HOT AND BOTHERED! LONG TERM IMPACTS OF LATE PREGNANCY HEAT STRESS ON SOWS AND PROGENY

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By

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Executive Summary

Heat stress is recognised as a significant and growing challenge for the Australasian pig industry, with negative impacts on sow welfare, reproductive performance and piglet survival particularly during the farrowing period. Previous research has established that even mild increases in ambient temperature around farrowing prolong parturition duration, reduce sow feed intake and impair farrowing outcomes. Extended farrowing is associated with elevated cortisol concentrations in the sow, increased risk of uterine infection, greater stillbirth incidence and compromised neonatal piglet health. Despite these well-documented consequences, the physiological mechanisms by which heat stress increases perinatal mortality and strategies to practically mitigate these effects remained incompletely understood. This project investigated the hypothesis that heat stress at farrowing extends parturition duration by limiting the energy available for uterine contractions, and that this physiological disruption impairs sow reproductive health and neonatal piglet gut development. A secondary objective was to evaluate whether in-water supplementation with betaine or sanguinarine (an extract from Macleaya cordata) could ameliorate the negative effects of heat stress in a commercial farrowing setting.

Heat-stressed gilts from Experiment 1 exhibited markedly elevated respiration rates, higher rectal temperatures and greater skin temperatures than controls. Feed intake was substantially reduced in the heat stress group. Venous blood analysis confirmed elevated plasma cortisol concentrations and altered acid–base status, consistent with physiological heat stress and metabolic compensation. The most striking outcome was a dramatically higher stillbirth rate in heat-stressed gilts. Risk analysis revealed that gilts experiencing cyclic heat stress were almost 15 times more likely to farrow stillborn piglets, with 93% of the stillbirths recorded attributable to heat stress. Umbilical cord rupture explained 55% of the stillbirths observed, and heat-stressed gilts were significantly more likely to produce piglets with ruptured cords. Placental expulsion interval was prolonged in heat-stressed gilts. Two gilts in the heat stress group experienced retained piglets and placentae post-partum, an event not observed in controls. Umbilical vein blood gas analysis demonstrated that piglets born to heat-stressed gilts had reduced oxygen supply at birth, evidenced by lower partial pressure of oxygen, reduced oxygen saturation and lower base excess, indicating foetal hypoxia as a key mechanism underlying the elevated perinatal mortality. This is the first study to demonstrate that cyclic heat stress of this magnitude at late gestation and farrowing negatively affects umbilical oxygen supply in pigs. In surviving piglets, maternal heat stress reduced small intestinal length and absolute brain weight at 48 hours of age, with a trend towards increased early liveborn mortality. Gut barrier function as assessed by transepithelial electrical resistance was not significantly different between groups, though jejunal crypt depth was reduced in piglets from heat-stressed gilts.

Neither betaine nor sanguinarine supplementation, administered individually or in combination via in-water dosing, improved farrowing performance or piglet survival outcomes above the control treatment in either commercial trial. In Experiment 3, litter weights at day 21 were significantly lower in the sanguinarine group compared to controls, though farrowing performance measures including total pigs born, pigs born alive, stillbirths and mortality rates were not significantly different between groups. The lack of treatment effect in Experiments 2 and 3 is most likely explained by the relatively mild heat stress conditions experienced by sows during both commercial trials. Internal farrowing shed

temperatures on the busiest farrowing days remained below 25°C across all replicate blocks in Experiment 3. As a result, the thermal challenge was insufficient to produce the magnitude of impairment observed under the controlled conditions of Experiment 1, limiting the opportunity to detect a treatment benefit.

The outcomes of this project have direct relevance to Australian pig producers managing sows through summer farrowing periods. The controlled experiment clearly demonstrated that heat stress at the levels readily experienced in Australian farrowing houses during summer significantly increases the risk of stillbirths and impairs neonatal piglet viability through reduced placental oxygen supply. These findings provide a strong evidence base for prioritising thermal management of farrowing facilities. Based on the results of this project, the following recommendations are made for industry:

- Sows are highly susceptible to heat stress during parturition, with negative and lasting impacts on piglet morphology, survival and growth. This period should be considered a priority for thermal management interventions.
- Farrowing houses should be climate controlled to maintain ambient temperatures below 24°C to limit heat stress experienced by sows during this critical reproductive stage.
- In-water supplementation with betaine or sanguinarine was not effective under the commercial conditions tested. However, the practicality of delivery via in-water dosing systems — which are now commonplace in Australian nursery and grower facilities — warrants continued investigation of this application strategy, particularly in naturally ventilated farrowing houses where pharmacological or nutritional mitigation of heat stress may offer a cost-effective management option.

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1. Introduction

Previous research has demonstrated that heat stress reduces sow feed intake during lactation, piglet growth and subsequent rebreeding success. There is now increasing evidence that heat stress experienced by sows farrowing over the summer months increases the expulsive phase of parturition, with lasting consequences to both the sow and progeny. Even at conditions that could be described as mild by Australian standards, increasing ambient temperature from 20°C to 25°C for four days around farrowing increased parturition length (Muns et al., 2016), with sows not exhibiting physical signs of heat stress until farrowing was completed. This indicates that the additional metabolic heat load of the piglets, and/or the exertion of farrowing make this a key period of heat stress susceptibility.

A possible explanation for this increased farrowing duration in the heat stressed sow is a state of metabolic depletion exacerbated by reduced feed intake and nutrient absorption (He et al., 2019) and the link between sow energy status and farrowing kinetics (Feyera et al., 2018). Put simply, farrowing duration is extended when time between last meal and piglet birth is increased, and so the decreased nutrient absorption of heat stressed sows to the point of farrowing may limit available energy for uterine contractions. This situation is likely exacerbated with increasing litter sizes.

The consequences of an extended farrowing duration on the sow are numerous. We have demonstrated that when farrowing duration exceeds 4h, circulating cortisol concentration in the sow increases exponentially, implying that welfare is impaired (Nowland et al., 2019). It is logical to assume that farrowing duration will impact uterine health as with an extended dilation of the cervix comes increased risk of bacterial infection (Peltoniemi et al., 2016). Previously it was demonstrated that as farrowing duration rose from less than 2h to 4-8h, the incidence of sow fever rose from 40 to 100% (Tummaruk and Sang-Gassanee, 2013). The most obvious impact of an extended farrowing duration on progeny is an increased risk of stillbirths. In a cross-herd evaluation, the study identified a lower stillbirth rate when farrowing house ambient temperature was set below 22°C (Vanderhaeghe et al., 2010). However in our review, we identified that the effect of an extended farrowing duration is far greater than this (Langendijk and Plush, 2019). Asphyxiated piglets that are born alive represent about 15-20% of the population under favourable climatic farrowing conditions (potentially exacerbated under heat stress), and they experience gastrointestinal tract (GIT) damage, poor colostrum intake, and reduced growth to ten weeks of age.

The application of sanguinarine, extracted from the plume poppy (*Macleaya cordata*), to alleviate the impacts of heat stress is relatively new, but presents exciting possibilities. For example *Macleaya cordata* extracts have been shown to counteract oxidative stress (Lima et al., 2019), including in heat stressed animals where improvements in feed conversion efficiency were observed (Estrada-Angulo et al., 2016). Additionally, sanguinarine has been shown to reduce cortisol concentrations in transported pigs (Artuso-Ponte et al., 2015). Sanguinarine also

improves GIT integrity and reduces Salmonella shedding (Robbins et al., 2013). In summary, the emerging evidence for sanguinarine to ameliorate heat stress is consistent with properties reducing stress and preserving GIT integrity.

Taken collectively, the sow is acutely sensitive to heat stress at farrowing and this has the potential to have far reaching consequences for both the sow and her progeny. In this series of experiments, we tested the hypotheses that; heat stress extends farrowing duration by limiting available energy sources required for uterine contractions, and this extended farrowing duration caused by heat stress impairs the reproductive physiology of the sow and gut health of the piglet. Due to the short duration of the farrowing period and long-lasting consequences, it is an ideal time to target with interventions such as sanguinarine to better manage heat stress at farrowing and reduce the negative impacts of seasonal infertility.

2. Methodology

These experiments were approved by the PIRSA Animal Ethics Committee (#28/20) and The University of Melbourne Animal Ethics Committee (20174). Experiment 1 took place at The University of Melbourne. Experiments 2 and 3 took place at SunPork Wasleys Piggery.

Experiment 1: We hypothesised that sows that experience heat stress during farrowing will result in a longer farrowing duration and will have an impact of the sow and piglet physiology.

