Australasian Pork Research Institute Ltd
APRIL

NOVEL ASPIRIN SUPPLEMENTATION DURING GESTATION TO IMPROVE FARROWING RATE AND PIGLET BIRTH WEIGHT OF SOWS MATED IN SUMMER

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Executive Summary

Sows mated in summer have a reduced farrowing rate, and the farrowed sows usually produce an increased percentage of born-light piglets with increased carcass fatness at slaughter. Previous studies have demonstrated that the loss of embryos during early gestation accounts for the majority of reproductive failure of sows that are mated in summer. Reduced piglet birth weight is likely to be a consequence of impaired fetal development caused by placental insufficiency. A low dose of Aspirin (e.g., 80 mg per day) has been used in human medicine to facilitate implantation and alleviate placental complications. We hypothesised that a low dose of Aspirin supplementation (240 ppm) during day 0 to 80 of gestation would improve the farrowing rate of sows mated in the summer and increase the piglet birth weight.

The research project consisted of two experiments: a safety study and an efficacy evaluation study. The safety study was conducted in the non-summer month. Sows were fed either a control gestation diet (n=20 sows) or an Aspirin-supplemented gestation diet (n=20 sows; 240 ppm) from day 1 to day 38 post-mating. The study showed that Aspirin supplementation did not affect pregnancy rate by day 38, body condition score or observed appetite. The main study was conducted on the sows that were lactated, weaned, and mated in summer. Sows were fed either a control gestation diet (n=197 sows) or an Aspirin-supplemented gestation diet (n=200 sows; 240 ppm) from day 1 to day 80 post-mating. Plasma samples were collected from day 30 of gestation. The plasma prostaglandin metabolite was measured as a biomarker of prostaglandin synthesis. Progesterone concentration was measured. The number of piglets born alive and stillborn was measured from farrowed sows. Individual piglet birth weight was measured from approximately 50% of randomly selected litters.

Results showed that Aspirin supplementation tended to reduce ($P<0.10$) plasma prostaglandin metabolite concentration, by 22%. However, plasma progesterone concentration was not affected. Aspirin supplementation did not affect the farrowing rate (av 70%) and average piglet birth weight (av. 1.3 kg). The farrowing rate and birth weight were lower than the farm records during the non-summer months.

In conclusion, 240 ppm supplementation of Aspirin during days 0-80 of gestation did not improve the farrowing rate or piglet birth weight of the sows lactated and mated in summer. Aspirin supplementation was shown in this study not to be an effective strategy to alleviate summer infertility or fetal growth restriction (as indicated by birth weight) in pigs. The research findings also indicated that the low dose of Aspirin (240 ppm) is safe for gestating sows in terms of reproductive performance, and it is a potential strategy for manipulating prostaglandin synthesis in gestating sows.

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1. Introduction

Sows mated in summer have a reduced farrowing rate, and the farrowed sows produce an increased percentage of born-light piglets with increased carcass fatness at slaughter (Liu *et al*. 2020). This seasonal phenomenon reduces the efficiency and profitability of commercial pig production. Research showed that the loss of embryos during early gestation accounts for the majority of reproductive failure of sows that are mated in summer (Love *et al*. 1993), and the reduced piglet birth weight is likely to be a consequence of impaired fetal development caused by placental insufficiency (Zhao *et al*. 2020). There have been no widespread solutions to consistently alleviate the above negative impacts of summer conditions on farrowing rate and progeny carcass characteristics.

Aspirin supplementation may be a novel strategy to prevent early embryo loss of sows mated in summer. Based on some indirect evidence, the early embryo loss of sows mated in summer may be a consequence of increased prostaglandin F2 alpha (PGF-2α) secretion. For example, an early study from pregnant cows showed that summer conditions can increase PGF secretion from the endometrium (Putney *et al*. 1989). This fact has recently been confirmed by an *in vitro* study using endometrium stromal cells (Sakai *et al*. 2018). PGF is known as a luteolysin that can cause regression of corporal luteal and thus interfere with progesterone secretion for embryo implantation and maintenance (Gordon 1997). It is known that Aspirin can inhibit PGF synthesis by inactivating the cyclooxygenase enzyme (Higgs *et al*. 1987). A literature review suggested that the inhibitors of PGF-2α have beneficial but inconsistent effect in improving fertility in cattle that have undergone embryo transfer (Spencer *et al*. 2020b; Jaśkowski *et al*. 2021).

Aspirin supplementation during gestation may improve fetal development. A study in sheep showed that heat stress conditions during gestation can cause fetal growth restriction by reducing umbilical blood flow rate by 23% (Limesand *et al*. 2007). A systematic review and meta-analysis showed that Aspirin oral administration at a low dose (50-150 mg daily) fed to mothers before 16 weeks of gestation can significantly reduce the risk of preeclampsia and fetal growth restriction in human infants (Roberge *et al*. 2017).

As PGF is required for normal reproductive processes, particularly during farrowing and ovulation, Aspirin administration should cease 28 days before farrowing. A Pork-CRC-funded study showed that injecting a PGF inhibitor in pre-farrowing sows increased stillborns and reduced the subsequent farrowing rate (Plush *et al*. 2021). The negative impacts were suspected to be related to the lack of endogenous PGF for inducing uterine muscle contraction and ovulation, respectively. The half-life of Aspirin is 5 days, so ceasing the administration from 28 days before farrowing is enough for complete clearance of Aspirin in animals before farrowing.

