Australian Government
Department of Agriculture

AUSTRALIAN Pork

Optimising the duration of betaine supplementation in pig production

Final Report
APL Project 2018-0023

January 2021

Rivalea Australia Pty Ltd
Fan Liu, David Henman, Chris Brewster, Robert Smits, Jessica Craig
Redlands Road
Corowa, NSW 2646

*** Commercial in Confidence**

Reports containing confidential information must have "Commercial in Confidence" marked on the cover page and supply a written explanation to APL. APL may request the Researcher to produce a non-confidential version of the Final Report in a form suitable for general distribution within a timeframe deemed suitable by APL.

Acknowledgements

This project is supported by funding from Australian Pork Limited.

We would like to thank Erin Ford, and Jake Walker, Steve Smith from Research and Innovation Unit, Rivalea for technical input in the experimental procedures.

We would also like to thank Dr Rebecca Athorn and Bryony Tucker for the technical support in using ultrasound for detecting ovulation.

Executive Summary

Betaine is used as a conditional supplement in pig diets and industry nutritionists have different recommendations on betaine usage for various stages of the pig lifecycle. The effective dosages of betaine reported are 0.1% in progeny diets and 0.2% in breeder diets, which increases the diet cost by $3.60 to $7.20 per tonne when supplemented at these dosages. The key factor that determines the cost-effectiveness of betaine application in pig production is when and for how long betaine is supplemented. In theory, betaine is more effective at improving performance when nutrient intake is limiting the performance of animals or if the body cells are under osmotic stress such as in heat stress conditions. Identifying the critical stages that require betaine supplementation can help us better utilise the function of betaine and shorten the duration of supplementation, thereby reducing the cost of production. We hypothesised that the critical stages for betaine supplementation are the first week post-weaning for weaner pigs (particularly for the light-weight weaners), the grower phase for grower/finisher pigs, and during summer lactation for breeders. Therefore, we conducted three separate experiments to evaluate the effects of duration of betaine supplementation in the weaner, grower/finisher, and lactation/gestation phases, respectively.

Betaine is not generally added to weaner pig diets due to a lack of knowledge on the duration of supplementation and effect on targeted body weight class. Light-weight weaners, known for their inferior growth performance, may benefit from betaine supplementation during the early post-weaning period. Experiment 1 followed a 2×2×2 factorial arrangement for studying the effects of 0.1% betaine supplementation during early (0-7 d post-weaning) and late (7-35 d post-weaning) weaner phase in light-weight and normal-weight weaners. One hundred and forty-four pens (n=18 pigs/pen) of weaned pigs (26 d age) were allocated into a 2×2×2 factorial arrangement based on weaning weight class ((light (3.6 ± 0.75 kg, mean ± SD) vs normal (6.6 ± 0.84 kg, mean ± SD)), early weaner phase diet (control vs 0.1% betaine) and late weaner phase diet (control vs 0.1% betaine). Basal diets contained sufficient methionine and choline as per industry practice. Growth performance during early, late and whole weaner phase were recorded. Blood IGF-1 concentration was measured on 7 d and 35 d post-weaning. Supplementing 0.1% betaine during the early weaner phase reduced ($P<0.05$) growth rate over the early post-weaning period (0-7 d) in both light- and normal-weight weaners, although blood IGF-1 concentration (7 d post-weaning) was not affected. Betaine supplementation during early or late weaner phase did not affect growth performance over the late weaner phase (7-35 d) or whole weaner phase (0-35 d), nor did it have an affect blood IGF-1 concentration (35 d) in light- or normal-weight weaners. Light-weight weaners had lower feed intake and a lower average daily gain than normal-weight weaners during the early, late and whole post-weaning period (all $P<0.01$), which was not improved by betaine supplementation. Therefore, betaine supplementation is not recommended in the weaner phase when other dietary methyl donors are sufficient.

In finishers, betaine is mainly used in summer as a means of reducing heat stress, but its function in reducing backfat thickness (Sales 2011) is rarely used nowadays due to the inconsistent responses in female pigs (Lawrence *et al.* 2002; Dunshea *et al.* 2009) and not being as cost effective as ractopamine (Mendoza et al. 2017). Experiment 2 aimed to optimise the duration of betaine supplementation for reducing carcass backfat thickness of finisher pigs. We hypothesised that 0.1% betaine supplementation within the grower phase (10-16 weeks old) would be more effective in reducing carcass backfat than supplementing in the finisher phase (16-22 weeks old), because grower pigs have a greater ratio of lean: fat deposition than finisher pigs. Thus, the betaine-spared energy can be deposited as more lean tissue. A total of 80 pens of pigs (40 pigs per pen) were allocated to a 2×2×2 factorial arrangement based on the grower phase diet (control vs 0.1% betaine during 10-16 weeks age), finisher phase diet

(control vs 0.1% betaine during 16-22 weeks age) and sex (castrated male vs female pigs). The experiment showed that betaine supplementation during the grower phase only (and not the finisher phase) can reduce backfat thickness in female pigs from 12.0 mm to 11.3 mm at standardised 80.5 kg hot standard carcass weight, whereas the effect was not seen in castrated male pigs (Grower diet × Finisher diet × Sex, $P<0.05$). Betaine supplementation during the finisher phase reduced ADG by 40 g per day regardless of sex (Finisher diet, $P<0.05$). Other growth performance parameters were not affected by the dietary treatments. It is estimated that the effect of betaine supplementation during the grower phase in reducing carcass backfat would increase net profit by $2.30/female pig (or by 2.90 c/kg of carcass) at a small investment in feed cost ($3.60 increase per tonne of feed; or equivalent to increase of cost of production by 0.60 c for each kg of carcass produced). The ratio of investment and net return is 1:4.8. In summary, betaine supplementation is recommended to be applied for 10-16 weeks age in female pigs as a means of reducing carcass backfat.

Lactating sows that are weaned and mated in summer usually have low farrowing rates and have piglets with lower birth weights. Betaine is usually added to sow diets to ameliorate the negative impacts of heat stress; however, it is unknown whether betaine supplementation should be practiced in the lactation diet and/or the gestation diet to achieve improved litter sizes and piglet birth weight. In addition, it is suspected that the low farrowing rate may be a consequence of mismatched timing of ovulation and artificial insemination. It is reported that lactational heat stress can prolong the oestrus-to-ovulation interval by 1.5 days, so the current industry practice, mating at the oestrus, may not be ideal for conception if the sows weaned in summer had a prolonged oestrus-to-ovulation interval. Betaine supplementation may normalize the prolonged oestrus-to-ovulation interval, as reported in a climatically controlled study. A total of 640 sows were allocated into a 2 × 2 factorial arrangement based on lactation diet fed during summer (control vs betaine) and gestation diet (control vs betaine diet, during the gestation prior to lactation (1st gestation) and during gestation subsequent to lactation (2nd gestation)). Betaine supplementation during summer lactation reduced the respiration rate of lactating sows on the 7th day of lactation and tended to reduce ($P=0.102$) backfat loss (-2.4 mm vs -2.0 mm) over summer lactation. Betaine supplementation during gestation reduced ($P<0.05$) body weight gain of sows by 3 kg in both the 1st and 2nd gestation. At the end of the subsequent (2^{nd}) gestation post-summer lactation, neither early reproductive (d 30 of gestation) failure rate, farrowing rate, litter size, or piglet birth weight were affected by betaine supplementation during either lactation or gestation. The duration of oestrus-to-ovulation interval was determined via transrectal ultrasound on a subset of sows that displayed behavioral oestrus on the 4^{th} and 5^{th} day post-weaning. Betaine supplementation did not affect the oestrus-to-ovulation interval (av. 1.2 days), implying that the current artificial insemination timing would still capture ovulation regardless of betaine supplementation. In other words, summer infertility (eg: 60% farrowing rate in this experiment) was not likely to be caused by missing the ovulation. In conclusion, betaine supplementation should be practiced in summer lactation diets but is not required in gestation diets on either side of a summer lactation.

To summarise, the following recommendations are made based on the experimental results:

- Betaine is not recommended to be added in weaner pig diets (4-10 weeks age) when other methyl donors are sufficient.
- Betaine is recommended to be added in the female grower phase diet (10-16 weeks age) as an economical strategy to reduce backfat thickness.
- Betaine is not recommended to be added in the gestation diet due to the negative impact on weight gain of sows during gestation.

- Betaine is recommended to be added in the lactation diet during summer as a strategy to alleviate heat stress.

Table of Contents

List of Tables

List of Figures

1. Background to Research

Betaine is used as a conditional supplement in pig diets and the industry nutritionists have different recommendations on betaine usage at various stages of the pig lifecycle. Betaine supplementation at 0.1% to 0.2% will increase diet cost by $3.6 to $7.2 per tonne which is not an insignificant cost. The key factor that determines the cost-effectiveness of betaine application in pig production is when and for how long betaine is supplemented, as betaine is more effective to improve productive performance when the nutrient intake is limiting the performance of pigs. Knowing the critical stages that require betaine supplementation will help pig producers use betaine more efficiently and cost effectively; however, there is not sufficient information in the scientific literature that identifies the critical stages that require betaine supplementation based on performance. Therefore, we designed these experiments to optimise the duration of betaine supplementation in different phases of pig production including weaners, grower/finishers and breeders by comparing the productive performance. The research project looked to answer the following questions:

(1) In weaners, to improve post-weaning growth performance, is betaine supplementation within the first seven days post-weaning more cost-effective than supplementing in the late weaner phase or throughout the whole weaner phase? Is the effect more significant in weaners with light body weights at weaning?

(2) In grower/finishers, to achieve a leaner carcass, is betaine supplementation within the grower phase more cost effective than the finisher phase?

(3) In breeders, to achieve greater reproductive performance in the sows mated in summer, is betaine supplementation within summer lactation more cost-effective than subsequent gestation? Can betaine supplementation in lactation diet during summer normalise heat-stress prolonged oestrus-to-ovulation intervals?

2. Objectives of the Research Project

This project set out to:

- Identify the critical stages required for betaine supplementation by studying productive performance;
- Optimise the duration of betaine supplementation in weaner, grower/finisher, and breeder diets to increase cost effectiveness.

3. Introductory Technical Information

Betaine, commonly referred to as N-tri-methyl glycine, is a known osmolyte and methyl donor, and was first discovered in sugar beets (reviewed by Craig (2004)). As an osmolyte, betaine can help maintain the water content in cells; as a methyl donor, it can facilitate the re-synthesis of methionine (an essential amino acid for muscle growth) from homocysteine. Betaine can be synthesised from choline oxidation and can be utilised to synthesis methionine using homocysteine, and the process happens in the liver and kidney of pigs. The main physiological effect of betaine discovered is it can reduce the maintenance requirement in pigs (Schrama *et al.* 2003) thus spares energy for productive performance.

Both of its functions may be beneficial to the newly weaned piglet because they usually experience a growth check as they transition from milk to solid feed, such that nutrient intake can be below maintenance requirements during the first week post-weaning. In addition, several studies have reported that betaine supplementation can increase secretion of insulin-like growth factor-1 (IGF-1), a key anabolic hormone that regulates muscle growth (Oksbjerg *et al.* 2004), in humans (Apicella *et al.* 2013), heifers (Lakhani *et al.* 2020) and finisher pigs (Huang *et al.* 2006; Lothong *et al.* 2016). The effects of betaine supplementation on IGF-1 secretion has not been studied in weaner pigs. Betaine supplementation does not occur widely in the formulation of weaner pig diets, because a critical phase or a class of body weight of weaners that require or may benefit from betaine supplementation has not been specified. A study showed that when fed experimental diets with deficient methionine (compared with NRC 2012 standard), supplementing 0.57% betaine numerically increased weaner growth rate by 47% within 0-7 d post-weaning and then the effect diminished, implying its effectiveness of betaine supplementation may be phase-dependent (Matthews *et al.* 2001). Weaners with a light body weight (eg: 4.1 to 5.0 kg; Mahan *et al.* 1991) had inferior subsequent growth performance than normal and heavy weight weaners, and it is known that infusing exogenous IGF-1 in neonatal born-light piglets can improve tissue growth (Schoknecht *et al.* 1997; Davis *et al.* 2002). Therefore, the potential effect of in-feed betaine to improving IGF-1 secretion has been proposed as a nutritional strategy for increasing growth rate of light-weight weaners. However, there have been no reports on whether betaine supplementation is more effective on light-weight, rather than normal-weight weaned pigs. Such information would be valuable to the pig industry particularly when the light-weight weaners can be sorted and separately fed and managed on some farms. Therefore, the experiment aimed to identify the duration of betaine supplementation (early (0-7 d) or late (7-35 d) weaner phase) and body weight class of weaners (light or normal weight) that responds best to betaine supplementation. We chose 0.1% inclusion rate for betaine in this experiment, because it was the effective dose that increased blood IGF-1 concentration in grower pigs (Huang *et al.* 2006; Lothong *et al.* 2016). In Experiment 1, we hypothesised that betaine supplementation (0.1%) in the weaner diet would increase IGF-1 and growth performance particularly when applied during the early weaner phase, and the effect would be more pronounced in the light-weight weaners.

In grower and finisher pigs, betaine is occasional used in summer as a means of reducing heat stress, but its function in reducing backfat thickness (Sales 2011) is rarely used nowadays due to the inconsistent responses in gilts and barrows (Lawrence *et al.* 2002; Dunshea *et al.* 2009) and not being as cost effective as ractopamine (Mendoza *et al.* 2017). The exact mechanism for betaine reducing fatness remains unclear, although several pathways were proposed including increasing fatty acid oxidation in muscles (Li *et al.* 2017) and improved insulin sensitivity (Du *et al.* 2018). The most common reported inclusion rate of betaine in grow/finish diets is 0.1%, which increases diet cost by approximately $3.6 per tonne. Optimising the duration of betaine supplementation during the grow/finish period, when most feed is consumed, will improve profitability. Grower pigs have a greater ratio of lean: fat deposition than finisher pigs (National Research Council 2012), therefore, the betaine-spared energy may be preferentially deposited as lean tissue. We hypothesised that 0.1% of betaine supplementation within grower phase (10-16 weeks old) is more effective in reducing carcass backfat than supplementing in finisher phase (16 weeks old to sale), and the effect maybe sex dependent.

In breeders, betaine is commonly used in lactation and gestation diets in summer, because research showed that 0.2% of betaine supplementation can increase number of piglets born alive when supplemented in the lactation diet (Ramis *et al.* 2011; Rivalea technical reports 97R12 and 94C56) or gestation diet (van Wettere et al. 2012; van Wettere et al. 2013). A recent study showed that lactational heat stress can delay the subsequent oestrus to ovulation interval by 1.5 days, and 0.2% betaine supplementation during the lactation period improved follicle development and numerically reduced oestrus-to-ovulation interval by 0.4 days (Cabezón *et al.* 2017). The key factor that determines the cost-effectiveness of betaine application in pig production is when and for how long betaine should be supplemented. The two questions we need to answer in regards to betaine supplementation in breeder diets are: (1) In breeders, to achieve a greater reproductive performance in the sows mated in summer, is betaine supplementation within summer lactation more cost-effective than the prior and subsequent gestation? (2) Can betaine supplementation in the lactation diet during summer normalise the heat-stress prolonged oestrus-to-ovulation intervals?