Sixteen pregnant primiparous sows were individually housed in plastic-slatted flooring pens (2.2m x 1.5m) for acclimation until day 110 of gestation, across two replicates (n = 8 per replicate). The temperature for the acclimatisation period was constant at 20ºC. The housing light period was 15 hours (6am to 9pm) and a 9 hour dark period (9pm to 6am) over the acclimatisation and experimental periods. All gilts were fed twice daily with a standard gestation diet as per farm practice at an average of 2.3kg per day and had ad libitum access to water over the acclimation period. On day 109 of gestation, farrowing crate infrastructure were installed into the individual pens.

Gilts were randomly allocated to one of two treatment groups; (1) exposed to thermoneutral control (CON; constant 20ºC, n = 8) or (2) cyclic heat stress conditions (HS, 30ºC between 9am and 5pm; 28ºC between 5pm and 9am, n = 8), from day 110 of gestation until farrowing completion. From day 110 of gestation until the day of farrowing gilts were fed a commercial transition diet (12.9 MJ DE/kg) twice daily at 4.0kg per day, receiving ad libitum access upon farrowing with ad libitum access to water throughout. Upon completion of farrowing, the HS gilts room temperature was downregulated and maintained at 20ºC during the postpartum period.

Gilt respiration rate, skin temperature and rectal temperature were recorded twice daily at 9am and 3pm from day 110 of gestation until farrowing. Respiration rate

was assessed by counting chest movement over a 20 second period and transformed into breaths per minute. Rectal temperature was measured using a digital thermometer (Surgipack, Vega Technologies Inc., Dongguan, China). Skin temperature was assessed by a non-invasive laser thermometer (Digitech Inc., Zurich, Swiss). Feed intake was recorded daily and reported as average daily feed intake.

A total of 13 gilts (n = 6 CON; n = 7 HS) had catheters inserted for repeated blood sampling. From day 110 of gestation, daily blood samples were collected using a 5ml syringe via the catheter until the day of farrowing. Approximately 1ml of the blood was immediately loaded into the blood gas analyser (Epoc; Alere, Waltham, MA, USA) to quantify oximetry and biochemistry of the blood. Remaining blood samples were immediately loaded into sodium heparin vacutainer (BD Vacutainer, Macquarie Park NSW, Australia) and centrifuged at 2000g for 15 minutes at 4^{o}C. The plasma was then collected and stored at -20^{o}C for analysis.

Farrowing duration, birth interval and placental expulsion interval were recorded via a camera (GoPro; CA, USA) placed above each farrowing crate. Gilts that had a birth interval greater than 90 minutes and received manual assistance as well as a gestation length greater than 119 days were excluded from the study (n = 1 CON; n= 1 HS). A colostrum sample was collected from a functional teat of each gilt during the parturition process and stored at -20^{o}C.

At the birth of each piglet, if an intact umbilical cord was present, a 1ml blood sample was collected from the umbilical vein via a 23G needle and heparinised syringe and immediately loaded into the blood gas analyser. Piglets were assessed and recorded for the following; status: birthweight, sex, birth order, rectal temperature, meconium staining and whether the cord was ruptured. Piglet bodyweight was recorded again at 24 and 48 hours of age. At 48 hours of age, a subset of piglets (n = 39 CON; n = 34 HS) with bodyweights closest to the litter average were euthanised. These piglets were then dissected for morphometric analysis of the small intestine, liver, heart, spleen, brain, quadriceps, muscle and femur. A section of jejunum and ileum was removed and rinsed in a pbs solution for mucosal barrier function measurements. A jejunum biopsy sample (5 cm long) was collected and fixed with 4% paraformaldehyde in 0.1 M PBS (pH = 7.4) for 24 h, then transferred to PBS/ 0.1% sodium azide and stored at 4 °C until processing for histological analysis. All gilts and the remaining piglets were euthanised subsequently.

As soon as the small intestine tissues were collected, the mucosal barrier function was assessed via transepithelial electrical resistance (TER) using methods previously described (Cottrell et al., 2020). Briefly, the proximal jejunum and distal ileum segments were opened along the mesenteric border, and the muscular layer from the serosal side was removed. The remaining tissue was pinned on a round aperture slider (0.3 cm2), which was then mounted into a Ussing chamber system (EasyMount Diffusion Chambers, Physiologic Instruments, San Diego, CA, USA). Following the tissue equilibration for 20 mins in the Krebs mannitol-glucose solutions, the tissues were clamped to the voltage of 0 V and administrated five pulses of 2 mV for a duration of 5 min. Changes in voltage (V) and current (I) were recorded for each

pulse over the 5 min period. The TER was calculated by Ohm's Law (R = V/I) multiplied by the surface area of the slider. Fixed jejunum biopsy samples were paraffin-embedded and cut via the cross-sectional area at the thickness of 5 µm using a rotary microtome (Leica biosystems, Victoria, Australia). The slides were then stained with haematoxylin and eosin (H&E). A total of eight images (four images per section) were obtained from each sample using a digital microscope (Zeiss, Oberkochen, Germany). Only intact (full finger-shaped) and well-orientated villi were chosen for the measurements. Intestinal villus height and crypt depth were measured using ImageJ software (NIH, USA) with the sample ID blinded. The villus height to crypt depth ratio was calculated.

Piglet plasma and colostrum immunoglobulin G (IgG) concentrations were analysed using a commercially available porcine IgG ELISA basic kit (Cat # 3151-1HD-6; Mabtech, Nacka Strand, Sweden), as per the manufacturer's instructions. The intra and inter-assay CVs were 5.1% and 7.1%, respectively. Total protein concentrations for colostrum and piglet plasma were analysed using the Pierce BCA assay kit (Cat # 23227; Thermo Fisher, Waltham, MA). The intra and inter-assay CVs were 2.6% and 2.1%, respectively. Gilt and piglet plasma cortisol concentrations were determined via radioimmunoassay methods described in a previous study (Lee et al., 2014). Each sample was assayed in duplicate, and the intra-assay CV was 3.1% and assay sensitivity was 0.17 ng/ml.

Data were analysed in Genstat (18th ed, VSN International, Hemel Hempstead, UK). Data normality was verified using Shapiro–Wilk's test. Log transformation was performed for parameters that were not normalised, and back-transformed means were reported. Each pregnant gilt was considered an experimental unit. For the repeated measurements of gilt thermal stress, data were analysed by linear mixed models (REML) with temperature treatment, day, time, and their interaction being fixed factors and gilt's ID and replicate being random factors. Sow venous blood data were analysed with temperature treatment and day as fixed factors and gilt's ID and replicate being random factors. Gilt reproductive performance data were analysed by two-way ANOVA. Piglet stillborn rate (stillbirth number / total born) and umbilical cord ruptured rate (ruptured umbilical cord number / total born) were analysed using Fisher's exact test. For piglet umbilical vein blood parameters at birth, data were analysed by REML. The statistical model included temperature treatment, sex and birth order as fixed effects with gilt's ID as a random factor. Other piglet data were analysed with temperature treatment, sex and their interaction being fixed effects and gilt's ID as a random factor. Birth order was removed as its effect was not significant. Attributable risk analysis was performed to evaluate the risk of being stillborn if piglets were born to a heat-stressed gilt and if the umbilical cord was ruptured. Pearson's correlation coefficient analysis was performed between appropriate continuous variables. A P-value ≤ 0.05 was considered significant, and a P-value ≤ 0.1 was considered a trend. The estimates were expressed as predicted means ± SEM.

Experiment 2: We hypothesised that in-water additives will aid in reducing heat stress in sows and therefore farrowing duration.

The experiment was conducted on a breeder unit with experimental sows (Camborough 42, PIC Australia, Grong Grong, NSW, Australia) over two replicates in December 2021 (maximum average daily temperature 29.2 ± 5.2ºC; minimum average daily temperature 11.3 ± 4.0ºC) and February 2022 (maximum average daily temperature 29.7 ± 4.4ºC; minimum average daily temperature 13.8 ± 3.7ºC).

One hundred and four multiparous pregnant sows (parity 3.5 ± 0.2) were moved from group gestational housing to the farrowing house 3.7 ± 0.2 days prior to farrowing. Sows were housed in conventional farrowing crates with a footprint of 1.8m x 2.4m, with fully slatted plastic flooring, across 3 rooms. Rooms were identical in design and were naturally cross-ventilated, with sows cooled via a temperature activated (>28^{O}C) evaporative dripper system installer over the head and flank of the sow. Each room was plumbed with a closed water system equipped with one water doser (Dosatron D25RE09, Feedworks, Lancefield Australia) for water treatment delivery. Treated water was delivered to two rows of crates, with the control row receiving untreated water via a bypass. Treatments were randomised across the three rows for each of the two replicates. Sows were fed 2 kg of a standard commercial transition diet (12.75 MJ DE/kg) twice daily at 7am and 2pm until parturition, when the diet was fed *ad libitum* until day 3 of lactation. Sows then received a standard commercial lactation diet (13.9 MJ DE/kg) in the form of a mash *ad libitum* until weaning. Feed disappearance was recorded twice daily from the completion of farrowing until weaning at approximately 21 days of lactation.