Based on the above evidence, we hypothesised that supplementing a low dose of Aspirin (240 ppm sodium salicylate; equivalent to 2 mg/kg body weight) during the first 80 days of gestation:

(1) Can prevent early embryo loss and thus improve the farrowing rate of sows mated in summer;
(2) Can increase piglet birth weight or reduce the within-litter proportion of piglets born below 1.1 kg.

2. Methodology

The research project consisted of a safety experiment (Experiment 1) and a main study (Experiment 2). All procedures that involved animals in Experiments 1 and 2 were in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (8th edition, 2013), and the protocol (ID:21-045) was approved by the Animal Ethics Committee of Rivalea Australia Pty Ltd, Corowa, NSW, Australia.

Experiment 1 (safety study)

Animal husbandry

To reduce the risk of any unexpected side effects of Aspirin treatment, a safety study (Experiment 1) was conducted using a small number of sows (n=20 sows on the control diet and 20 sows on the Aspirin diet). Experiment 1 started on 13 September 2021 and concluded on 24 December 2021. Sows were allocated to either a control diet or an Aspirin diet after mating. The gestation diet formulation is shown in Table 1. The Aspirin diet contained 240 mg of sodium salicylate (Abbey Animal Health Pty Ltd, Glendenning, NSW, Australia). Gestating sows were housed in pens of 7 or 6 with manual feeding. Feeding was conducted by dropping the calculated amount of feed on the concrete floor in the morning (i.e., 2.4 kg feed per sow).

Measurements

Sow body weight and backfat thickness were monitored at the entry to the gestational shed and on the 38th day of gestation. Body condition score was assessed once per week on individual sows (Pairis-Garcia and Moeller 2017). Briefly, the score 1 means emaciated, score 2 means thin, score 3 is ideal, score 4 means fat, and score 5 means overly fat. Visual examination was conducted once per week to determine whether blood existed in the faeces. The presence of faecal blood was used as indication of ulcers developed in the gastrointestinal tract. The pregnancy of sows was checked using transabdominal ultrasound at the fourth and sixth week of gestation as per commercial practice.

Experiment 2

Animal husbandry

Sows that were weaned and mated in summer were allocated to the control (n=200 sows) and Aspirin-supplemented diet (n=197 sows) on the second day of mating over 5 weeks of mating batches (15 January- 13 February 2023). The experiment was conducted in a commercial piggery (Rivalea Australia Pty Ltd, Corowa, NSW, Australia). The experimental sows were mixed with commercial (non-trial) sows and housed in one large gestation pen with six electronic feeders for a total capacity of feeding 200 sows. All the feeders have two overhead hoppers for storing control and Aspirin diet. The allocated diet (either control or Aspirin diet) dropped into the feeder when the feeder sensor detected the presence of the radio frequency identification ear tag of the sow. Gestating sows were given a 2.4 kg diet allowance per day until the 108th day of gestation. The gestation diet formulation is shown in **Table 1**. The Aspirin diet contained 240 mg of sodium salicylate (Abbey Animal Health Pty Ltd, Glendenning, NSW, Australia). Experimental sows were moved to farrowing houses on average of 108 days in gestation and then farrowed in individual farrowing crates.

Farrowing outcomes

The pregnancy of sows was checked using transabdominal ultrasound as described above. Sows that returned to oestrus, found to have a negative pregnancy test, and showed abortions were counted as reproductive failure. Sows that were dead, lame or refused to eat from the ESF feeders were recorded as off-trial for non-reproductive reasons. The reproductive failure rate was calculated as the ratio of the number of reproductive failures and the number of sows mated in each group. The farrowing rate of sows was counted as a ratio between the number of farrowed sows and the number of sows mated in each group. The number of piglets born alive, stillborn and mummified were recorded within the first 24 hours post-farrowing before cross-fostering. Meanwhile, the newborn piglets were individually weighed using a digital scale. Individual newborn piglets (excluding mummies) were weighed using a digital scale in 92 randomly selected litters per treatment. The percentage of piglets that weighed ≤ 1.1 kg at birth and the coefficient of variation (CV) were calculated on a litter basis.

Physiological monitoring for signs of heat stress

A total of 32 focal gestating sows from each treatment were monitored for rectal temperature and respiration rate as the physiological signs of heat stress. The physiological monitoring was conducted one day per week at 13:00 h for the first 7 weeks of gestation. Rectal temperature was measured using a digital thermometer (Model DT-K01A; Liberty Health Products, VIC, Australia). The respiration rate was monitored by visually counting the number of chest contractions during a 30-second duration.

Blood sampling, Progesterone and PGFM assay

Blood samples were obtained from 12 mixed-parity sows from each treatment group via jugular venipuncture on d 30 of gestation. Blood samples were collected in heparinised vacutainers (BD Vacutainers, 10 mL, Item Number 367883, BD Diagnostics, Preanalytical Systems, Oxford, UK). Then, plasma samples were separated after centrifugation at 1,600 × g for 10 min under 4°C (Heraeus Megafuge 16R, Item Number 50122064, Thermo Fisher Scientific, North Ryde, NSW, Australia). The plasma samples were stored at -20 °C and later used to measure progesterone and 3, 14-dihydro-15-keto-prostaglandin F2-alpha metabolite (PGFM) concentrations.

