercetin

in milk was sufficient to have an impact on the piglets' health. Lastly, a study in humans which examined the absorption of flavonols after drinking tea with or without milk, found no differences in the absorption of quercetin [179]. Thus, even though further research on the bioavailability of quercetin would be useful, it appears that using milk replacer as a solvent does not result in a reduced bioaccessibility, and does not explain the absence of any impact on the LBW piglets' performance during this chapter's study.

4.5 Conclusions

The present study showed no beneficial effects of any of the supplemented compounds (i.e. bovine colostrum, scFOS, and quercetin) on the zootechnical performance or survival of LBW piglets. The supplementation of scFOS surprisingly showed a negative impact on the LBW piglets' survival. Thus, more research is required to evaluate the impact of birth weight on the efficacy and possible detrimental effects of scFOS. Moreover, a drenching period of seven days is very labour-intensive. Additionally, it could be interesting to examine not only the dosages but also the combination of different compounds as pre-weaning mortality has a complex, multifactorial origin, as each compound can only tackle one potential underlying cause.

Chapter 5.

The effect of drenching (very) low birth weight piglets with a dense, concentrated milk replacer at farms with differing farrowing management

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Abstract

*Introducing hyperprolific sows has led to proportionally more (very) low birth weight ((V)LBW) piglets, accompanied by higher mortality. To improve the survival of (V)LBW piglets, drenching a dense milk replacer (DMR) could be applied. A first experiment evaluated the effect of drenching DMR (1 or 3 doses within 24 h after birth) to LBW ((mean litter birth weight−1*SD) and weighing between 1 kg and 750 g) and VLBW piglets ((mean litter birth weight−1.5*SD) and weighing less than 750 g). On days 1, 2, 3, 9, and two days post-weaning, body weight, growth, skin lesions, and mortality were monitored. No effect of DMR was observed on any of the parameters. In a second experiment, LBW piglets were supplemented with DMR (similarly to experiment 1) at two farms differing in the level of perinatal care. The same parameters were evaluated, and again none were affected by drenching DMR. Overall survival of the LBW piglets was significantly higher at the farm with high perinatal care. It can be concluded that good perinatal management is more effective in enhancing the survival of LBW piglets than drenching.*

5.1 Introduction

A management strategy that aims to improve the survival and performance of LBW piglets, and one that has been the subject of several studies, is manually providing neonatal piglets with a nutritional supplement via drenching. Different compounds, such as colostrum, prebiotics, antioxidants, or energy boosters, can be drenched [60, 96, 98, 99, 101-103, 180, 181]. However, the lack of a consistent across-studies criterion to identify the targeted LBW piglets limits the comparison of results between studies and obscures drawing a conclusion on the effect of these compounds (reviewed by [64, 72, 172]). While some authors apply birth weight cut-offs up to 1.00–1.35 kg to define LBW piglets [99, 101-103], others use lower birth weights [104, 180, 182, 183]. Moreover, some studies even consider piglets merely below one kilogram as very low birth weight piglets [103] while other studies consider piglets in this weight range as LBW piglets [15,17,19,26,27]. Thus, it remains to be determined in what conditions (e.g., birth weight, perinatal management) and which compounds exert a beneficial effect on piglet performance. Moreover, in the case that drenching would significantly improve piglet performance, additional factors should be taken into account before drenching can be advised as a pre-weaning strategy, i.e., the labour costs and intensity (individual and/or repeated handling of supplemented piglets), and financial costs (supplemented products, spillage) associated with the nutritional support via drenching [172].

The present study was designed to evaluate the supplementation of a dense, concentrated milk replacer on the survival and performance of LBW piglets.

The first experiment examined whether drenching a dense milk replacer affected the performance (growth, survival) of low birth weight piglets and what frequency of drenching was optimal. It was hypothesised that the milk replacer would improve their performance indirectly by supplying enough energy so the piglet can achieve a (first) suckle. Additionally, it was tested whether this boosting effect would be higher when the milk replacer was drenched three times versus only once. Simultaneously, the existence of a lower limit – in terms of birth weight – up to which drenching would have an effect was assessed. It was hypothesised that piglets with a birth weight below 750 g were too weak to experience any benefits from drenching a milk replacer.

In a second step, the experiment was repeated at another farm. This allows us to check the reproducibility of the results at the first farm and to assess the impact of possible farm-specific conditions. In this experiment both farms differed in their perinatal management (partus induction, neonatal supervision, heat provision). It was hypothesised that, in the case of a higher level of perinatal care, drenching a dense milk replacer would improve the performance of LBW piglets more than in the case of lower perinatal care.

5.2 Materials and methods

5.2.1 Ethical approval

This study was reviewed and approved by the Ethical Committee for Animal Experimentation of the University of Antwerp (ECD 11/2018) and was compliant with national legislation and European guidelines (2010/63/EC).

5.2.2 Farms and animals

The study consisted of two consecutive field trials that were conducted on a commercial farm in Meer (Farm A, Hoogstraten, Belgium) and Loenhout (Farm B, Wuustwezel, Belgium). The main animal and management traits of the two farms are presented in Table 5.1.
All piglets included in the study, as well as their littermates, were subjected to the standard handling procedures in the farm: before the age of one week, all piglets were ear-tagged, tail-docked, received a 200 mg iron dextran intramuscular injection, and all male piglets were castrated using meloxicam analgesics. Sows that had been used for piglet selection during previous farrowing rounds were not considered in later farrowing rounds, thus each sow was only included once. Each of the treatment groups comprised equal numbers of sex and birth weight category piglets.

5.2.3 Piglet selection

Experiment 1: Farm A
All piglets were weighed within four hours after birth. When parturition was finished, the mean birth weight of each litter (mean BW_{litter}) and the SD were calculated. Additionally, the mean birth weight of all piglets that were born from 139 sows ($BW_{population}$, n = 2337) and the SD were calculated. When considering $BW_{population}$–1 SD, the resulting birth weight was less than 1 kg. When considering $BW_{population}$–1.5 SD, the birth weight was less than 750 g. Subsequently, LBW and VLBW piglets were selected using two criteria, based on both the farm population's and the litter's mean birth weight:

Piglets with a birth weight between (mean BW_{litter}–1 SD) and (mean BW_{litter}–1.5 SD) and weighing between 750 g and 1 kg were categorised as LBW piglets.
Piglets with a birth weight between (mean BW_{litter}–1.5 SD) and (mean BW_{litter}–2.5 SD) and weighing less than 750 g were categorised as VLBW piglets.
A sample size calculation was performed, using G*Power [114]. In total, 80 LBW and 80 VLBW piglets were selected during seven farrowing rounds from 92 sows (Table 5.1). An equal number of male and female piglets was selected.

Experiment 2: Farm B

Following the poor survival of VLBW piglets at Farm A (see results section), only LBW piglets were selected at Farm B (*n* = 150) (Table 5.1), spread over eight farrowing rounds and 98 sows and having an equal number of male and female piglets. Like Farm A, the mean birth weight of all piglets that were born from 230 sows was calculated ($BW_{population}$, *n* = 4473) which – after deducting 1 SD and 1.5 SD – resulted in birth weights less than 1 kg and 750 g, respectively. Thus, the same selection criteria for LBW piglets that were used at Farm A were applied during the selection at Farm B.

Table 5.1. Farrowing management at the two farms, the sows, the very low birth weight (VLBW), and low birth weight (LBW) piglets that were used during the study.

Parameter	***Farm A***	***Farm B***
Breed (sow)	TN70: Topigs Norsvin x Norsvin Landrace	Danbred YL hybrid: Danbred Yorkshire x Danbred Landrace
Parity (mean ± SD)	4.31 ± 2.52	3.23 ± 1.67
Average litter size (mean ± SD)	17.14 ± 2.96	19.92 ± 3.24
Birth weight VLBW piglets (kg; mean ± SD)	0.64 ± 0.10	Not applicable
Birth weight LBW piglets (kg; mean ± SD)	0.86 ± 0.07	0.87 ± 0.06
Birth weight all weighed piglets ($BW_{population}$; kg; mean ± SD)*	1.28 ± 0.35	1.17 ± 0.32
Weaning age (days; mean ± SD)	20 ± 0.79	23 ± 0.00
Farrowing induction	Only in case of prolonged or no labour	All sows
Monitoring during farrowing	Twice a day (morning and evening round)	Constant supervision throughout day
Drying new-born piglets	No	Sometimes
Assistance first suckle	No	Sometimes
Heat provision	No, plastic cover over creep area	Heated floor in creep area
Cross-fostering	No	Yes
Milk supplementation	No	Yes
Hygiene lock	Yes, no shower	Yes, with shower
Selected piglets	160	150

**$BW_{population}$ = all piglets (normal, LBW, and VLBW) that were born from 139 and 230 sows at farms A and B, respectively.*

*** During every farrowing round, sows farrowed over a time course of 3 days at Farm A, while all piglets were born and selected on the same day at Farm B (all sows farrowed in the course of 1 day).*

5.2.4 Experimental treatments

All piglets were randomly allocated to one of five different treatments (by using ear tags) after they were categorised as LBW or VLBW piglets in the case of Farm A and LBW piglets only in the case of Farm B.

The first treatment group received a single oral supplementation of a dense milk replacer (DMR) providing nutrients and energy in a low volume (5 mL). The DMR was prepared by dissolving 6 g of a plain milk replacer (Piglet Milk R714®, Table 5.2) in 4 mL of water (40 °C) at the time of drenching. Every 5 mL dose of milk replacer provided 59.61 kJ metabolizable energy to the drenched piglet. This group of piglets was drenched with 5 mL of DMR only once and immediately after being ear-tagged. A second treatment group was drenched three times with DMR: immediately after ear-tagging on day 1, in the evening of day 1, and in the morning of day 2. Two sham treatments were used as control groups. In the first group, animals were sham-drenched once by holding an empty 5 mL syringe in the mouth for 20 seconds (duration of drenching), immediately after ear-tagging. In a second sham group, the piglets were also sham drenched, but three times (morning day 1, evening day 1, and morning day 2). After (sham) drenching, each piglet was returned to the litter in a standardised way: every piglet was returned to a teat or, if the sow was not lying down on her side, amongst its siblings. To ensure any observed effect could be attributed to the product rather than the act of drenching, and to evaluate any direct influence of handling during drenching, a negative control group was added in which the animals were not drenched and only handled during data collection. This was a fifth treatment group. Within each treatment group, the number of female and male piglets (and birth weight category in the case of Farm A) was equal. The litter sizes were not standardised in size. Moreover, since the treatments were allocated on the piglet level, a single sow could have 1 up to 5 piglets belonging to the different experimental groups. The same treatments were used during both experiments.

Table 5.2. Nutrient, chemical, and energetic composition of the milk replacer in the supplemented concentration (6 g milk powder in 4 mL water).

Analytical Constituents		***Nutritional Additives***	
Crude protein (%)	13.1	***Vitamin A (IU/100 mL)***	1,666.5
Crude fat (%)	10.5	***Vitamin D3 (IU/100 mL)***	333.3
Crude ash (%)	5.0	***Vitamin E (mg/100 mL)***	5
Crude fibre (%)	0	***Vitamin K (mg/100 mL)***	0.3
Moisture (%)	35.3	***Vitamin C (mg/100 mL)***	10.5
Lactose (%)	35.4	***Vitamin B1 (mg/100 mL)***	0.4
Lysine (%)	1.2	***Vitamin B2 (mg/100 mL)***	0.4
Methionine (%)	0.4	***Vitamin B6 (mg/100 mL)***	0.3
Cystine + Methionine (%)	0.7	***Vitamin B12 (µg/100 mL)***	2.6
Calcium (%)	0.4	***Iodine (mg/100 mL)***	0.1
Sodium (%)	0.4	***Manganese (mg/100 mL)***	3.0
Phosphorus (%)	0.3	***Zinc (mg/100 mL)***	5.6
Magnesium (%)	0.1	***Selenium (mg/100 mL)***	0.02
Iron (mg/100 mL)	5.1	***Propyl gallate (mg/100 mL)***	0.2
Copper (mg/100 mL)	10.3	***Butylated hydroxyanisole (mg/100 mL)***	0.2
Energetic value			
Metabolizable energy (kJ/100 mL|kcal/100 mL)		1,192.1 | 285.3	
Net energy (MJ/100 mL|kcal/100 mL)		953.2 | 228.0	

5.2.5 Data collection

5.2.5.1 *Body weight and growth*

All piglets were weighed on the day of birth (day 1), day 2, day 3, day 9, and two days after weaning (day 24 and day 26 at farms A and B, respectively). All animals were weighed before drenching. Average daily growth (ADG) was calculated from day 1 until two days post-weaning, using the following formula: (body $weight_{day\ x}$ – birth weight)/(x-1). Additionally, to determine growth relative to the individual birth weight, the factorial growth (body $weight_{day\ x}$/birth weight), metabolic weight (body $weight^{0.75}$), and factorial metabolic rate (metabolic $weight_{day\ x}$/metabolic $weight_{day\ 1}$) were calculated.

5.2.5.2 *Colostrum intake*

Drenching DMR could result in an energy boost that allows piglets to obtain their first suckle faster, or by contrast, result in a filled stomach and a satiated feeling, resulting in a delayed first suckle. Thus, DMR could affect colostrum intake and given the importance of colostrum for the performance of the piglet [21, 145], affect their subsequent performance. To determine whether supplementing 5 mL of DMR would have any of the effects, the colostrum intake (CI) was determined whenever the exact time of birth and the body weight immediately after birth were known of both the firstborn piglet and the (V)LBW piglet. This resulted in the estimation of CI of a subset of VLBW (33 piglets, 18 females, 15 males) and LBW (Farm A: 38 piglets, 17 females, 21 males; Farm B: 33 piglets, 21 females, 12 males) piglets. To calculate the CI, the mechanistic model as described by Theil et al. [184] was applied, using the weight gain after 24 hours (WG, g), the body weight at birth (BWB, kg), and the suckling duration from birth until 24 hours after the birth of the litter's first piglet (D, min):

$$CI = -106 + (2.26*WG) + (200*BWB) + (0.111*D) - (1414*(WG/D)) + (0.0182*(WG/BWB))$$

5.2.5.3 *Skin lesion scoring*

A skin lesion score (for the entire body) was given using the scoring system according to Rundgren and Löfquist [118], Pluske and Williams [119], and Parrat et al.[120]:

0: no lesions
1: < 5 superficial lesions (skin unbroken)
2: 5-10 superficial lesions or < 5 deep lesions (skin broken and evidence of haemorrhage)
3: > 10 superficial lesions or > 5 deep lesions

The skin lesion scoring was performed on day 1, day 2, day 3, day 9, and two days post-weaning.

5.2.5.4 *Mortality*

The number of piglets that died was inventoried 24 hours after birth (day 2), on day 3, day 9, and two days after weaning. An animal was registered as deceased (day of death) on the day it was no longer observed (discarded by the farmer). Given the fixed time point at which the animals were checked, mortality could only be registered by the absence of a piglet and not by the cause of death.

5.2.5.5 *Cortisol and chromogranin A*

At Farm B, saliva was sampled from 30 LBW piglets at the age of 47 days to determine whether sham drenching would induce an acute stress response, and consequently, have an impact on any potential effect of the DMR supplementation (results presented in supplementary material (Supplement 1 [185-188]).

5.2.6 Statistical analysis

To meet normality and/or homoscedasticity, body weight, factorial growth, metabolic weight, and factorial metabolic rate were log-transformed, while the other outcome variables required no transformations. Effects were considered statistically significant if $p \leq 0.05$. Post-hoc analysis with Tukey's correction was used to compare different groups. All values are presented as median ± SD.

Experiment 1: Farm A

To evaluate the potential effect of drenching DMR on body weight, growth and colostrum intake, linear mixed models were fitted in JMP Pro 15.2 (SAS Institute Inc., Cary, NC, USA). Treatment, age, sex, and birth weight category (LBW or VLBW) were included as fixed effects. In addition, all relevant interactions between treatment, age, birth weight category, and sex were added to the model. Given that the piglets were selected over several farrowing rounds, farrowing round was added as a random effect. To account for the dependence between littermates or between measurements on the same piglet (but at a different age), random factors for sow (nested in the farrowing round) and piglet (nested in sow) were included. This starting model was simplified using stepwise backward modelling, during which all non-significant effects were removed from the starting model.
To create a ranking for the probability of more severe skin lesions occurring in certain groups, an ordinal logistic regression model was used in which treatment, age, sex, birth weight category, and their interactions were considered model effects. This model was simplified using stepwise backward modelling by only retaining significant ($p \leq 0.05$) effects.
The probability of higher mortality between the different groups was evaluated by Cox's proportional hazard model. Treatment, age, sex, birth weight category, and their interactions were added as fixed factors. This model was simplified using stepwise backward modelling ($p \leq 0.05$). A post-hoc analysis was performed using risk ratios. Additionally, mortality was visualized using Kaplan-Meier curves.

Experiment 2: Farm B

To analyse the potential effect of drenching DMR on body weight, growth and colostrum intake, linear mixed models were fitted in JMP Pro 15.2 (SAS Institute Inc., Cary, NC, USA). In this second experiment we only drenched LBW piglets. As a result, treatment, age, and sex, were included as fixed effects. In addition, all relevant interactions between these fixed factors were added to the model. Given that the piglets were selected over several farrowing rounds and, to account for the dependence between littermates and between measurements on the same piglet (but at a different age), random factors for farrowing round, sow (nested in the farrowing round) and piglet (nested in sow) were included. This starting model was simplified using stepwise backward modelling, during which all non-significant effects ($p > 0.05$) were removed from the starting model.
To create a ranking for the probability of more severe skin lesions occurring in certain groups, an ordinal logistic regression model was used in which treatment, age, sex, and

their interactions were considered model effects. This model was simplified using stepwise backward modelling by only retaining significant ($p \leq 0.05$) effects.
The probability of higher mortality between the different groups was evaluated by Cox's proportional hazard model. Treatment, age, sex, and their interactions were added as fixed factors. This model was simplified using stepwise backward modelling using the stepwise backward method ($p \leq 0.05$). A post-hoc analysis was performed using risk ratios. Additionally, mortality was visualized using Kaplan-Meier curves.