Upon entry into the farrowing house, sows were weighed and P2 backfat depth was recorded. Sow were randomly allocated to crates in one of the 3 rooms containing the following experimental treatments delivered via water:

1. Control (CON); no additive (n=26).
2. Betaine (BET); natural betaine (Betafin, Feedworks Lancefield AU) at 2.5L/1000L water (n=26).
3. Sanguinarine (SAN); extract from *Macleaya cordata* (Sangrovit, Phytobiotics EltvilleDEU) at 100g/1000L water (n=26).
4. Betaine + Sanguinarine (BET+SAN).; both natural betaine and sanguinarine at dosages listed above (n=26).

Rectal temperature and respiration rates were recorded twice daily (8am and 2pm) upon entry into the farrowing shed, at farrowing, day 1 of lactation and day 7 of lactation (following same procedure as stated in Exp 1).

Each sow was monitored during farrowing via hourly observation, with inter-piglet birth interval and total farrowing duration recorded via camera analysis (GoPro Hero4; California USA). Farrowing performance (intervention required, total pigs born, pigs born alive, pigs stillborn, mummies, 24 hour mortality) were all recorded. Upon birth sex and weight were recorded from piglets (n=2,156). All piglets were fostered within 24 hours of farrowing, with all piglet movements staying within treatment. On day 2 of lactation piglets were tail docked using cauterisation, administered oral coccidiostat (1 mL Baycox Piglet Coccidiocide (50mg/mL Toltrazuril) Elanco Australiasia Pty Ltd., Macquarie Park AU) and an iron injection

(1mL Feron 200 + B12 (200mg/mL iron as iron dextran, 40 µg/mL cyanocobalamin) Elanco Australasia Pty Ltd., Macquarie Park, AU). At weaning, survival status and weight at 21 days of age were recorded individually, with number of pigs weaned recorded for the litter. Sows were weighed and P2 backfat depth was recorded upon exit of the farrowing house.

Data were analysed (IBM SPSS Statistics for Windows, Version 28.0, Armonk, NY, United States) with a p-value < 0.05 considered significant and <0.10 a tendency. Farrowing performance and growth data were analysed using negative binomial regression and general linear mixed models with treatment as a fixed effect and replicate as a random term. Data presented are mean ± standard error of the mean (SEM).

Experiment 3: The results of Experiment 2 showed that sanguinarine was the most effective in-water treatment. We hypothesised that the addition of sanguinarine in the sows drinking water would reduce farrowing duration in times of heat stress, improving sow and piglet performance.

The experiment was conducted on a breeder unit with experimental sows (Camborough 42, PIC Australia, Grong Grong, NSW, Australia) over three replicates in January 2023 (maximum average daily temperature 32.5°C ± 5.4; minimum average daily temperature 14.6 ± 4.4°C), February 2023 (maximum average daily temperature 30.9 ± 6.6°C; minimum daily temperature 14.1 ± 5.6°C) and March 2023 (maximum average daily temperature 26.7 ± 3.9°C; minimum daily temperature 11.7 ± 3.0°C).

A total of 528 multiparous pregnant sows (parity 2.8 ± 0.12) was moved from group gestation housing to farrowing housing 4.6 ± 0.35 days prior to farrowing. Sows were housed and managed as mentioned in Experiment 2. Upon entry into the farrowing house, sows were weighed and P2 backfat depth was recorded. Sows were randomly allocated to crates in one of the three rooms containing the following experimental treatments delivered via water:

1. Control (CON); no additive (n = 223).
2. Sanguinarine (SAN); extract from *Macleaya cordata* (Sangrovit, Phytobiotics Eltville DEU) at 100g/1000L water (n = 305).

Similar to Experiment 2, alternating water lines were placed on treatment water system, with remaining lines receiving untreated mains water. The lines allocated to treatment changed between rooms and replicates. Each sow was monitored during farrowing via hourly observation. All measures were taken as per Experiment 2. Sows' subsequent reproductive performance was collected from the farm performance software (wean-to-service interval, pregnancy rate).

Data were analysed (IBM SPSS Statistics for Windows, Version 28.0, Armonk, NY, United States) with a p-value < 0.05 considered significant and <0.10 a tendency. Farrowing performance and growth data were analysed using negative binomial regression for binary data and general linear regression for continuous data with

parity and treatment as fixed effects and replicate as a random term. Data presented are mean ± standard error of the mean (SEM).

3. Outcomes

Experiment 1

Thermal stress of the gilts

Heat stress increased average gilt respiration rates across the experimental period (HS = 104 ± 4.1 vs. CON = 32 ± 4.1 breaths per min $P < 0.001$; Fig. 1A). Respiration rates were higher at 1500 h than 0900 h (72 ± 3.4 vs. 64 ± 3.4 breaths per min, $P < 0.001$; data not shown in the Fig.1). No main effect of day on respiration rates ($P = 0.49$) was observed, but there was a significant interaction ($P = 0.032$) between temperature treatment and day for respiration rates, such that gilts under HS conditions had a lower respiration rate on the day of farrowing (77 ± 8.1 breaths per min) than other days, whereas gilts from the CON group had a consistent respiration rate over the experimental period. Gilts exposed to HS had higher rectal (HS = 39.5 ± 0.19 vs CON = 38.1 ± 0.19 °C, $P < 0.001$; Fig. 1B) and skin temperatures (HS = 38.1 ± 0.26 vs. CON = 34.0 ± 0.26 °C, $P < 0.01$; Fig. 1C) than gilts from the CON group across the experimental period. Gilt rectal temperatures (39.0 ± 0.14 vs. 38.6 ± 0.14 °C, $P < 0.001$) and skin temperatures (36.4 ± 0.20 vs. 35.7 ± 0.19 °C, $P < 0.001$) were higher at 1500 h than 0900 h (data not shown on the Fig.1). Furthermore, there was an interaction ($P = 0.006$) between temperature treatment and day for rectal temperatures as gilts under CON conditions had higher rectal temperatures when approaching the due day, whereas heat-stressed gilts showed the opposite trend (Fig. 1B).

The effect of day on skin temperatures was significant ($P < 0.001$), with the highest being observed on d -1 and the lowest being observed on d -6 relative to the farrowing day. A temperature treatment by day interaction for skin temperatures was also significant ($P < 0.001$), such that gilts from the CON group had increased skin temperatures as gestation advanced, whereas heat-stressed gilts had consistent skin temperatures over the experimental period (Fig. 1C).

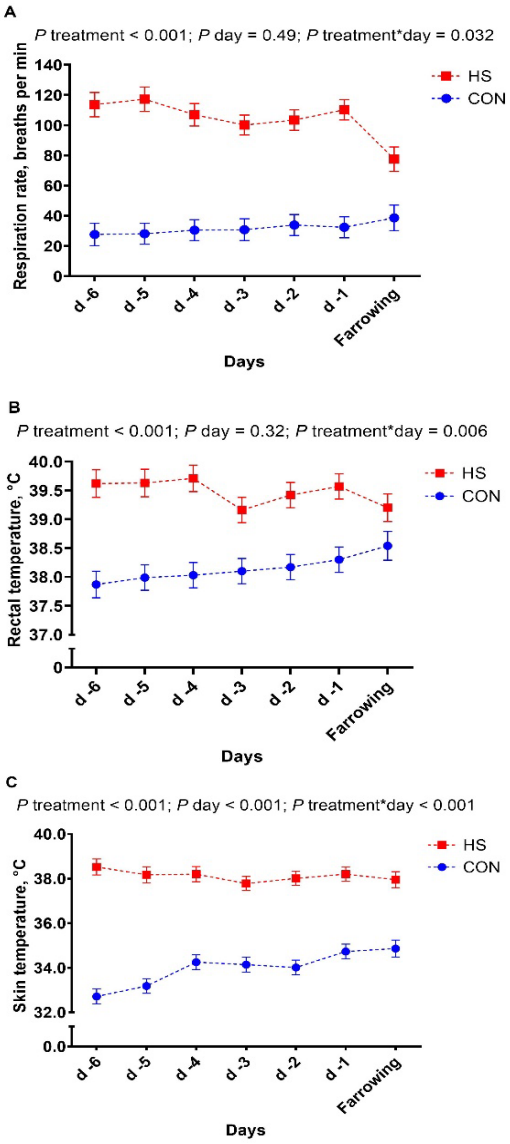

Figure 1. Predicted means (± SEM) for respiration rate (A), rectal temperature (B) and skin temperature (C) of pregnant gilts exposed to control (CON, n = 8 gilts) or cyclical heat stress (HS, n = 8 gilts) conditions.