4. Research Methodology

4.1 Experiment 1 (weaner phase)

All animal procedures had prior institutional ethical approval from the Rivalea Animal Ethics Committee (protocol ID:18N070C) under the requirement of the New South Wales Prevention of Cruelty to Animals Act (1979) in accordance with the National Health and Medical Research Council/Commonwealth Scientific and Industrial Research Organisation/Australian Animal Commission Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013).

4.1.1 Animals and experimental design

The experiment followed a 2×2×2 factorial arrangement to investigate the effects of betaine supplementation during the early (0-7 d) and late (7-35 d) post-weaning period in weaned piglets with normal and light body weight. A total of 2592 piglets (Primegro™ genetics, Corowa, NSW) were weaned from mixed parity sows at 26 days of age and sorted into two weight classes- normal (6.6 ± 0.84 kg, mean ± standard deviation) and light (3.6 ± 0.75 kg, mean ± standard deviation). Pigs were housed in group pens (18 weaners per pen; 0.29 m2 pen space per pig; 144 pens in total) with the pen used as the experimental unit for measuring growth performance. Weaners were allocated into 2×2×2 treatments at weaning, based on wean weight class (light vs normal weight) × early phase betaine supplementation (control vs 0.1% betaine) × late phase betaine supplementation (control vs 0.1% betaine). There were nine male pens and nine female pens for each treatment group. As per common industry practice, the 35 d weaner phase was divided into two phases- early (0-7 d) and late (7-35 d) post-weaning period with different basal diets offered in each phase. The basal (control) diets were formulated to meet or exceed NRC requirement (NRC 2012) with sufficient methyl donors including methionine and choline (Table 1.1). The betaine diets were formulated to contain 0.1% crystalline betaine (Vistabet™, ABVista, UK) which replaced 0.1% wheat in the basal diets. Ambient temperature of the weaner shed was controlled at 28°C when the weaners arrived in the shed and was decreased by 2°C weekly until d 35 post-weaning. Pigs were fed *ad libitum* and had *ad libitum* access to water via nipple drinkers. The number of pigs that died during the trial were within normal commercial observations. For light weight weaners, 24 and 82 pigs died out of 1296 pigs during the early phase and the whole weaner phase (0-35 d), respectively. For normal- weight weaners, 3 and 16 pigs died out of 1296 pigs during the early phase and the whole weaner phase, respectively.

4.1.2 Growth performance

The pens of pigs were weighed at weaning (0 d; same day as entry to the weaner shed), 7 d and 35 d post-weaning and average daily gain (ADG) was calculated. Feed delivery and refusals were weighed once or twice per week to calculate average daily feed intake (ADFI). Feed conversion ratio (FCR) was calculated as the ratio between ADFI and ADG. The growth performance was reported for the early, late and whole weaner phase.

4.1.3 Blood sampling and IGF-1 assay

Forty-eight pigs (n=3 male and 3 female pigs per treatment group) were blood sampled via jugular venepuncture on 7 and 35 d post-weaning for IGF-1 measurement. Pigs were randomly chosen from each pen on each of the blood sampling days. The blood sample was collected via heparinised vacutainers and a drop of whole blood sample were stained on the IGF-1 sample card (PrimeGro™) pending analysis. The IGF-1 in blood samples were extracted and measured using a commercial kit (Quantikine® Human IGF-1 immunoassay kit, R and D Systems, USA), with samples assayed in singlicate.

4.1.4 Statistical analysis

All data were analysed using the Univariate procedure for the effects of diet (early weaner phase; control vs betaine), diet (late weaner phase; control vs betaine), weaning weight class (normal vs light), the interaction between diet (early weaner phase) and diet (late weaner phase), and the three-way interaction. Sex of the pigs were set as a block factor. All the analysis was conducted in SPSS (version 24; IBM, Chicago, IL, USA). Data were reported as the mean ± standard error (se) of the main effect of wean weight class, diet (early weaner phase), and diet (late weaner phase), as none of the interactive effects were significant. The main dietary effect (late weaner phase) was not reported for the growth performance that was evaluated during early weaner phase and IGF-1 measured on 7 d, because the effect of diet (late weaner phase) was not significant as expected. Means were considered to differ significantly when $P \leq 0.05$, and a trend was identified when $P \leq 0.10$.

Table 1.1 Composition of basal diets

Ingredient (% of fed basis)	Early weaner phase (0-7 d) diet	Late weaner phase (7-35 d) diet
Wheat	52	34
Barley	15	0
Lupin	0	5
Canola meal	0	4
Soybean meal	3.0	6.7
Meat meal	2.5	6.7
Fish meal	7.3	2.5
Blood meal	1.0	1.5
Soy protein concentrate	0	0.8
Lactose	10.7	8.3
Tallow	1.6	1.0
Molasses	1.0	1.0
Lysine	0.65	0.55
DL-Methionine	0.26	0.14
Choline chloride	0.017	0
Threonine	0.28	0.22
Isoleucine	0.18	0.13
Tryptophan	0.10	0.06
Phytase and NSP enzymes	0.03	0.03
Organic acids [A]	0.6	0.6
Antibiotics [B]	0.8	0.5
Salt	0.2	0.2
Premix [C]	0.30	0.30
Calculated nutrients		
DE (MJ/kg)	15	15.1
Protein (%)	19.1	22.2
Lysine (%)	1.5	1.5
SID lysine (%)	1.36	1.36
Methionine (%)	0.60	0.48
Cysteine (%)	0.30	0.26
Choline, ppm	1250	1440
Calcium (%)	0.7	0.79
available phosphorus (%)	0.56	0.53

[A] 2000 ppm medium chain fatty acids and 4000 ppm benzoic acid in both 0-7 d and 7-35 d post-weaning period

[B] 400 ppm lincomycin during 0-7 d post-weaning; 1000 ppm chlortetracycline hydrochloride during 7-35 d post-weaning

[C] supplied per kg of diet: copper, 20.5 mg; manganese, 20 mg; zinc, 470 mg; iron, 100 mg; iodine, 0.5 mg; selenium, 0.3 ppm; vitamin A, 10000 IU; vitamin D_3, 2000 IU; vitamin K, 1.5 mg; vitamin B-1, 1.0 mg; vitamin B-2, 5.0 mg; vitamin B-6, 4.0 mg; vitamin B-12, 20 µg; Niacin, 15 mg; pantothenic acid, 10 mg; vitamin C, 100 mg; biotin, 0.05 mg; vitamin E, 100 I

4.2 *Experiment 2 (grower/finisher phase)*

4.2.1 Experimental design

The procedures that involved animals in the current study were in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (8th edition) (National Health and Medical Research Council 2013) and approved (Protocol ID 19N003C) by the Animal Ethics Committee of Rivalea Pty Ltd, Corowa, NSW, Australia. The experiment followed a 2×2×2 factorial design (n=10 pens per treatment) for studying the effect of sex, grower phase diet (control vs 0.1% betaine), finisher diet (control vs 0.1% betaine) and their interactions on growth performance and carcass traits. The live animal phase of the experiment was conducted in group pens fitted with an automatic feeding system at the Research and Innovation Unit of Rivalea Australia Pty Ltd (Corowa, NSW, Australia) between 13 January and 16 April 2020. Pigs were housed in pens (39 pigs per pen) with a solid concrete floor and walls, and partially slatted areas. A total of 80 pens of Landrace × Large White × Duroc male and female pigs (Primegro™ Genetics, Corowa, NSW) were selected into the experiment at 10 weeks of age with an average body weight of 26.6 ± 3.19 kg (mean ± standard deviation), and randomly allocated into the 2×2×2 arrangement (n=10 pens per treatment group) based on average pen weight. The control diets were formulated to contain similar or excess nutrients levels recommended by NRC 2012 (Table 2.1). The betaine supplementation was achieved by replacing 0.25% wheat with 0.25% liquid betaine (the liquid betaine product contained 40% natural betaine) or equivalent to 0.10% pure betaine. Pigs had *ad libitum* access to feed and free access to water via nipple drinkers. Male pigs received the second vaccination dose of Improvac™ (Zoetis) during the experiment at 18 weeks age. Pigs were sold by pen at an average of 22 weeks of age.

Table 2.1. Composition of experimental diets (% as fed-basis) used in Experiment 2

Ingredient	Grower Control	Grower Betaine	Finisher Control	Finisher Betaine
Wheat	43.0	43.0	40.3	40.3
Barley	33.5	33.5	41.8	41.8
Canola meal	15	15	12.0	12.0
Meat meal	4.3	4.3	1.7	1.7
Tallow	1.0	1.0	0.5	0.5
Limestone	0.97	0.97	1.2	1.2
Dicalcium Phosphate	0	0	0.4	0.4
Lysine-HCL	0.5	0.5	0.45	0.45
DL-Methionine	0.11	0.11	0.06	0.06
Threonine	0.14	0.14	0.13	0.13
Mineral premix[1]	0.11	0.11	0.11	0.11
Vitamin premix[2]	0.06	0.06	0.035	0.035
Feed emzymes[3]	0.12	0.12	0.12	0.12
Salt	0.33	0.33	0.33	0.33
Liquid betaine (40%)	0	0.25	0	0.25
Calculated nutrients				
Digestible energy, MJ/kg	13.8	13.8	13.6	13.6
Protein, %	18.1	18.1	16.3	16.3
Fat, %	3.0	3.0	2.3	2.3
Fibre, %	4.9	4.9	4.8	4.8
Calcium, %	0.90	0.90	0.92	0.92
Available phosphorus, %	0.43	0.43	0.42	0.42
Lysine, %	1.14	1.14	0.98	0.98
SID lysine%	0.98	0.98	0.84	0.84
Methionine, %	0.40	0.40	0.32	0.32
Choline, %	0.18	0.18	0.16	0.16

[1] Supplied per kg of grower diets: copper, 101 mg; manganese, 46.8 mg; zinc, 73.0 mg; iron, 56.0 mg; iodine, 0.675 mg; selenium, 0.30 mg; chromium 0.16 mg. Supplied per kg of finisher diets: copper, 100 mg; manganese, 28.0 mg; zinc, 50.0 mg; iron, 70.0 mg; iodine, 0.50 mg; selenium, 0.20 mg; chromium 0.20 mg.

[2] Supplied per kg of grower diets: vitamin A, 5000 IU; vitamin D3, 1000 IU; vitamin K, 0.66 mg; vitamin B-1, 1.0 mg; vitamin B-2, 3.3 mg; vitamin B-6, 2.0 mg; vitamin B-12, 6.7 µg; niacin, 20 mg; pantothenic acid, 10.0 mg, Vitamin E 31.7 IU. Supplied per kg of finisher diets: vitamin A, 3000 IU; vitamin D3, 600 IU; vitamin K, 0.40 mg; vitamin B-1, 0.6 mg; vitamin B-2, 2.0 mg; vitamin B-6, 1.2 mg; vitamin B-12, 4.0 µg; niacin, 12 mg; pantothenic acid, 6.0 mg, Vitamin E 19 IU.

[3] Supplied per kg of grower and finisher diets: phytase, 100 mg; xylanase, 50 mg

4.2.2 *Measurements on growth performance and carcass traits*

Growth performance was evaluated between 10-16 weeks age, 16 weeks to sale and 10 weeks- sale. The total body weight and number of pigs in each pen was recorded at 10 weeks age, 16 weeks age and the day before sale. Any mortalities and removals were recorded daily. The daily feed intake (ADFI), average daily gain (ADG) and feed conversion rate (FCR; feed consumed: live weight gained) were calculated based on the total weight of pigs in the pen, total feed allocation, and the number of pigs in the pen. The hot standard carcass weight (HSCW; head on, visceral tissue off, trotters on), backfat thickness at the P2 site (65 mm from the midline over the last rib; Hennessy Chong fat depth probe measurement), and loin depth (at P2 site) from each individual pig were measured in the abattoir (Rivalea Australia Pty Ltd, Corowa, NSW, Australia). Average dressing percentage for each pen was calculated by the ratio between average HSCW and average live weight of the pen.

4.2.3 *Statistical Analysis*

Numerical outcome variables such as growth performance and carcass traits (using the average of each pen as an experimental unit) were analysed using the UNIVARIATE procedure for the effect of Sex (female vs castrated male), Grower diet (control vs betaine), Finisher diet (control vs betaine), Grower diet × Finisher diet and Sex × Grower diet × Finisher diet. Mortality rate was analysed by Pearson's *Chi*-squared analysis and are expressed as percentages. $P \leq 0.05$ was regarded as statistically significant. All the statistical analyses were conducted in SPSS (IBM SPSS Statistics for Windows, v25, Armonk, NY).

4.3 *Experiment 3 (gestation/lactation phase)*

4.3.1 *Animals and experimental design*

The experimental procedures in Experiment 3 were in accordance with Australian Code for the Care and Use of Animals for Scientific Purposes (8th edition, 2013), and the protocol (ID:19N025C) was approved by the Animal Ethics Committee of Rivalea Australia Pty Ltd, Corowa, NSW, Australia.

A total of 640 multiparous mated sows were allocated to a 2×2 factorial arrangement based on summer lactation diet (control vs betaine) and gestation (prior and post summer lactation) diet (control vs betaine) over 8 batches (Figure 3.1). Betaine supplementation in the gestation and lactation diets were both achieved by replacing 0.5% wheat with 0.5% liquid betaine (equivalent to 0.16% tri-methyl-glycine) in the basal diet (Table 3.1). The whole experimental duration consisted of an initial gestation (1st gestation), a summer lactation, and the subsequent gestation (2nd gestation). During gestation, sows were housed in group pens (40 sows per pen from mating to until 4th week of gestation, then 80 sows per pen) and fed using electronic sow feeders (40 sows per feeding station) which allowed for individual feeding. Sows were restrict-fed with an average allowance of 2.3 kg/d during gestation. On the 108th day of gestation, sows were moved to farrowing houses and then housed in individual farrowing crates. Sows were offered *ad libitum* access to the lactation diet on the 2nd day post-farrowing up until weaning. The lactation period of the sows from the 8 batches took place during the season of summer in Australia (December 2019-March 2020). The average recorded temperature inside the farrowing shed was 25.4 ± 5.01°C (mean ± standard deviation) over the duration of the season.