Farm A vs. Farm B
In a second step, the data of both farms were combined in one large dataset to evaluate any differences between the two farms in the potential effect of drenching DMR. For possible differences in view of body weight and colostrum intake, linear mixed models were fitted in JMP Pro 15.2 (SAS Institute Inc., Cary, NC, USA) where farm was included as a fixed effect (next to treatment, age, and sex). In addition, all relevant interactions between farm, and the other fixed factors were included. Similar to the other analyses, farrowing round, sow, and piglet were added as random effects. This starting model was simplified using stepwise backward modelling, by removing those factors that had non-significant effects ($p > 0.05$) from the starting model.
The probability of more severe skin lesions in one of the farms was assessed using an ordinal logistic regression model with farm, treatment, age, sex, and their interactions considered model effects. This model was analysed using stepwise backward modelling by only retaining significant ($p \leq 0.05$) effects.
The probability of higher mortality in one of the farms was evaluated by Cox's proportional hazard model. Farm, treatment, age, sex, and their interactions were added as fixed factors using the stepwise backward method ($p \leq 0.05$). A post-hoc analysis was performed using risk ratios. Additionally, mortality was visualized using Kaplan-Meier curves.

5.3 Results

5.3.1 Experiment 1

5.3.1.1 *Body weight and growth*
None of the fixed factors (treatment, age, sex, and birth weight category) showed a significant interaction for the different parameters related to body weight and growth. These interactions were removed as fixed factors from the linear mixed model, retaining only the individual fixed factors: treatment, age, sex, and birth weight. There was no effect of drenching DMR on the body weight ($p = 0.179$) (Figure 5.1), ADG ($p = 0.091$), factorial growth ($p = 0.130$), metabolic weight ($p = 0.430$) or factorial metabolic rate ($p = 0.149$). As expected, body weight increased during the experimental period ($p < 0.001$), as did factorial growth ($p < 0.001$), metabolic weight ($p < 0.001$), and factorial metabolic rate ($p < 0.001$). Understandably, the body weight of VLBW piglets was lower than that of LBW piglets ($p < 0.001$). It remained lower throughout the investigated period (since there was no

significant interaction between birth weight category and age) (Figure 5.1). Consequently, the VLBW piglets also had a significantly lower ADG (p = 0.022) and metabolic weight (p < 0.001) than LBW piglets, and this throughout the study period. However, the factorial growth and factorial metabolic rate did not differ between the two birth weight categories (p = 0.683 and p = 0.685, respectively) (Table 5.3). No sex effect was observed for body weight: p = 0.595, ADG: p = 0.527, factorial growth: p = 0.452, metabolic weight: p = 0.709, and factorial metabolic rate: p = 0.452 (Supplementary material, Table S5).

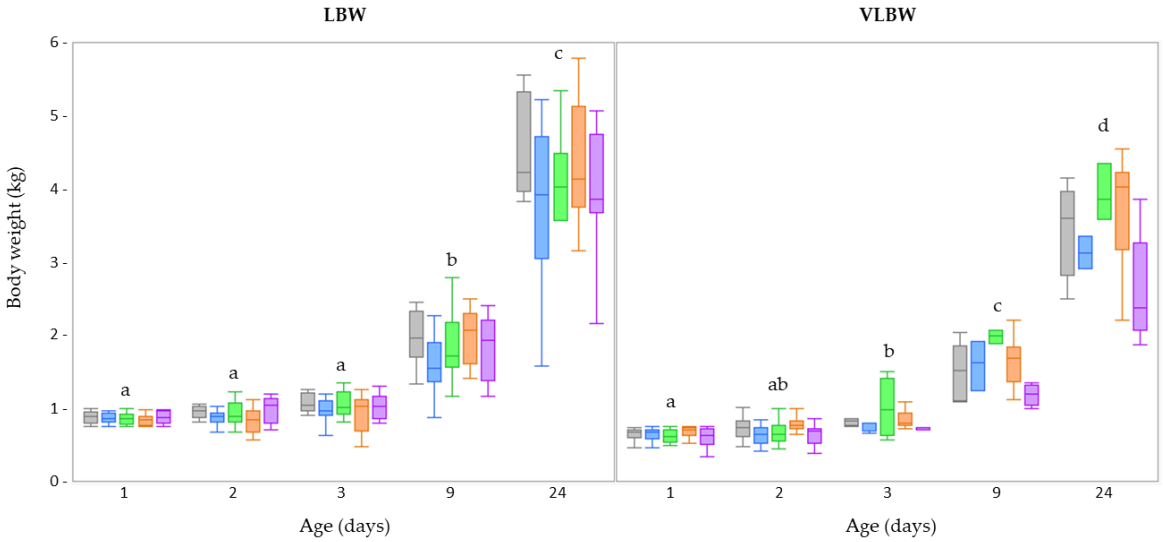

Figure 5.1. . Boxplots of the body weight at different time points (day of birth (day 1), day 2, day 3, day 9, and day 24 (two days after weaning)) of very low birth weight (VLBW; n = 80) and low birth weight (LBW; n = 80) piglets per treatment (dense milk replacer (DMR) one dose (grey box), DMR three doses (blue box), no treatment (green box), sham one dose (orange box), sham three doses (purple box)) at Farm A. There was no effect of drenching DMR on body weight (p = 0.179). Body weight increased during the experimental period independent of treatment and birth weight category (p < 0.001). Ages carrying a different subscript letter were significantly different.

Table 5.3. Comparison of average daily growth (ADG), factorial growth, metabolic weight and factorial metabolic rate of low birth weight (LBW) with very low birth weight (VLBW) piglets at days 1, 2, 3, 9, and 2 days post-weaning (24 days) at the farm with low perinatal management (Farm A (median ± SD)).

		VLBW	***n***	***LBW***	***n***	***p-value***
ADG (kg)	Day 2	0.10 ± 0.15	26	0.08 ± 0.12	56	0.783
	Day 3	0.10 ± 0.06	26	0.10 ± 0.07	56	0.854
	Day 9	0.11 ± 0.05	26	0.12 ± 0.05	55	0.274
	Day 24	0.12 ± 0.03	24	0.14 ± 0.04	53	0.022
Factorial growth	Day 2	1.02 ± 0.22	59	1.06 ± 0.18	72	0.303
	Day 3	1.14 ± 0.31	33	1.20 ± 0.22	62	0.938
	Day 9	2.25 ± 0.63	26	2.12 ± 0.56	56	0.828
	Day 24	4.91 ± 1.31	24	4.94 ± 1.39	53	0.957
Metabolic weight ($kg^{0.75}$)	Day 1	0.74 ± 0.08	80	0.89 ± 0.10	80	< 0.001
	Day 2	0.77 ± 0.12	59	0.93 ± 0.13	72	< 0.001
	Day 3	0.85 ± 0.13	33	1.01 ± 0.15	62	0.002
	Day 9	1.44 ± 0.25	26	1.56 ± 0.27	56	0.001
	Day 24	2.54 ± 0.43	24	2.84 ± 0.52	53	0.018
Factorial metabolic rate	Day 2	1.01 ±0.16	59	1.05 ± 0.13	72	0.303
	Day 3	1.11 ± 0.21	33	1.15 ± 0.16	62	0.938
	Day 9	1.84 ± 0.38	26	1.76 ± 0.38	56	0.828
	Day 24	3.30 ± 0.66	24	3.31 ± 0.82	53	0.957

5.3.1.2 *Colostrum intake*

Colostrum intake was only measured at day 1. Therefore, age was not included as a fixed factor in the statistical analysis. None of the fixed factors (treatment, sex, and birth weight category) showed a significant interaction effect on colostrum intake. These interactions were removed as fixed factors from the linear mixed model, retaining only the individual fixed factors: treatment, sex, and birth weight. No effect of treatment (p = 0.575) (Figure 5.2) or sex (p = 0.295) (Supplementary material, Table S5) was observed for the colostrum intake. The VLBW piglets ingested significantly less colostrum than LBW piglets (p = 0.001) (Figure 5.2).

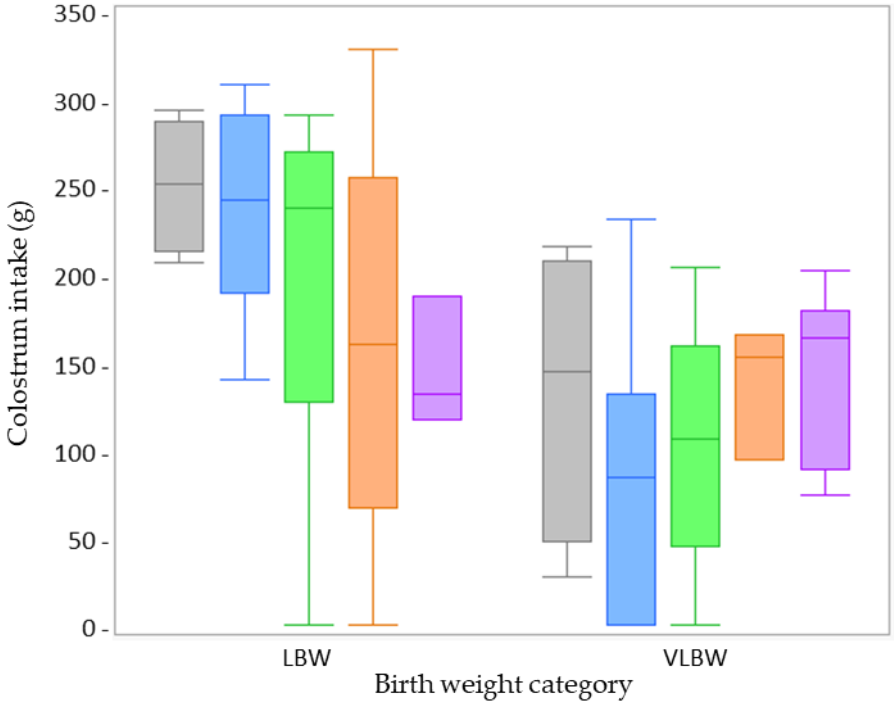

Figure 5.2. Boxplots of the colostrum intake at Farm A from low birth weight (LBW; n = 38) and very low birth weight (VLBW; n = 33) piglets per treatment (dense milk replacer (DMR) one dose (grey box; LBW n = 4, VLBW n = 5), DMR three doses (blue box; LBW n = 11, VLBW n = 9), no treatment (green box; LBW n = 9, VLBW n = 6), sham one dose (orange box; LBW n = 11, VLBW n = 3), sham three doses (purple box; LBW n = 3, VLBW n = 10)). There was no effect of treatment on colostrum intake (p = 0.575). Irrespective of treatment, colostrum intake was significantly less in VLBW when compared to LBW piglets (p = 0.001).

5.3.1.3 *Skin lesion scores*

None of the fixed factors (treatment, age, sex, and birth weight category) showed a significant interaction regarding skin lesions scores. These interactions were removed as fixed factors from the linear mixed model, retaining only the individual fixed factors: treatment, age, sex, and birth weight. No treatment (p = 0.187) (Figure 5.3) or sex effect (p = 0.204) (Supplementary material, Figure S2) was observed on the probability of having more severe skin lesions. Birth weight category had no effect on the severity of skin lesions (p = 0.295) (Figure 5.3). An age effect was observed (p < 0.0001). The highest risk of observing skin lesions was on day 24 (2 days after weaning), followed by day 1, day 2, day 9 and day 3 (Supplementary material, Figure S3).

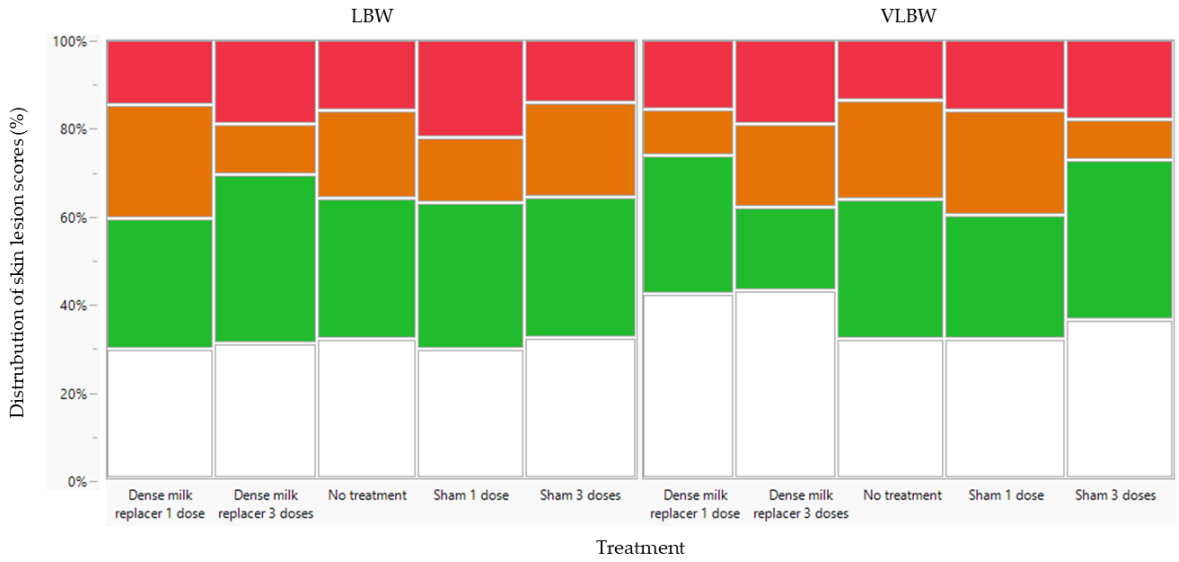

Figure 5.3. Distribution of skin lesion (SL) scores at Farm A (low perinatal management) of the selected low birth weight (LBW, n = 80) and very low birth weight (VLBW, n = 80) piglets per treatment (dense milk replacer (DMR) one dose, DMR three doses, no treatment, sham one dose, sham three doses). There was a no significant effect of treatment or birth weight category on the probability of having more severe SL. The following scoring system was applied: 0: no lesions (white); 1: < 5 superficial lesions (skin unbroken) (green); 2: 5-10 superficial lesions or < 5 deep lesions (skin broken and evidence of haemorrhage) (orange); 3: > 10 superficial lesions or > 5 deep lesions (red).

5.3.1.4 *Mortality*

None of the fixed factors (treatment, age, sex, and birth weight category) showed a significant interaction in view of mortality. These interactions were removed as fixed factors from the linear mixed model, retaining only the individual fixed factors: treatment, age, sex, and birth weight. Treatment had no effect on the probability of dying ($p = 0.572$) (Figure 5.4). No sex effect was observed ($p = 0.395$) (Supplementary material, Figure S4). The VLBW piglets had a significantly higher risk of dying than LBW piglets (Risk ratio: 2.39; $p < 0.001$) (Figure 5.4). The animals had the greatest risk of dying during the first day after birth, with the risk decreasing over the following time points at Farm A ($p < 0.001$) (Figure 5.4).

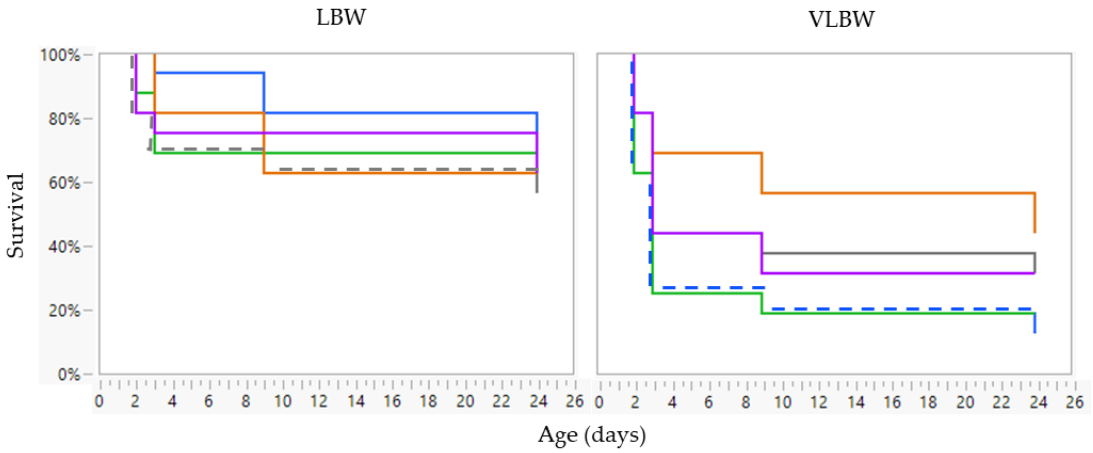

Figure 5.4. Cumulative mortality at Farm A of very low birth weight (VLBW; n = 80) and low birth weight (LBW; n = 80) piglets per treatment (no treatment (green line), sham 1 dose (orange line), sham 3 doses (purple line), dense milk replacer 1 dose (grey line) or dense milk replacer 3 doses (blue line)). Cox's proportional hazard model showed that VLBW piglets had a significantly higher risk of dying than LBW piglets (Risk ratio: 2.39; p < 0.001). The animals had the greatest risk of dying during the first day after birth, with the risk decreasing over the following time points (p < 0.001).