Gilt feed intake and venous blood parameters

Heat stress decreased gilt average daily feed intake (HS = 1.4 ± 0.25 vs. CON = 3.5 ± 0.24 kg/d, P < 0.001) across the experimental period. The effects of HS on gilt venous blood parameters are presented in Table 1. Heat stress increased gilt plasma cortisol concentrations (P = 0.01). Gilts from the HS group had higher whole blood pH (P < 0.001), creatinine (P = 0.02), while having lower partial pressure of carbon dioxide (pCO_2, P < 0.001), bicarbonate ($cHCO_3$, P = 0.02), and calcium (P = 0.04). Heat stress tended to increase oxygen saturation rate (P = 0.06) and tended to decrease sodium (P = 0.09) and base excess (P = 0.07) in the gilt venous blood.

Table 1. Predicted means (± pooled SEM) for blood parameters of gilts exposed to control (CON) or heat stress (HS) conditions between d110 of gestation until farrowing completion.

Variable	Treatment[1]		SEM	*P* value
	CON	HS		
Glucose, mmol/L	5.40	5.12	0.195	0.35
Cortisol[2], ng/mL	1.32	1.60	0.062	0.01
	(20.9)	(39.8)	-	-
pH	7.47	7.53	0.008	<0.001
Partial pressure of CO_2, mmHg	43.9	32.7	1.79	0.002
Partial pressure of O_2, mmHg	35.5	37.4	1.50	0.50
Sodium, mmol/L	144	142	0.8	0.09
Potassium, mmol/L	4.26	4.07	0.075	0.11
Calcium, mmol/L	1.28	1.21	0.022	0.04
Lactate, mmol/ L	1.20	1.20	0.115	0.90
Creatinine, mg/dL	2.87	3.50	0.170	0.02
Hematocrit, %	33.7	34.7	1.14	0.57
Hemoglobin, g/dL	11.4	11.8	0.39	0.50
Bicarbonate, mmol/L	31.4	27.6	1.02	0.02
Base excess, mmol/L	7.75	4.95	0.971	0.07
Oxygen saturation, %	67.8	77.4	2.91	0.06

[1]Treatment: CON = control (n = 6 gilts; constant 20 °C); HS = cyclic heat stress (n=7 gilts, 0900 h to 1700 h, 30°C; 1700 h to 0900 h, 28°C).

[2] Cortisol was analysed from the plasma while other parameters were analysed from the whole blood. Log transformation was performed before analysis, and figures in parentheses are back-transformed means.

Gilt farrowing performance

The gestation length was similar between the treatment groups (P = 0.70; Table 2). A total of 88 piglets were born to gilts in the CON group, of which two male piglets were stillborn. A total of 93 piglets were born to gilts in the HS group, of which 31 piglets (n = 13 females; n = 18 males) were stillborn. Thus, the overall stillborn rate was significantly higher in the HS group than the CON group (HS = 33.3% vs. CON = 2.3%, P < 0.001). It is important to note that one gilt from the HS group farrowed nine piglets that were all stillborn. Gilts from the HS group tended to farrow less liveborn piglets (P = 0.09) than the CON group (Table 2). Heat-stressed gilts took longer to start expelling the placentae (P = 0.003), although the active farrowing duration was not statistically significant (P = 0.21; Table 2). There was a positive correlation between farrowing duration and placental expulsion interval (r = 0.60, P = 0.02). Piglet birth interval was not different between the treatment groups (P = 0.16; Table 2). Irrespective of treatments, there was an effect of birth order on piglet birth interval (P < 0.001) as piglets born late in the litter had a greater birth interval compared to the early and middle groups (early = 14.3 ± 3.22, middle = 12.3 ± 2.98 and late = 21.1 ± 3.03 min; data not shown in the Table).

The total born number was similar between the treatment groups (Table 2). Liveborn litter weights were reduced by HS (P = 0.02), although total litter weights (incl. stillbirth) were similar between treatments (Table 2). The odds for a piglet to be born with a ruptured umbilical cord was higher in the HS group than the CON group (P < 0.001; Table 2). Among the 31 stillborn piglets in the HS group, 16 piglets were born with a ruptured umbilical cord. The attributable risk analysis revealed that cord rupture explained 55% of the stillbirths. The association between birth order and stillbirth was significant (P = 0.01), such that the odds for stillbirth increased with birth order (early = 11.5%, middle = 13.8% and late = 30.5%), regardless of temperature treatments. Risk analysis revealed that the relative risk factor of stillbirth associated with HS was 14.7, meaning that gilts in the HS group were almost 15 times more likely as gilts in the CON group to farrow stillborn piglets. In addition, attributable risk analysis indicated that 93% of stillbirth observed in the current study could be attributed to HS. Of note, two gilts from the HS group had three piglets and their associated placentae retained at the birth canal found on d 3 post-partum (piglets were not included for birth interval and farrowing duration analyses). In contrast, retained piglets or placentae did not occur in gilts from the CON group.

Table 2. Predicted means (± pooled SEM) for reproductive performance of gilts expose to control (CON) or heat stress (HS) conditions between d 110 of gestation until farrowing completion.

Variable	Treatment[1]		SEM	P value
	CON	HS		
Gestation length, d	115.6	116.0	0.66	0.70
Farrowing duration[2], min	2.21	2.32	0.061	0.21
	(162)	(209)	-	-
Birth interval[2], min	1.09	1.23	0.086	0.16
	(12.3)	(17.0)	-	-
Placental expulsion interval[2], min	2.11	2.25	0.026	0.003
	(129)	(178)	-	-
Total born, n	13.3	13.8	1.07	0.76
Liveborn piglets, n	12.60	8.60	1.510	0.09
Stillbirths, %	2.30	33.3	-	< 0.001
Umbilical cord ruptured, %	10.2	30.6	-	< 0.001
Mummified fetus, %	8.30	4.10	-	0.180
Litter weight (incl. stillborn), kg	16.7	16.5	1.63	0.96
Litter weight (liveborn), kg	16.5	10.1	1.61	0.02
Coefficient of variation for liveborn birth weight	0.16	0.18	0.023	0.37

[1] Treatment: CON = control (n = 7 gilts; constant 20 °C); HS = cyclic heat stress (n=7 gilts, 0900 h to 1700 h, 30°C; 1700 h to 0900 h, 28°C).

[2] Log transformation was performed before analysis, and figures in parentheses are back-transformed means.

Piglet umbilical vein parameters and meconium staining at birth

Piglets born to heat-stressed gilts had lower birth umbilical vein partial pressure of oxygen (pO_2, P = 0.04), oxygen saturation rate (P = 0.03), base excess (P = 0.02) and pCO_2 (P = 0.01) than piglets from the CON group (Fig. 2). Piglets from the HS group tended to have higher lactate (P = 0.07) and lower bicarbonate (P = 0.07) concentrations in the umbilical vein (Fig. 2). Other umbilical blood variables, such as pH and haematocrit, were not significantly different between temperature treatments (Fig. 2). Piglets born late in the litter had lower umbilical vein pH (P = 0.05), base excess (P = 0.02) and higher lactate levels (P = 0.002) than piglets born earlier in the litter. In addition, piglets born early in the litter had higher sodium (P = 0.02), calcium (P = 0.005), but lower potassium ion (P = 0.005) concentrations than piglets born late in the litter, regardless of temperature treatments. There were no main effects of sex on any of the cord blood parameters. At birth, piglets born to heat-stressed gilts had greater skin meconium staining scores (CON = -1.09 ± 0.424 (back-transformed mean, 0.47) vs. HS = 0.06 ± 0.424 (back-transformed mean, 1.04), P = 0.022).