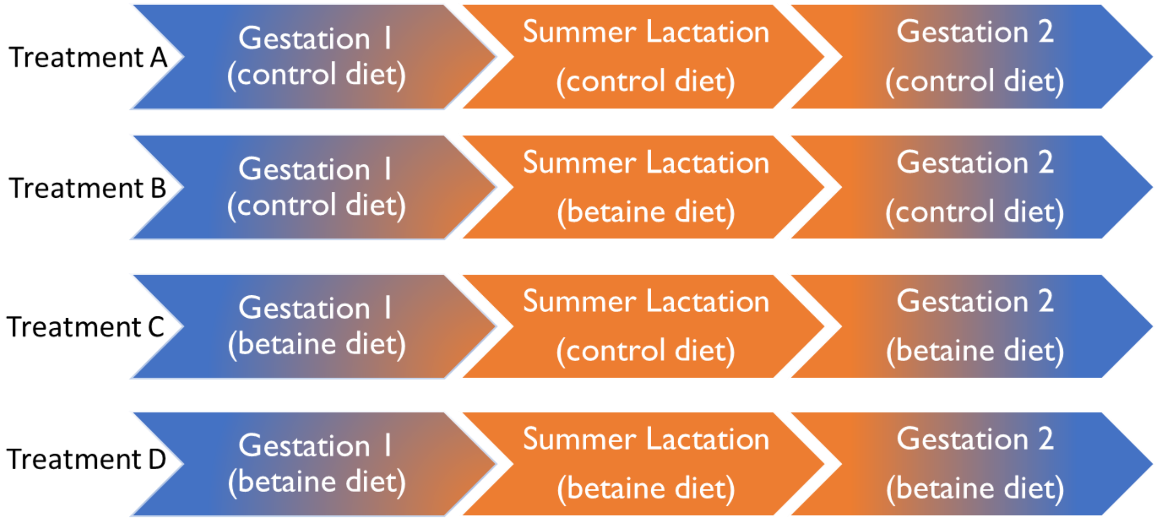


Figure 3.1 Experimental design of Experiment 3

Table 3.1 Feed formulation of basal diets used in Experiment 3

Ingredients (% as fed-basis)	Gestation	Lactation
Wheat	41	57
Barley	40	10
Mill mix	6.7	6.7
Canola meal (38% CP)	6.7	10
Soya bean meal (46% CP)	0	2.5
Meat meal (60% CP)	0	3.3
Fish oil	0.2	0.4
Tallow	1	6
Limestone	1.3	1
Dicalcium Phosphate	0.8	0
Phytase	0.01	0.01
Lysine	0.25	0.41
Threonine	0.03	0.13
MgSO4	0	0.4
Salt	0.3	0.35
Mineral premix	0.2	0.25
Vitamin premix	0.13	0.13
Calculated nutrients		
Dry matter	88.6	90.5
Digestible energy	13.3	15.0
Crude protein	13.3	16.9
Total Calcium	0.93	0.84
Available Phosphorus	0.44	0.41
Lysine	0.69	1.00
SID lysine	0.59	0.87
Methionine	0.27	0.27
Cysteine	0.30	0.34
Choline, ppm	1329	1537

[1] Supplied per kg of gestation diets: copper, 12 mg; manganese, 24 mg; zinc, 50 mg; iron, 78 mg; iodine, 1.6 mg; selenium, 0.25 mg; chromium 0.35 mg. Supplied per kg of lactation diets: copper, 9 mg; manganese, 30 mg; zinc, 60 mg; iron, 80 mg; iodine, 2.0 mg; selenium, 0.3 mg; chromium 0.4 mg.

2 Supplied per kg of gestation diets: 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, 96 µg; niacin, 30 mg; pantothenic acid, 15.0 mg, Vitamin E 82 IU. Supplied per kg of lactation diets: 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, 140 µg; niacin, 30 mg; pantothenic acid, 15 mg, Vitamin E 95 IU.

4.3.2 Body conditions and farrowing outcomes of the 1st gestation

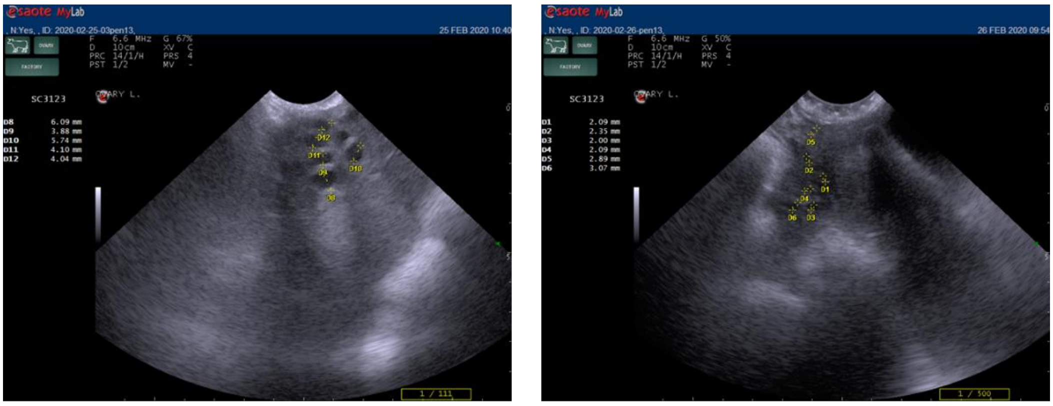
The body weight and backfat thickness of sows were measured on day 1 and day 108 of gestation. Backfat thickness was measured using an ultrasound machine (CTS-900 V; Shantou Institute of Ultrasonic Instruments, China) at the P2 site (65 mm from the midline over the last rib). At the end of the 1st gestation, the number of born-alive, stillborn piglets and mummified fetuses were recorded once the sows finished farrowing. Feed intake data over both gestations was retrieved from the electronic sow feeding records (ESF).

4.3.3 Lactation performance

As a commercial practice to ensure the number of piglets matches the number of functional teats a sow has, cross-fostering was practiced within the dietary treatment group on the 2nd day post-farrowing. Litter size and weight were record post-fostering and then at 21-days of age. Litter weight gain was calculated by subtracting post-foster litter weight from the 21-day litter weight. The average lactation length was 28 days. The body weight and backfat thickness of all sows were measured at weaning. The daily feed intake of individual sows was calculated using the amount of feed delivered to the individual sow and subtracting the amount of daily feed refusal.

4.3.4 Oestrus and ovulation detection

Oestrus detection was conducted from the 3rd day until the 7th day post-weaning and the sows were determined to be in oestrus when the displayed the behavioural signs of oestrus (exhibiting standing oestrus when back pressure was applied in the presence of a boar). At the first sign of behavioural oestrus sows were artificially inseminated (AI) by post-cervical AI. Twenty-four hours after the first insemination sows were inseminated again, and were then moved to gestation accommodation within two days of the second AI. Sows showing delayed behavioural oestrus (>7 days post-weaning) were excluded from the 2nd gestation in this experiment. For quantifying oestrus-to-ovulation interval, a subset of sows were selected from those exhibiting behavioural oestrus on the 4th day and 5th day post-weaning (representing 88% of weaned sows). Transrectal ultrasound (OneLab™ Esaote) of the ovaries was conducted daily on the subset of sows from the 2nd day after detected behavioural oestrus until the pre-ovulatory follicles (diameter ≥5 mm) disappeared (Figure 3.2). The setup of the ultrasound machine and the scanning probe was referenced from Knox and Althouse (1999). Ovulation was assumed to have occurred 12 hours before the pre-ovulatory follicles were no longer seen on the ovaries.



(A) An ovary with pre-ovulatory follicles

(B) An ovary post-ovulation

Figure 3.2 Ultrasound images of ovaries with pre-ovulatory follicles (A) and post-ovulation (B)

4.3.5 Body condition and farrowing outcomes of the 2nd gestation

Body weight and backfat thickness were measured using the same method as in the 1st gestation. The number of born-alive, stillborn piglets and mummified fetuses were recorded once the sows finished farrowing. At the end of the 2nd gestation, birth weights of born alive and stillborn piglets were recorded after farrowing and before cross-fostering.

4.3.6 Statistical analysis

Data of continuous variables were analysed using linear mixed models for the effect of lactation diet (control vs betaine during summer lactation) and gestation diet (control vs betaine during the prior and subsequent gestation) and their interaction. The parity of sows at entry to this experiment was used as a block factor. Dietary treatments were equally allocated into each parity. Farrowing fate (classified as farrowed or not farrowed due to reproductive reasons) was analysed using Chi-squared analysis. All analyses were conducted in SPSS (24th edn, IBM, Chicago, IL, USA). Values are presented in the result tables as mean ± s.e. unless noted otherwise. The treatment effect was considered significant when $P \leq 0.05$, and a trend was considered when $P \leq 0.10$.

5. Results

5.1 Experiment 1 (weaner phase)

5.1.1 Growth performance

Pigs were allocated to the experiment with a similar body weight between dietary treatments (Table 1.2). Betaine supplementation during the early weaner phase tended to reduce growth rate (P=0.088) and significantly increased (P=0.037) FCR in both normal- and light-weight classes (Table 1.2). Feed intake during the early weaner phase was not affected by betaine supplementation. The interaction between weaning weight class and diet (early weaner phase) was not significant for any measurement during early weaner phase.

Over the late weaner phase (7-35 d post-weaning period), ADFI, ADG or FCR was not affected by betaine supplementation during early or late weaner phase (Table1.3). The interaction between weaning weight class, diet (early weaner phase) and diet (late weaner phase) was not significant for any measurement during late weaner phase (7-35 d post-weaning period).

Over the entire weaner phase (0-35 d post-weaning), ADFI, ADG or FCR was not affected by betaine supplementation during early or late weaner phase (Table 1.3). The interaction between weaning weight class, diet (early weaner phase) and diet (late weaner phase) was not significant for any measurement during the whole weaner phase.

As per the experimental design, the light-weight weaners entered the experiment with lighter body weights than the normal-weight weaners (3.6 vs 6.6 kg, se=0.10, P<0.001). The light-weight weaners had reduced ADFI (116 vs 143 g, se=3.0, P<0.001) and ADG (92 vs 109 g, se=4.0, P=0.003), and tended to have lower FCR (1.34 vs 1.44, se=0.042, P=0.071) than the normal-weight weaners during the early weaner phase (Table 1.2). The light-weight weaners had lower body weight than normal-weight weaners on the 7 d post-weaning (4.3 vs 7.4 kg, se=0.10, P<0.001). The light-weight weaners had reduced ADFI (479 vs 659 g, se=7.9, P<0.001), ADG (352 vs 473 g, se=5.2, P<0.001) and FCR (1.36 vs 1.39, se=0.010, P=0.020) than the normal-weight weaners during the late weaner phase (Table 3). The light-weight weaners had lower body weight than normal-weight weaners on d 35 post-weaning (14.1 vs 20.7 kg, se=0.24, P<0.001). Over the entire weaner phase, the light-weight weaners had reduced ADFI (396 vs 547 g, se=7.0, P<0.001), ADG (302 vs 352 g, se=5.0, P<0.001) and FCR (1.31 vs 1.36, se=0.012, P<0.001) than normal-weight weaners (Table 1.3).

5.1.2 Blood IGF-1 concentration

Betaine supplementation during early weaner phase did not affect the blood IGF-1 concentration measured in light- or normal-weight weaners on the 7 d post-weaning. The weaners with light weaning weight had reduced blood IGF-1 concentration compared to those with normal body weight (42 vs 65 ng/mL, se=4.8, P<0.001) (Figure 1.1). The interaction between diet (early weaner phase) and weaning weight class was not significant.

Betaine supplementation during the early or the late weaner phase did not affect blood IGF-1 concentration measured at 35 d post-weaning (Figure 1.2). The interaction between betaine supplementation during early and late weaner phase was not significant. Weaning weight class did not affect 35 d blood IGF-1 concentration. The three-way interaction between diet (early weaner phase), diet (late weaner phase) and weaning weight was not significant on this measurement.

Table 1.2 Growth performance (early weaner phase 0-7d post weaning) of weaners fed control or 0.1% betaine diet (mean ± se)

Variables	**Wean weight class**		**Diet (early weaner phase)**		**P-values**		
	Light (n=72 pens)	**Normal (n=72 pens)**	**Control (n=72 pens)**	**Betaine (n=72 pens)**	**Wean weight class**	**Diet (early weaner phase)**	**Two-way interaction**
Weight d 0, kg	3.6 ± 0.09	6.6 ± 0.09	5.1 ± 0.09	5.1 ± 0.09	<0.001	0.99	0.97
Weight d 7, kg	4.3 ± 0.10	7.4 ± 0.10	5.9 ± 0.10	5.7 ± 0.10	<0.001	0.63	0.91
ADFI, g	116 ± 3.3	148 ± 3.3	134 ± 3.3	130 ± 3.3	<0.001	0.42	0.84
ADG, g	92 ± 4.0	109 ± 4.0	105 ± 4.0	95 ± 4.0	0.003	0.080	0.75
FCR	1.34 ± 0.042	1.44 ± 0.042	1.33 ± 0.042	1.45 ± 0.042	0.071	0.037	0.92

Table 1.3 Growth performance (late and whole weaner phase) of weaners fed control or 0.1% betaine diet during 0-7 d or 7-35 d post weaning (mean ± se)

Variables	Wean weight class		Diet (early weaner phase)		Diet (late weaner phase)		P-values				
	Light (n=72 pens)	Normal (n=72 pens)	Control (n=72 pens)	Betaine (n=72 pens)	Control (n=72 pens)	Betaine (n=72 pens)	Wean weight class	Diet (early weaner phase)	Diet (late weaner phase)	Diet (early weaner phase) × Diet (late weaner phase)	Three-way interaction
Weight d 7, kg	4.3 ± 0.10	7.4 ± 0.10	5.9 ± 0.10	5.7 ± 0.10	5.8 ± 0.10	5.8 ± 0.10	<0.001	0.74	0.87	0.96	1.00
Weight d 35, kg	14.1 ± 0.24	20.7 ± 0.24	17.4 ± 0.24	17.4 ± 0.24	17.5 ± 0.24	17.3 ± 0.24	<0.001	0.91	0.72	0.87	0.99
ADFI (7-35 d), g	476 ± 8.3	656 ± 8.3	566 ± 8.3	567 ± 8.3	575 ± 8.3	558 ± 8.3	<0.001	0.88	0.27	0.96	1.00
ADG (7-35 d), g	352 ± 5.2	474 ± 5.2	412 ± 5.2	414 ± 5.2	416 ± 5.2	410 ± 5.2	<0.001	0.66	0.48	0.81	0.94
FCR (7-35 d)	1.35 ± 0.012	1.39 ± 0.012	1.37 ± 0.012	1.36 ± 0.012	1.38 ± 0.012	1.35 ± 0.012	0.020	0.70	0.29	0.83	0.76
ADFI (0-35 d), g	396 ± 7.4	547 ± 7.4	471 ± 7.4	471 ± 7.4	478 ± 7.4	465 ± 7.4	<0.001	0.99	0.24	0.91	0.97
ADG (0-35 d), g	302 ± 4.6	402 ± 4.6	352 ± 4.6	352 ± 4.6	350 ± 4.6	353 ± 4.6	<0.001	0.97	0.64	0.59	0.92
FCR (0-35 d)	1.31 ± 0.012	1.36 ± 0.012	1.34 ± 0.012	1.33 ± 0.012	1.35 ± 0.012	1.32 ± 0.012	0.003	0.95	0.12	0.71	0.94