5.3.2 Experiment 2

5.3.2.1 *Body weight and growth*

The performance data of LBW piglets receiving the 5 treatments at Farm B were similar to those observed at Farm A in experiment 1. There were no significant interaction effects between treatment, age, and sex on the variables related to body weight and growth. Therefore, only the individual fixed factors of treatment, age, and sex were retained. Treatment did not affect the body weight (p = 0.345) (Figure 5.5), ADG (p = 0.207), factorial growth (p = 0.241), metabolic weight (p = 0.307) or factorial metabolic rate (p = 0.477) (Supplementary material, Table S5). There was no difference between male and female LBW piglets for any of the parameters: body weight: p = 0.716, ADG: p = 0.301, factorial growth: p = 0.602, metabolic weight: p = 0.812, factorial metabolic rate p = 0.777 (Supplementary material, Table S5). Body weight increased over time (p < 0.001) (Figure 5.5), as did the factorial growth (p < 0.001), metabolic weight (p < 0.001) and factorial metabolic rate (p < 0.001) (Supplementary material, Table S5).

In comparing both farms, none of the fixed factors (treatment, age, sex) showed a significant interaction with 'farm'. In the analysis of the individual fixed factors, only age (see above) had a significant impact. Thus, no difference in the LBW piglets' body weight (p = 0.439) (Figure 5.1, 5.5), ADG (p = 0.062), factorial growth (p = 0.095), metabolic weight (p = 0.051) or factorial metabolic rate (p = 0.956) was observed when comparing Farm A with Farm B (Supplementary material, Table S5, S6).

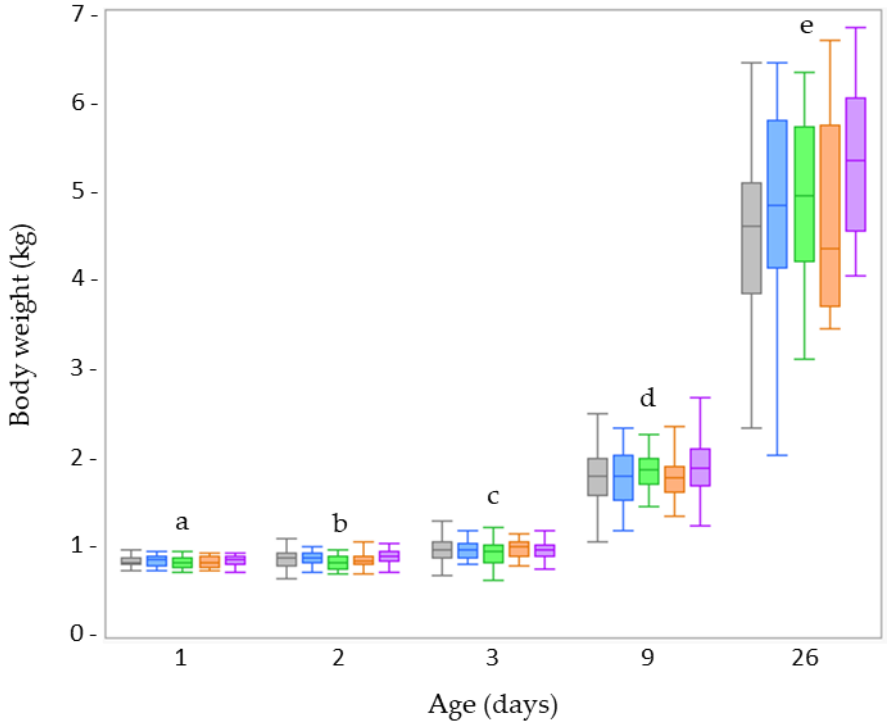

Figure 5.5. Boxplots of the body weight at different time points (day of birth (day 1), day 2, day 3, day 9, and day 26 (two days after weaning)) of low birth weight (LBW; n = 150) piglets per treatment (dense milk replacer (DMR) one dose (grey box), DMR three doses (blue box), no treatment (green box), sham one dose (orange box), sham three doses (purple box)) at Farm B. There was no effect of drenching DMR on body weight (p = 0.345). Body weight increased during the experimental period independent of treatment and birth weight category (p < 0.001). Ages carrying a different subscript letter were significantly different.

5.3.2.2 *Colostrum intake*

As in experiment 1, there were no significant interaction effects on colostrum intake between treatment, age, and sex. Therefore, only the individual fixed factors of treatment and sex were retained. In addition, at Farm B, colostrum intake did not differ significantly between the different treatment groups (p = 0.277) (Figure 5.6) or between males and females (p = 0.825) (Supplementary material, Table S5).

In comparing both farms, none of the fixed factors (treatment, age, sex) showed a significant interaction with 'farm'. Next to treatment and sex, there was no difference in the LBW piglets' colostrum intake between Farm A (n = 38) and Farm B (n = 33) (p = 0.421) (Figure 5.2, 5.6; Supplementary material, Table S5, S6).

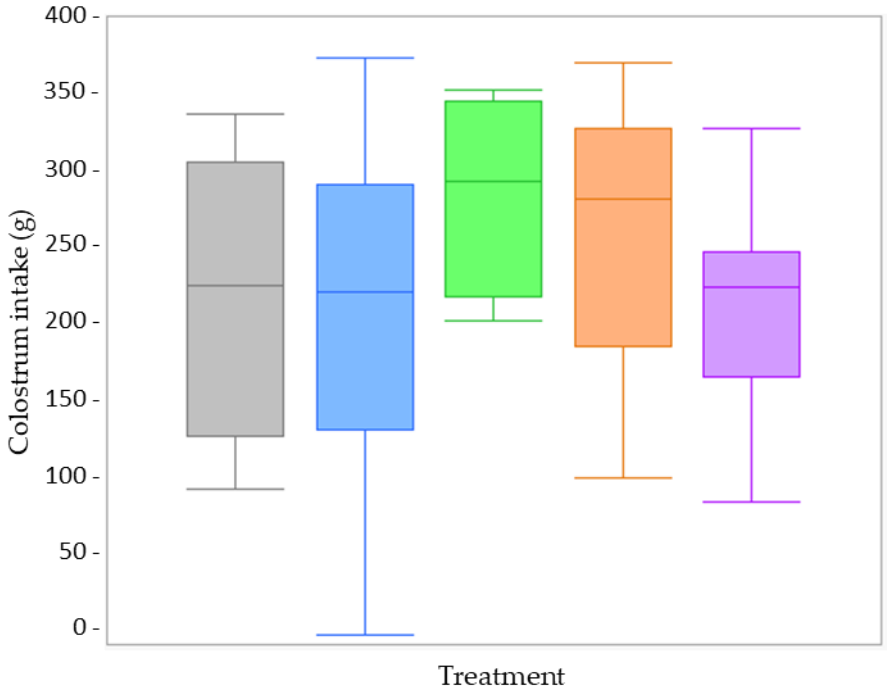

Figure 5.6. Boxplots of the low birth weight piglets' colostrum intake per treatment (dense milk replacer (DMR) one dose (grey box), DMR three doses (blue box), no treatment (green box, sham one dose (orange box), sham three doses (purple box)) at Farm B (high perinatal management; n = 33). Colostrum intake did not differ significantly between the different treatment groups (p = 0.277).

5.3.2.3 *Skin lesion scores*

As in experiment 1, none of the fixed factors (treatment, age, and sex) showed a significant interaction effect on skin lesion scores. These interactions were removed as fixed factors from the model, retaining only the individual fixed factors: treatment, age, and sex, which showed similar effects as in experiment 1. Treatment did not affect the risk of having more severe skin lesions (p = 0.352) (Figure 5.7). No sex effect was observed either (p = 0.364) (Supplementary material, Figure S5). An age effect was observed (p < 0.001): the highest risk of observing skin lesions was on day 26 (2 days after weaning), followed by day 9, day 3, day 2, and day 1 (Supplementary material, Figure S6).

When comparing both farms, none of the other fixed factors (treatment, age, and sex) showed an interaction with farm as a fixed factor. However, at Farm A, the probability of having more severe skin lesions was higher compared to Farm B (p < 0.001) (Figure 5.3, 5.7).

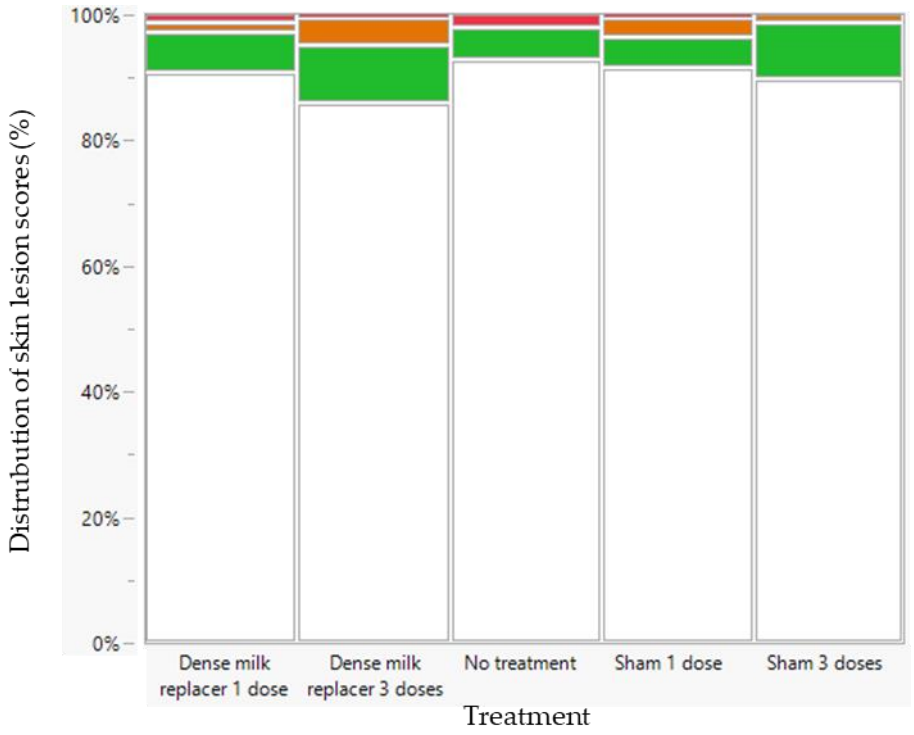

Figure 5.7. Distribution of skin lesion (SL) scores per treatment at Farm B (low perinatal management) of the selected low birth weight piglets (n = 150) per treatment (dense milk replacer (DMR) one dose, DMR three doses, no treatment, sham one dose, sham three doses). The following scoring system was applied: 0: no lesions (white); 1: < 5 superficial lesions (skin unbroken) (green); 2: 5-10 superficial lesions or < 5 deep lesions (skin broken and evidence of haemorrhage) (orange); 3: > 10 superficial lesions or > 5 deep lesions (red).

5.3.2.4 *Mortality*

As in the data on mortality in experiment 1, the data from experiment 2 showed no significant interactions between treatment, age, and sex. Thus, in the statistical analysis, only the fixed factors were retained. As in experiment 1, treatment did not affect the mortality of LBW piglets (p = 0.999) (Figure 5.8). No sex effect was observed either (p = 0.886) (Supplementary material, Figure S7). There was an age effect that affected the risk of dying (p < 0.001). The highest risk of dying was between day 3 and day 9 (p < 0.001).
When comparing both farms (no interaction was observed for the other fixed factors), the risk of dying for LBW piglets was significantly higher at farm A (Risk ratio 10.05; p < 0.001 (Figure 5.4, 5.8).

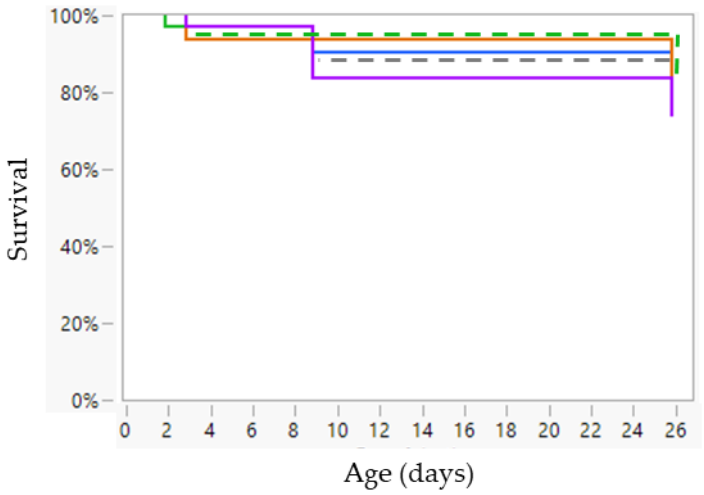

Figure 5.8. Cumulative mortality of low birth weight (LBW) piglets from Farm B (n = 150) at different time points (day 1, 2, 3, 9, and two days after weaning (day 26)) per treatment: no treatment (green line), sham one dose (orange line), sham three doses (purple line), dense milk replacer one dose (grey line) or dense milk replacer three doses (blue line). Cox's proportional hazard model showed no effect of treatment (p = 0.999). The animals had the greatest risk of dying between day 3 and 9 (p < 0.001).

5.4 Discussion

This study was designed to evaluate whether the oral supplementation of a dense, concentrated milk replacer to LBW piglets affected their performance (growth, survival). Simultaneously, the existence of a lower limit (in terms of birth weight) up to which drenching would have an effect was assessed. The rationale was that, by concentrating the milk replacer, more energy and nutrients could be provided to the LBW piglets per supplementation and would provide the piglets with the necessary boost to start ingesting more colostrum and milk themselves.

The results from our first experiment showed no effect of drenching DMR on either the LBW or VLBW piglets' growth or survival, and thus, a very low birth weight (< 750 g) cannot be used as a criterion to refine the target group for drenching. However, the overall performance and survival of VLBW piglets, even after one or three doses of DMR, were much lower than that of LBW piglets. This suggests that VLBW piglets might be too weak to benefit from interventions such as drenching, and consequently, should not be considered for supplementation. This accords with Paredes et al. [189]. They concluded that piglets that deviate more than 2.5 SD from the mean birth weight of the farm population exhibit no potential to compensate their growth and performance under practical farm conditions. On average, VLBW piglets weighed 26% less than LBW piglets at birth and reduced the weight difference to 17% on the second day post-weaning. This was illustrated by the fact that LBW piglets increased their weight by a factor of 4.7 on average from birth to weaning, while VLBW piglets increased their body weight by a factor of 5.2. However, no statistically significant difference in growth was observed. Whereas some

authors did not see any compensatory growth [190-192], others observed an increased body weight gain (relative to their birth weight) in lighter piglets [28], depending on their body mass index [193]. However, the aforementioned studies applied different definitions or selection protocols for (V)LBW piglets, based solely on the individual birth weight [28, 190], the individual and litter birth weight [191], the morphology [193], or in some instances, even removed piglets with a birth weight below 750 g from the experiment [190, 192], making it difficult to compare. Although the compensatory growth of VLBW piglets remains debatable, our results showed an explicitly inferior performance compared to LBW piglets. The VLBW piglets consumed considerably lower amounts of colostrum (below the required 250 g to survive [43]). They were characterised by very high mortality rates, mainly during the first week after birth. The cumulative mortality of VLBW piglets rose to 68%, whereas that of LBW piglets was limited to 30% at the farm with low perinatal farrowing management. Similar differences in mortality between these birth weight categories were found by Quiniou et al. [28]. In that study, the post-weaning mortality of piglets with a birth weight between 610 g and 800 g rose to 52%, whereas that of piglets, born between 810 g and 1 kg, was limited to 29%. Contrary to our study, Quiniou and colleagues [28] found lower mortality rates in the very small piglets, which could be attributed to the applied management strategies, such as cross-fostering and the provision of a heat lamp.

When considering the LBW piglets (piglets with a birth weight between 750 g and 1 kg and not deviating more than 1.5 SD from the litter's mean birth weight), the DMR did not affect any of the assessed parameters, regardless of whether the animals were drenched once or three times. Following these results, another study in which DMR with a similar energy density was supplemented to piglets did not find an improved colostrum intake or survival. However, the authors did observe an increased body weight and small intestine development in the DMR-fed piglets at weaning age (21 days) [143]. In contrast to the current study (60 kJ per dose of 5 mL DMR), the authors provided the DMR ad libitum to the piglets twice a day, enabling the piglets to acquire a higher caloric intake. The importance of providing enough calories during the first hours after birth was illustrated in similar studies where fat-based supplements were drenched, and no effect on mortality was found when only 71-74 kJ was given to the animals [101]. In another study by Declerck and colleagues [103], a decrease in mortality was observed in piglets below 1 kg when the piglets were provided with 156-165 kJ within the first day after birth. Furthermore, the piglets used in the study by de Greeff et al. [143] had an average birth weight of 1.3 kg, whereas the LBW piglets in our experiments had an average birth weight of 0.86 kg. Thus, not only the amount of ingested DMR but also the difference in birth weight can explain these contradictory results, since small, low-viability piglets often have impaired gut development and nutrient absorption [194]. Consequently, LBW piglets require not only a high-caloric energy boost and nutrient supplementation, but also a supplement that can improve gut function (reviewed by [194]), a factor that is absent in a plain (concentrated) milk replacer.

A second experiment was conducted to evaluate the reproducibility of the results from experiment 1 at a farm with more intensive farrowing management. The comparison between two different management strategies would allow us to determine whether a lack

of good perinatal care might act as a confounding factor and might mask any potential effect of drenching DMR. It was hypothesised that in the case of a higher level of perinatal care (supervision, supplemental heat), DMR improved the performance of LBW piglets more than in the case of a lower level of perinatal care.