The progesterone assay was conducted using a radioimmunoassay kit (cat. # IM1188; Beckman Coulter, Brea, CA, USA) with a minimum detection limit of 0.11 ng/mL. The progesterone assay was conducted in duplicate in one run, and the intra-assay CV was 6.7%. The PGFM assay was conducted using an ELISA kit (cat. # MBS7214882; MyBiosource, San Diego, CA, USA) with a minimum detection limit of 50 pg/mL. The PGFM assay was duplicated in one run, and the intra-assay CV was 5.8%.

Statistical analyses

Sow body weight, feed intake, litter measurements, and blood analysis data were analysed using the UNIVARIATE procedure of SPSS (IBM SPSS Statistics for Windows, v27, Armonk, NY, USA) for the main effect of sow treatments (Control vs Aspirin) with the parity of sows (defined as Parity 2, 3, 4, and 5+) as a blocking factor. Results are presented as mean ± standard error (SE). Reproductive failure (a total count of return, abortion, negative pregnant test) and the farrowing fate of sows (defined as farrowed or not farrowed) were analysed by Pearson's Chi-square analysis and reported as a

percentage of the total distribution. Means were considered significantly different when $P \leq 0.05$, and a trend was considered when $P \leq 0.10$.

3. Outcomes

Experiment 1 Results

Weekly visual assessment did not find any faecal blood (Table 2). Two sows from the control group showed temporary signs of lameness during the weekly monitoring but recovered the week after (**Table 2**). One sow from the Aspirin group had reduced appetite in the third week of gestation but regained appetite the week after (**Table 2**).

The pregnancy rate by the 38th day post-mating did not differ (P = 0.38) between control and Aspirin treatment (**Table 3**). The body weight gain or backfat gain was not significantly affected by the Aspirin supplementation (both P>0.1) (**Table 3**). The body condition score of gestating sows was not different between treatments (Diet, P = 0.24; Diet*Week=0.79) during the first six weeks of gestation (**Figure 1**).

Experiment 2 Results

Feed intake and body condition of gestating sows

After mating, sows that were allocated to Aspirin and Control groups had a similar parity (P = 0.96), body weight (P = 0.91) and backfat (P = 0.51) (**Table 4**). At entry to the farrowing house (approximately 108th day of gestation), body weight (P = 0.40) and backfat (P = 0.98) were both similar between the two treatment groups. The change in body weight (P = 0.16) and backfat (P = 0.60) in this duration was similar between the control and aspirin groups (**Table 4**).

Farrowing outcomes

Aspirin supplementation did not affect the percentage of sows that showed reproductive failure or farrowing rate (both P > 0.1) (**Table 5**). Aspirin supplementation tended to alter (Chi-square = 4.5, df = 2, P = 0.106) the distribution of reproductive failures among the early, middle and late gestational stages. Specifically, the sows supplemented with Aspirin tended to have a lower percentage of reproductive failure in early gestation but a higher percentage in late gestation than the control sows (**Figure 2).**

Total number of piglets born, born alive, stillborn and mummified fetuses did not differ between treatments (all P>0.1). Average birth weight, percentage of pigs less than 1.1 kg, and within-litter CV of birth weight was similar between treatments (all P>0.1).

Plasma concentration of prostaglandin metabolite (PGFM) and progesterone

Aspirin supplementation tended to reduce plasma PGFM concentration (P = 0.104) from 300 pg/mL to 235 pg/mL (**Figure 3**). The Aspirin supplementation did not significantly affect plasma progesterone concentration (**Figure 4**).

Physiological signs of heat stress of gestating sows

The respiratory rate was linearly reduced with gestational weeks (Week, P = 0.011) (**Figure 5 A**). Aspirin supplementation did not affect respiration rate (Aspirin, P = 0.63). The interaction between Aspirin and gestational weeks was insignificant (P = 0.72).

Rectal temperature linearly declined with gestational weeks (Week, P < 0.001) (**Figure 5 B**). The main effect of Aspirin on rectal temperature was not significant (Aspirin, P = 0.32). However, the interaction between Aspirin and gestational weeks tended to be significant (P = 0.070), such that the sows supplemented with Aspirin had lower rectal temperature than the control group on the second week of gestation, but sows on Aspirin had a higher rectal temperature than control sows on the fourth and fifth week of gestation.

Table 1. Formulation of basal diet (as-fed).

Ingredient	Inclusion rate
Wheat, %	31
Barley, %	44
Millmix, %	10
Canola meal, %	6
Soybean meal, %	3
Fish oil, %	0.2
Tallow, %	1.0
Limestone, %	1.5
Di-Calcium Phosphate, %	1.0
Lysine-HCl, %	0.16
Premix, %	0.3
Calculated energy and nutrients:	
Digestible energy, MJ/kg	13.3
Crude protein, %	12.2
Fat, %	2.8
Calcium, %	1.00
Available phosphorus, %	0.48
Available lysine, %	0.66

[a] Supplied per kg of diet: copper, 15 mg; manganese, 25 mg; zinc, 55 mg; iron, 91 mg; iodine, 1.7 mg; selenium, 0.27 mg; chromium, 0.56 mg; vitamin A, 15000 IU; vitamin D3, 3200 IU; vitamin K, 1.0 mg; vitamin B-1, 1.5 mg; vitamin B-2, 5.0 mg; vitamin B-6, 3.0 mg; vitamin B-12, 81 µg; niacin, 30 mg; pantothenic acid, 15 mg, folic acid, 2.7 mg; vitamin C, 55 mg; biotin, 0.16 mg; vitamin E, 77 IU.