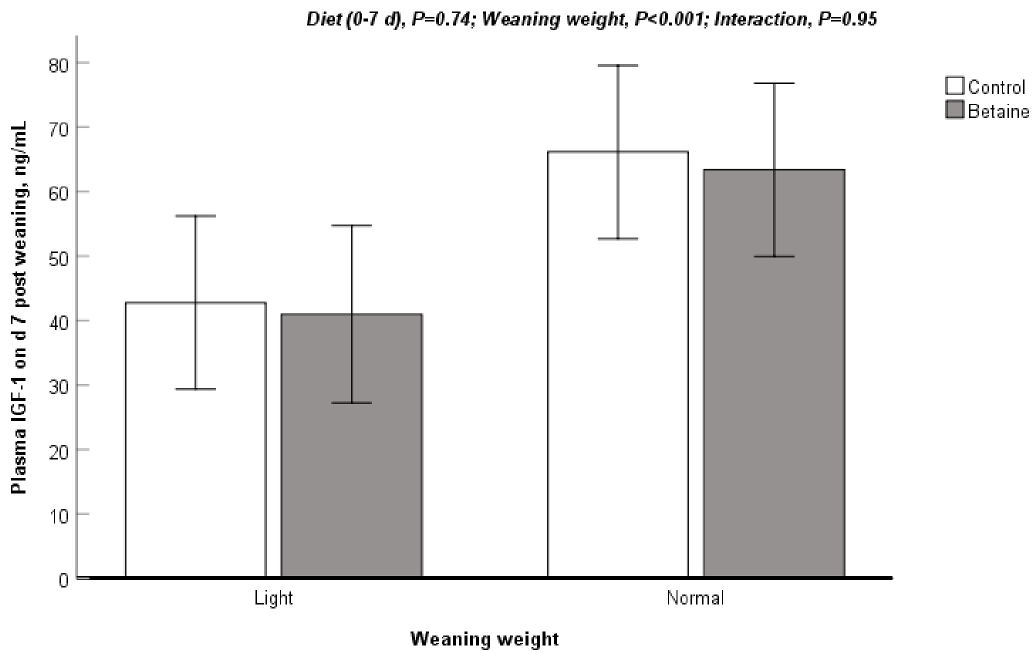


Figure 1.1 Effects of betaine supplementation and duration on blood IGF-1 concentration (mean ± se) measured on the 7 d post weaning

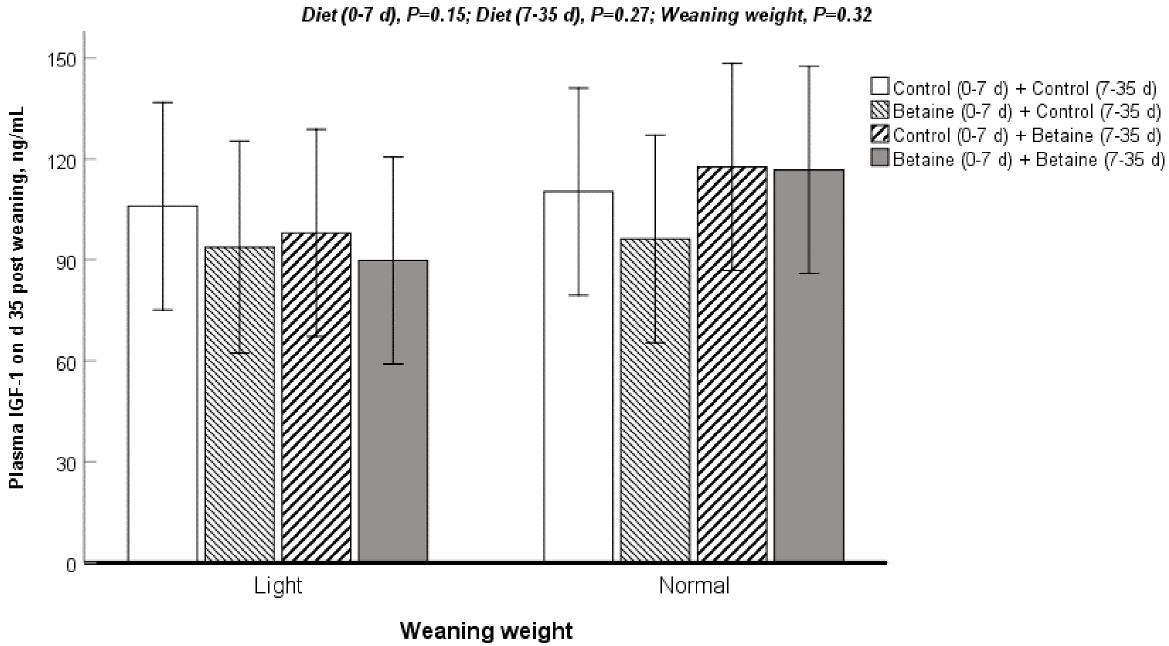


Figure 1.2 Effects of betaine supplementation and duration on blood IGF-1 concentration (mean ± se) measured on the 35 d post weaning

5.2 Experiment 2 (grower/finisher phase)

5.2.1 Growth performance (10-16 weeks age)

The ADFI, ADG and FCR evaluated for 10-16 weeks of age period was not affected by betaine supplementation (Table 2.2). Female pigs had greater ADFI (P<0.001), higher FCR (P<0.001) but similar ADG compared with castrated male pigs for 10-16 weeks of age period. The interaction between sex and diet was not significant for ADFI, ADG or FCR for 10-16 weeks of age period.

5.2.2 Growth performance (16 weeks-sale and 10-weeks-sale)

The total days from entry (10 weeks age) to sale (approximately 22 weeks age) was controlled similarly among treatment groups (Table 2.3). Body weight at 42 d and at slaughter was not affected by betaine supplementation in either the grower or finisher phase. Feed intake (ADFI; 42 d to slaughter) and FCR (42 d to slaughter) were not affected by betaine supplementation during the grower or finisher phase. Betaine supplementation during the finisher phase had the main effect (P=0.046) of reducing ADG (42 d to slaughter) from 1.08 kg to 1.04 kg per day. Neither betaine supplementation during the grower or the finisher phase affected ADFI, ADG or FCR evaluated from 10 weeks age to sale. The interaction between Grower diet × Finisher diet or the interaction among Sex × Grower diet × Finisher diet was not significant for any of the above measurements. Female pigs had lower ADG (P<0.001), higher FCR (P=0.003), and similar ADFI compared with castrated male pigs during the period from d 42 to sale. Female pigs had lower ADG (P<0.001), higher FCR (P<0.001), and tended to have greater ADFI (P=0.055) compared with castrated male pigs when the whole experimental period was evaluated.

5.2.3 Carcass traits

Carcass weight, dressing percentage, and loin depth were not affected by betaine supplementation during the grower or finisher phases (Table 2.4). The interaction between Grower diet × Finisher diet and the interaction between Sex × Grower diet × Finisher diet were not significant for HSCW, dressing percentage, or loin depth. The interaction among Sex × Grower diet × Finisher diet was significant (P=0.015) for backfat when HSCW was used as a covariate. Specifically, backfat thickness of female pigs was reduced from 12.0 mm to 11.3 mm when betaine supplementation was applied in the grower phase only, whereas the backfat of castrated male pigs was not affected by betaine supplementation during any phase.

Table 2.1 Growth performance of grower pigs (10-16 weeks) supplemented with vs without 0.1% betaine (mean ± se)

Variables	**Sex**	**Control**	**Betaine**	**P-values**		
				Sex	**Betaine**	**Interaction**
Body weight at entry, kg	female	26.8 ± 0.71	26.7 ± 0.77	0.79	0.88	0.98
	castrates	26.6 ± 0.69	26.5 ± 0.69			
Body weight 42 d, kg	female	61.0 ± 1.33	61.2 ± 1.41	0.96	0.68	0.79
	castrates	60.7 ± 1.26	61.6 ± 1.30			
ADFI, kg	female	1.77 ± 0.034	1.79 ± 0.037	<0.001	0.56	0.91
	castrates	1.64 ± 0.033	1.66 ± 0.033			
ADG, kg	female	0.82 ± 0.018	0.82 ± 0.019	0.88	0.51	0.55
	castrates	0.83 ± 0.016	0.81 ± 0.017			
FCR	female	2.18 ± 0.042	2.15 ± 0.039	<0.001	0.97	0.44
	castrates	2.03 ± 0.037	2.01 ± 0.038			

Table 2.2 Growth performance of pigs (16 weeks -slaughter) supplemented with vs without 0.1% betaine during grower (10-16 weeks) vs finisher phase (16-22 weeks) (mean ± se)

Variables	**Sex**	**Grower-Control**		**Grower- Betaine**		**P-values**				
		Finisher-control	**Finisher-betaine**	**Finisher-control**	**Finisher-betaine**	**Sex**	**Grower diet**	**Finisher diet**	**Grower* Finisher diet**	**Sex*Grower diet*Finisher diet**
Days from entry to slaughter, days	female	83.7 ± 2.38	84.4 ± 2.27	85.0 ± 2.71	84.9 ± 2.39	0.96	0.79	0.93	0.90	0.90
	castrates	84.4 ± 2.27	84.4 ± 2.27	84.4 ± 2.27	84.4 ± 2.27					
Body weight, entry, kg	female	26.8 ± 0.82	26.7 ± 0.77	27.4 ± 0.92	26.3 ± 0.81	0.75	0.92	0.57	0.76	0.80
	castrates	26.6 ± 0.77	26.6 ± 0.77	26.6 ± 0.77	26.5 ± 0.77					
Body weight, 42 d, kg	female	60.8 ± 1.36	61.1 ± 1.21	61.7 ± 1.45	61.1 ± 1.28	0.95	0.65	0.49	0.87	0.62
	castrates	60.2 ± 1.81	61.2 ± 1.81	60.2 ± 1.81	63.1 ± 1.91					
Body weight, sale#, kg	female	105.1 ± 1.60	104.1 ± 1.51	105.9 ± 1.81	104.3 ± 1.60	0.004	0.61	0.47	0.81	0.96
	castrates	108.0 ± 1.51	107.8 ± 1.51	108.8 ± 1.51	108.2 ± 1.51					
ADFI, 42 d-sale*, kg	female	2.51 ± 0.071	2.42 ± 0.068	2.53 ± 0.081	2.47 ± 0.071	0.73	0.97	0.45	0.43	0.33
	castrates	2.47 ± 0.068	2.56 ± 0.068	2.53 ± 0.068	2.44 ± 0.068					
ADG, 42 d-sale*, kg	female	1.04 ± 0.031	1.01 ± 0.028	1.03 ± 0.033	1.02 ± 0.029	<0.001	0.89	0.046	0.55	0.31
	castrates	1.12 ± 0.028	1.09 ± 0.028	1.15 ± 0.028	1.05 ± 0.029					
FCR, 42 d-sale*, kg	female	2.42 ± 0.070	2.41 ± 0.063	2.46 ± 0.075	2.42 ± 0.066	0.003	0.95	0.22	0.88	0.84
	castrates	2.23 ± 0.063	2.36 ± 0.063	2.21 ± 0.063	2.35 ± 0.066					
ADFI, d 0-sale#, kg	female	2.13 ± 0.046	2.09 ± 0.044	2.16 ± 0.052	2.12 ± 0.046	0.055	0.66	0.83	0.51	0.50
	castrates	2.03 ± 0.044	2.10 ± 0.044	2.07 ± 0.044	2.05 ± 0.044					
ADG, d 0-sale#, kg4	female	0.93 ± 0.011	0.92 ± 0.011	0.93 ± 0.013	0.93 ± 0.011	<0.001	0.45	0.50	0.98	0.65
	castrates	0.96 ± 0.011	0.96 ± 0.011	0.98 ± 0.011	0.97 ± 0.011					
FCR, d 0-sale#, kg4	female	2.30 ± 0.036	2.28 ± 0.034	2.33 ± 0.040	2.29 ± 0.036	<0.001	0.99	0.84	0.38	0.63
	castrates	2.11 ± 0.034	2.18 ± 0.034	2.12 ± 0.034	2.12 ± 0.034					

Days from entry to sale (84.4) was used as a covariate; *Days from 16 weeks age to sale (42.6) was used as a covariate

Table 2.3 Carcass traits of pig supplemented with vs without 0.1% betaine during grower (10-16 weeks) vs finisher phase (16-22 weeks) (mean ± se)

Variables	**Sex**	**Grower -Control**		**Grower- Betaine**		**P-values**				
		Finisher-control	**Finisher-betaine**	**Finisher-control**	**Finisher-betaine**	**Sex**	**Grower diet**	**Finisher diet**	**Grower*Finisher diet**	**Sex*Grower diet*Finisher diet**
HSCW, kg	female	80.2 ± 1.81	79.3 ± 1.72	81.2 ± 2.06	79.6 ± 1.81	0.51	0.6	0.67	0.75	0.96
	castrates	80.3 ± 1.72	80.9 ± 1.72	81.5 ± 1.72	81.2 ± 1.72					
Backfat, mm	female	12.0 ± 0.34	11.4 ± 0.32	11.4 ± 0.38	11.6 ± 0.34	0.28	0.96	0.73	0.72	0.18
	castrates	11.1 ± 0.32	11.4 ± 0.32	11.5 ± 0.32	11.3 ± 0.32					
Backfat#, mm	female	12.0 ± 0.18	11.5 ± 0.17	11.3 ± 0.20	11.7 ± 0.18	0.003	0.35	0.98	0.23	0.015
	castrates	11.2 ± 0.17	11.4 ± 0.17	11.3 ± 0.17	11.2 ± 0.17					
Loin depth, mm	female	56.8 ± 0.92	56.5 ± 0.87	56.2 ± 1.04	55.9 ± 0.92	<0.001	0.73	0.37	0.95	0.96
	castrates	54.0 ± 0.87	53.2 ± 0.87	54.1 ± 0.87	53.4 ± 0.87					
Loin depth#, mm	female	56.9 ± 0.58	56.9 ± 0.55	55.9 ± 0.65	56.2 ± 0.58	<0.001	0.23	0.37	0.62	0.88
	castrates	54.1 ± 0.55	53.0 ± 0.55	53.7 ± 0.55	53.1 ± 0.55					
Dressing, %	female	76.8 ± 0.30	76.2 ± 0.29	76.4 ± 0.34	76.1 ± 0.30	<0.001	0.99	0.91	0.72	0.32
	castrates	74.4 ± 0.29	75.1 ± 0.29	74.9 ± 0.29	75.0 ± 0.29					

Hot standard carcass weight (HSCW; 80.5 kg) was used as a covariate

5.3 Experiment 3 (gestation/lactation phase)

5.3.1 Farrowing outcomes of the 1st gestation

Sows were allocated into the control and betaine treatments at the start of the 1st gestation with a similar body weight and backfat thickness (Table 3.2). Betaine reduced body weight gain over the first gestation (44.7 vs 41.8 kg, P<0.007), resulting in a lower body weight (278.6 vs 274.7 kg, P=0.018) at entry to farrowing house (day 108 of gestation) for sows on the betaine diet although gestational ADI was similar. Backfat thickness gain over the first gestation was not affected by betaine supplementation during the first gestation. The percentage of sows failed the pregnancy during early gestation (by day 30) was small (<2.0%), and it was not affected by betaine supplementation during the 1st gestation (Figure 3.2). Betaine supplementation during the first gestation did not affect farrowing rate (Figure 3.3), number of piglets born alive, stillborn or number of mummied fetuses (Table 3.2).