When comparing the results of LBW piglets between the two farms, the birth weight and subsequent growth performance did not differ, regardless of whether the animals were drenched with DMR once or three times or not supplemented at all. Thus, it would appear the farm's perinatal management did not influence any potential effect of the DMR. However, the mortality and the risk of skin lesions of LBW piglets were significantly lower at the farm with more labour-intensive perinatal management. Additionally, despite no effect of DMR on mortality at any of the farms, there was more variation between treatment groups at the farm with low perinatal care. Good farrowing and neonatal management have been shown to have an important effect on the survival of piglets. Nevertheless, little is known about good perinatal care for (very) small piglets since most studies and reviews have focused on piglets with a birth weight above 1 kg [72, 194-200]. Therefore, it remains difficult to attribute the higher survival and lesser skin lesions at the second farm to one distinct management strategy. Moreover, the differences in genetics, feeding strategy, etc. could have influenced these results as well. An important difference between the two farms was the presence of staff during farrowing at Farm B. This was possible since most sows farrowed in one day, via the use of farrowing induction. At Farm A, farrowing was much less induced, and sows farrowed during a period of three days. Farrowing supervision, accompanied by regular drying and assistance to the udder, can improve the colostrum intake and the survival of piglets, increasing the survival chances of LBW piglets [23,40,42,43,47]. However, no difference in colostrum intake was observed between the two farms. The mechanistic model by Theil et al. [184] requires the time of birth of the litter's first piglet, the birth weight of the piglet of interest, and the body weight of the latter, 24 hours after the birth of the first piglet. Potentially, the low number of piglets we could include in the calculation can explain why we could not detect a difference in colostrum intake between the two farms. A higher colostrum intake was to be expected at the farm with higher perinatal care, given the occasional drying and assistance to the teat. Secondly, no cross-fostering of the LBW piglets was performed at Farm A. Whereas some authors found a positive effect of cross-fostering on growth [195] or survival [200], others found no positive results [81, 200]. This, combined with a lack of knowledge on the exact effect of cross-fostering on LBW piglets, often results in the application of intermediate protocols in practice [196]. Another management strategy that was only applied at Farm B was the supplementary feeding of a milk replacer via milk dishes (starting 48 hours after farrowing). The positive effect of providing a milk replacer is also debatable. Some studies have shown a positive effect on growth and survival [195], while others did not observe such an effect [199]. In general, it remains difficult to attribute an exact effect of one specific intervention on the performance and survival of piglets. More research, mainly on LBW piglets, is needed regarding the effect of individual or combined perinatal management strategies.

5.5 Conclusions

The present study found a significantly lower performance and survival of (V)LBW piglets at the farm with low perinatal care. Drenching DMR had no effect on the performance or survival of LBW piglets, regardless of the quality of the perinatal care. It is challenging to provide LBW piglets with enough calories within a practically-achievable number of drenching applications. Good farrowing management and neonatal care improved the survival level, but not growth performance, of LBW piglets. It remains difficult to attribute this positive effect to one or more interventions. Our experimental set-up only allowed us to evaluate the perinatal management as a confounding factor on the effect of drenching. Thus, more research on a good perinatal protocol for LBW piglets is needed, as it appears to be more beneficial for LBW piglets than supplementing a dense milk replacer.

Chapter 6.

General Discussion

6.1 Overview study results

The first aim of this PhD-thesis was to assess the safety of drenching to ensure that the associated handling did not harm the already weakened LBW piglets and would not nullify any effect of the supplemented substance. To that end, a sham-drenched group was compared with a non-handled group of LBW piglets with the following conclusions:

Handling associated with drenching:

- had no impact on the body weight of LBW piglets,
- did not affect the measured biochemical parameters (glucose, NEFA, urea, IgG and IGF-1),
- did not influence the haematology (RBC, HCT, HGB, WBC, thrombocytes),
- did not influence the probability of having more severe skin lesions,
- did not impact the pre-weaning mortality of LBW piglets.

Thus, drenching can be applied safely in underprivileged piglets to enhance their survival chances.

Following the verification of drenching's safety, a study was conducted to examine whether bioactive compound supplementation could assist LBW piglets in their performance and survival. Three different compounds – bovine colostrum, scFOS and quercetin – were dissolved separately in a plain milk replacer and drenched once per day during seven days to LBW piglets. The results were as follows:

In the applied dosage:

- the milk replacer did not affect the performance or survival and was, consequently, considered a valid, neutral dissolvent,
- none of the substances affected the body weight,
- no effect of the compounds was observed on the biochemistry (same parameters as above),
- the compounds did not affect the haematology (same parameters as above),
- the treatments did not influence the probability of having more severe skin lesions,
- bovine colostrum and quercetin had no effect on the LBW piglets' mortality,
- scFOS supplementation resulted in an increased mortality.

Hence, the bioactive compounds were not able to improve the LBW piglets' resilience, in terms of performance and survival.

The scFOS supplementation requires further examination to determine whether it has a detrimental effect on LBW piglets.
A drenching period of seven days is too labour-intensive under practical circumstances.

During a third and final field study, a dense, concentrated milk replacer was evaluated on its effect on the resilience of LBW piglets by drenching it one or three times. Additionally, a lower birth weight limit of 750 g was assessed to determine whether piglets below this threshold (VLBW piglets) were too weak to benefit from drenching. This experiment was conducted at two different farms to confirm repeatability and any confounding, farm-specific influences.

- the dense milk replacer did not affect body weight or growth,
- drenching dense milk replacer did not have an impact on the colostrum intake,
- there was no difference in skin lesions between the treatment groups,
- the dense milk replacer had no effect on the pre-weaning mortality,
- drenching one or three times did not result in different outcomes,
- VLBW piglets had a lower body weight and growth than LBW piglets,
- VLBW piglets ingested significantly less colostrum,
- the birth weight category did not affect the probability of having more severe skin lesions,
- VLBW piglets had a significantly higher risk of dying than LBW piglets,
- the risk of having more severe skin lesion was higher at the farm with low perinatal management,
- the LBW piglets were at a higher risk of dying at the farm with low perinatal management.

In summary, the dense milk replacer was not able to improve the LBW piglets' performance or survival, regardless of whether it was supplemented three times or only once.
The VLBW piglets showed an inferior performance and survival compared to LBW piglets and can be considered an unavailing category for drenching.
Perinatal management was not a confounding factor in the milk replacer's drenching results but did appear to have an impact on the animals' survival (higher than that of supplementing milk replacer).

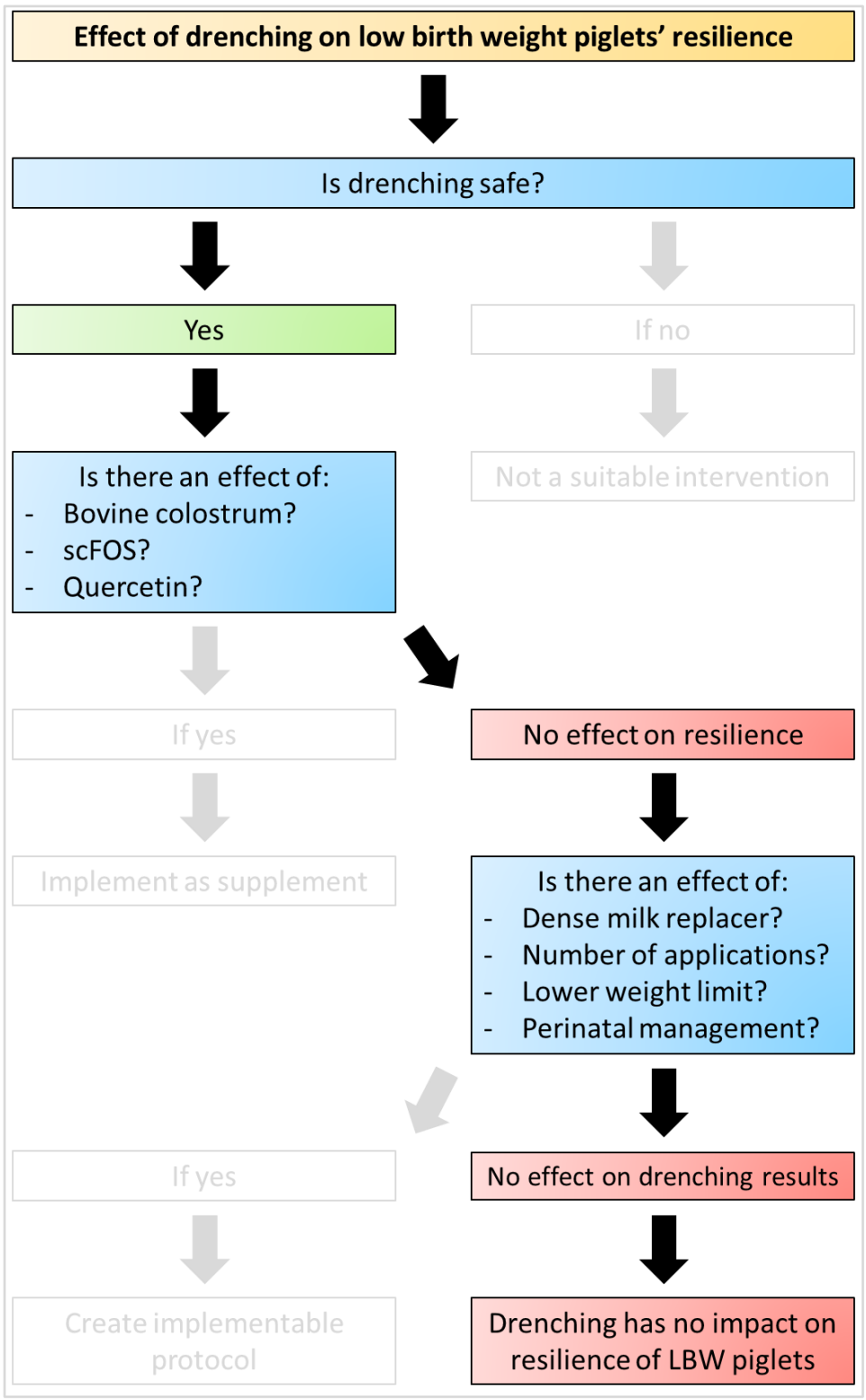

Figure 6.1. Overview of this PhD's main objectives and results.

6.2 Drenching as a tool to improve LBW piglets' resilience

This thesis has confirmed the safety of drenching as a tool in the neonatal management of LBW piglets. None of the included studies found any impact of handling during drenching that might aggravate the performance or survival of underprivileged piglets. These results do not only endorse a safe implementation of drenching as an intervention in LBW piglets, but also eliminate the likelihood that standardised handling during drenching could act as a confounder during experiments. Consequently, different outcomes in drenching studies with a similar setup cannot be attributed to the act of drenching. For instance, in a study by Santos et al. [104], no effect of coconut oil supplementation on the pre-weaning mortality was found in piglets with a birth weight between 600 and 900 g. Declerck et al. [103], on the other hand, did observe a reduction in the pre-weaning mortality after drenching piglets (< 1.00 kg) with a coconut oil booster. An explanation for these different results should, thus, be sought in factors, other than handling during drenching, such as the genotype, housing, composition of the compound, etc.
Nevertheless, the results from this thesis' study (Chapter 3) do suggest that a treatment group with minimal human interaction should always be included in the experimental setup to eliminate any confounding effect of handling, given that not all piglets might be handled in a standardised manner.
Even though drenching can be regarded as a safe intervention, positive and consistent effects of bioactive compound supplementation on the resilience of LBW piglets have yet to be found. Only two studies have observed an improved survival when supplementing bovine colostrum [99] or the abovementioned commercial booster containing coconut and soybean oil [103]. The first study [99] (positive effect of bovine colostrum on pre-weaning mortality) might seem to contradict the lack of positive results described in Chapter 4 of this thesis. However, these differences can be attributed to the higher birth weights (1.13 kg vs. 0.86 kg), and potentially, to the larger volumes of bovine colostrum (per administration 5 mL vs. 2 mL) in the study by Muns et al. [99]. Moreover, the number of piglets within the supplemented group was much larger than in this thesis' study (218 vs. 38), highlighting a potential limitation of calculating the sample size using body weight instead of mortality (preliminary power calculation). In general though, the results from this thesis are in alignment with most literature and confirm that – in terms of energy supplementation – drenching cannot consistently improve LBW piglets' survival during the entire pre-weaning period, regardless of the administered product (bovine colostrum, scFOS, quercetin, milk replacer (Chapter 4), dense milk replacer (Chapter 5)) or dosage. Similarly, none of the studies in this dissertation could confirm a positive effect on the performance of LBW piglets, in terms of body weight and growth. These results confirm the conclusions of previous drenching studies that were unable to improve the growth of LBW piglets by drenching one or several compounds.
Perhaps most remarkable, was the negative effect of scFOS on the survival of LBW piglets. As elaborated in Chapter 4, the positive effect of scFOS appears to be birth weight-related, with only beneficial results in piglets with a birth weight higher than 1.00 kg

(when scFOS are drenched in the dosage as applied in this thesis' study). A plausible explanation for the negative impact of scFOS is the often-impaired gut health (higher intestinal permeability, reduced antioxidant response [65]) and a less stable and diverse microbiota [149-151, 201]. As discussed by Wang et al. [202], the effect of prebiotics in new-born animals can differ, depending on the abundance of bacteria before the prebiotic supplementation. Hence, it could be assumed that the prebiotic effect of scFOS in LBW piglets was not large enough to result in any positive results due to an inadequate amount of bifidobacterial species. Additionally, it could also be assumed that an impaired gut health affects the tolerance for scFOS. Prebiotic supplementation can result in negative effects, due to their osmotic potential and/or excessive or unwanted fermentation [203]. Although the tolerance for scFOS is usually very high in healthy individuals [203], it would be interesting to evaluate their direct effect at the gut level in LBW piglets. Furthermore, it would be interesting to evaluate whether other prebiotics also have a negative impact on LBW piglets. One such alternative could be inulin, a prebiotic that is extracted from chicory root and Jerusalem artichoke. In a study by Li et al. [204] inulin – composed of fructose units with one terminal glucose and a polymerisation degree of 2 to 60 – supplementation in piglets with a normal birth weight resulted in an improved growth and gut development, and consequently, an increased resilience during weaning. It would be interesting to compare the effects of inulin and scFOS in LBW piglets. Inulin might stimulate other bacterial (sub)species, ferment into other short-chain fatty acids or have less osmotic properties than scFOS in LBW piglets, and thus, not have a negative impact on the piglets' survival. Another alternative for scFOS could be polydextrose, a glucose polymer attached with sorbitol and citric acid. Polydextrose has already been examined for its safety in human milk formula, using a suckling piglet model. No toxicological effect was observed in neonatal piglets (with a normal birth weight), thus, it would be interesting to examine the effect of polydextrose supplementation in LBW piglets.

So far, only subcutaneous glucose injections – whether or not combined with drenching – have resulted in increased body weight at the age of 21 days ([71], reviewed by [64]). This suggests that small piglets – particularly IUGR piglets – might need a more direct energy source to have long-term effects on their growth. Drenching however, requires the animal to swallow, digest and absorb the administered product. It can be presumed that within the included LBW piglets of this thesis' studies, a significant fraction consisted of IUGR piglets. IUGR piglets have been shown to have an underdeveloped gastrointestinal system (e.g. thinner and shorter small intestines [66, 153], less and smaller intestinal villi [205], reviewed by [64]). A recent study observed a reduction in enzyme activity and transporter molecules that are essential for the absorption of glucose in IUGR piglets. Thus, IUGR piglets suffer from a decreased glucose absorption capacity [206]. This could explain why an indirect energy source (oral supplementation) could not increase the LBW piglets' blood glucose level practically or swiftly. This delay in energy boost could have been too long for the LBW piglets to overcome other problems, such as hypothermia, hypoglycaemia or crushing. As shown by Engelsmann et al. [71], IUGR piglets that received a glucose injection and an oral colostrum supplementation, obtained the

quickest increase in blood glucose levels during the first 24 hours. The glucose injection circumvented the intestinal absorption, resulting in a rapid increase in glucose levels. Consequently, the delayed or impaired glucose supply through colostrum was buffered. Additionally, the authors hypothesised that a glucose injection enabled the piglets to compete more at the udder and that the extra energy from glucose injections potentially slowed down the utilisation of the scarce glycogen reserves. Thus, higher glucose concentrations could be maintained for a more extended period [71]. This hypothesis [71] highlights a limitation of the studies from Chapters 4 and 5: by not differentiating between LBW and IUGR piglets it is difficult to attribute the lack of results to the supplemented product (or its dosage) or to the fraction of IUGR piglets. However, the feasibility of glucose injections is something that requires further examination, given that administering an injection does not come without risk of injury and requires trained personnel or veterinarian assistance. As an alternative to injection, Jarratt et al. [207] supplemented glucose orally to LBW piglets to evaluate whether similar results could be achieved. Again, with the oral supplement no effect was observed on the long-term growth of the animals. Moreover, LBW piglets that were drenched with glucose showed a reduced growth from day one until day 3 of life. Similar to the studies in this thesis, the authors did not register the proportion of IUGR piglets within the LBW group. Therefore, it was not possible to determine whether an impaired absorption of glucose could explain the lack of positive effects and whether the oral supplementation of a simple energy source, such as glucose, might have benefitted the animals if only non-IUGR LBW piglets would have been included.

In conclusion, the studies in this dissertation, combined with recent literature, have shown that drenching is a safe intervention to be used in LBW piglets (Chapter 3), but drenching alone is not able to improve the resilience of LBW piglets during the entire pre-weaning period. It remains challenging to translate positive in vitro results from compounds, such as scFOS or quercetin, into field-trials (Chapter 4 and 5). Unlike in vitro studies, on-farm circumstances are difficult to standardise, resulting in complex, multifactorial experimental setups. Additionally, this thesis attempted to create practically achievable drenching protocols, thus, the number of drenching applications to suppress the labour costs were limited. However, the (lack of positive) results from the conducted experiments do emphasise the importance to differentiate between LBW and IUGR piglets in future studies and in perinatal farm management. Moreover, even though this thesis was not able to prove drenching as a valuable, single tool to improve the resilience of LBW piglets, it would be interesting to evaluate the combination of drenching with other interventions (e.g., glucose injections) to determine whether such combinations could result in long-term effects by eliminating the weaknesses of drenching.