Table 2. Weekly health assessment record (Exp 1. Safety study).

			Control							Control							Control							Aspirin							Aspirin							Aspirin						
Days in gestation	Date	Measurements	Sow 37	Sow 40	Sow 28	Sow 38	Sow 14	Sow 18	Sow 25	Sow 9	Sow 29	Sow 8	Sow 20	Sow 33	Sow 13	Sow 1	Sow 23	Sow 10	Sow 7	Sow 32	Sow 4	Sow 22	Sow 39	Sow 35	Sow 3	Sow 19	Sow 5	Sow 21	Sow 36	Sow 15	Sow 26	Sow 2	Sow 24	Sow 31	Sow 12	Sow 6	Sow 11	Sow 16	Sow 17	Sow 27	Sow 30	Sow 34
2	15/9/21	Lameness (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		Evidence of fecal blood (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		Body condition score	3	3	3	3	3	2	3	3	4	4	4	3	1	3	4	2	3	3	3	3	3	3	2	3	4	3	2	2	2	2	4	3	2	4	3	4	4	4	4	4	4
		Not interested in feed (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	21/9/21	Lameness (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		Evidence of fecal blood (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		Body condition score	3	3	3	3	3	2	4	3	3	3	4	4	2	3	4	3	3	3	3	3	3	4	2	4	4	3	3	3	4	3	4	3	3	4	3	4	3	4	4	4	4
		Not interested in feed (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	N
14	28/9/21	Lameness (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		Evidence of fecal blood (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		Body condition score	3	4	3	3	3	2	4	3	3	4	4	4	2	4	4	3	3	3	3	3	3	4	3	4	4	4	3	3	4	3	4	4-lost	3	3	4	3	4	4	4	4	4-lost tag
		Not interested in feed (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
21	10/06/2021	Lameness (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	N	N		N	N		N	N	N		N	N		N	N	N	N	N	N	N	N	N
		Evidence of fecal blood (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		N	N		N	N	N		N	N		N	N	N	N	N	N	N	N	N
		Body condition score	3	4	3	3	3	2	4	3	4	4	4	4	2	3	4	3	3	3	3	3	3		2	3		3	3	3		3	4		3	3	3	4	4	4	4	4	4
		Not interested in feed (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		N	N		N	N	N		N	N		N	N	N	N	N	N	N	N	N
28	13/10/21	Lameness (Y/N)	N	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		N	N		N	N	N		N	N		N	N	N	N	N	N	N	N	N
		Evidence of fecal blood (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		N	N		N	N	N		N	N		N	N	N	N	N	N	N	N	N
		Body condition score	3	4	3	3	3	2	4	3	3	4	4	4	2	3	4	3	3	4	3	3	4		2	3		3	3	3		3	4		3	4	3	4	3	4	4	4	4
		Not interested in feed (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		N	N		N	N	N		N	N		N	N	N	N	N	N	N	N	N
35	19/10/21	Lameness (Y/N)	N	N		N	N	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N		N	N		N	N	N		N	N		N	N	N	N	N	N	N	N	N
		Evidence of fecal blood (Y/N)	N	N		N	N	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N		N	N		N	N	N		N	N		N	N	N	N	N	N	N	N	N
		Body condition score	4	4		3	3	2	4	3	3	3	4	4	2	3	4		3	4	3	3	3		3	3		4	3	3		4	4		3	4	3	4	4	4	4	4	4
		Not interested in feed (Y/N)	N	N		N	N	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N		N	N		N	N	N		N	N		N	N	N	N	N	N	N	N	N

[1]Notes: No fecal blood was detected in any sows (n=20 control and 20 Aspirin sows). Two sows from the control group showed temporary sign of lameness during the weekly monitoring but recovered the week after. One sow from the Aspirin group was not interested in feed in the 3rd week of gestation but regained appetite the week after.

Table 3. Effects of Aspirin supplementation during d 0-38 on pregnancy rate, body weight and backfat of gestating sows (Exp 1. safety study).

Measurements	Control (n=20)	Aspirin (n=20)	SEM	P-values
% Pregnant sows by d 38[1]	90% (18/20)	80% (16/20)		0.38
Body weight, d 0, kg	226	238	0.68	0.16
Body weight, d 38, kg	244	252	6.6	0.39
Change of body weight, kg	18	13	3.5	0.25
Backfat, d 0, mm	13.1	14.1	0.65	0.24
Backfat, d38, mm	14.6	15.9	0.44	0.03
Change of backfat, mm	1.5	1.8	0.55	0.70

[1] Two sows from the control group were found not pregnant on the 35th day after mating (irregular returns); four sows (including one sow with a history of a return) from the Aspirin group were found not pregnant on the 21st day after mating (regular returns).