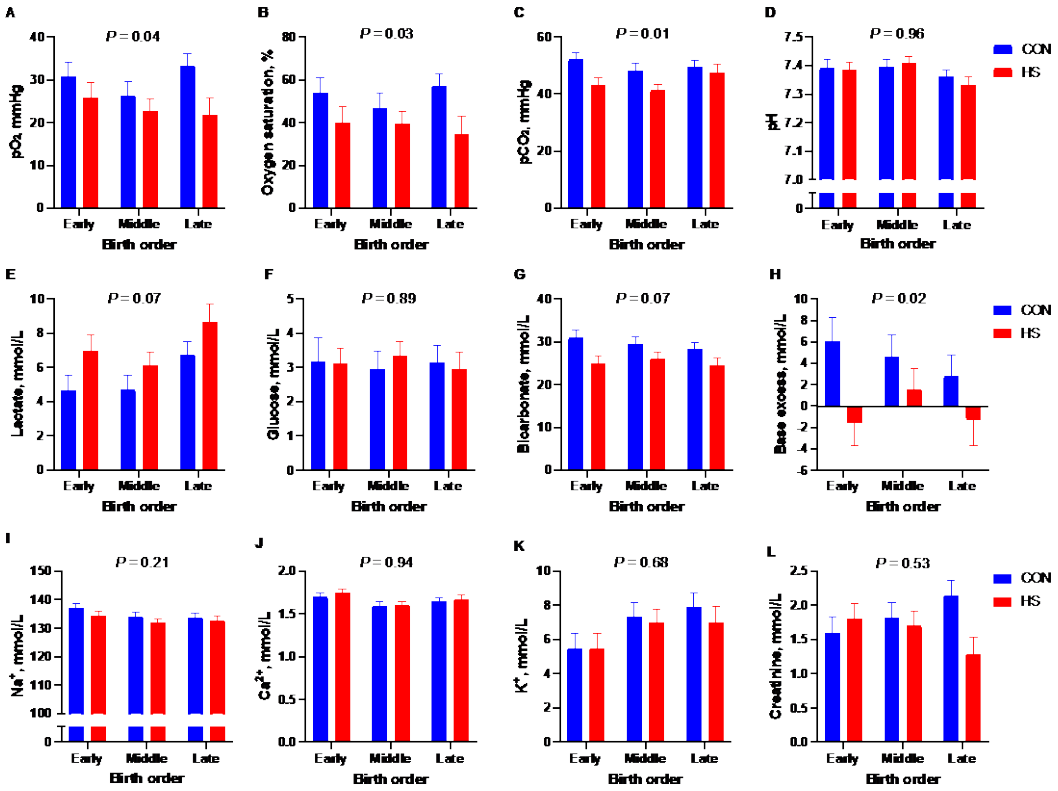

Figure 2. Predicted means (± SEM) for piglet umbilical vein parameters (A-L) from control (CON, n = 33 umbilical blood samples) or heat stress (HS, n = 28 umbilical blood samples) groups. Piglet birth order (first piglet to last piglet in the litter) was divided into thirds based on the total litter size of each gilt and classified as 'early' (first 1/3), 'middle' (middle1/3) and 'late' (last 1/3). P values represent the statistical significance of the main effect of temperature treatment from the linear mixed model.

Neonatal piglet development and morphology

The incidence of liveborn mortality within 48 h of age showed a trend to be higher in the HS group (HS = 8.1% vs. CON = 2.3%, P = 0.10). The impacts of maternal HS and gender on piglet morphology are presented in Table 3 (no significant interactions were identified). There were no differences between the CON and the HS groups for average piglet bodyweight at birth (P = 0.37), 24 h (P = 0.29) and 48 h of age (P = 0.29; Table 3). Piglet 24 h and 48 h average growth rates were not significantly different between groups (Table 3). Piglet rectal temperature at birth tended to be higher in the HS group (HS = 38.5 ± 0.37 vs. CON = 37.6 ± 0.33 °C, P = 0.09), but was not different between groups at 48 h of age (CON = 38.5 ± 0.18 vs. HS = 38.4 ± 0.18 °C, P = 0.83) (data not shown in the Table). Piglets born to gilts exposed to HS had a shorter small intestine than piglets born to CON gilts (P = 0.02; Table 3). However, the small intestine weight relative to length remained similar between groups (Table 3). The absolute brain weight was lower in piglets from the HS group (P = 0.001). However, relative brain weight was not different between groups (P = 0.83). There was an interaction (P = 0.02) between treatment and sex for relative muscle weight, such that the weight percentage was higher in females than males in the HS group (female = 0.26 ± 0.009 vs. male = 0.24 ± 0.009 %) but was similar between sexes (female = 0.25 ± 0.009 vs. male = 0.25 ± 0.008 %) in the CON group (data not shown in the Table). The effect of temperature treatments on other absolute or relative organ weights were not significant (Table 3). Regardless of temperature treatments, male piglets had heavier absolute spleen (P = 0.02) and brain (P = 0.004) weights but had a lighter relative heart weight (P = 0.04) than female piglets (Table 3). In addition, the male piglets had a longer small intestine than females (P = 0.03; Table 3).

Table 3. Predicted means (± pooled SEM) for piglet bodyweight and gross morphology.

Variable	Treatment[1]		SEM	Sex		SEM	*P* value	
	CON	HS		Female	Male		Treatment	Sex
Piglet bodyweight and growth rate								
Birth (incl. stillborn), kg	1.29	1.22	0.065	1.24	1.27	0.048	0.50	0.33
Birth (liveborn), kg	1.29	1.20	0.068	1.24	1.25	0.052	0.37	0.69
24 h, kg	1.41	1.29	0.074	1.35	1.36	0.057	0.29	0.94
48 h, kg	1.54	1.41	0.076	1.44	1.51	0.059	0.29	0.13
24 h growth rate, g/d	103	89	30.8	93	99	22.4	0.77	0.55
48 h growth rate, g/d	113	94	23.3	95	113	17.5	0.63	0.12
Piglet gross morphology at 48 h of age								
Small intestine								
Length, cm	393.6	351.7	10.84	364.4	381.0	8.48	0.02	0.03
Weight, g	66.8	56.4	4.94	61.5	61.7	3.67	0.17	0.95
Weight/length, g/cm	0.171	0.159	0.012	0.168	0.163	0.009	0.50	0.31
Length/bodyweight, cm/kg	255.4	256.9	11.44	255.1	257.1	8.67	0.93	0.78
Weight/bodyweight, %	4.39	3.98	0.176	4.27	4.11	0.141	0.13	0.21
Organ absolute weight & length								
Liver weight, g	57.6	50.2	4.95	53.4	54.4	3.61	0.32	0.50

Brain weight, g	31.8	28.3	0.57	29.1	31.0	0.51	0.001	0.004
Muscle weight, g	3.90	3.48	0.313	3.66	3.72	0.232	0.38	0.55
Heart weight, g	13.8	12.2	0.65	13.2	12.8	0.55	0.12	0.53
Spleen weight, g	2.61	2.33	0.226	2.32	2.63	0.174	0.41	0.03
Femur weight, g	3.95	3.36	0.251	3.62	3.71	0.191	0.12	0.61
Femur length, cm	4.15	3.99	0.077	4.06	4.08	0.061	0.18	0.63
Relative weight								
Femur weight/length, g/cm	0.947	0.827	0.048	0.876	0.899	0.038	0.10	0.53
Brain/liver weight, g/g	0.576	0.620	0.061	0.596	0.600	0.045	0.62	0.85
Muscle/femur weight, g/g	0.99	1.05	0.058	1.03	1.02	0.043	0.53	0.92
Muscle/bodyweight, %	0.249	0.250	0.008	0.254	0.246	0.006	0.91	0.20
Liver/bodyweight, %	3.74	3.56	0.170	3.68	3.62	0.134	0.45	0.60
Brain/bodyweight, %	2.12	2.09	0.119	2.09	2.11	0.093	0.83	0.78
Heart/bodyweight, %	0.904	0.869	0.031	0.924	0.849	0.028	0.43	0.04
Spleen/bodyweight, %	0.165	0.165	0.007	0.158	0.172	0.006	0.96	0.06
Femur/bodyweight, %	0.258	0.238	0.009	0.252	0.244	0.008	0.13	0.32

[1] Treatment: CON = control (constant 20 °C); HS = cyclic heat stress (0900 h to 1700 h, 30 °C; 1700 h to 0900 h, 28 °C). Piglet bodyweights were assessed for all newborn piglets, whereas morphology data were obtained from a subset of piglets (CON, n = 39 piglets; HS, n = 34 piglets) at 48h of age.

Piglet small intestinal barrier function and histology

There were no differences in jejunal (P = 0.56; Fig. 3A) or ileal (P = 0.30; Fig. 3B) TER between treatment groups. The jejunal villus height was similar between treatments (P = 0.26; Fig. 3C). Jejunal crypt depth was lower in piglets from the HS group compared to the CON group (P = 0.02; Fig. 3D). The villus height to crypt depth ratio was similar between treatment groups (P = 0.89; Fig. 3E). None of those variables were affected by piglet sex or treatment by sex interaction.