6.3 The importance of vitality

The vitality of an animal represents its strength or vigour and is important for the animal to survive. In pigs, it is essential for the new-born animals to move around and obtain a first suckle bout as quickly as possible. Therefore, vitality is an important trait for survival in piglets. Vitality is not to be confused with viability which represents the general ability of an animal to thrive and survive. In other words, a good neonatal vitality attributes to the viability of a new-born animal ([208], reviewed by [209]).
Vitality can be assessed by using the Apgar scoring system. This system was created by Virginia Apgar in 1952 as a rapid method to determine the clinical status of new-born infants and the need of intervention to establish a normal breathing. The system uses five different criteria that are each scored between 0 and 2: the skin colour (Appearance), the heart rate (Pulse), the reflex irritability (Grimace), the muscle tone (Activity) and the respiratory effort (Respiration). These scores are reported at one and five minutes after birth, followed by assessments at five-minute intervals until 20 minutes for infants with a score below 7 (reviewed by [209, 210]. Revermann et al. [211] adjusted the Apgar system to a scoring method that could be applied to piglets using the following criteria: skin colour (normal/pink, pale, abnormal/blue), respiration (within 15 sec, after 15 sec, irregular after 15 sec), latency to first movement (within 15 sec much movement, within 15 sec less movement, no movement within 15 sec), latency to first attempt to stand up (within 1 min, 1-5 min, after 5 min), latency to first teat contact (within 10 min, 10-30 min, after 30 min), meconium stained skin (no meconium, less, much) and condition of umbilical cord (connected, ruptured ≥15 cm, ruptured <15 cm). Stillborn piglets were scored with a 0 and a threshold of 6 was applied to define a piglet clinically viable. However, the adjusted Apgar system requires the measurement of many parameters and trained personnel to result in an objective and correct end score which is often not feasible. In that respect, an Austrian study by Shodl et al. [212] validated a vitality scoring system at the litter level by comparing the vitality score with the pre-weaning mortality. The authors created a system that scored the litter's vitality from one to four:

- 1: More than 4 piglets in the litter show signs of reduced vitality
- 2: 3 to 4 piglets in the litter show signs of reduced vitality
- 3: 1 to 2 piglets in the litter show signs of reduced vitality
- 4: No piglet shows signs of reduced vitality

The litters were assessed within 24 hours after farrowing was over (starting from the expulsion of the placenta). Signs of reduced vitality were defined as weak appearance, pale skin, reduced activity and insufficient suckling. The study found a decreasing pre-weaning mortality of 29%, 23%, 16% and 8% with an increasing vitality score of 1, 2, 3 and 4, respectively. Thus, the authors succeeded in developing a valid vitality scoring system that requires much less labour and training from farmers.

As mentioned in the previous section, drenching cannot improve the performance and survival of LBW piglets during a longer period (pre-weaning period). However, the main idea of drenching is often to provide LBW piglets with a first energy boost to allow them to reach the udder and ingest adequate amounts of colostrum from the sow, rather than providing the animals with the entire energetic requirements (which is often not feasible due to gastric volume limitations, labour costs, etc.). Put differently, drenching might rather aim to improve the vitality (short-term) than the resilience (long-term) of LBW piglets. During the studies of this thesis, the vitality of LBW piglets before and after drenching was not assessed. The experiments did rule out any boosting effect that was large enough for the animals to improve their survival or performance without any further interventions. However, if there was a limited effect on the vitality, perhaps this response could have been large enough to have beneficial effects on the piglets if there had been a second intervention. All the interventions that have been described in the introduction (nurse sows, cross-fostering, split suckling, artificial rearing, supplementary milk) have one thing in common: they require a certain basic vigour from the animals. If drenching (with or without glucose injections [71]) was able to improve the vitality of LBW, and an intervention – such as split suckling – had subsequently been applied, the two strategies could have a complementary to even a synergistic effect that might result in a long-term improvement of the animals' resilience. Therefore, it would be interesting to examine such combinations in the future.
An additional benefit of incorporating vitality assessment in future drenching studies, would be to evaluate the existence of a threshold vitality score for supplementation. It can be presumed that piglets with a very low vitality will not be able to improve their vigour – through a limited effect of drenching – sufficiently to reach the udder, and thus, will still have a (too) large latency to suckle. These animals would, consequently, not be a target group for drenching.

6.4 Defining (very) low birth weight piglets

It has already been described in the introduction how the definition for LBW piglets often varies between different studies (Figure 6.2). Given the short- and long-term consequences of LBW, it is important that these animals are identified as soon as possible and in a correct way to allow appropriate interventions to be applied. Additionally, it is important that LBW piglets are defined in a similar way during different experiments to allow the comparison of results. At the moment, there is no consensus about the precise definition of LBW piglets, or for that matter, of VLBW or IUGR piglets [213]. Many studies apply an arbitrary body weight value – often based on previous literature – without taking other factors into account [28, 36, 79, 94, 96, 99, 183, 190, 214]. Others have not only relied on previous research examples, but have used a criterion that was based on the study population's birth weight. Different approaches have been used, such as the tenth centile of the mean birth weight [29], a deduction of the standard deviation from the mean birth weight [31, 93], the lowest quartile(s) of the birth weights [82, 98, 103, 193,

215] or a statistical analysis of the population's weight (Akaike information criterion) [102, 216]. Alternatively, other authors have opted to define LBW piglets based on the mortality [30, 45, 217-219]. The disadvantage of these characterisations – apart from resulting in different LBW piglet thresholds – is that there is no comparison within the litters (the litter heterogeneity). If a litter consists of only small piglets, the competition amongst siblings will be different from litters that have both small and larger piglets. Additionally, by only including the birth weight or mortality from one study population, other factors such as geographical area, sex or breed are not considered. In the studies by Calderón Diaz et al. in Ireland [218] and Feldpausch et al. in Spain and the USA [30], the cut-offs for LBW White x Landrace piglets differed by 20%, even though the same breed and segmented regression model (difference in slope, based on mortality) were used. Moreover, when the relationship between birth weight and mortality was used exclusively to define LBW piglets, only short-term consequences were considered. Given that LBW also has an impact on later health outcomes [28, 125], this method might be too narrow (reviewed by [213]).
The studies within this thesis have avoided these problems by using the mean birth weight of the litters, thus, defining LBW piglets that are born to the same sow and have developed under the same environmental conditions during the intrauterine period (reviewed by [213]). In the first and second study (Chapter 3 and 4), piglets that deviated between 1 and 2.5 SD from the litter's mean birth weight were classified as LBW piglets. This method allowed to incorporate the heterogeneity and competitive disadvantage of small piglets within the litter. However, this selection procedure did result in a large fraction of VLBW piglets that had very low survival chances. Furthermore, when a litter consisted of piglets of different size, but all animals had a heavy birth weight, animals were wrongly defined as (V)LBW (e.g. litters with a few piglets weighing 1.3 kg and all others weighing 1.5 kg could result in LBW piglets with a birth weight of 1.3 kg). Therefore, the selection procedure was optimised in a following study (Chapter 5) to include the population's mean birth weight to determine weight values for LBW cut-offs, and a distinction between LBW and VLBW piglets was created. The adjusted selection procedure led to the following definitions for (V)LBW piglets:

- Piglets with a birth weight between (mean BW_{litter}–1 SD) and (mean BW_{litter}–1.5 SD) and weighing between 750 g and 1 kg were categorised as LBW piglets.
- Piglets with a birth weight between (mean BW_{litter}–1.5 SD) and (mean BW_{litter}–2.5 SD) and weighing less than 750 g were categorised as VLBW piglets.

This method resulted in a truly individualised birth weight threshold, distinguishing between small and extremely small piglets, and considering the heterogeneity of the litter, all while taking a larger population's mean birth weight into account (reviewed by [213]).

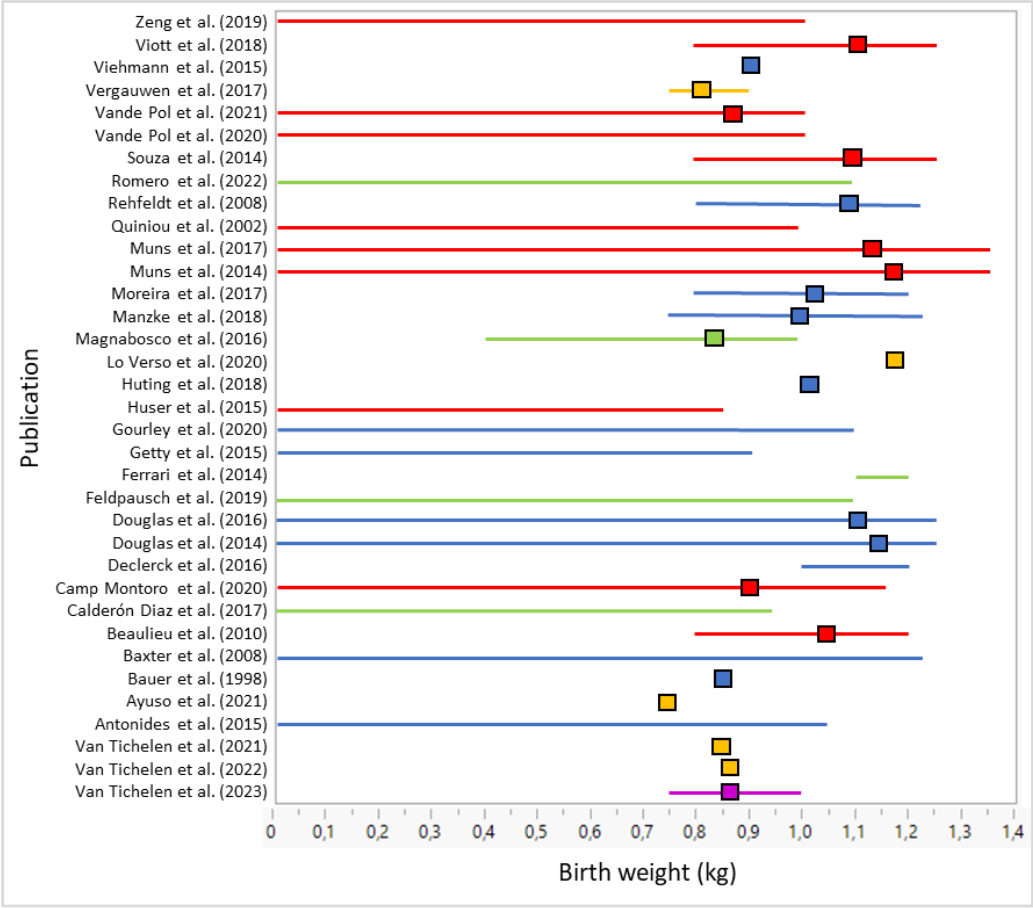

Figure 6.2. Birth weights that were applied to define low birth weight (LBW) piglets in different studies, including the studies of this thesis. Squares present the mean birth weight of the LBW piglets (when mentioned in the study). Minimum and maximum birth weight ranges of LBW piglets are presented by lines (minimum was set at 0 kg when no lower limit was mentioned in the study). The LBW piglets were classified, based on arbitrarily chosen thresholds (primarily based on previous literature) (red), based on the population's weight (blue), based on the mortality (green), based on the litter's weight (orange) or based on a combination of the litter's and the population's weight (purple).

The results from Chapter 5 showed that VLBW had very abysmal performance and survival. Nearly 70% of all VLBW piglets did not survive the pre-weaning period, whereas the LBW piglets' mortality stagnated at 30%. Consequently, the study concluded that VLBW are not a target group for drenching and the focus should instead be on LBW piglets. This conclusion does impose the question of what should be done with VLBW piglets. Unfortunately, the study did not include IUGR differentiation. Nonetheless, it can be presumed that many of the VLBW piglets were IUGR piglets. Van Ginneken et al. [64] reviewed the difficulties and knowledge gaps (e.g. potential sex bias) that researchers and farmers are faced with when trying to improve the survival and long-term consequences of IUGR. Additionally, the question of what to do with non-IUGR and/or low-vitality piglets below 750 g remains unanswered. Paredes et al. [189] concluded that piglets with a birth weight of 2.5 SD below the mean of the total population (which coincides with less than 750 g) have no potential to thrive under practical circumstances. The authors also

described piglets above the mean birth weight minus 2 SD (which can be translated into weights above 750 g) as the most interesting subpopulation for supportive interventions. Similarly, Beaulieu et al. [190] and Rehfeldt et al. [192] considered piglets with a birth weight of 750 g and 800 g, respectively, as non-viable and excluded them from their experiments. Although it appears that a consensus could be found on considering very small piglets as non-viable, precise and objective criteria to classify piglets as too small or non-viable are still lacking. It would be very interesting for future studies to determine which parameters could be used for classification, and what should, subsequently, be done with these animals. In other words, whether non-viable piglets should be humanely killed, in what way they should be humanely killed (aiming for instant loss of consciousness and avoiding pain and stress [37]), and what the humane endpoints should be, such as:

- Birth weight (in relation to the mean birth weight of the litter)
- Vitality scoring
- Rectal temperature
- IUGR classification

6.5 Perinatal management

The last study in this thesis (Chapter 5) added the perinatal management as a possible factor into the experimental set-up. Although the management did not appear to influence any potential effect of the supplemented milk replacer, the pre-weaning mortality was much lower at the farm with higher perinatal care (18% vs. 31% at the farm with lower neonatal care). The largest differences in management between the two included farms were the farrowing environment (in terms of heat provision) and the farrowing supervision.
Given their high susceptibility to hypothermia, creating an optimal, thermoneutral environment is essential for piglets to survive. However, this ideal ambient temperature cannot be achieved by simply increasing the farrowing room's temperature to 32-38°C (thermoneutral for new-born piglets). The thermoneutral zone for sows is much lower, with an upper limit of 22°C (reviewed by [72]). In a study by Muns et al. [220], it was observed that even a relatively small increase in room temperature (to 25°C) around farrowing resulted in heat-stressed sows. The animals increased their respiratory rate and lay down more often in a lateral position to lose heat through conduction, but failed in reducing their body temperature. Consequently, the stressed animals had a longer farrowing duration – which has a negative impact on (LBW) piglets – and a reduced feed intake. Not only did this have a detrimental effect on the sows' welfare, but also – through the reduced feed intake and the subsequent reduction in milk yield – on the weaning weights of the piglets. Additionally, the reduced milk production can result in the piglets spending more time close to the sow, due to hunger. This can increase the risk of crushing, especially for the less vital LBW piglets (reviewed by [72]). Muns and colleagues

[220] suggested that crated sows have less opportunities to thermoregulate through altered behaviour, and thus, have a lower critical temperature compared to sows that are kept in loose farrowing systems. Therefore, loose pens might appear to be a good management strategy to improve the sows' thermoregulation, and consequently, the piglets' performance and survival. However, outdoor and loose farrowing systems have an increased risk of crushing, resulting in pre-weaning mortalities of 21% and 14-20%, respectively. In contrast, the average pre-weaning mortality in traditional farrowing crates ranges between 10 and 12% (reviewed by [37, 72]). This emphasises the difficulty of creating a good farrowing environment for both sows and piglets. In order to minimise the negative impact on the welfare of sows, it is generally recommended to keep the farrowing room's temperature between 20 and 22°C (reviewed by [72]).
Even though maintaining an ambient temperature below 22°C will benefit the sows' welfare, for the piglets this temperature is far below their lower critical threshold. When piglets are born, they experience a dramatic drop in temperature of 15-20°C from the intrauterine to the extrauterine environment. Consequently, the piglets' body temperature will drop 2-4°C within 20-60 minutes after birth ([214], reviewed by [72]). In LBW piglets, this decline in body temperature is even more severe and appears to last longer than in larger piglets [214, 215]. As mentioned during the introduction, piglets – and especially LBW piglets – are very cold-sensitive animals due to their hairlessness and very limited fat and glycogen reserves, and rely on an adequate colostrum intake for their thermoregulation [40-42, 221]. Under natural circumstances, nesting material can be used to provide piglets with a warmer environment, but this is often not feasible in traditional farrowing crates (e.g. because of the manure handling system [222]). Therefore, alternative managerial interventions are necessary to prevent hypothermia in (LBW) piglets (reviewed by [72]). One possible method is to reduce the temperature gradient by providing a localised heated area. This can be achieved by providing a covered creep area to contain some warmth. This method was applied at the farm with lower perinatal management in the study from Chapter 5. Only covering the creep area does not provide the animals with an external heat source, however, but relies on the body warmth of the animals when they huddle together. More efficient is providing floor heating (or a heating lamp) in the creep area (applied at the farm with higher perinatal management) [59, 221, 222]. Despite that these localised areas reduce the temperature gradient in the farrowing pen, they do not confine the animals and new-born piglets appear to be more attracted by the sow [221, 223]. Vande Pol et al. [214] confined piglets in a box with a heating lamp for 30 minutes and observed that this technique had a larger effect on the animals' rectal temperature than studies that did not apply heated confinement. Simultaneously, the authors examined the effect of drying the piglets with a desiccant which also proved to be efficient. Moreover, combining both methods appeared to result in the highest rectal temperatures. It would, however, be interesting for future studies to evaluate the impact on the colostrum intake of this method. Vande Pol et al. [214] dried and confined the piglets immediately after birth which inevitably delays the first suckle. Hence, it would be interesting to determine whether drying, followed by assistance to the teat and later heated confinement would be better.