5.3.2 Lactation performance during summer

Litter weight and litter size (post-foster within treatment group) was similar between treatments; however, the average piglet weight (post-foster) was reduced by betaine supplementation during the 1st gestation (1.55 kg vs 1.49 kg, P=0.006) (Table 3.3). The litter weight and litter size measured on the 21st day of lactation was not affected by betaine supplementation during the prior gestation or over the summer lactation period. The ADFI of sows, weaning body weight of lactating sows, or the weaning weight of piglets was not affected by betaine supplementation during the prior gestation or over the summer lactation period. Sows fed betaine supplementation during the summer lactation tended to lose less backfat thickness than those did not receive betaine in lactation diet (-2.0 vs -2.4 mm, P=0.102) (Table 3.3).

5.3.3 Weaning-to-oestrus interval and oestrus-to-ovulation intervals after summer lactation

Betaine supplementation during the prior gestation or summer lactation did not affect the weaning-to-oestrus interval (Table 3.3) or oestrus-to-ovulation interval (av. 1.2 d; Figure 3.4) after the summer lactation.

5.3.4 Farrowing outcomes of the 2nd gestation

At the start of the 2nd gestation, body weight and backfat thickness were both similar among the treatment groups (Table 3.4). Again, sows fed betaine during the 1st and 2nd gestation had a reduced body weight gain over the 2nd gestation (44.5 vs 40.9 kg, P=0.029), resulting in a lower body weight at the end of the 2nd gestation (295 vs 290 kg, P=0.005) compared with the sows did not receive betaine during the gestations. The gain of backfat over the 2nd gestation was not affected by betaine supplementation in gestations or the summer lactation (Table 3.4). Neither the reproductive failure during early gestation (d 30) (Figure 3.5) or the farrowing rate (Figure 3.6) was not affected by betaine supplementation in the gestations or the summer lactation. Number of born-alive or stillborn piglets after the 2nd gestation was not affected by betaine supplementation in the gestations or the summer lactation; however, betaine supplementation during the summer lactation reduced the number of mummified fetuses after the 2nd gestation (0.41 vs 0.24 mummified foetuses per litter, P=0.017). Litter birth weight or average piglet birth weight after the 2nd gestation was not affected by betaine supplementation in the gestations or the summer lactation.

5.3.5 Physiological signs of heat stress of sows during summer lactation and subsequent gestation (2nd gestation)

During summer lactation, sows had a faster respiration rate at 4 PM than at 9 AM (Time, P<0.001) (Figure 3.7). The interaction between lactation diet*day*time was significant (P=0.040), such that betaine supplementation during lactation reduced respiration rate at 4 PM on the 7th day of lactation. Betaine supplementation during the prior gestation did not affect respiration rate at any timepoints. Rectal temperature of lactating sows was greater at 4 PM than at 9 AM (Time, P<0.001; Figure 3.8). Betaine supplementation during the 1st gestation or the summer lactation did not affect rectal temperature. No interactive effects were found significant.

In the subsequent gestation after the summer lactation, sows had a greater respiration rate (Figure 3.9) and rectal temperature (Figure 3.10) at 4 PM than at 9 AM (Time, both P<0.001). Both respiration rate and rectal temperature declined with week of gestation (Weeks, both P<0.001). The interaction among gestation diet*lactation diet*time was significant (P=0.022), such that the sows offered betaine during summer lactation but not during gestations (1st and 2nd) had a greater respiration rate than other dietary treatment groups at 4 PM during the 2nd gestation (Figure 3.9). Betaine supplementation during gestations (1st and 2nd) or summer lactation did not affect the rectal temperature of sows during the 2nd gestation (Figure 3.10). None of interactive effects were significant for rectal temperature.

Table 3.2 Body conditions and farrowing outcomes of sows fed control vs betaine over the 1st gestation (prior to summer lactation)

Variables	**Control (n=320)**	**Betaine (n=320)**	**SEM**	**P-values**
Parity of sows	3.4	3.4	0.081	0.98
Body weight at d 0 of first gestation, kg	234.9	234.9	1.24	0.99
Backfat at d 0 of first gestation, mm	19.00	19.00	0.26	0.94
Body weight at d 108 of first gestation, kg	278.6	274.7	1.24	0.018
Backfat at d 108 of first gestation, mm	21.3	21.1	0.30	0.53
Body weight change d0-d108 first gestation, kg	44.7	41.8	0.80	0.007
Back fat change d0-d108 first gestation, kg	2.4	2.3	0.15	0.77
ADI, kg	2.31	2.31	0.005	0.88
Number of piglets born alive per litter	12.4	12.7	0.19	0.22
Number of stillborn piglets per litter	1.0	1.0	0.09	0.60
Number of mummified piglets per litter	0.3	0.3	0.04	0.46
Number of total piglets per litter	13.8	14.0	0.21	0.46

Table 3.3 Lactation performance of sows fed control vs 0.2% betaine during summer lactation vs the prior gestation

Variables	Gestation diet (G)		SEM	Lactation diet (L)		SEM	P-values		
	Control (n=272)	Betaine (n=269)		control (n=276)	betaine (n=265)		G	L	G x L
Litter weight post-foster, kg	18.2	17.9	0.20	18.2	17.9	0.2	0.25	0.35	0.28
Litter size post-foster	11.9	12.0	0.12	12	11.9	0.12	0.43	0.37	0.44
Average piglet weight post-foster, kg	1.55	1.49	0.015	1.52	1.52	0.014	0.006	0.91	0.82
Litter weight (d 21), kg	61.3	62.3	0.99	62.3	61.2	0.99	0.44	0.41	0.64
Litter size (d 21)	9.9	10.1	0.16	10	10	0.16	0.22	0.96	0.79
Average piglet weight (d 21), kg	6.2	6.2	0.061	6.3	6.2	0.061	0.63	0.21	0.41
Litter weight gain (d 21- post-foster), kg	43.0	44.3	0.96	44.0	43.3	0.96	0.30	0.56	0.50
Weaning age of first lactation, days	28	28	0.1	28	28	0.1	0.71	0.59	0.89
Average daily feed intake (lactation), kg	5.86	5.84	0.052	5.84	5.85	0.052	0.75	0.95	0.35
Wean-to-oestrus interval, d	6.0	5.8	0.38	5.6	6.2	0.38	0.69	0.26	0.31
Body weight at weaning, kg	255	254	1.5	253	256	1.5	0.53	0.21	0.46
Backfat at weaning, mm	18.9	19.1	0.3	18.6	19.3	0.3	0.55	0.082	0.56
Body weight change during lactation, kg	-21.3	-23.3	0.99	-21.4	-23.2	0.99	0.14	0.18	0.38
Backfat change during lactation, kg	-2.2	-2.2	0.19	-2.4	-2.0	0.19	0.76	0.102	0.74

Table 3.4 Body conditions and farrowing outcomes (2^{nd} gestation) for sows fed control vs 0.2% betaine during summer lactation vs gestations (prior and subsequent)

Variables	**Gestation diet (G)**		**SEM**	**Lactation diet (L)**		**SEM**	**P-values**		
	control (n=152)	**betaine (n=152)**		**control (n=145)**	**betaine (n=159)**		**G**	**L**	**G x L**
Parity of sows	4.4	4.6	0.09	4.4	4.4	0.09	0.60	0.91	0.63
Body weight (d 0 of second gestation), kg	249	249	1.50	248	250	1.46	0.84	0.59	0.46
Backfat (d 0 of second gestation), mm	18.6	18.6	0.3	18.4	18.8	0.3	0.97	0.36	0.21
Body weight (d 108 of second gestation), kg	295	290	1.7	293	291	1.7	0.005	0.33	0.64
Backfat (d 108 of second gestation), mm	21.2	20.6	0.4	20.7	21.1	0.4	0.21	0.43	0.95
Body weight change (d 108-d 0), kg	44.5	40.9	1.25	43	42.6	1.25	0.029	0.69	0.58
Backfat (d 108-d 0), mm	2.5	2.0	0.27	2.3	2.1	0.27	0.15	0.59	0.08
ADI (2nd gestation), kg	2.29	2.30	0.005	2.3	2.29	0.005	0.18	0.59	0.19
Number of piglets born alive	12.7	12.5	0.25	12.8	12.4	0.24	0.41	0.19	0.49
Number of stillborn piglets	1.1	1.2	0.12	1.01	1.27	0.12	0.91	0.11	0.062
Number of mummified foetuses	0.37	0.29	0.054	0.41	0.24	0.052	0.26	0.017	0.32
Number of total piglets	14.2	13.9	0.27	14.6	13.9	0.27	0.35	0.32	0.097
Litter birth weight, kg	18.8	18.4	0.35	18.6	18.5	0.34	0.34	0.78	0.88
Average piglet birth weight, kg	1.40	1.39	0.022	1.39	1.39	0.022	0.66	0.92	0.086

Only sows that farrowed at the end of 2nd gestation are included in the table.

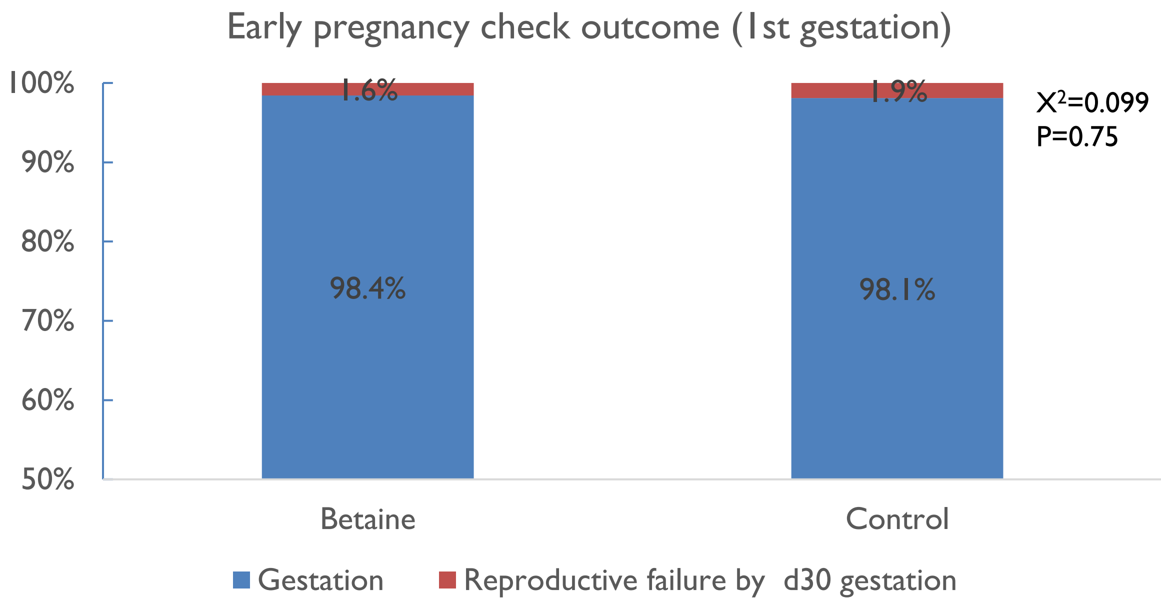


Figure 3.2 Outcomes of early pregnancy check (d 30 of gestation) of sows fed control vs 0.2% betaine diet during the gestation in prior to a summer lactation

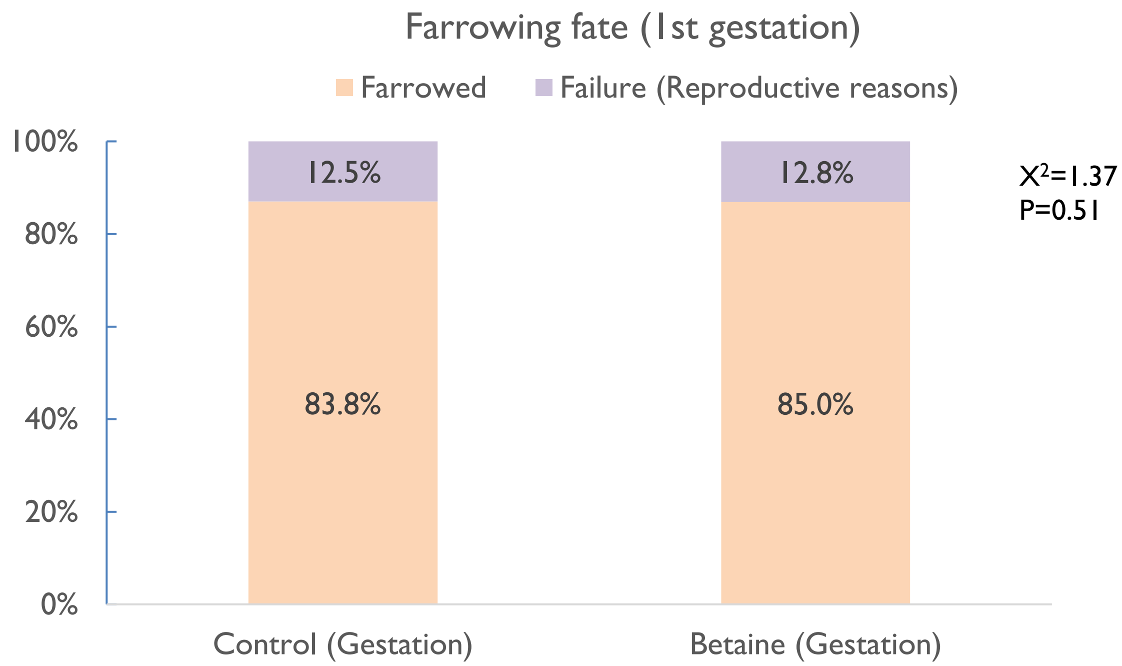


Figure 3.3 Farrowing rate of sows fed control vs 0.2% betaine diet during the gestation in prior to a summer lactation

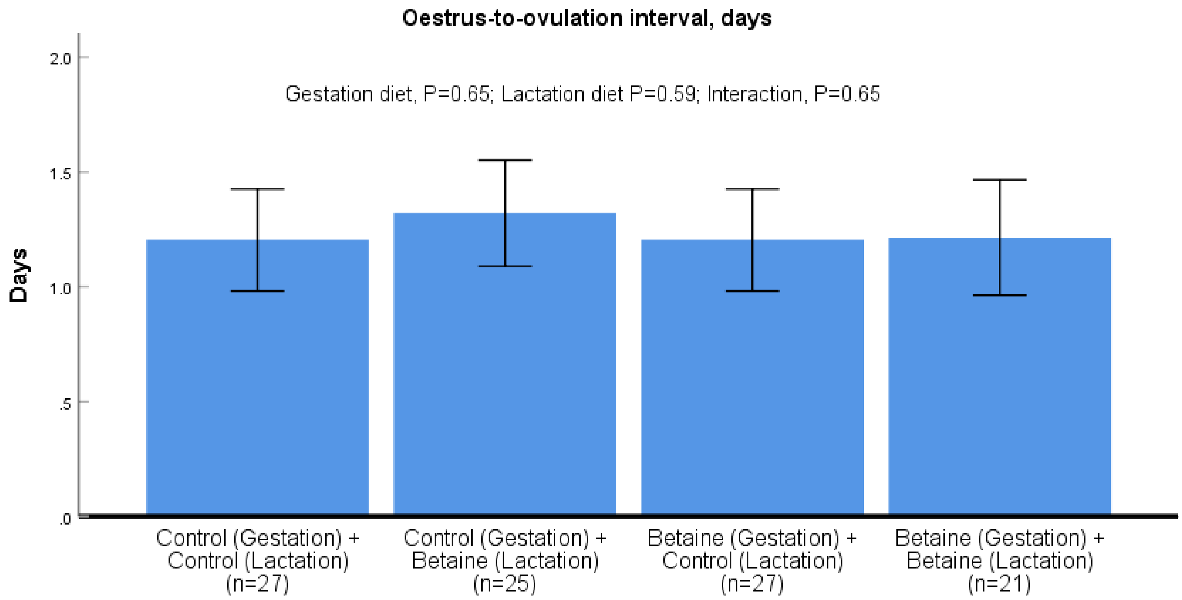


Figure 3.4 Oestrus-to-ovulation interval of sows fed control vs 0.2% betaine diet during summer lactation vs the prior gestation