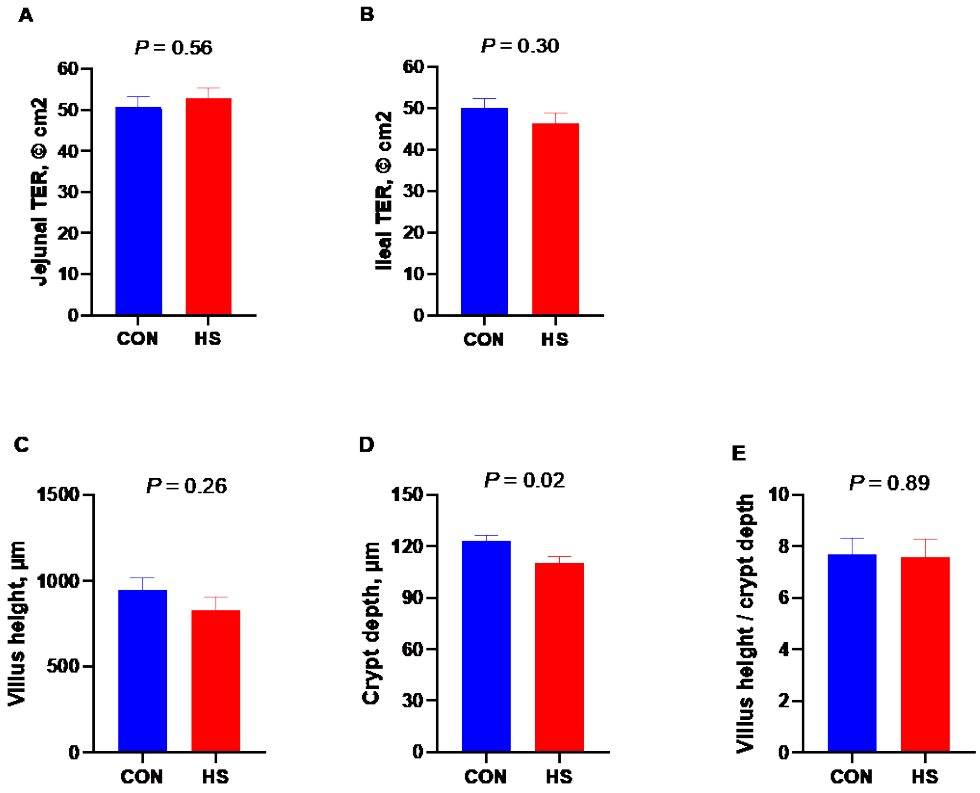

Figure 3. Predicted means (± SEM) for jejunal transepithelial electrical resistance (TER) (A), ileal TER (B), jejunal villus height (C), jejunal crypt depth (D) and villus height to crypt depth ratio (E) of piglets from control (CON, n = 39 piglets) or heat stress (HS, n = 34 piglets) groups.

Piglet plasma and colostrum biochemistry

Maternal HS did not change piglet plasma cortisol (CON = 1.75 ± 0.059 (back-transformed mean, 56.2) vs. HS = 1.83 ± 0.063 (back-transformed mean, 67.6) ng/mL, P = 0.39), IgG (CON = 21.9 ± 3.02 vs. HS = 18.7 ± 3.17 mg/mL, P = 0.50) or total protein (CON = 47.5 ± 2.81 vs. HS = 47.5 ± 2.99 mg/mL, P = 0.99) concentrations at 48 h of age. Gilts had similar colostrum IgG (CON = 41.4 ± 5.04 vs. HS = 43.4 ± 5.82 mg/mL, P = 0.80) and total protein (CON = 174.8 ± 8.62 vs. HS = 170.3 ± 9.95 mg/mL, P = 0.77) concentrations between treatments. There was a positive correlation between IgG and total protein concentration in piglet plasma at 48 h of age (r = 0.66, P = 0.002). However, there was no significant correlation between IgG and total protein concentration in the colostrum (r = 0.27, P = 0.35).

Experiment 2

There was no significant difference in rectal temperature on days -3 and 0 relative to farrowing (Figure 4). On day 1, a lower temperature was identified for the BET sows, with a trend was also noted for SAN sows on day 7.

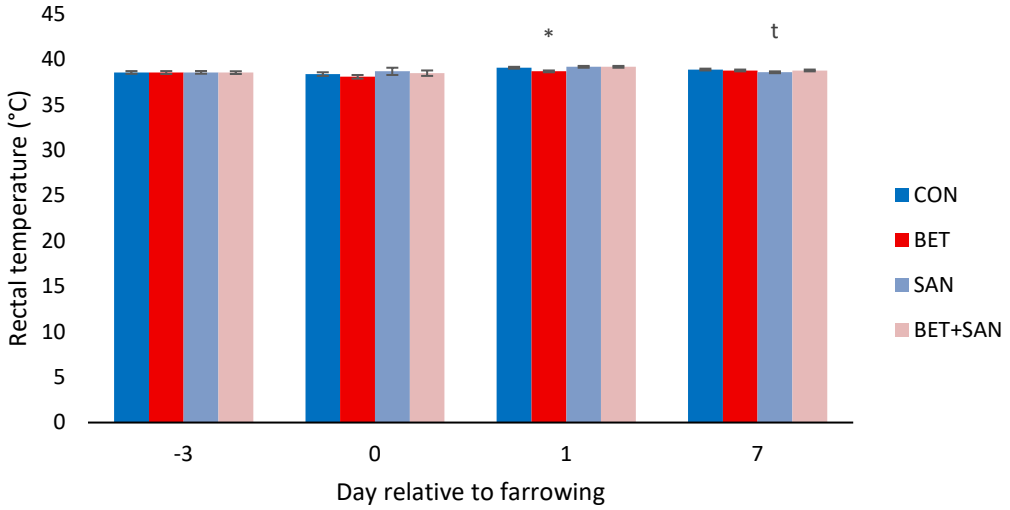

Figure 4. Predicted means (± SEM) for rectal temperature (°C) of sows supplied with water that contained no treatment (CON; n = 26), betaine (BET; 2.5L/1000L n = 26), sanguinarine (SAN; 100g/1000L n = 26) or a mixture of betaine and sanguinarine (BET+SAN; n = 26). * $P < 0.05$, ^{t} $P < 0.1$

There was no significant difference between any treatments in respiration rate at any day relative to farrowing (Figure 5).

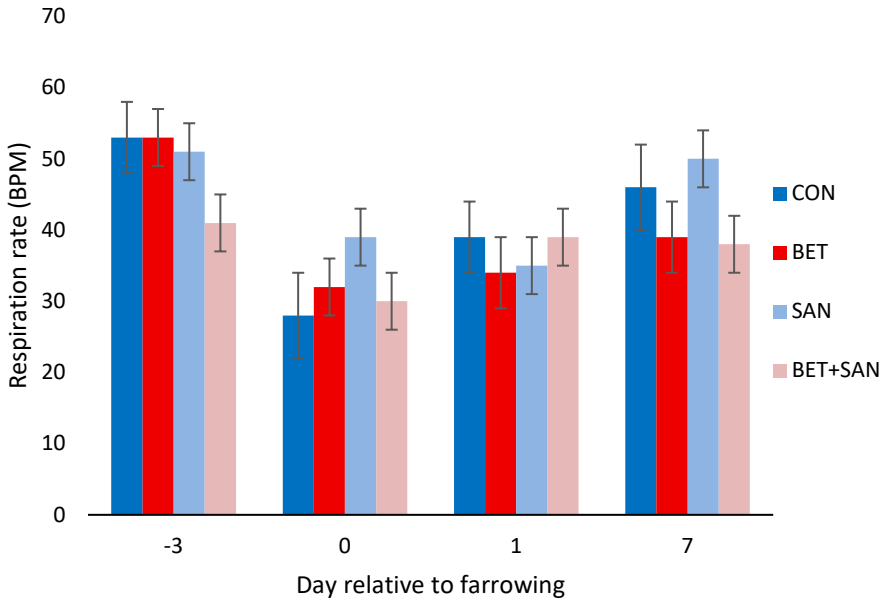

Figure 5. Predicted means (± SEM) for respiration rate (breaths per minute, BPM) of sows supplied with water that contained no treatment (CON; n = 26), betaine (BET; 2.5L/1000L n = 26), sanguinarine (SAN; 100g/1000L n = 26) or a mixture of betaine and sanguinarine (BET+SAN; n = 26).

There was a difference (P < 0.001) in the time sows spent on treatment before farrowing, with BET+SAN group spending significantly less time on treatment than the BET treatment group. No differences in any sow parameters were noted (Table 4).

A higher proportion (P < 0.001) of sows were induced to farrow in the SAN and BET+SAN treatment when compared to the CON sows, with BET sows having the lowest induction frequency of all groups (Table 5). Fewer BET sows, and more BET+SAN sows (P = 0.052), were observed to farrow during working hours than CON and SAN groups. Litters born in the CON treatment group were significantly heavier than other treatment groups, with a higher average pig and minimum weight. Coefficients of variation also showed a trend (P < 0.096) that control and BET + SAN sows had significantly less variation in litter size than BET or SAN treatments alone. Furthermore, sows in the CON and BET + SAN treatment groups gave birth to less piglets that were < 1.1 kg (Table 5) .

After 21 days, heavier litter weights still tended (P = 0.071) to be observed in the CON group. Average pig weight was higher (P = 0.007) in the Betaine + Sangrovit treatment group (Table 6)

Table 4. Predicted means (± SEM) for sow measures of sows supplied with water that contained no treatment (CON; n = 26), betaine (BET; 2.5L/1000L n = 26), sanguinarine (SAN; 100g/1000L n = 26) or a mixture of betaine and sanguinarine (BET+SAN; n = 26). Superscripts [a,b,c] indicate means significantly different from one another.