Nevertheless, preventing heat loss through evaporation (drying), convection and radiation (confined heating) can be a good perinatal management strategy to improve the survival of LBW piglets. A different approach to assist LBW piglets in their thermoregulation is to provide the animals with an internal source of heat. In a recent study, heated saline (45°C) was injected intraperitoneally in piglets immediately after birth [100]. The warm saline injection resulted in an increased rectal temperature, a higher colostrum intake and an improved survival in LBW piglets. However, the weight gain was lower in piglets that had received the saline injection, suggesting that the handling and injection could have stressed the animals. The inclusion of a sham group would have allowed the authors to determine whether injecting could have disadvantaged the piglets. Although these results seem very promising, the feasibility of injecting LBW animals remains arguable, as mentioned earlier.

The second managemental intervention that differed between the two farms in this dissertation's study was the level of farrowing surveillance (Chapter 5). Regular supervision during farrowing can allow additional help to the piglets, such as the abovementioned assistance to a heated area, to a teat or more intensive interventions (e.g. clearing airways, administering oxygen, glucose injections, etc.). It is crucial, however, to maintain a good balance between assisting LBW piglets and disturbing the litter. Too much disturbance might alter the maternal behaviour and eventually result in counterproductive outcomes (reviewed by [59, 72]). Additionally, this supervision is ideally performed continuously during 3 hours after the start of farrowing. An uninterrupted observation results in fewer stillbirths and improved survival, precisely that of LBW piglets. On the other hand, when only occasional supervision is applied, more stillbirths have been observed than in continuous or no surveillance, illustrating a complex relationship between staff assistance and fear/stress responses in the animals (reviewed by [10]).

The difficulty of applying good farrowing supervision often lies in the amount of labour costs. Given the upscaling which has more than doubled the number of animals per farm, the challenge of monitoring both sows and new-born piglets has only increased. Fortunately, currently ongoing technological advances in machine learning and artificial intelligence could reduce some of the human workload and be incorporated into commercial farm settings soon. Several promising computer vision techniques have already been demonstrated to provide reliable real-time, continuous monitoring. To monitor physiological parameters, such as the body temperature or the respiration rate, invasive and complex techniques are often applied. For instance, rectal probes or implanted sensors for the rectal temperature, vests that must be worn by piglets to count the respiration, etc. Through thermal imaging, a continuous body surface temperature can be assessed around the ear base and eyes – which are known to correlate with the body core temperature – and sudden changes can be detected immediately. For the respiration rate, a computer vision system has been developed to assess the respiratory rate automatically. Determining the body weight of piglets is usually a rather labour-intensive task that requires individual weighing. Recently tested automated systems that use digital colour and depth images, combined with machine learning techniques have

shown promising results. Moreover, computer vision-based machine learning algorithms can accurately detect piglets' feeding and nursing behaviour, recognise reduced activity, and consequently, alert farmers when there are indications of sickness or thermal stress. In combination with automated monitoring of the environment (humidity, radiant temperature, air speed, etc.), these continuous, real-time observations could provide farmers with accurate and early feedback, allowing them to modify the farrowing management, tailored to the needs of that moment, at a low labour cost (reviewed by [221]).

6.6 Conclusions and future perspectives

Within this dissertation, three field-studies have been conducted on drenching LBW piglets.

The first experiment (Chapter 3) succeeded in validating drenching as a safe technique to be applied in LBW piglets. The effect of handling during oral supplementation (catching, fixating, drenching) had not been tested up until this study. The results of this study provide authors of future drenching trials with the confirmation that the act of drenching has no effect on the performance or survival of LBW piglets, and thus, any observed effect can be attributed completely to the supplemented substance.

In Chapter 4, the effect of three bioactive substances – bovine colostrum, scFOS and quercetin – was evaluated in LBW piglets. None of the substances was able to positively affect the resilience of LBW piglets at the doses given after a drenching period of seven days. Consequently, in the applied dosages, these products are not suitable to assist LBW piglets during the neonatal period.
Additionally, drenching a concentrated milk replacer (once or three times) did not improve the performance and survival of LBW piglets either (Chapter 5). In conclusion, these results indicate that drenching – without any additional intervention – cannot improve LBW piglets' resilience. It would, however, be interesting for future studies to evaluate the combination of drenching with other interventions that require a basic vigour, such as split suckling.

Throughout the different experiments, the selection of LBW piglets has been optimised to create an objective, individual definition for LBW piglets, based on the mean birth weight of both the litter and the study population. This definition allows to incorporate the heterogeneity, and thus sibling competition, into the LBW classification.

The VLBW – most likely IUGR – piglets showed very high mortality rates and were classified as a non-suitable group for drenching (Chapter 5). These results raise the question on what the correct management of these animals should be. If VLBW/IUGR

piglets could be considered as non-viable or impossible to be assisted (e.g., too long latency before suckling or unable to suckle, even with interventions), then perhaps a protocol with humane endpoints and humane killing should be created.

The results from Chapter 5 excluded perinatal management as a confounding factor for drenching. However, the mortality in LBW piglets was much lower when an intensive neonatal care was applied. These results indicated the importance of a high-quality perinatal management that might be more efficient than supplementation of substances.

In summary:

- Drenching is safe, but does not suffice as a single intervention to improve the resilience of LBW piglets.
- Researchers and farmers need to differentiate between VLBW (IUGR) and LBW piglets, given that only the latter are a target group for interventions.
- The development of a protocol on humane endpoints and humane killing of VLBW (IUGR) piglets is necessary.
- For future studies, it would be interesting to evaluate the long-term effects of the following combination: drenching, combined with secondary interventions (split suckling, cross-fostering…) in a high-quality perinatal management setting (Figure 6.3).

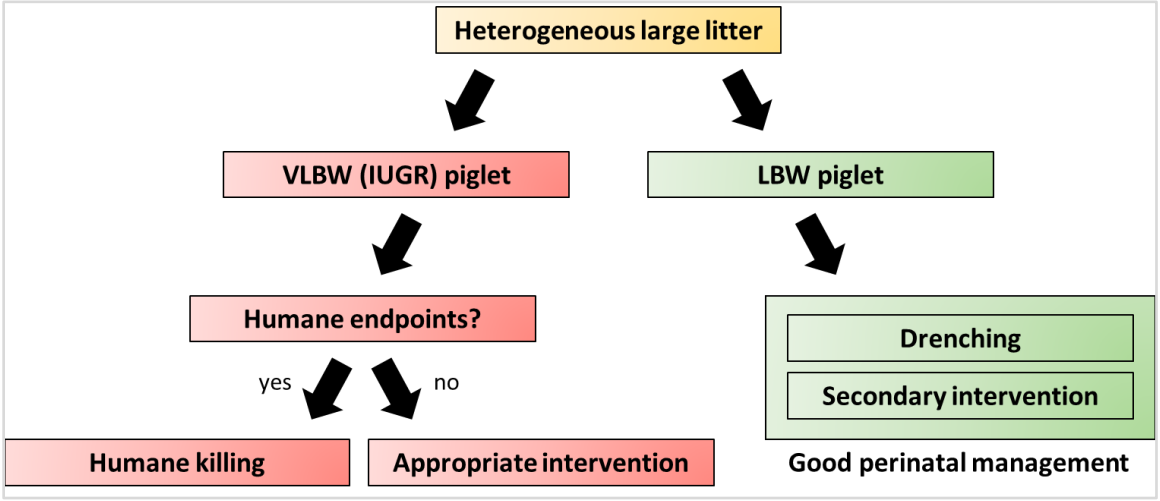

Figure 6.3. Future perspectives for studies focusing on improving the survival and performance of low birth weight (LBW), very low birth weight (VLBW) and intrauterine growth restricted (IUGR) piglets.

Curriculum vitae

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- Supervisor Honours College project Nils Hafenscher, entitled: 'Effect van het hanteren tijdens drenchen op de acute stressrespons bij biggen met een laag geboortegewicht'.
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Systematic Review, Cochrane 2021

Longitudinal data & Mixed models, University of Antwerp 2021

Multiple regression & ANOVA, University of Antwerp 2020

Basic principles of Statistics, University of Antwerp 2019

Laboratory Animals Sciences, Category C, University of Ghent 2014

Conferences & Study meetings

15th International Symposium on Digestive Physiology of Pigs 2022
Rotterdam, Netherlands
Poster presentation: 'Improving survival of low birth weight piglets – What is more important: farrowing care or drenching a milk replacer?'

International Pig Veterinary Society – Belgian Branch 2019
Merelbeke, Belgium
Oral presentation: 'Drenching low birth weight piglets: Friend or Foe?'

70th Annual meeting of the European Federation of Animal Science 2019
Ghent, Belgium
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Supplementary material

Table S 1. Blood values (median ± SD) of glucose, non-esterified fatty acids (NEFA), urea, immunoglobulin G (IgG), insulin-like growth factor 1 (IGF-1), red blood cells (RBCs), haematocrit (HCT), haemoglobin (HGB), white blood cells (WBCs), lymphocytes, monocytes, neutrophils, eosinophils, basophils and thrombocytes, presented by age, sex and treatment from selected low birth weight piglets (linear mixed models, Tukey post-hoc analysis, $p \leq 0.05$).

	Age					***Sex***					***Treatment***				
	Day 9		**Day 38**			**Female**		**Male**			**No treatment**		**Sham**		
	n	**Median ± SD**	***n***	**Median ± SD**	***p*-value**	***n***	**Median ± SD**	***n***	**Median ± SD**	***p*-value**	***n***	**Median ± SD**	***n***	**Median ± SD**	***p*-value**
Glucose (mmol/L)	20	6.87 ± 1.64	16	6.40 ± 1.70	0.300	24	6.40 ± 1.92	12	6.75 ± 1.03	0.960	16	6.55 ± 2.02	20	6.56 ± 1.32	0.341
NEFA (mmol/L)	20	0.58 ± 0.79	14	0.17 ± 0.18	<0.001	23	0.46 ± 0.47	11	0.45 ± 1.05	0.925	15	0.47 ± 0.52	19	0.33 ± 0.82	0.125
Urea (mmol/L)	19	3.66 ± 0.88	15	1.66 ± 0.69	<0.001	22	2.84 ± 1.41	12	2.32 ± 1.13	0.479	14	3.24 ± 1.13	20	2.32 ± 1.40	0.081
Ig G (mg/mL)	11	3.31 ± 4.93	11	2.97 ± 1.02	0.344	16	3.24 ± 4.27	6	2.55 ± 1.13	0.773	10	3.39 ± 5.50	12	2.55 ± 1.79	0.560
IGF-1 (ng/mL)	11	8.95 ± 15.99	11	38.61 ± 21.02	0.008	16	23.48 ± 25.05	6	15.03 ± 14.48	0.298	10	23.48 ± 27.05	12	20.17 ± 17.62	0.175
RBC (10^{12}/L)	10	4.27 ± 0.42	15	5.69 ± 0.56	<0.001	17	5.23 ± 0.96	8	5.33 ± 0.89	0.687	9	5.11 ± 1.04	16	5.46 ± 0.88	0.769
HCT (%)	10	30.45 ± 2.50	15	35.9 ± 3.51	0.005	17	33.5 ± 3.58	8	32.30 ± 4.96	0.600	9	32.60 ± 3.88	16	33.20 ± 4.15	0.759
HGB (g/dL)	10	8.40 ± 0.71	15	10.40 ± 1.12	0.001	17	9.20 ± 1.43	8	9.35 ± 1.26	0.814	9	9.20 ± 1.70	16	9.65 ± 1.17	0.418
WBC (10^3/µL)	10	10.73 ± 5.10	15	17.95 ± 4.20	0.021	17	13.18 ± 5.61	8	18.24 ± 4.11	0.039	9	12.66 ± 6.58	16	17.81 ± 4.39	0.908
Lymphocytes (10^3/µL)	10	4.18 ± 1.75	15	7.96 ± 1.62	0.001	17	6.15 ± 2.42	8	7.06 ± 1.87	0.616	9	4.94 ± 2.82	16	6.93 ± 1.90	0.950
Monocytes (10^3/µL)	10	0.89 ± 0.37	15	1.55 ± 0.57	<0.001	17	0.94 ± 0.52	8	1.47 ± 0.70	0.086	9	1.08 ± 0.51	16	1.12 ± 0.64	0.952
Neutrophils (10^3/µL)	10	5.83 ± 3.15	15	8.33 ± 2.90	0.172	17	6.14 ± 3.22	8	9.70 ± 2.24	0.005	9	5.49 ± 3.77	16	8.40 ± 2.52	0.612
Eosinophils (10^3/µL)	10	0.10 ± 0.20	15	0.19 ± 0.13	0.817	17	0.13 ± 0.11	8	0.27 ± 0.20	0.038	9	0.12 ± 0.12	16	0.19 ± 0.17	0.913
Basophils (10^3/µL)	10	0.02 ± 0.06	15	0.01 ± 0.02	0.177	17	0.01 ± 0.05	8	0.02 ± 0.01	0.552	9	0.01 ± 0.06	16	0.02 ± 0.01	0.440
Thrombocytes (10^3/µL)	10	1087.50 ± 374.48	15	444.00 ± 173.61	<0.001	17	646.00 ± 458.02	8	560.00 ± 283.45	0.461	9	764.00 ± 535.69	16	596.00 ± 334.41	0.689

Table S 2. Blood values (median ± SD) of glucose, non-esterified fatty acids (NEFA), urea, immunoglobulin G (IgG), insulin-like growth factor 1 (IGF-1), red blood cells (RBC), haematocrit (HCT), haemoglobin (HGB), white blood cells (WBCs), lymphocytes, monocytes, neutrophils, eosinophils, basophils and thrombocytes, presented by age, sex and treatment from selected low birth weight piglets (linear mixed models, $p \leq 0.05$).

	Age			***Sex***			***Treatment***		
	Day 9	**Day 38**		**Female**	**Male**		**Milk replacer**	**Sham**	
	Median ± SD (*n*)	**Median ± SD (*n*)**	***p*-value**	**Median ± SD (*n*)**	**Median ± SD (*n*)**	***p*-value**	**Median ± SD (*n*)**	**Median ± SD (*n*)**	***p*-value**
Glucose (mmol/L)	6.77 ± 0.98 (25)	6.30 ± 0.93 (21)	0.125	6.45 ± 1.00 (24)	6.56 ± 0.94 (22)	0.479	6.30 ± 0.86 (27)	6.56 ± 1.32 (20)	0.400
NEFA (mmol/L)	0.52 ± 0.47 (25)	0.08 ± 0.28 (22)	<0.001	0.33 ± 0.44 (27)	0.32 ± 0.51 (20)	0.882	0.32 ± 0.56 (29)	0.33 ± 0.82 (19)	0.562
Urea (mmol/L)	3.21 ± 1.02 (24)	1.58 ± 1.29 (23)	<0.001	2.91 ± 1.46 (25)	2.00 ± 1.18 (22)	0.077	2.46 ± 1.37 (27)	2.32 ± 1.40 (20)	0.495
IgG (mg/mL)	4.77 ± 2.97 (12)	2.79 ± 1.75 (12)	0.020	3.70 ± 2.92 (14)	3.55 ± 2.14 (10)	0.957	5.41 ± 2.81 (12)	2.55 ± 1.79 (12)	0.057
IGF-1 (ng/mL)	14.96 ± 8.70 (12)	24.01 ± 25.43 (12)	0.001	25.75 ± 19.26 (14)	20.13 ± 12.19 (10)	0.137	27.46 ± 15.31 (12)	20.17 ± 17.62 (12)	0.647
RBC (10^{12}/L)	4.24 ± 0.57 (14)	5.60 ± 0.55 (22)	<0.001	5.37 ± 0.92 (20)	5.25 ± 0.96 (16)	0.923	5.29 ± 0.99 (20)	5.46 ± 0.88 (16)	0.151
HCT (%)	29.75 ± 4.10 (14)	35.10 ± 3.62 (22)	0.001	32.90 ± 3.59 (19)	33.50 ± 6.15 (17)	0.445	33.20 ± 5.51 (20)	33.20 ± 4.15 (16)	0.665
HGB (g/dL)	8.10 ± 1.37 (15)	10.05 ± 0.87 (22)	<0.001	9.65 ± 1.31 (20)	9.20 ± 1.63 (17)	0.996	9.40 ± 1.67 (21)	9.65 ± 1.17 (16)	0.858
WBC (10^3/µL)	13.38 ± 4.38 (15)	18.24 ± 4.01 (22)	<0.001	15.67 ± 5.55 (20)	18.20 ± 4.59 (17)	0.533	16.30 ± 5.74 (21)	17.81 ± 4.39 (16)	0.606
Lymphocytes (10^3/µL)	4.95 ± 1.62 (15)	7.92 ± 1.34 (22)	<0.001	7.12 ± 1.95 (20)	7.17 ± 2.06 (17)	0.358	7.17 ± 2.11 (21)	6.93 ± 1.90 (16)	0.960
Monocytes (10^3/µL)	0.90 ± 0.32 (15)	1.74 ± 0.70 (22)	<0.001	1.13 ± 0.66 (20)	1.49 ± 0.78 (17)	0.839	1.49 ± 0.78 (21)	1.12 ± 0.64 (16)	0.600
Neutrophils (10^3/µL)	6.25 ± 2.98 (15)	8.89 ± 3.28 (22)	0.054	7.03 ± 3.73 (20)	9.31 ± 2.91 (17)	0.700	7.23 ± 3.93 (21)	8.40 ± 2.52 (16)	0.984
Eosinophils (10^3/µL)	0.07 ± 0.16 (15)	0.21 ± 0.12 (22)	0.200	0.15 ± 0.08 (20)	0.21 ± 0.18 (17)	0.008	0.16 ± 0.12 (21)	0.19 ± 0.17 (16)	0.361
Basophils (10^3/µL)	0.02 ± 0.01 (15)	0.01 ± 0.01 (22)	0.537	0.01 ± 0.01 (20)	0.02 ± 0.01 (17)	0.299	0.01 ± 0.01 (21)	0.02 ± 0.01 (16)	0.752
Thrombocytes (10^3/µL)	846 ± 421.24 (15)	443 ± 168.39 (22)	0.002	586 ± 351.91 (20)	468 ± 304.67 (17)	0.189	404 ± 329.13 (21)	596 ± 334.41 (21)	0.053

Table S 3. Blood values (median ± SD) of glucose, non-esterified fatty acids (NEFA), urea, immunoglobulin G (IgG), insulin-like growth factor 1 (IGF-1), red blood cells (RBC), haematocrit (HCT), haemoglobin (HGB), white blood cells (WBCs), lymphocytes, monocytes, neutrophils, eosinophils, basophils and thrombocytes, presented by treatment (pooled data from day 9 and day 38) from selected low birth weight piglets (linear mixed models, $p \leq 0.05$).