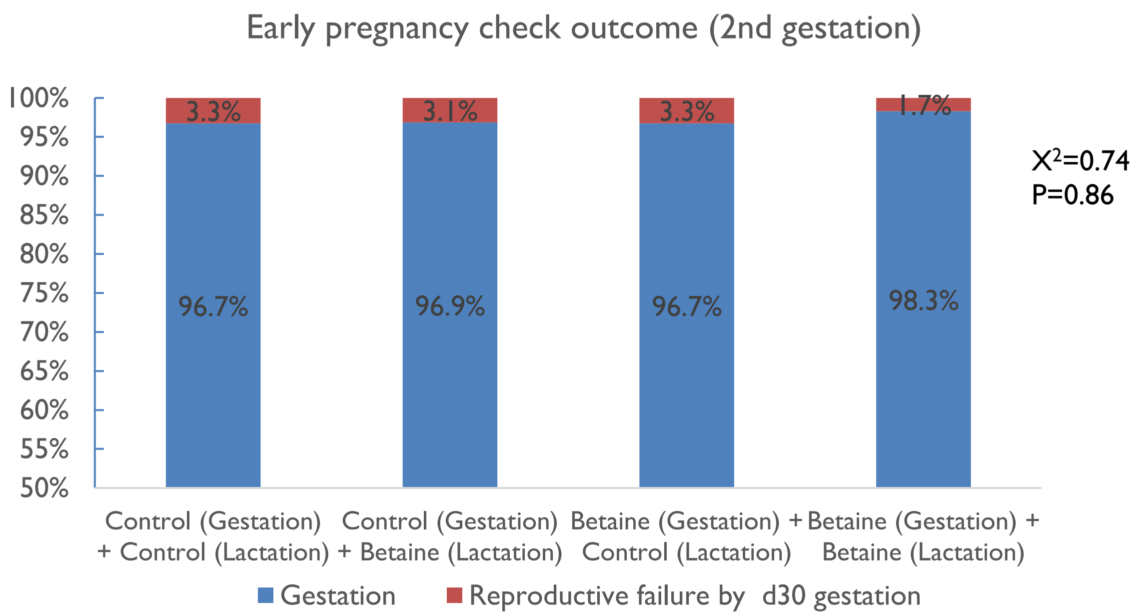


Figure 3.5 Outcomes of early pregnancy check (d 30 of gestation) of sows fed control vs 0.2% betaine diet during summer lactation vs gestations (prior and subsequent)

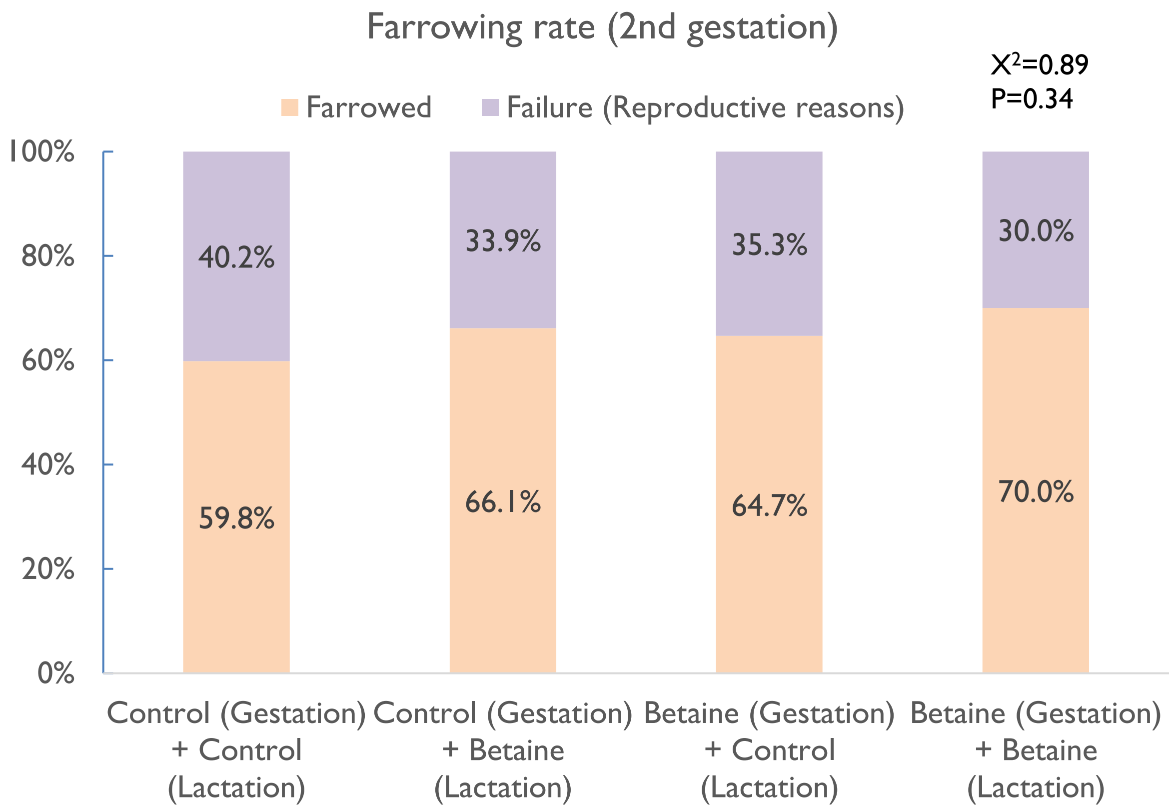
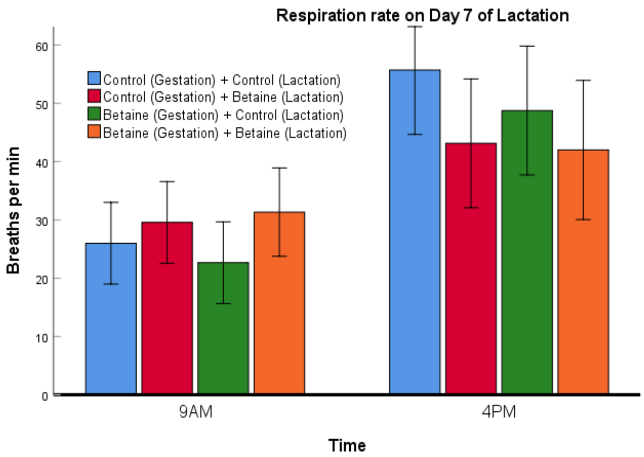


Figure 3.6 Farrowing rate of sows fed control vs 0.2% betaine diet during summer lactation vs gestations (prior and subsequent)

The farrowing rate was from the sows weaned and mated in summer. The main effect of betaine supplementation during summer lactation or the gestations (prior and subsequent) was not significant (P>0.10).

A



B

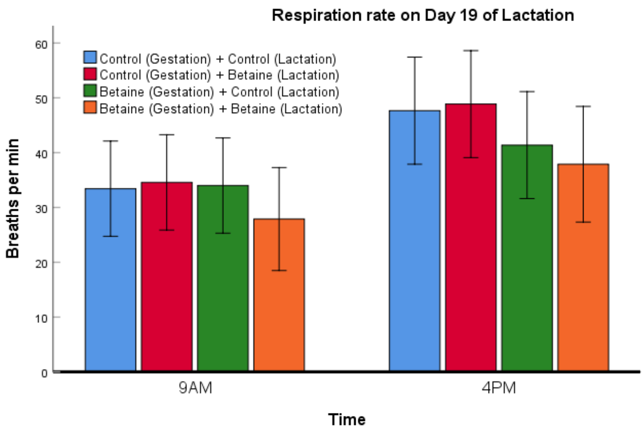
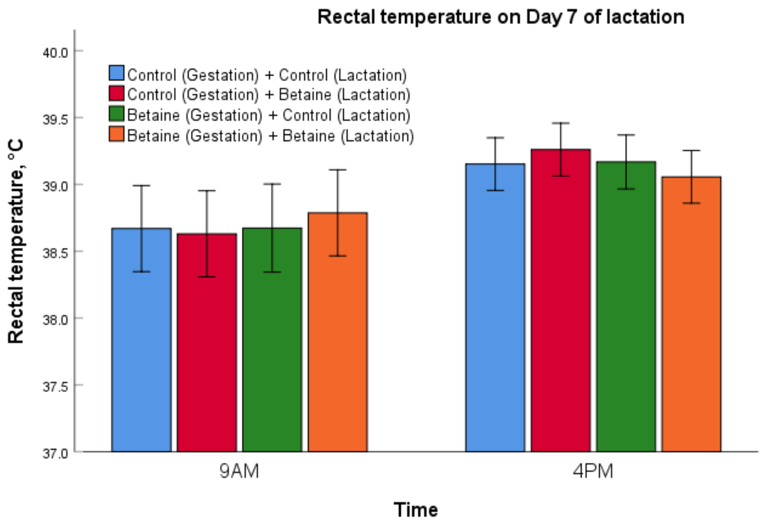


Figure 3.7 Respiration rate of lactating sows on day 7 (A) and 19 (B) during summer lactation (mean ± se)

The P-values are 0.75 (day), <0.001 (time), 0.13 (gestation diet), 0.50 (lactation diet), 0.96 (gestation diet*lactation diet), 0.31 (gestation diet*time), 0.102 (lactation diet*time), 0.49 (gestation diet*day), 0.99 (lactation diet*day), 0.78 (gestation diet*day*time), 0.040 (lactation diet*day*time), 0.91 (gestation diet*lactation diet*day*time).

A



B

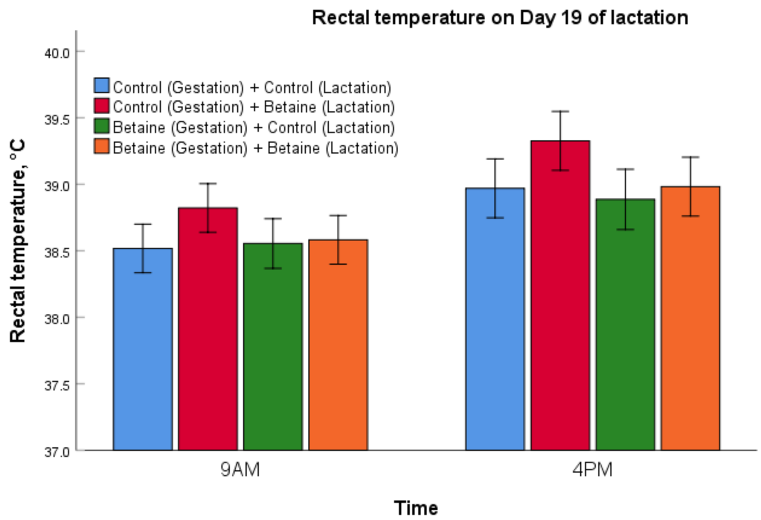
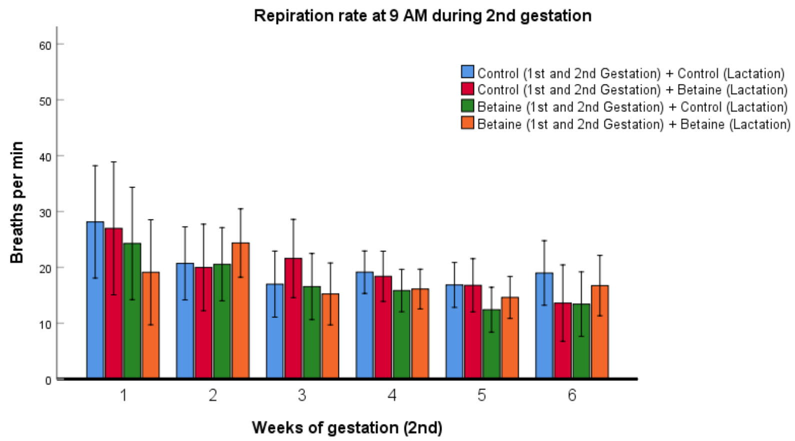


Figure 3.8 Rectal temperature of lactating sows on day 7 (A) and 19 (B) during summer lactation (mean ± se)

The P-values are 0.12 (day), <0.001 (time), 0.30 (gestation diet), 0.18 (lactation diet), 0.34 (gestation diet*lactation diet), 0.12 (gestation diet*time), 0.91 (lactation diet*time), 0.22 (gestation diet*day), 0.14 (lactation diet*day), 0.33 (gestation diet*day*time), 0.62 (lactation diet*day*time), 0.33 (gestation diet*lactation diet*day*time).

A



B

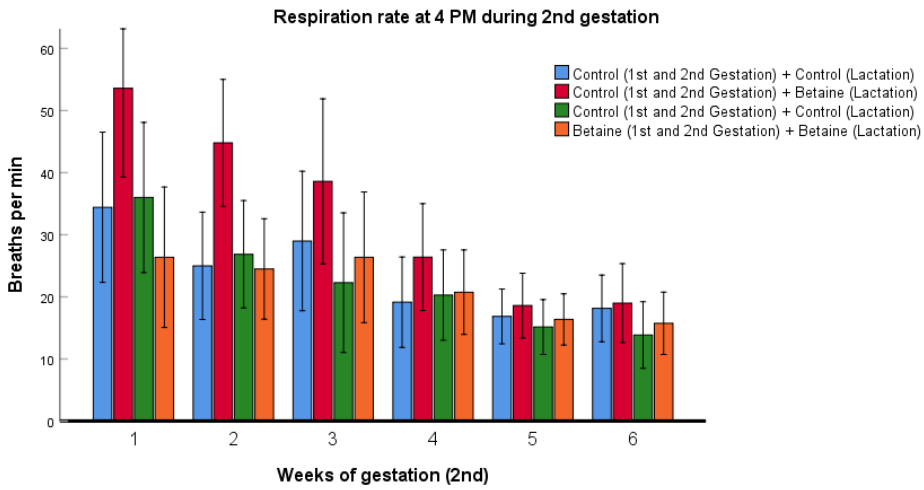
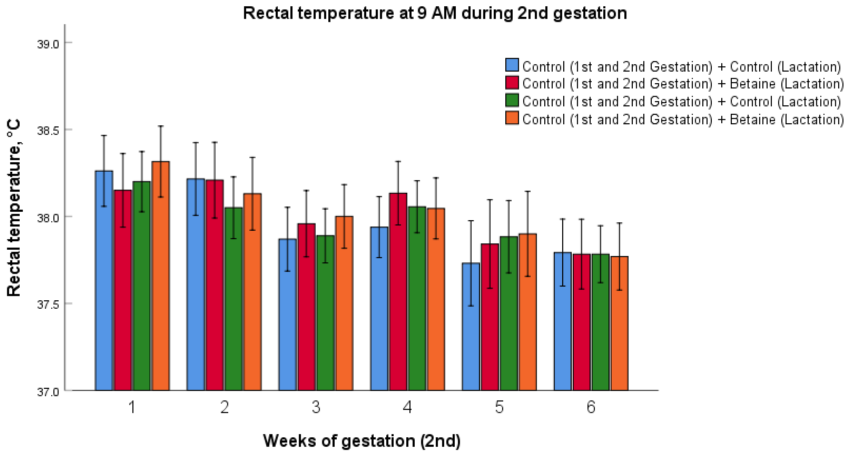


Figure 3.9 Respiration rate of gestating sows at 9 AM (A) and 4 PM (B) during the first six weeks of the second gestation (mean ± se)

The P-values are <0.001 (week), <0.001 (time), 0.052 (gestation diet), 0.32 (lactation diet), 0.30 (gestation diet*lactation diet), 0.090 (gestation diet*time), 0.067 (lactation diet*time), 0.022 (gestation diet*lactation diet*time), 0.21 (gestation diet*week), 0.60 (lactation diet*week), 0.18 (gestation diet*week*time), 0.32 (lactation diet*week*time), and 0.13 (gestation diet*lactation diet*day*time).