	CON		BET		SAN		BET + SAN		P value
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Parity	3.6	0.4	3.3	0.4	3.6	0.4	3.5	0.4	0.85
Time on treatment before farrowing (days)	3.8[a]	0.2	4.1[a]	0.2	3.6[a]	0.2	3.3[b]	0.2	< 0.001
Sow condition									
Entry weight (kg)	262.5	8.0	268.1	8.3	270.2	8.1	264.1	8.6	0.62
Exit weight (kg)	241.7	4.5	242.6	4.6	249.9	4.4	248.2	5.0	0.52
Weight change (kg)	18.4	7.4	18.6	7.6	17.2	7.4	16.0	7.7	0.92
Entry P2 (mm)	18.3	0.3	18.7	0.3	18.7	0.3	19.0	0.3	0.33
Exit P2 (mm)	18.0	0.3	18.4	0.3	18.7	0.3	18.6	0.3	0.28
P2 change (mm)	0.3	0.3	0.1	0.3	0.1	0.3	0.5	0.3	0.52
Total feed intake to 21 days (kg)	143.5	7.9	151.7	8.2	144.9	7.9	143.4	8.1	0.77

Table 5. Predicted means (± SEM) for farrowing performance of sows supplied with water that contained no treatment (CON; n = 26), betaine (BET; 2.5L/1000L n = 26), sanguinarine (SAN; 100g/1000L n = 26) or a mixture of betaine and sanguinarine (BET+SAN; n = 26). Superscripts [a,b,c] indicate means significantly different from one another.

Farrowing performance	**CON**		**BET**		**SAN**		**BET + SAN**		**P value**
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	
Induced (%)	53.9[a]	34.7-72.0	15.6[b]	5.7-36.1	84.7[c]	65.1-94.3	76.9[c]	55.7-89.8	< 0.001
Observed (%)	65.8[a]	44.8-82.0	40.7[b]	22.0-62.5	65.3[a]	44.3-81.7	84.2[c]	65.4-93.7	0.052
Assisted (%)	41.5	20.7-65.8	45.9	19.1-75.2	49.1	25.9-72.7	35.3	16.8-59.6	0.88
Number of assists per sow	0.9	0.3	0.7	0.3	1.1	0.3	0.9	0.3	0.80
Number of piglets removed per sow	1.4	0.6	0.7	0.4	1.1	0.5	0.7	0.3	0.69
Farrowing duration (min)	284	32	239	42	303	32	321	46	0.58
Inter-piglet birth interval (min)	19.8	3.5	20.0	4.0	22.3	3.4	24.6	4.3	0.70
Sows that gave birth to piglets born dead (%)	50.6	31.7-69.3	51.0	30.9-70.7	60.3	40.4-77.4	38.2	21.2-58.8	0.49

*95% confidence interval presented rather than SEM for binary data.

Table 6. Predicted means (± SEM) for litter performance of sows supplied with water that contained no treatment (CON; n = 26), betaine (BET; 2.5L/1000L n = 26), sanguinarine (SAN; 100g/1000L n = 26) or a mixture of betaine and sanguinarine (BET+ SAN; n = 26). Superscripts [a,b,c] indicate means significantly different from one another.

Litter weight	**CON**		**BET**		**SAN**		**BET + SAN**		**P value**
Day 1	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	
Litter weight (kg)	17.1	0.9	15.6	1.0	14.5	0.9	15.4	1.0	0.027
Average pig weight (kg)	1.43[a]	0.06	1.32[a]	0.06	1.26[b]	0.06	1.40[a]	0.06	0.031
Minimum pig weight (kg)	0.99[a]	0.07	0.86[ab]	0.07	0.84[b]	0.06	1.02[a]	0.06	0.024
Maximum pig weight (kg)	1.81	0.07	1.74	0.07	1.65	0.07	1.84	0.07	0.14
Standard deviation litter weight (kg)	0.25	0.02	0.27	0.02	0.26	0.02	0.25	0.25	0.96
Co-efficient of variation pig weight (%)	13.8	1.1	15.6	1.1	15.8	1.0	13.8	1.1	0.096
Number of pigs < 1.1kg	2.2[a]	0.6	3.3[ab]	0.9	4.0[b]	1.0	2.0[a]	0.6	0.046
Day 21									
Litter weight (kg)	59.2	4.9	51.0	5.0	50.3	4.9	53.6	5.0	0.071
Average pig weight (kg)	5.6[ab]	0.2	5.3[b]	0.2	5.3[b]	0.2	5.8[ab]	0.2	0.007
Minimum pig weight (kg)	3.8	0.2	3.6	0.2	3.5	0.2	4	0.2	0.18
Maximum pig weight (kg)	7.00	0.3	6.8	0.3	6.9	0.3	7.3	0.3	0.26
Standard deviation litter weight (kg)	1.07	0.1	1.05	0.1	1.11	0.1	1.13	0.1	0.85

Co-efficient of variation pig weight (%)	19.0	1.3	20	1.4	21.1	1.3	19.6	1.4	0.62
Number of pigs < 4kg	1.3	0.3	1.5	0.3	1.7	0.3	0.8	0.2	0.11

Experiment 3

Figure 6 outlines the environmental conditions experienced by the sows on the day of farrowing. On the busiest farrowing days, internal shed temperature was less than 25°C in all three replicate blocks.

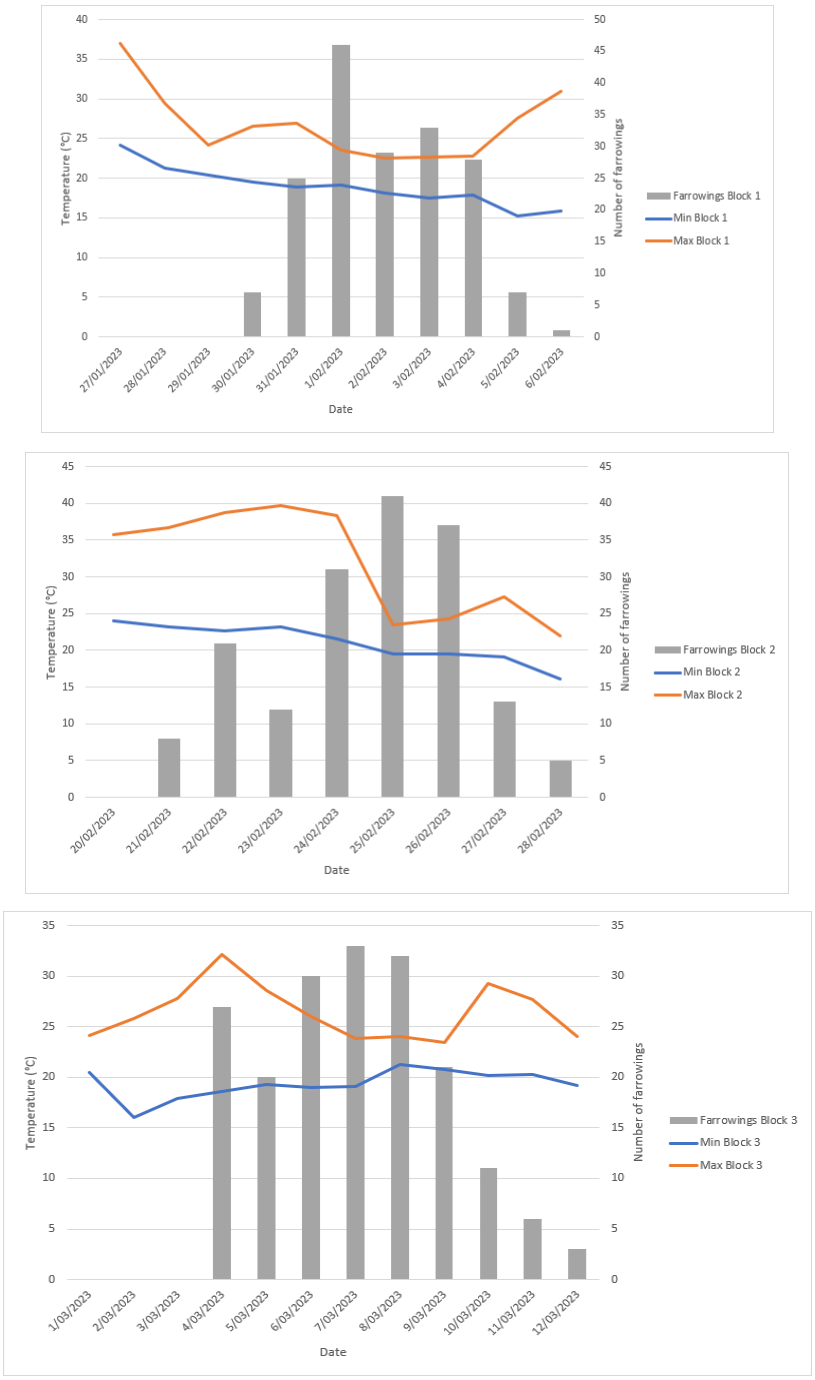

Figure 6. Average minimum (blue line) and maximum (orange line) daily temperatures and the number of sows farrowed (grey bar) for each date on the three time replicates (blocks).