	Treatment				
	Milk replacer	**Colostrum**	**Quercetin**	**scFOS**	***p*-value**
	Median ± SD (*n*)	**Median ± SD (*n*)**	**Median ± SD (*n*)**	**Median ± SD (*n*)**	
Glucose (mmol/L)	6.30 ± 0.86 (27)	6.00 ± 1.20 (24)	6.17 ± 1.37 (23)	6.23 ± 1.07 (15)	0.466
NEFA (mmol/L)	0.32 ± 0.56 (29)	0.38 ± 0.56 (24)	0.45 ± 0.42 (22)	0.46 ± 0.72 (16)	0.799
Urea (mmol/L)	2.46 ± 1.37 (27)	2.73 ± 1.23 (23)	2.32 ± 1.09 (21)	1.84 ± 1.60 (15)	0.121
IgG (mg/mL)	5.41 ± 2.81 (12)	3.11 ± 1.17 (10)	4.07 ± 3.09 (12)	2.62 ± 2.37 (14)	0.146
IGF-1 (ng/mL)	27.46 ± 15.31 (12)	16.87 ± 15.55 (9)	18.59 ± 13.39 (11)	13.51 ± 17.73 (14)	0.292
RBC (1012/L)	5.29 ± 0.99 (20)	5.37 ± 0.96 (15)	5.01 ± 1.11 (18)	5.28 ± 1.07 (10)	0.580
HCT (%)	33.20 ± 5.51 (20)	35.50 ± 3.82 (15)	33.90 ± 4.59 (18)	33.90 ± 4.23 (10)	0.096
HGB (g/dL)	9.40 ± 1.67 (21)	9.80 ± 1.32 (15)	9.05 ± 1.80 (18)	9.85 ± 1.25 (10)	0.151
WBC (103/µL)	16.30 ± 5.74 (21)	19.45 ± 6.39 (15)	16.50 ± 5.27 (18)	15.50 ± 6.07 (10)	0.324
Lymphocytes (103/µL)	7.17 ± 2.11 (21)	8.71 ± 2.10 (15)	6.84 ± 2.23 (18)	6.91 ± 3.31 (10)	0.362
Monocytes (103/µL)	1.49 ± 0.78 (21)	1.06 ± 0.43 (15)	1.39 ± 0.75 (18)	1.18 ± 0.64 (10)	0.295
Neutrophils (103/µL)	7.23 ± 3.93 (21)	8.82 ± 3.30 (14)	8.25 ± 3.47 (18)	8.42 ± 3.02 (10)	0.833
Eosinophils (103/µL)	0.16 ± 0.12 (21)	0.14 ± 0.10 (15)	0.10 ± 0.10 (18)	0.23 ± 0.16 (9)	0.158
Basophils (103/µL)	0.01 ± 0.01 (21)	0.01 ± 0.01 (15)	0.02 ± 0.01 (18)	0.01 ± 0.01 (9)	0.823
Thrombocytes (103/µL)	404 ± 329.13 (21)	546 ± 316.93 (15)	484 ± 335.40 (18)	402 ± 346.78 (10)	0.590

Table S 4. Blood values (median ± SD) of glucose, non-esterified fatty acids (NEFA), urea, immunoglobulin G (IgG), insulin-like growth factor 1 (IGF-1), red blood cells (RBC), haematocrit (HCT), haemoglobin (HGB), white blood cells (WBCs), lymphocytes, monocytes, neutrophils, eosinophils, basophils and thrombocytes, presented by age and sex (pooled data from day 9 and day 38) from selected low birth weight piglets (linear mixed models, $p \leq 0.05$).

	Age			***Sex***		
	Day 9	**Day 38**	***p*-value**	**Female**	**Male**	***p*-value**
	Median ± SD (*n*)	**Median ± SD (*n*)**		**Median ± SD (*n*)**	**Median ± SD (*n*)**	
Glucose (mmol/L)	6.38 ± 1.14 (54)	5.90 ± 1.07 (35)	0.009	6.17 ± 1.10 (42)	6.18 ± 1.16 (47)	0.466
NEFA (mmol/L)	0.57 ± 0.58 (55)	0.08 ± 0.24 (36)	<0.001	0.43 ± 0.59 (44)	0.39 ± 0.54 (47)	0.974
Urea (mmol/L)	2.65 ± 1.19 (49)	1.76 ± 1.29 (37)	<0.001	2.47 ± 1.29 (40)	2.47 ± 1.34 (46)	0.409
IgG (mg/mL)	4.27 ± 2.66 (24)	2.63 ± 2.37 (24)	0.029	3.91 ± 2.90 (18)	3.22 ± 2.42 (30)	0.843
IGF-1 (ng/mL)	9.19 ± 12.11 (24)	25.35 ± 20.13 (22)	<0.001	20.81 ± 23.12 (18)	15.66 ± 16.17 (28)	0.363
RBC (1012/L)	4.27 ± 0.62 (30)	6.09 ± 0.64 (33)	<0.001	5.35 ± 1.03 (32)	5.19 ± 1.03 (31)	0.631
HCT (%)	31.90 ± 4.17 (31)	37.50 ± 3.83 (33)	<0.001	33.55 ± 4.31 (32)	33.90 ± 5.28 (32)	0.359
HGB (g/dL)	8.50 ± 1.26 (31)	10.40 ± 1.07 (33)	<0.001	9.55 ± 1.46 (32)	9.35 ± 1.68 (32)	0.255
WBC (10^3/µL)	13.51 ± 4.13 (31)	20.38 ± 4.95 (33)	<0.001	16.21 ± 5.66 (32)	17.76 ± 5.98 (32)	0.771
Lymphocytes (10^3/µL)	5.52 ± 2.00 (31)	8.58 ± 1.70 (33)	<0.001	7.58 ± 2.39 (32)	7.33 ± 2.31 (32)	0.363
Monocytes (10^3/µL)	0.91 ± 0.33 (31)	1.56 ± 0.72 (33)	<0.001	1.33 ± 0.72 (32)	1.20 ± 0.65 (32)	0.283
Neutrophils (10^3/µL)	6.85 ± 2.94 (31)	9.42 ± 3.41 (32)	0.001	8.11 ± 3.48 (32)	8.04 ± 3.51 (31)	0.942
Eosinophils (10^3/µL)	0.08 ± 0.07 (31)	0.21 ± 0.12 (32)	<0.001	0.16 ± 0.12 (31)	0.10 ± 0.12 (32)	0.553
Basophils (10^3/µL)	0.01 ± 0.01 (31)	0.01 ± 0.01 (32)	0.762	0.02 ± 0.01 (31)	0.01 ± 0.01 (32)	0.064
Thrombocytes (10^3/µL)	815 ± 367.24 (31)	364 ± 204.30 (33)	0.001	529 ± 330.26 (32)	463 ± 321.85 (32)	0.235

Supplement: Cortisol and chromogranin A

Material & Methods

At farm B, saliva was sampled from 30 LBW piglets at the age of 47 days to determine whether sham drenching would induce an acute stress response, and consequently, have an impact on any potential effect of the DMR supplementation. Attempts to collect sufficient volumes of saliva at an earlier age (day 1, day 9, day 27, and day 31 at farm A) were unsuccessful. Saliva samples were analysed to determine the concentration of two known acute stress markers: cortisol and chromogranin A [185]. The LBW piglets were divided into three groups: sham-drenched piglets, piglets that were picked up during 20 sec, and piglets that were snared during 30 sec (positive control) [186]. Saliva samples were collected by picking up the piglets (the animals were not conditioned and would not chew on the pads voluntarily, thus, had to be picked up) and gently inserting a synthetic cylindrical collection pad (MicroSAL, Oasis Diagnostics [187]) into the mouth, before and after the applied treatment. To ensure that the acute stress-induced cortisol release had occurred, saliva samples were collected 30 minutes after the treatment [188]. All samples were kept on ice before being stored at -80 °C until further analysis. The samples were analysed using commercially available ELISA kits for cortisol (IBL-International, RE52611) and Chromogranin A (CgA, MyBioSource, MBS288843) [185].

Statistical analysis

To evaluate the effect of handling piglets during drenching on the cortisol and CgA response, a linear mixed model was used. Treatment, sex, and their interaction were added as fixed factors. The time between the first and second sampling (immediately before and 30 minutes after the treatment, respectively), and the time of day when saliva was collected were added as random factors. The model was simplified using the stepwise backward method ($p \leq 0.05$). A post hoc analysis was performed using Tukey's correction.

Results

There was no significant interaction between treatment and sex. A treatment effect was observed on the cortisol response ($p = 0.001$). Snaring the LBW piglets resulted in a higher cortisol response, compared to sham-drenching or picking up the animals (Figure S1). No treatment effect was observed for the CgA response ($p = 0.829$) (Figure S1). Sex did not affect the cortisol ($p = 0.765$) or CgA ($p = 0.166$) response.

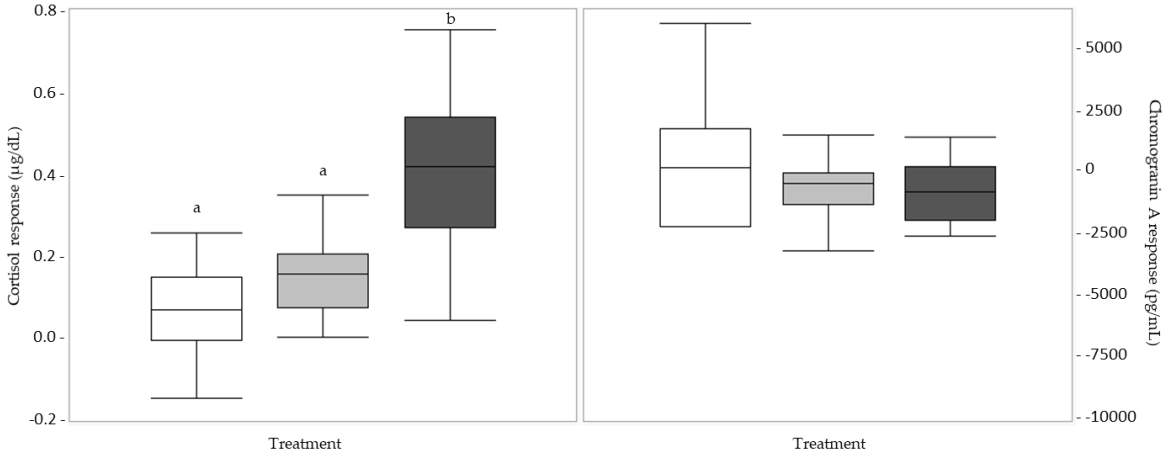

Figure S 1. Cortisol response and Chromogranin A (CgA) response in low birth weight piglets after picking up (white box; n = 10), sham drenching (light grey box, n = 10) or snaring (dark grey box; n = 10). Significant differences between treatments are indicated by a different letter.

Table S 5. Comparison of body weight, average daily growth (ADG), factorial growth, metabolic weight, factorial metabolic rate and colostrum intake of low birth weight (LBW) and very low birth weight (VLBW) piglets at days 1, 2, 3, 9, and 2 days post-weaning (PW) at the farm with low (farm A) and high perinatal management (farm B) (median ± SD).*

		Farm A						***Farm B***		
		VLBW			LBW			LBW		
		Female	Male	VLBW (all)	Female	Male	LBW (all)	Female	Male	LBW (all)
Body weight (kg)	Day 1	0.67 ± 0.10	0.67 ± 0.10	0.67 ± 0.10	0.87 ± 0.08	0.85 ± 0.08	0.86 ± 0.08	0.88 ± 0.07	0.87 ± 0.06	0.87 ± 0.06
	Day 2	0.69 ± 0.15	0.71 ± 0.14	0.70 ± 0.14	0.93 ± 0.15	0.89 ± 0.19	0.91 ± 0.17	0.89 ± 0.11	0.88 ± 0.09	0.89 ± 0.10
	Day 3	0.80 ± 0.16	0.78 ± 0.20	0.80 ± 0.18	1.03 ± 0.18	0.97 ± 0.20	1.01 ± 0.19	1.02 ± 0.13	0.97 ± 0.12	1.00 ± 0.13
	Day 9	1.66 ± 0.36	1.24 ± 0.38	1.63 ± 0.36	1.99 ± 0.39	1.71 ± 0.45	1.81 ± 0.42	1.88 ± 0.31	1.87 ± 0.28	1.87 ± 0.29
	2 days PW	3.73 ± 0.78	3.07 ± 0.78	3.47 ± 0.77	4.14 ± 0.97	4.00 ± 0.94	4.02 ± 0.95	4.85 ± 1.00	4.98 ± 1.01	4.90 ± 1.01
ADG (kg)	Day 2	0.09 ± 0.18	0.10 ± 0.08	0.10 ± 0.15	0.08 ± 0.10	0.07 ± 0.15	0.08 ± 0.12	0.04 ± 0.09	0.03 ± 0.08	0.04 ± 0.09
	Day 3	0.06 ± 0.09	0.04 ± 0.12	0.10 ± 0.06	0.10 ± 0.10	0.08 ± 0.08	0.10 ± 0.07	0.07 ± 0.05	0.07 ± 0.08	0.07 ± 0.07

	Day 9	0.12 ± 0.05	0.08 ± 0.05	0.11 ± 0.05	0.13 ± 0.05	0.11 ± 0.05	0.12 ± 0.05	0.13 ± 0.04	0.13 ± 0.04	0.13 ± 0.04
	2 days PW	0.13 ± 0.03	0.10 ± 0.03	0.12 ± 0.03	0.15 ± 0.04	0.14 ± 0.04	0.14 ± 0.04	0.15 ± 0.04	0.16 ± 0.04	0.16 ± 0.04
Factorial growth	Day 2	1.01 ± 0.27	1.02 ± 0.12	1.02 ± 0.22	1.07 ± 0.14	1.05 ± 0.21	1.06 ± 0.18	1.03 ± 0.11	1.02 ± 0.10	1.02 ± 0.11
	Day 3	1.18 ± 0.33	1.06 ± 0.29	1.14 ± 0.31	1.22 ± 0.18	1.17 ± 0.26	1.20 ± 0.22	1.16 ± 0.13	1.13 ± 0.14	1.16 ± 0.14
	Day 9	2.31 ± 0.66	1.83 ± 0.55	2.25 ± 0.63	2.14 ± 0.59	1.99 ± 0.54	2.12 ± 0.56	2.16 ± 0.34	2.16 ± 0.33	2.16 ± 0.34
	2 days PW	5.47 ± 1.43	4.22 ± 1.09	4.91 ± 1.31	4.95 ± 1.38	4.92 ± 1.44	4.94 ± 1.39	5.29 ± 1.21	5.72 ± 1.19	5.68 ± 1.21
Metabolic weight ($kg^{0.75}$)	Day 1	0.74 ± 0.09	0.74 ± 0.08	0.74 ± 0.08	0.92 ± 0.05	0.90 ± 0.05	0.89 ± 0.10	0.91 ± 0.05	0.90 ± 0.05	0.90 ± 0.05
	Day 2	0.76 ± 0.13	0.77 ± 0.12	0.77 ± 0.12	0.97 ± 0.09	0.91 ± 0.07	0.93 ± 0.13	0.92 ± 0.08	0.91 ± 0.07	0.92 ± 0.07
	Day 3	0.85 ± 0.12	0.83 ± 0.15	0.85 ± 0.13	1.03 ± 0.09	0.98 ± 0.09	1.01 ± 0.15	1.01 ± 0.10	0.98 ± 0.09	1.00 ± 0.09
	Day 9	1.46 ± 0.24	1.23 ± 0.25	1.44 ± 0.25	1.64 ± 0.39	1.59 ± 0.26	1.56 ± 0.27	1.61 ± 0.20	1.59 ± 0.26	1.59 ± 0.23
	2 days PW	2.68 ± 0.44	2.32 ± 0.44	2.54 ± 0.43	2.90 ± 0.76	3.33 ± 0.51	2.84 ± 0.52	3.27 ± 0.52	3.33 ± 0.51	3.29 ± 0.52
Factorial metabolic weight	Day 2	1.01 ± 0.19	1.01 ± 0.09	1.01 ±0.16	1.06 ± 0.07	1.03 ± 0.14	1.05 ± 0.13	1.02 ± 0.08	1.02 ± 0.08	1.02 ± 0.08
	Day 3	1.13 ± 0.22	1.05 ± 0.20	1.11 ± 0.21	1.16 ± 0.08	1.10 ± 0.17	1.15 ± 0.16	1.12 ± 0.09	1.10 ± 0.10	1.11 ± 0.10
	Day 9	1.87 ± 0.40	1.57 ± 0.34	1.84 ± 0.38	1.77 ± 0.41	1.65 ± 0.29	1.76 ± 0.38	1.78 ± 0.21	1.78 ± 0.30	1.78 ± 0.26
	2 days PW	3.58 ± 0.72	2.95 ± 0.56	3.30 ± 0.66	3.27 ± 0.82	3.20 ± 0.84	3.31 ± 0.82	3.49 ± 0.60	3.70 ± 0.58	3.68 ± 0.60
Colostrum intake (g)		200.51 ± 156.43	173.78 ± 121.09	199.18 ± 140.30	230.97 ± 76.74	189.83 ± 99.85	223.37 ± 90.57	227.29 ± 97.18	230.58 ± 68.66	227.29 ± 86.77

**2 Days post weaning was on day 24 and day 26 at farm A and B, respectively.*

Table S 6. Comparison of body weight, average daily growth (ADG), factorial growth, metabolic weight, factorial metabolic weight and colostrum intake of low birth weight (LBW) piglets at the farm with low (farm A) and high perinatal care (farm B) for the different treatments (no treatment, sham one dose, sham three doses, dense milk replacer (DMR) one dose and DMR three doses) (median ± SD).