Table 4. Effects of Aspirin supplementation during 0-80 d gestation on body weight, backfat and feed intake of gestating sows (Exp. 2 main study).

Measurements	Control (n=146)	Aspirin (n=154)	SEM	P-values
Parity at entry	2.81	2.84	0.11	0.96
Body weight (d 0), kg	217	217	2.0	0.91
Body weight (d 110), kg	254	257	2.1	0.40
Body weight gain, kg	35	39	1.4	0.16
Backfat (d 0), mm	17.2	17.0	0.32	0.51
Backfat (d 110), mm	21.4	21.4	0.48	0.98
Backfat change, mm	3.6	3.7	0.34	0.60
Average daily feed intake, kg/d	2.4	2.4	0.26	0.86

Table 5. Effects of Aspirin supplementation during 0-80 d gestation on farrowing outcomes of sows (Exp. 2 main study).

Measurements	Control (n=139)	Aspirin (n=137)	SEM	P-values
Gestational length, days	115.8	115.8	0.20	0.83
Reproductive failure[1], %	28.0% (56/200)	27.9% (55/197)		0.92
Farrowing rate[2], %	69.5% (139/200)	69.5% (137/197)		0.91
Total born	13.4	13.7	0.44	0.64
Born alive	12.1	12.1	0.38	0.93
Stillborn	1.2	1.3	0.15	0.53
Mummified fetuses	0.16	0.26	0.06	0.23

[1]Reproductive failures include the sows that were tested negative during routine pregnancy checks (fourth and sixth weeks in gestation), abortions, and sows that were assessed as non-pregnant at entry to the farrowing house.
[2]There were 6 and 7 sows from the control and Aspirin groups, respectively were removed for non-reproductive reasons (i.e., sudden deaths, lame, and non-eater).

Table 6. Effects of Aspirin supplementation during 0-80 d gestation on birth weight measurements of focal litters (Exp. 2 main study).

Measurements	Control (n=92 litters)	Aspirin (n=92 litters)	SEM	P-values
Birth weight, kg	1.33	1.34	0.12	0.65
% piglets ≤1.1 kg	25.7	24.9	2.4	0.83
Within-litter CV birth weight, %	22.3	22.0	0.92	0.83

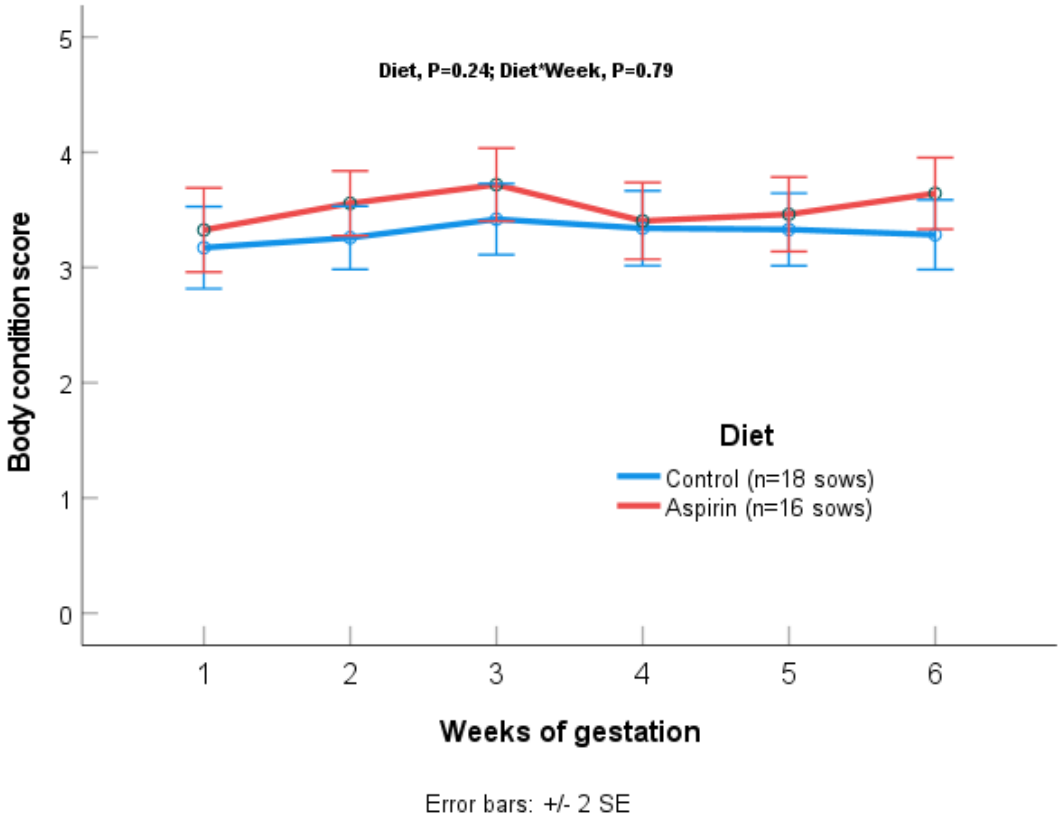


Figure 1. Body condition score of sows fed on control vs Aspirin supplemented diet (Exp 1. safety study).