There was no significant difference in farrowing performance between CON and SAN sows (Table 7).

Table 7. Predicted means (± SEM) for farrowing performance measures from sows supplied with water that contained no treatment (CON) or sanguinarine (SAN; 100g/1000L).

	CON		SAN		P value
	Mean	SEM	Mean	SEM	
n	223		305		
Previous performance					
Total pigs born	12.2	2.07	12.9	1.57	0.68
Pigs born alive	11.4	1.95	12.2	1.48	0.75
Pigs born dead	0.8	1.18	0.8	0.14	0.93
Number of pigs weaned	10.9	1.86	10.6	1.29	0.85
Current performance					
Parity	2.8	0.13	2.8	0.12	0.93
Gestation length (days)	116.4	0.86	116.2	0.73	0.99
On treatment (days)	4.6	0.37	4.6	0.32	0.95
Observed (%)	22	16-29	24	18-30	0.65
Assisted (%)	31	18-48	21	11-48	0.27
Total pigs born	13.2	1	13	0.84	0.86
Pigs born alive	12.4	0.95	12	0.78	0.77
Pigs born dead	0.8	0.09	0.9	0.08	0.42
Sows with ≥ 1 piglet born dead (%)	53	45-60	47	41-53	0.25
Litter size after fostering	11.8	0.9	11.5	0.75	0.78
Number of pigs weaned	9.6	0.74	9.4	0.62	0.89
Pre-foster deaths	0.5	0.06	0.5	0.05	0.78
Post-foster deaths	1.0	0.11	1.0	0.09	0.53
Piglets removed	1.5	0.14	1.5	0.12	0.94
Liveborn mortality	1.5	0.14	1.4	0.12	0.59
Total mortality	2.4	0.21	2.4	0.18	0.98

*95% confidence interval presented rather than SEM for binary data.

An increase in the maximum piglet weight (P = 0.045) was noted in the CON group the day after farrowing (Table 8). After 21 days, litter weights were significantly heavier in the CON litters compared to SAN.

Table 8. Predicted means (± SEM) for litter weight measures from sows supplied with water that contained no treatment (CON) or sanguinarine (SAN; 100g/1000L).

Piglet Weights	CON		SAN		P value
	Mean	SEM	Mean	SEM	
n	223		305		
Day 1					
Number	12.1	2.18	12.0	1.94	0.99
Total weight (kg)	16.9	0.52	16.0	0.47	0.19
Minimum weight (kg)	1.0	0.04	1.0	0.04	0.61
Maximum weight (kg)	1.8	0.06	1.7	0.05	0.045
Average weight (kg)	1.3	0.04	1.3	0.04	0.24
Co-efficient of Variation (%)	17.8	0.90	16.2	0.8	0.17
Number of pigs < 1.1kg	2.3	0.52	2.9	0.53	0.46
Day 21					
Number	10.5	1.91	9.9	1.61	0.79
Total weight (kg)	59.7	2.06	54.2	1.84	0.044
Minimum weight (kg)	3.9	0.14	3.8	0.13	0.39
Maximum weight (kg)	7.3	0.17	7.0	0.16	0.31
Average weight (kg)	5.7	0.13	5.5	0.11	0.34
Co-efficient of Variation (%)	18.9	0.9	18.8	0.8	0.93
Number of pigs < 4kg	0.9	0.24	1.1	0.29	0.52

There was no significant effect of treatment on lactation failure rate, wean to service interval, the incidence of sows bred within seven days of weaning or pregnancy rate (Table 9).

Table 9. Predicted means (± SEM) for rebreeding of sows supplied with water that contained no treatment (CON) or sanguinarine (SAN; 100g/1000L).

	CON		SAN		
	Mean	SEM	Mean	SEM	P value
Subsequent Reproduction					
Lactation failure (%)*	4.0	2-8	7.0	4-10	0.25
Lactation length*	25.0	1.88	25.2	1.6	0.95
WSI (days)	6.8	0.59	6.4	0.47	0.57
Bred < 7 days (%)*	84	77-89	87	82-91	0.41
Pregnancy rate (%)*	84	77-89	85	78-90	0.79

*95% confidence interval presented rather than SEM for binary data.

4. Application of Research

The overarching hypothesis of this project was that heat stress at farrowing would have negative impacts on sow farrowing performance leading to impaired piglet

survival and growth, and sow subsequent reproduction, and that in water additives previously shown to reduce heat stress would reverse these negative effects.

The principal findings of the first experiment were that heat exposure of pregnant gilts during the last week of gestation and farrowing upregulated maternal stress responses and altered farrowing physiology. The extended farrowing durations in heat stressed sows resulted in increased antenatal piglet stress, as evidenced by increased meconium staining, reduced placenta oxygen and dramatically increased stillbirth rates. Of note, although the stillbirth rate for the heat stress cohort was 30% overall, this was not evenly distributed and there was a propensity for certain sows to have larger proportional losses, whereas other sows were less effected. In itself this indicates that there are opportunities through selection of husbandry interventions to make a meaningful reduction in the levels of stress experienced by sows during heat waves and the subsequent effects on reproductive performance. To the best of our knowledge, this is the first study to demonstrate that cyclic heat stress imposed during late gestation and farrowing (30ºC between 9am and 5pm and 28ºC between 5pm and 9am) negatively affects oxygen supply through the umbilical cord in pigs. These findings emphasize the detrimental effects of sow heat at these levels in the farrowing shed on sow welfare and piglet survival.

In Experiment 2, there were very few impacts of treatment on markers of heat stress, farrowing performance and so piglet survival. This is likely explained by the mild conditions experienced at the experimental site during the farrowing period. Using a recently published paper that defines heat stress for late gestation sows (McConn et al., 2022), 38% of the sows experienced mild heat stress (24˚C), 32% moderate heat stress (25.5˚C) and 18% severe heat stress (30.8˚C) on the day of farrowing. There was a difference in day 1 litter weight, average piglet weight, and number of piglets < 1.1kg, with the SAN piglets being the smallest. This is unlikely due to the actual treatments applied, but rather an artifact of low numbers. Despite piglets from the SAN treatment being at a severe weight disadvantage, peri-natal mortality was numerically the lowest in this treatment.

Because of the mild conditions, and similar performance of SAN litters despite the smaller piglet size, Experiment 3 tested SAN over a larger sample size. Unfortunately, similar environmental conditions to the year prior were experienced, and there was no advantage of the in-water additive tested. What should be noted was the ease in which the in waterline dosers could be set up, turned on/off and the fact that no specialised diet was required with associated milling, transport and storage implications. With dosers now commonplace in nursery and grower facilities, the last two experiments successfully administered in water additive to sows, and work testing this application strategy should continue.

5. Conclusion

The current data demonstrated that pregnant gilts were susceptible to elevated temperatures in the last week leading up to and during farrowing, as evidenced by

higher body temperatures, a marked reduction in feed intake, increased plasma cortisol concentrations and poor farrowing outcomes. Gilts exposed to cyclic heat stress farrowed more stillborn piglets and fewer liveborn piglets. Maternal heat stress reduced oxygen supply from the placenta to the piglet through the umbilical cord, explaining the increased stillborn rates recorded. However, the causes of heat stress-induced umbilical oxygen insufficiency remain complicated but seem to be linked to placental hypoxia. In those liveborn piglets, maternal heat stress had an immediate impact on the length and crypt depth of piglet small intestine, although changes in other phenotypes were less evidenced within the first 48 h of postnatal life.

The two in-water strategies, betaine and sanguinarine, did not improve sow and piglet farrowing performance above the control treatment, but it could be argued that the sows experienced low levels of heat stress. Future work should continue testing in water means of reducing late gestation and farrowing heat stress.

6. Limitations/Risks

The obvious limitation of this project was the mild conditions experienced by the sows in late gestation and at farrowing during Experiments 2 and 3. There may be merit in screening other in-water strategies when sows are housed in climate controlled experimental facilities.

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

1. Sows are suggestible to heat stress during parturition, and this has negative impacts on piglet morphology, survival and growth.
2. Farrowing houses should be climate controlled (< 24°C) to limit the heat stress experienced by the sow during this crucial reproductive stage.
3. None of the in-water treatments were effective at improving sows farrowing performance, but efforts should continue as in-water means of ameliorating heat stress may be a simple strategy in naturally ventilated farrowing houses.

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Appendices

Weicheng Zhao completed his Doctor of Philosophy in 2022 at The University of Melbourne with thesis titled *Impact of gestational heat stress on the fetus and neonatal piglet* https://hdl.handle.net/11343/326444