A



B

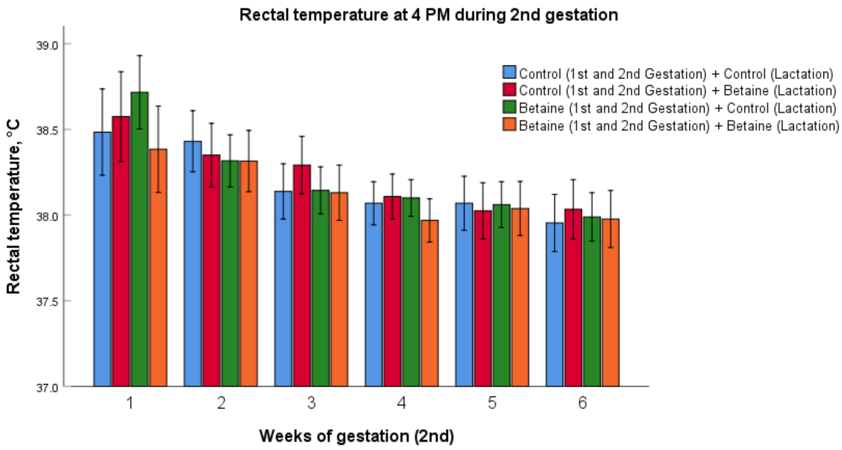


Figure 3.10 Rectal temperature of gestating sows at 9 AM (A) and 4 PM (B) during the first six weeks of the second gestation (mean ± se)

The P-values are <0.001 (week), <0.001 (time), 0.81(gestation diet), 0.78 (lactation diet), 0.49 (gestation diet*lactation diet), 0.41 (gestation diet*time), 0.19 (lactation diet*time), 0.70 (gestation diet*week), 0.55 (lactation diet*week), 0.76 (gestation diet*week*time), 0.42 (lactation diet*week*time), and 0.13 (gestation diet*lactation diet*day*time).

6. Discussion

6.1 Experiment 1 (weaner phase)

The hypothesis tested in Experiment 1 was that 0.1% betaine supplementation can increase IGF-1 secretion and growth performance in weaned piglets particularly when applied within early weaner phase (0-7 d), and the effect would be more pronounced in light-weight weaners. The experiment found that betaine supplementation during either the early or late weaner phase or over the whole weaner did not affect the growth performance over the late weaner phase (7-35 d) or the whole weaner phase (0-35 d). Adversely, the ADG and FCR of the early weaner phase were both compromised by betaine supplementation. Blood IGF-1 concentrations measured on 7 d and 35 d were not affected by betaine supplementation. Therefore, the hypothesis was rejected.

The basal diet used in the experiment was formulated with high levels methionine (0.60% and 0.48%), cysteine (0.30% and 0.26%) and choline (1250 and 1440 ppm) for the early and late weaner phases respectively. The levels of methionine and choline in our basal diets were well above the requirements for 5-25 kg weaners as recommended in NRC 2012 (0.49%-0.40% methionine and 600-400 ppm choline), to ensure that the effect of betaine on growth performance observed in the current study was independent of its methionine- or choline- sparing effect. In fact, the ability of weaner pigs to use betaine for sparing methionine is inconsistently reported (Emmert *et al.* 1998; Matthews *et al.* 2001). Our results showed that betaine supplementation increased FCR by 9% and reduced ADG by 11% during the early weaner phase. Similarly, a previous study showed betaine supplementation numerically reduced ADG during 0-7 d post-weaning when the basal diet contained sufficient (0.40%- 0.45%) methionine (Matthews *et al.* 2001). The negative impacts of betaine supplementation on growth performance may be due to an imbalanced ratio between amino acids and dietary energy. For example, betaine supplementation at 0.1%-0.2% negatively affected growth performance of finisher pigs when the basal diets contained relatively high ratio of amino acids and dietary energy (eg: 0.55 g standardised ileal digestible (SID) lysine to MJ digestible energy (DE) and 0.20-0.30 g SID methionine to MJ DE in these studies) (Matthews et al. 1998; Mendoza et al. 2017). The diets used in our weaner experiment also contained high levels of dietary amino acids (0.90 g SID lysine per MJ DE and 0.36 g SID methionine per MJ DE), which may result in betaine supplementation inhibiting growth performance particularly in the early weaner phase. To understand the exact mechanism, future experiments should investigate the interactive effects between betaine and amino acids: DE ratio on growth performance of weaner pigs. Our results showed that growth performance during late weaner phase was not affected by the betaine supplementation during the early or late weaner phase, which agreed with the previous findings that betaine supplementation did not improve growth performance when methionine level was sufficient (Matthews *et al.* 2001).

Unlike the studies that have reported that betaine supplementation increased IGF-1 secretion in finisher pigs (Huang *et al.* 2006; Lothong *et al.* 2016), our results showed that betaine supplementation did not affect blood IGF-1 concentration measured at 7 d and 35 d post weaning. The disparity in responses may be due to the age difference of pigs used in these studies. In other words, weaner pigs have an immature somatotropin-IGF axis. For example, a study showed that IGF-1 secretion was not responsive to exogenous somatotropin treatment until pigs reached 43 d age and then the response increased with age (Harrell *et al.* 1999). The exact mechanism of betaine to stimulate IGF-1 secretion is not understood. We speculate that betaine may be able to stimulate somatropin secretion, given IGF-1 is a down-stream hepatic hormone stimulated by somatotropin produced from the pituitary gland. One study reported that 0.125% betaine supplementation can increase the pulsatile secretion of somatotropin in finisher pigs (Huang *et al.* 2007). Another explanation is that betaine has a similar effect to methionine in upregulating IGF-1 via activating Peroxisome Proliferator-Activated Receptors (PPAR) pathway in hepatocytes (Wan *et al.* 2017) . For example, an *in vitro* study showed that adding betaine in cultured rat hepatocytes can increase the IGF-1 protein secretion (Lee *et al.* 2006), suggesting the effect of betaine in improving IGF-1 secretion can be independent of somatotropin. It is possible that the basal diet was already sufficient in methionine and choline, such that betaine supplementation did not increase IGF-1 production further. A limitation of the current experiment is that the sample size used for IGF-1 comparison was relatively small (n=6 per treatment on each day point), thus may not be sufficient for detecting the true difference among all treatment groups. This should be investigated in future studies.

Our study confirmed that the body weight class at weaning can significantly affect the entire weaner phase performance, which agrees with previous findings (Slifierz *et al.* 2013; Collins *et al.* 2017). Our results showed that the weaners with a light weaning weight (av. 3.6 kg) had a 14% lower ADG than those in the normal weaning weight class (av. 6.6 kg) over the 0-35 d weaner phase. Blood IGF-1 measured 7 d post-weaning was 35% lower in light- compared to normal-weight weaners, which reflects the inferior production performance of the light-weight weaners. Interestingly, we observed that the blood IGF-1 concentration became similar between the two weaning weight classes on 35 d post-weaning even though the body weight was still 34% lower for the light-weight weaners. Similarly, a human study showed that IGF-1 was lower in light-weight infants at birth as a consequence of inferior fetal development, but it exceeded that of normal-weight infants from one-year age when insulin becomes the main determinant for IGF-1 secretion (Iñiguez *et al.* 2006). The overall feed intake of piglets increased dramatically over the weaner phase, therefore the role of insulin in determining IGF-1 secretion might become more obvious in the late weaner phase. Light-weight weaners are usually born with a light body weight, and born-light piglets can develop insulin resistance in later life (Niu *et al.* 2019), then hyperinsulinemia can lead to an increased concentration of circulating IGF-1. To summarise, we speculate that the inferior growth performance of the light-weight weaners during the early weaner phase may be due to the inadequate IGF-1, but the poor growth performance of light-weight weaners during the late weaner phase may be a consequence of insulin resistance which could gradually develop with age. Light-weight weaners may require different nutritional interventions during early and late weaner phase in order to improve growth performance in the future.

In conclusion, 0.1% betaine supplementation is not recommended in the weaner diet as specified in this study, particularly when other dietary methyl donors are adequate. Strategies to reduce mortality and improve growth performance of light-weight weaners should be explored in future.

6.2 Experiment 2 (grower/finisher phase)

The hypothesis tested in Experiment 2 was that betaine supplementation during the grower phase is more effective than during the finisher phase in reducing carcass backfat. The main finding of the study was that supplementing 0.1% betaine during the grower phase (10-16 weeks age) can reduce backfat from 12.0 mm to 11.3 mm in female pigs at an 80.5 kg standardised carcass weight. The finding has provided evidence that betaine supplementation during the grower phase may be used as a partial solution to reducing carcass backfat thickness.

The current study is the first to explore the effects of different durations of betaine supplementation in fattening pigs. The three-way interaction between sex and betaine supplementation during either the grower or finisher phase indicated betaine supplementation during the grower phase (but not finisher phase) reduced carcass backfat thickness in female pigs but this effect was not found in castrated male pigs. The results partially supported our hypothesis that betaine is more effective in reducing carcass backfat when applied during a younger phase (10-16 weeks) compared with an older phase. Dunshea *et al.* (2009) found 0.15% betaine supplementation during the finisher phase (from 15 weeks of age) did not affect backfat thickness at slaughter. The duration dependent effects of betaine in reducing carcass fatness may be related to the age-dependent ratio of muscle to fat deposition. For example, the ratio of muscle to fat deposition rate reduces when body weight increases in the range of 55 kg to 127 kg (Friesen et al. 1994a; Friesen et al. 1994b). With regard to the sex-dependent effect of betaine on reduce carcass backfat thickness, contrary to our result, an earlier study showed that betaine reduced backfat thickness in castrated male pigs but not female pigs (Lawrence *et al.* 2002). The disparity between results may be due to the different basal fatness between sexes in these two experiments. Specifically, the experiment conducted by Lawrence *et al*. (2002) found that the castrated male had 30% greater backfat thickness than the female pigs at slaughter; whereas, conversely in our current study the castrated males had lower carcass backfat thickness than the female pigs. Combining the two studies, it is consistent that betaine supplementation is effective in reducing carcass fatness in the sex that had the greater fat deposition, implying a potential interactive effect with the basal fat deposition rate. The exact mechanism of how betaine reduces carcass backfat thickness remains unclear, however possible modes of action include effects of betaine in increasing fatty acid oxidation in muscles (Li *et al.* 2017) and improved insulin sensitivity (Du *et al.* 2018). It is also worthwhile to mention the grower/finisher experiment was conducted during summer conditions (January-March 2020), and although this was unintentional, it is known that hot conditions can attenuate lipolysis in grower pigs (Pearce *et al.* 2013), thus it is possible that the betaine-reduced carcass backfat was a reflection of the attenuated lipolysis being improved by betaine supplementation. Future experiments should be conducted to confirm such effect in cooler seasons.

From the results of the present study, betaine supplementation during the finisher phase (16 weeks to slaughter) is not recommended, because of its negative effect in reducing ADG by 40 g, regardless of sex. This finding is in agreement with a study by Mendoza et al. (2017), in which 0.2% betaine reduced ADG by 50 g when supplemented to pigs between 90 kg-120 kg live weight. The ADG reduction was associated with > 100 g/d reduction in ADFI in that study, whereas the ADFI was not significantly affected by betaine supplementation in our current study. Experiment 1 conducted in this project showed that 0.1% betaine can reduce ADG and increase FCR when supplemented in the first seven days post-weaning. As discussed in Experiment 1, there seems to be an interactive effect between betaine and lysine: energy ratio on growth performance – e.g. betaine reduced growth performance when the SID lysine: DE ratio in basal diet was higher than approximately 0.50 g (without ractopamine) (Matthews *et al.* 1998; Mendoza *et al.* 2017). The SID lysine: DE used in our in our finisher phase diet was as high as 0.62 g/MJ. Interestingly, the reduction in growth rate was not seen when betaine was supplemented during the grower phase even when the lysine: DE ratio was similar between the grower and finisher phase diets, indicating that such an effect is age-dependent. Further experiments are required to investigate the interactive effects between betaine, lysine: energy ratio and age of pigs.

From an economic aspect, the effect of betaine supplementation during the grower phase in reducing carcass backfat would increase net profit by $2.30/female pig (or by 2.90 cents per kg of carcass weight) at a small investment in feed cost ($3.60 increase per tonne of feed, or equivalent to 0.30 cents increase in producing a kg of HSCW). The ratio of investment and net return is 1:9.6. In summary, dietary betaine supplementation is recommended to be applied at 10-16 weeks age in female pigs as a means of reducing carcass backfat.

6.3 Experiment 3 (gestation and lactation phase)

The questions that we answered in the Experiment 3 was whether sow diets should be supplemented with betaine during lactation or gestation to improve piglet birth weight and litter size of the sows lactating and being re-mated in summer. We found that betaine supplementation during two gestations (the prior gestation (1st) and subsequent gestation (2nd)) or a summer lactation did not affect the number of born-alive piglets or birth weight of piglets born to sows lactating and re-mated in summer. However, betaine supplementation during summer lactation exhibited some beneficial effects, such as reducing backfat loss over lactation and ameliorating physiological signs of heat stress in lactating sows (reduction in respiration rate). These findings support the practice of betaine supplementation during summer lactation. Noticeably, betaine supplementation during the 1st gestation (pre-summer lactation) and 2nd gestation (post-summer lactation) reduced body weight gain of gestating sows in the 1st gestation as well as in the 2nd gestation. Moreover, the reduction of gestational weight gain during the 1st gestation led to a reduced litter birth weight, therefore betaine supplementation should not be practiced during gestation. Overall, betaine supplementation had some benefits when fed during a summer lactation, and it is recommended for supplementation in lactation only (not gestation).