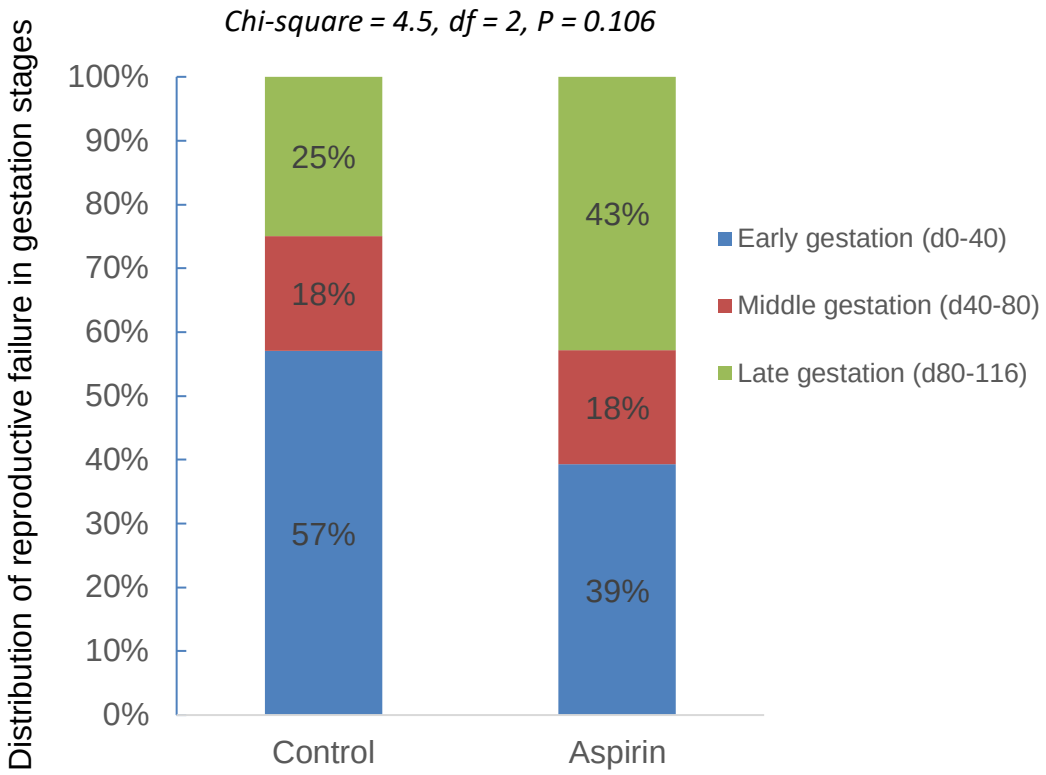


Figure 2. Distribution of reproduction failures among the early, middle and late gestational stage of sows fed control vs Aspirin diet during day 0-80 of gestation (Exp. 2 main study).

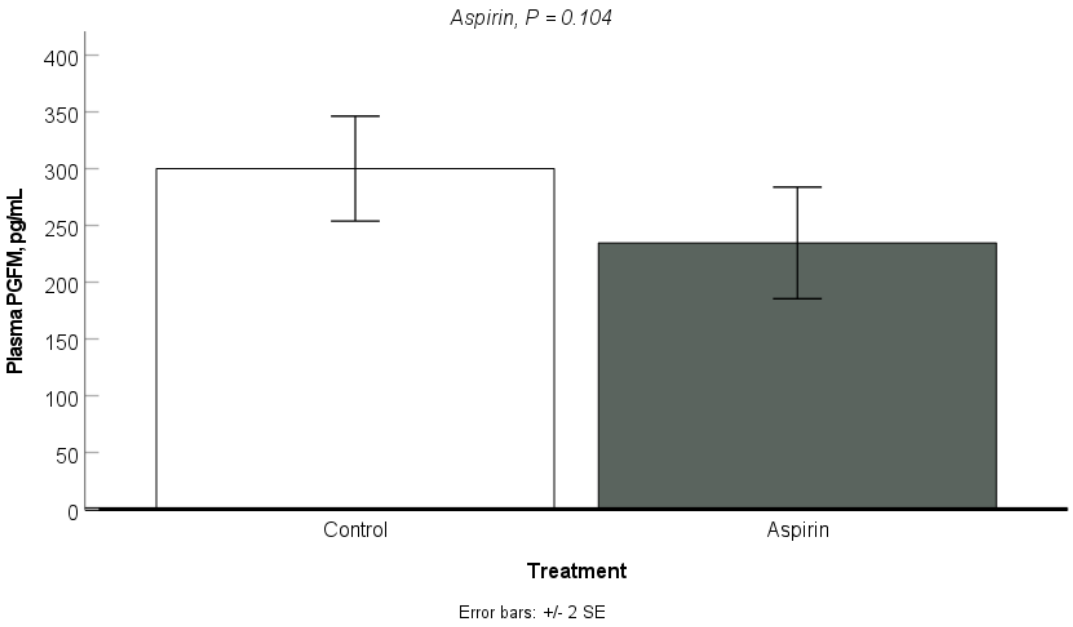


Figure 3. Effects of Aspirin supplementation on plasma PGFM concentration of gestating sows on day 30 (n=12 mixed-parity sows per treatment) (Exp. 2 main study).