	Treatment	***Farm A***	***Farm B***	***Farm A + B***
Body weight	No treatment	1.01 ± 1.20	0.98 ± 1.60	0.99 ± 1.49
	Sham 1 dose	0.99 ± 1.33	1.02 ± 1.46	1.02 ± 1.42
	Sham 3 doses	1.09 ± 1.22	0.99 ± 1.65	1.01 ± 1.54
	DMR 1 dose	1.04 ± 1.39	1.00 ± 1.42	1.03 ± 1.41
	DMR 3 doses	0.98 ± 1.14	1.02 ± 1.59	0.99 ± 1.47
ADG (kg)	No treatment	0.14 ± 0.04	0.16 ± 0.03	0.15 ± 0.04
	Sham 1 dose	0.14 ± 0.04	0.14 ± 0.04	0.14 ± 0.04
	Sham 3 doses	0.14 ± 0.04	0.17 ± 0.03	0.17 ± 0.04
	DMR 1 dose	0.15 ± 0.03	0.15 ± 0.04	0.15 ± 0.04
	DMR 3 doses	0.12 ± 0.04	0.15 ± 0.04	0.15 ± 0.04
Factorial growth	No treatment	1.18 ± 1.39	1.13 ± 1.88	1.16 ± 1.74
	Sham 1 dose	1.15 ± 1.55	1.15 ± 1.55	1.15 ± 1.68
	Sham 3 doses	1.16 ± 1.32	1.16 ± 1.32	1.12 ± 1.78
	DMR 1 dose	1.17 ± 1.59	1.17 ± 1.59	1.14 ± 1.62
	DMR 3 doses	1.11 ± 1.27	1.11 ± 1.27	1.12 ± 1.65
Metabolic weight ($kg^{0.75}$)	No treatment	1.01 ± 0.75	0.98 ± 0.94	0.99 ± 0.88
	Sham 1 dose	0.99 ± 0.82	1.01 ± 0.86	1.01 ± 0.84
	Sham 3 doses	1.07 ± 0.74	0.99 ± 0.95	1.01 ± 0.90
	DMR 1 dose	1.03 ± 0.83	1.00 ± 0.84	1.02 ± 0.84
	DMR 3 doses	0.98 ± 0.71	1.00 ± 0.94	0.98 ± 0.87
Factorial metabolic weight	No treatment	1.13 ± 0.83	1.10 ± 1.06	1.11 ± 0.99
	Sham 1 dose	1.11 ± 0.92	1.11 ± 0.98	1.11 ± 0.96
	Sham 3 doses	1.12 ± 0.78	1.08 ± 1.08	1.09 ± 1.00
	DMR 1 dose	1.13 ± 0.92	1.09 ± 0.93	1.10 ± 0.93
	DMR 3 doses	1.08 ± 0.76	1.09 ± 1.02	1.09 ± 0.95
Colostrum intake (g)	No treatment	235.87 ± 95.91	279.75 ± 62.69	244.83 91.55
	Sham 1 dose	159.08 ± 108.15	268.89 ± 88.17	204.92 ± 107.19
	Sham 3 doses	130.86 ±36.50	214.57 ±67.71	198.75 ± 65.26
	DMR 1 dose	249.81 ± 38.00	215.18 ± 89.86	231.84 ± 70.93
	DMR 3 doses	240.06 ± 83.10	211.85 ± 1.07.33	240.06 ± 93.45

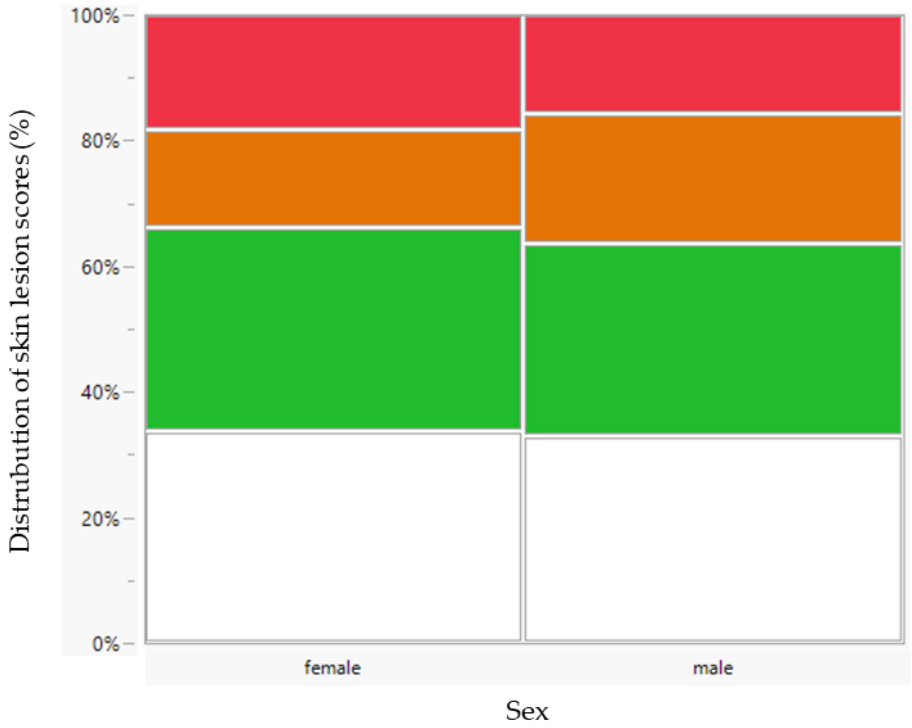

Figure S 2. Distribution of skin lesion (SL) scores at farm A (low perinatal management) of the selected low birth weight (LBW) and very low birth weight (VLBW) piglets per sex (female: n = 80; male: n = 80). There was a no significant effect of sex on the probability of having more severe SL. The following scoring system was applied:

0: no lesions (white)

1: < 5 superficial lesions (skin unbroken) (green)

2: 5-10 superficial lesions or < 5 deep lesions (skin broken and evidence of haemorrhage) (orange)

3: > 10 superficial lesions or > 5 deep lesions (red)

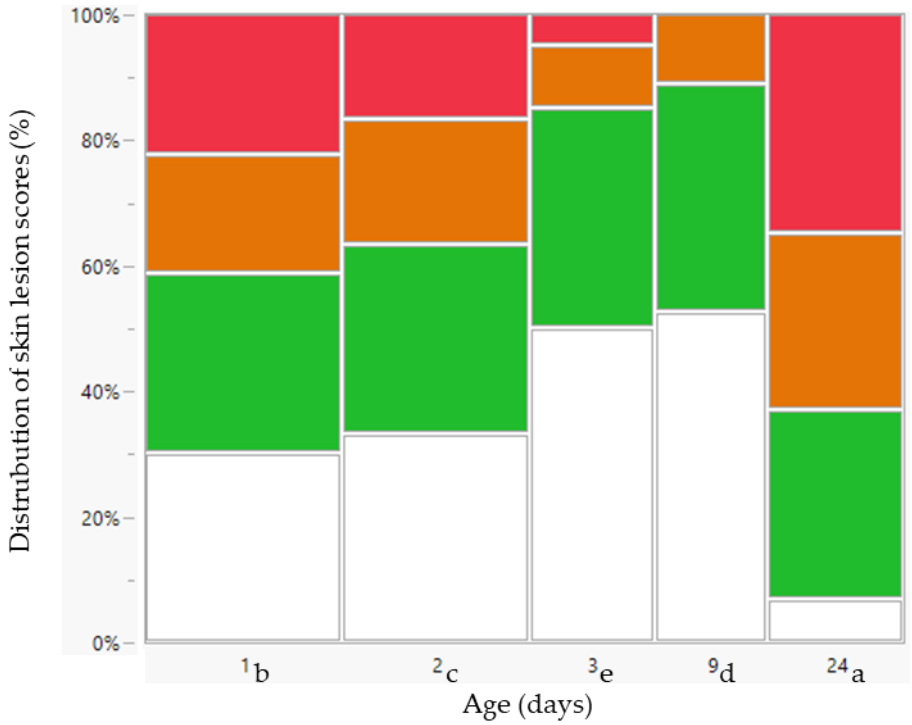

Figure S 3. Distribution of skin lesion (SL) scores at farm A (low perinatal management) of the selected low birth weight (LBW, n = 80) and very low birth weight (VLBW, n = 80) piglets per time point. There was a significant age effect on the SL ($p < 0.001$) The probability of having more severe skin lesions is presented by subscripts (from high to low probability, in alphabetical order). The following scoring system was applied:

0: no lesions (white)

1: < 5 superficial lesions (skin unbroken) (green)

2: 5-10 superficial lesions or < 5 deep lesions (skin broken and evidence of haemorrhage) (orange)

3: > 10 superficial lesions or > 5 deep lesions (red)

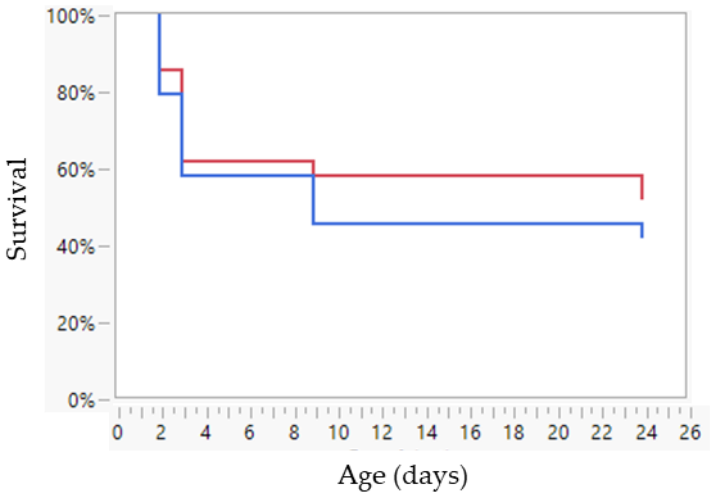

Figure S 4. Cumulative mortality of female (red line; n = 80) and male (blue line; n = 80) low birth weight (LBW) and very low birth weight (VLBW) piglets at farm A (low perinatal care). Cox's proportional hazard model showed no sex effect (p = 0.395).

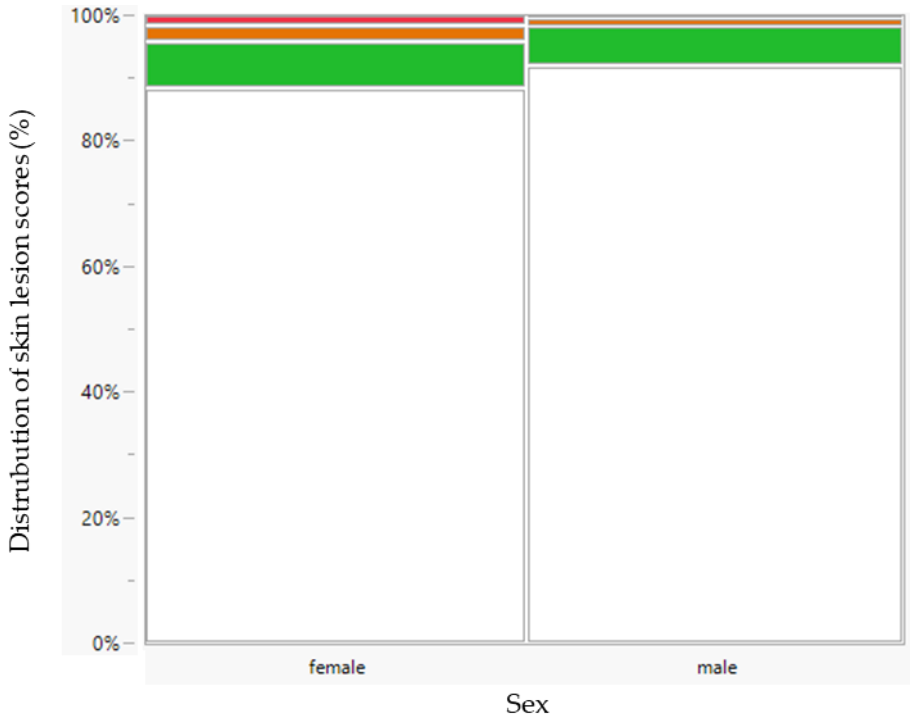

Figure S 5. Distribution of skin lesion (SL) scores at farm B (high perinatal management) of the selected low birth weight (LBW) per sex (female: n = 75; male: n = 75). There was a no significant effect of sex on the probability of having more severe SL. The following scoring system was applied:

0: no lesions (white)

1: < 5 superficial lesions (skin unbroken) (green)

2: 5-10 superficial lesions or < 5 deep lesions (skin broken and evidence of haemorrhage) (orange)

3: > 10 superficial lesions or > 5 deep lesions (red)

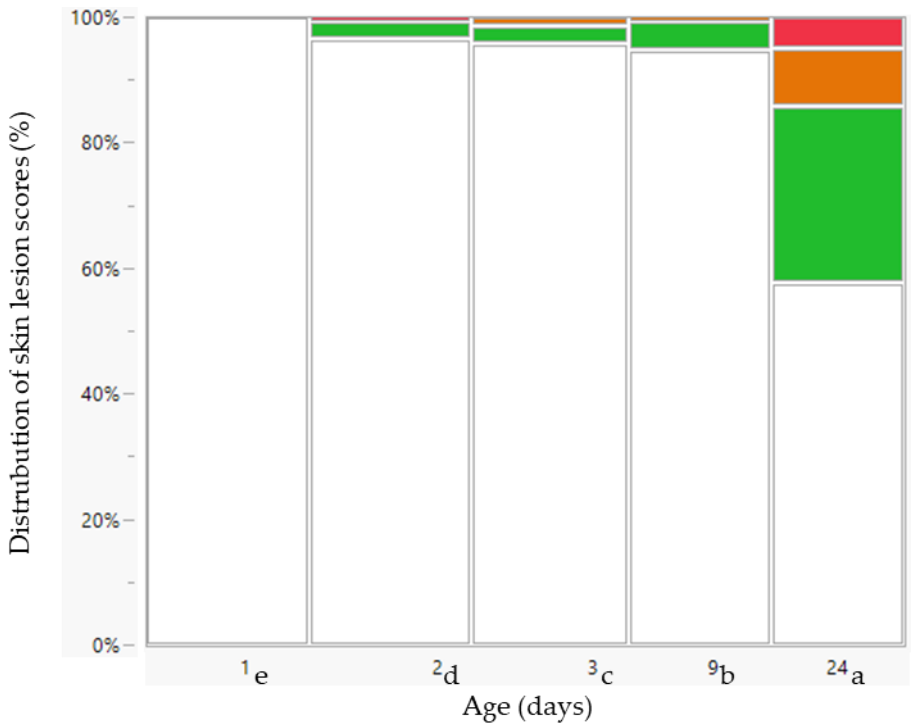

Figure S 6. Distribution of skin lesion (SL) scores at farm B (high perinatal management) of the selected low birth weight (LBW, n = 150) piglets per time point. There was a significant age effect on the SL ($p < 0.001$) The probability of having more severe skin lesions is presented by subscripts (from high to low probability, in alphabetical order). The following scoring system was applied:

0: no lesions (white)

1: < 5 superficial lesions (skin unbroken) (green)

2: 5-10 superficial lesions or < 5 deep lesions (skin broken and evidence of haemorrhage) (orange)

3: > 10 superficial lesions or > 5 deep lesions (red)

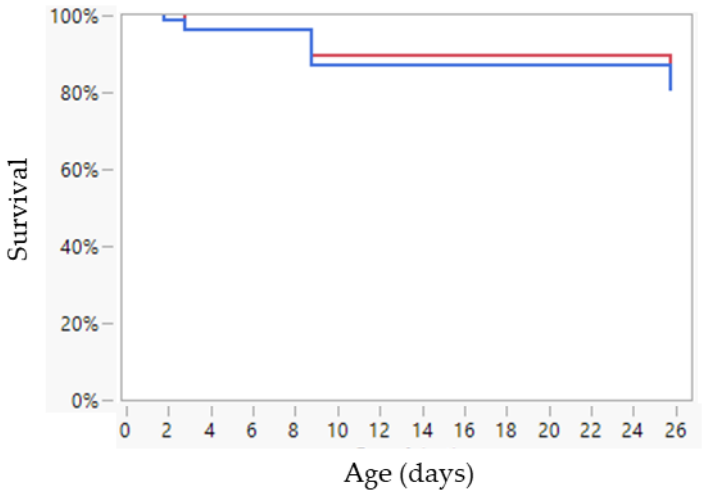

Figure S 7. Cumulative mortality of female (red line; n = 75) and male (blue line; n = 75) low birth weight (LBW) piglets at farm B (high perinatal care). Cox's proportional hazard model showed no sex effect (p = 0.886).