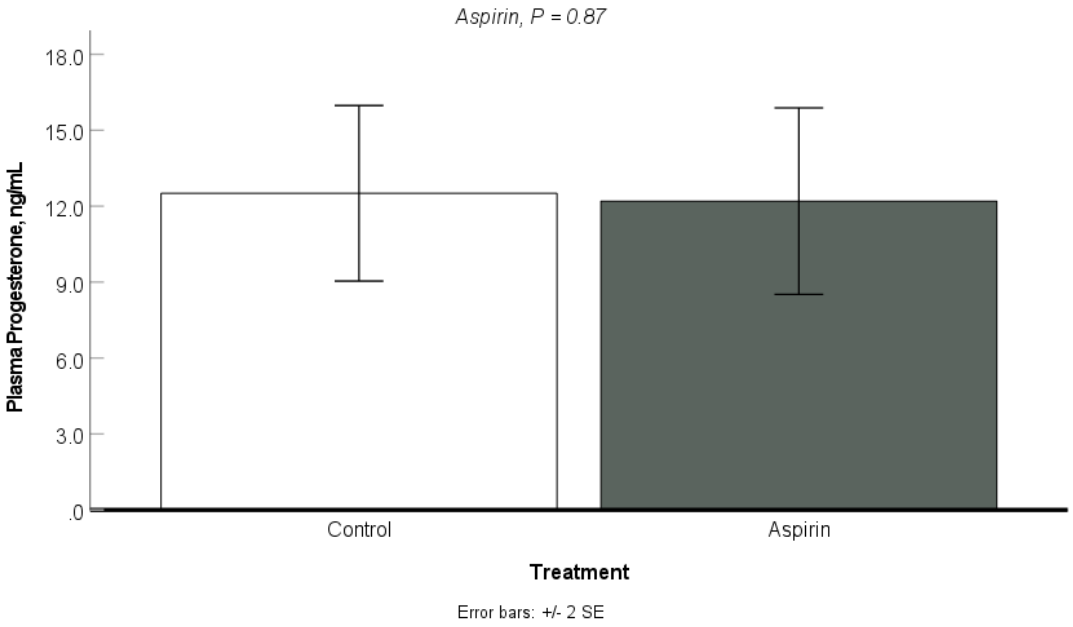
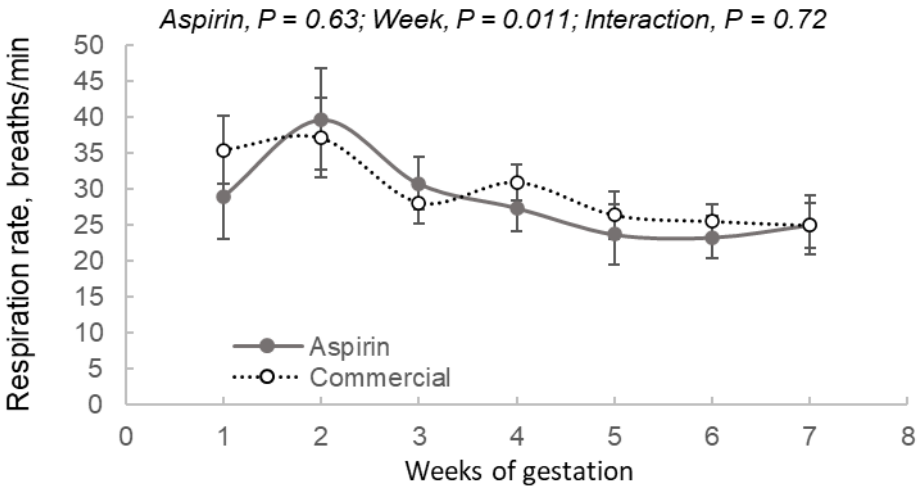
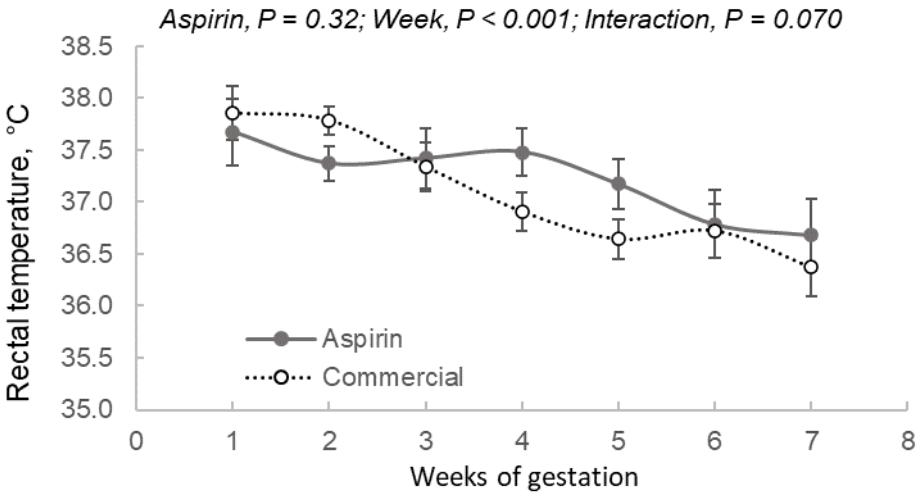


Figure 4. Effects of Aspirin supplementation on plasma progesterone concentration of gestating sows on day 30 (n=12 mixed-parity sows per treatment) (Exp. 2 main study).



(A)



(B)

Figure 5. Effects of Aspirin supplementation on (A) respiration rate and (B) rectal temperature of gestating sows in the first seven weeks post-mating (n=32 mixed-parity sows per treatment) (Exp. 2 main study). Data are shown as mean ± standard error.

4. Application of Research

The key finding of the research is that the low dose of Aspirin supplementation (240 ppm) fed during days 0-80 of gestation tended to reduce prostaglandin metabolite concentration by 22%, but did not affect the reproductive performance of sows that were mated in summer. The experimental sows mated in summer exhibited a low farrowing rate (av. 70%), a typical sign of summer infertility that has been reported before (Liu *et al*. 2020). The birth weight of piglets in this experiment averaged 1.3 kg, which is similar to what we reported in the sows mated in summer, and it was lower than that of piglets born to the sows mated in the cooler season (av. 1.55 kg) (Liu *et al*. 2020). A recent study revealed that the low birth weight of piglets born to the sows mated in summer may be due to heat stress-induced placental insufficiency during early and mid-gestation (Zhao *et al*. 2020).

Aspirin supplementation tended to delay pregnancy failure in the current experiment. Specifically, the percentage of pregnancy loss (out of the total number of reproductive loss) observed during the first trimester, when the majority of pregnancy loss happened, reduced from 57% to 43% in the Aspirin group. However, Aspirin increased the percentage of pregnancy loss in the third trimester from 25% to 43%. It remains unknown how Aspirin delayed pregnancy failure, or conversely, increased failure later in gestation. However, the overall farrowing rate was not improved by Aspirin supplementation in the current study. Similarly, a recent study showed that oral administration of a bolus of 187 g Aspirin on Day 14 and 15 post-insemination did not improve the pregnancy rate or plasma progesterone of cows mated in summer (Spencer *et al*. 2020a).

However, it is worthwhile to emphasise that there is a pig-specific knowledge gap on whether heat stress can increase endometrial PGF 2α production. Furthermore, it remains unclear whether such an upregulation of PGF 2α (if it existed) is the main cause of the early embryo loss of gestating sows. Early studies in pregnant gilts revealed that the prostaglandin PGF 2α is mainly produced from uterine endometrium (Schomberg 1967). A pig-specific review of the role of PGF 2α in regulating reproduction is reviewed by De Rensis *et al*. (2012). In that review, the role of PGF 2α in inducing abortion in mid-term pregnant sows and inducing parturition of near-term pregnant sows are well-known due to the luteolytic function of PGF 2α. According to a theory developed by Bazer and Thatcher (1977), pregnant sows can avoid the luteolytic effect of PGF 2α by re-directing PGF 2α secretion into the uterine lumen, thus preventing PGF 2α from entering the utero-ovarian vein and causing CL regression. The blastocyst-secreted estrogen plays a role in switching PGF 2α from endocrine secretion to exocrine secretion (Bazer 1989). An *in vitro* study demonstrated that increasing the incubation temperature of bovine uterus stromal cells from 38.5 to 40.5°C upregulated the prostaglandin synthase mRNA expression and more than doubled the PGF 2α secretion (Sakai *et al*. 2018); however, such a topic has not been studied in sows. Hence, it is worthwhile to investigate the effect of heat stress on prostaglandin synthesis of gestating sows under climatic controlled conditions. Such evidence would increase our understanding on the role of PGF 2α in summer infertility of sows and set directions for the development of mitigation strategies.

The efficacy of Aspirin supplementation may be finetuned via a dose-response experiment. The magnitude of reduction in plasma prostaglandin by Aspirin is lower than that reported in humans. For example, a similar dose of Aspirin orally administered (80 mg/d; a commonly prescribed low dose of Aspirin) to healthy humans for 2 weeks can reduce plasma prostaglandin synthesis by approximately 45% (Boutaud *et al*. 2016) and placental prostaglandin by approximately 50% (Walsh *et al*. 2020). By comparison, our current experiment found a tendency of a 22% reduction in PGFM in pregnant sows supplemented with a similar dose of Aspirin. The efficacy of Aspirin in reducing fetal growth restriction was dose-dependent, according to the literature review in humans (Roberge *et al*. 2017). The effective dose of Aspirin for facilitating fetal growth may be different between human and sows due to the morphological difference of placenta type and number of fetuses in the uterus. Novel applications of the inhibitive effect of gestational Aspirin supplementation in prostaglandin synthesis reported in the current experiment may be developed for other veterinary purposes.

5. Conclusion

Supplementation of 240 ppm dietary Aspirin during d 0 to 80 of gestation did not positively impact the low farrowing rate or birth weight observed in sows mated in summer, although plasma concentration of prostaglandin 2α tended to be reduced by Aspirin supplementation, by 22%, indicative of a mild inhibition on prostaglandin synthesis.

6. Limitations/Risks

None.

7. Recommendations

Supplementing 240 ppm Aspirin during d 0-80 of gestation is not recommended as a strategy to improve the farrowing rate and birth weight of sows mated in summer.

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Appendix 1 - Notes

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