Betaine supplementation during lactation ameliorated the backfat loss and physiological signs of heat stress, namely respiration rate, in lactating sows over summer. In the current study, the ADFI of sows during summer lactation was 5.8 kg per day or, equivalent to 87 MJ DE/d, and could not satisfy the calculated energy requirement for milk production (NRC 2012 model) and maternal body maintenance, thus may have resulted in a negative energy balance over lactation, and hence a loss of backfat. The reduction in sow body weight and backfat thickness in lactation of all sows (measured at entry to the farrowing house and at weaning in this experiment) support the theory of a negative energy balance in sows over a summer lactation, which is in agreement with the findings from previous, climatically controlled experiments (Messias de Bragan ca *et al.* 1998; Quiniou and Noblet 1999). The effect of betaine in ameliorating lactation backfat loss in sows may be related to its effect in reducing heat stress, which is indicated by the reduction in respiration rate in the current study. It has been previously reported that betaine supplementation was able to reduce heat production in grower pigs (Schrama *et al.* 2003), which may support our findings in lactating sows. Betaine supplementation may reduce heat production which may have reduced the heat load in lactating sows in summer, thus less respiration was required for heat dissipation. Similarly betaine supplementation reduced respiration rate of dairy cows under hot conditions (Hall *et al.* 2016). Betaine supplementation during the prior gestation did not achieve the above beneficial effects in lactating sows, indicating that the legacy effect of betaine supplementation during the prior gestation is not effective for lactating sows in summer. In other words, supplementing betaine directly during a summer lactation is required to positively impact sow performance.

Betaine supplementation during summer lactation or gestation (the prior and subsequent gestation in relative to the summer lactation) did not improve the number of piglets born alive, total born, or piglet birth weight in litters of sows mated in summer (i.e. the sows farrowing at end of the 2nd gestation) in the current experiment. It has been reported that sows mated in summer have lower piglet birth weights than those mated during cooler seasons (Liu *et al.* 2019). The seasonal effect was also reflected from the numerical comparison on birth weight between the 1st gestation (mated in cool season; av. 1.5 kg birth weight) and 2nd gestation (mated in summer; av. 1.4 kg) in the current experiment. The exact reason for the low birth weight piglets born to sows mated in summer remains unclear. Some evidence suggests that heat stress conditions during the first half of gestation can reduce birth weight of piglets (Lucy *et al.* 2012). More evidence has showed that gestational heat stress can reduce foetal development in pregnant sheep (Bell *et al.* 1989; Early *et al.* 1991). A previous study reported that betaine supplementation during gestation can increase the number of piglets born alive in Parity 3+ sows mated in summer (van Wettere *et al.* 2012), but such effect was not observed in our experiment (the interactive effect between diet and parity on number of piglet born alive, stillborn or total born was not significant).

Interestingly, betaine supplementation during the summer lactation reduced the number of mummified foetuses from 0.41 to 0.24 per litter in the subsequent farrowing, implying a carry-over effect of betaine supplementation during summer lactation on the subsequent reproductive performance. Mummification occurs when foetuses die after approximately day 35-40 of gestation when bone calcification has occurred (Lefebvre 2015). There has not been enough research to date studying the effects of heat stress during gestation or lactation on foetal mortality rate, because such experiments require a large number of litters. We cannot explain why betaine supplementation during summer lactation can reduce the number of mummified fetuses in the subsequent parity.

Betaine supplementation during gestation consistently reduced the body weight gain of sows over the two gestation periods in the current study. The reduction in body weight gain of betaine supplemented sows over gestation is likely to compromise foetal development. For example, in our current study, the average piglet birth weight (measured post-fostering) after the 1st gestation was reduced by 60 g in the gestational betaine supplementation treatment, implying that betaine supplementation during a cool gestation period may have negative impacts on foetal development. This is the first experiment to report such an effect. The expected reduction in piglet birth weight after the 2nd gestation was not observed, possibly because the effect was overwhelmed by the overall lower piglet birth weight from the sows mated in summer. Specifically, the overall average piglet birth weight after the 2nd gestation was more than 100 g lower than the birth weight from the 1st gestation which is may be related to the hot conditions during summer lactation or early phase of the 2nd gestation. The negative impact of betaine supplementation during gestation may be also related to the lysine to energy ratio used in the basal diet that was discussed in the Experiment 1 and 2, which is worthwhile investigating in future.

A previous study showed that supplementing 0.2% betaine during a summer lactation can shorten the weaning-to-remating interval from 4.5 to 4.2 d, which was associated with a 4% increase in ADFI (Cabezón *et al.* 2016). However, we did not observe any effect of betaine supplementation on ADFI in lactating sows or the subsequent weaning-to-oestrus interval in the current study. The average weaning-to-remating interval after the summer lactation was 6 days, and this was not affected by betaine supplementation at any stage. The oestrus-to-ovulation interval quantified in the subset of sows that showed behavioural oestrus on the 4th and 5th day post-weaning averaged at 1.2 d, and it was not affected by betaine supplementation during gestation or lactation. This oestrus-to-ovulation interval in our study indicated that the current insemination timing (1st serve at detection of oestrus then 2nd serve 24 h later) enabled a timely conception. In other words, the low farrowing rate after summer lactation (60%-70%) in the current study, reflecting summer infertility, was not likely to be a consequence of incorrect timing of AI. Therefore, other factors causing summer infertility should be explored in future studies.

In conclusion, betaine is recommended to be included in the summer lactation diet given its effects in reducing backfat loss, mitigating physiological signs of heat stress, and possibly reducing the number of mummified foetuses in subsequent gestation. Betaine supplementation during the prior or subsequent gestation (in relation to the summer lactation) provides no benefit to sow performance and reduced sow body weight gain during gestation, and thus is not recommended.

7. Implications & Recommendations

All the experiments in this project used commercial diets which contained sufficient methionine and choline as per industry practise, thus the overall effects of betaine was centred around its osmoregulatory properties or other novel functions etc rather than its methyl donor properties. Based on the findings conducted under the above experimental conditions, the following recommendations were made:

1. Betaine is not recommended to be added in weaner pig diets (4-10 weeks age) when other dietary methyl donors are sufficient.
2. Betaine is recommended to be added in the female grower phase diet (10-16 weeks age) as an economical strategy to reduce backfat thickness.
3. Betaine is not recommended to be added in the gestation diet due to the negative impact on weight gain of sows during gestation.
4. Betaine is recommended to be added in the lactation diet during summer as a strategy to alleviate heat stress.
5. Further investigation is required to confirm the interactive effects of betaine supplementation in pigs with dietary lysine to DE ratios.

8. Intellectual Property

There is no intellectual property arising from the research. The findings from the project are publishable with the permission from APL.

9. Technical Summary

All experiments followed a classical design, and conventional measurements (eg: growth and reproductive performance) were used.

10. Literature cited

Apicella J.M., Lee E.C., Bailey B.L., Saenz C., Anderson J.M., Craig S.A.S., Kraemer W.J., Volek J.S., Maresh C.M. (2013). Betaine supplementation enhances anabolic endocrine and Akt signaling in response to acute bouts of exercise. *European Journal of Applied Physiology* 113, p. 793-802.

Bell A.W., McBride B.W., Slepetis R., Early R.J., Currie W.B. (1989). Chronic heat stress and prenatal development in sheep: I. Conceptus growth and maternal plasma hormones and metabolites. *Journal of Animal Science* 67, p. 3289-3299.

Cabezón F.A., Schinckel A.P., Richert B.T., Stewart K.R., Gandarillas M., Pasache M., Peralta W.A. (2016). Effect of betaine supplementation during summer on sow lactation and subsequent farrowing performance. *The Professional Animal Scientist* 32, p. 698-706.

Cabezón F.A., Stewart K.R., Schinckel A.P., Richert B.T. (2017). Effects of betaine and heat stress on lactation and postweaning reproductive performance of sows. *The Professional Animal Scientist* 33, p. 241-253.

Collins C.L., Pluske J.R., Morrison R.S., McDonald T.N., Smits R.J., Henman D.J., Stensland I., Dunshea F.R. (2017). Post-weaning and whole-of-life performance of pigs is determined by live weight at weaning and the complexity of the diet fed after weaning. *Animal Nutrition* 3, p. 372-379.

Craig S.A. (2004). Betaine in human nutrition. *The American Journal of Clinical Nutrition* 80, p. 539-549.

Davis T., Fiorotto M., Burrin D., Vann R., Reeds P., Nguyen H., Beckett P., Bush J. (2002). Acute IGF-I infusion stimulates protein synthesis in skeletal muscle and other tissues of neonatal pigs. *American journal of physiology. Endocrinology and metabolism* 283, p. E638-47.

Du J., Shen L., Tan Z., Zhang P., Zhao X., Xu Y., Gan M., Yang Q., Ma J., Jiang A.a., Tang G., Jiang Y., Jin L., Li M., Bai L., Li X., Wang J., Zhang S., Zhu L. (2018). Betaine supplementation enhances lipid metabolism and improves insulin resistance in mice fed a high-fat diet. *Nutrients* 10, p. 131.

Dunshea F.R., Cadogan D.J., Partridge G.G. (2009). Dietary betaine and ractopamine combine to increase lean tissue deposition in finisher pigs, particularly gilts. *Animal Production Science* 49, p. 65-70.

Early R.J., McBride B.W., Vatnick I., Bell A.W. (1991). Chronic heat stress and prenatal development in sheep: II. Placental cellularity and metabolism. *Journal of Animal Science* 69, p. 3610-3616.

Emmert J.L., Webel D.M., Biehl R.R., Griffiths M.A., Garrow L.S., Garrow T.A., Baker D.H. (1998). Hepatic and renal betaine-homocysteine methyltransferase activity in pigs as affected by dietary intakes of sulfur amino acids, choline, and betaine. *Journal of Animal Science* 76, p. 606-610.

Friesen K., Nelssen J., Unruh J., Goodband R., Tokach M. (1994a). Effects of the interrelationship between genotype, sex, and dietary lysine on growth performance and carcass composition in finishing pigs fed to either 104 or 127 kilograms. *Journal of Animal Science* 72, p. 946-54.

Friesen K.G., Nelssen J.L., Goodband R.D., Tokach M.D., Unruh J.A., Kropf D.H., Kerr B.J. (1994b). Influence of dietary lysine on growth and carcass composition of high-lean-growth gilts fed from 34 to 72 kilograms. *Journal of Animal Science* 72, p. 1761-1770.

Hall L.W., Dunshea F.R., Allen J.D., Rungruang S., Collier J.L., Long N.M., Collier R.J. (2016). Evaluation of dietary betaine in lactating Holstein cows subjected to heat stress. *Journal of Dairy Science* 99, p. 9745-9753.

Harrell R., Thomas M., Boyd R., Czerwinski S., Steele N., Bauman D. (1999). Ontogenic maturation of the somatotropin/insulin-like growth factor axis. *Journal of Animal Science* 77, p. 2934-41.

Huang Q.C., Xu Z.R., Han X.Y., Li W.F. (2006). Changes in hormones, growth factor and lipid metabolism in finishing pigs fed betaine. *Livestock Science* 105, p. 78-85.

Huang Q.C., Xu Z.R., Han X.Y., Li W.F. (2007). Effect of betaine on growth hormone pulsatile secretion and serum metabolites in finishing pigs. *Journal of Animal Physiology and Animal Nutrition* 91, p. 85-90.

Iñiguez G.n., Ong K., Bazaes R., Avila A., Salazar T., Dunger D., Mericq V.n. (2006). Longitudinal changes in insulin-like growth factor-I, insulin sensitivity, and secretion from birth to age three years in small-for-gestational-age children. *The Journal of Clinical Endocrinology & Metabolism* 91, p. 4645-4649.

Knox R., Althouse G. (1999). Visualizing the reproductive tract of the female pig using real- time ultrasonography. *Journal of Swine Health and Production* 7, p. 207-215.
Lakhani P., Kumar P., Alhussien M.N., Lakhani N., Grewal S., Vats A. (2020). Effect of betaine supplementation on growth performance, nutrient intake and expression of IGF-1 in Karan Fries heifers during thermal stress. *Theriogenology* 142, p. 433-440.
Lawrence B.V., Schinckel A.P., Adeola O., Cera K. (2002). Impact of betaine on pig finishing performance and carcass composition. *Journal of Animal Science* 80, p. 475-482.
Lee M.S., Kim M.-S., Park S.Y., Kang C.-W. (2006). Effects of betaine on ethanol-stimulated secretion of IGF-I and IGFBP-1 in rat primary hepatocytes: involvement of p42/44 MAPK activation. *World journal of gastroenterology* 12, p. 1718-1722.
Lefebvre R.C. (2015). Fetal mummification in the major domestic species: current perspectives on causes and management. *Veterinary medicine (Auckland, N.Z.)* 6, p. 233-244.
Li S., Wang H., Wang X., Wang Y., Feng J. (2017). Betaine affects muscle lipid metabolism via regulating the fatty acid uptake and oxidation in finishing pig. *Journal of Animal Science and Biotechnology* 8, p. 72.
Liu F., Ford E., Smits R., Morrison R., Brewster C., Henman D., Zhao W., Leury B., Cottrell J., Dunshea F., Bell A. KH Plush, R (Ed.) (2019) 'A comparison of placenta and piglet birth weight of sows mated in summer and autumn, Seventeenth Biennial Conference of the Australasian Pig Science Association (APSA).' Adelaide, Australia. (Animal (Cambridge University Press):
Lothong M., Tachampa K., Assavacheep P., Angkanaporn K. (2016). Effects of dietary betaine supplementation on back fat thickness and serum IGF-1 in late finishing pigs. *The Thai Journal of Veterinary Medicine* 46, p. 427-434.
Lucy M.C., Safranski T.J., Rhoades J.N., Ross J.W., Gabler N.K., Rhoads R.P., Baumgard L.H. (2012). Litter characteristics and thermoregulatory behavior of first parity sows exposed to a controlled heat stress (HS) during gestation. *Journal of Animal Science* 90, p. 731.
Matthews J.O., Southern L.L., Bidner T.D. (2001). Estimation of the total sulfur amino acid requirement and the effect of betaine in diets deficient in total sulfur amino acids for the weanling pig2. *Journal of Animal Science* 79, p. 1557-1565.
Matthews J.O., Southern L.L., Pontif J.E., Higbie A.D., Bidner T.D. (1998). Interactive effects of betaine, crude protein, and net energy in finishing pigs2. *Journal of Animal Science* 76, p. 2444-2455.
Mendoza S.M., Boyd R.D., Zier-Rush C.E., Ferket P.R., Haydon K.D., van Heugten E. (2017). Effect of natural betaine and ractopamine HCl on whole-body and carcass growth in pigs housed under high ambient temperatures1. *Journal of Animal Science* 95, p. 3047-3056.
Messias de Bragan ca M., Mounier A.M., Prunier A. (1998). Does feed restriction mimic the effects of increased ambient temperature in lactating sows? *Journal of Animal Science* 76, p. 2017-24.
National Health and Medical Research Council (2013) 'Australian code for the care and use of animals for scientific purposes ' (National Health and Medical Research Council: Canberra)
National Research Council (2012) 'Nutrient Requirements of Swine.' (Natl Acad Press: Washington, DC)
Niu Y., He J., Zhao Y., Shen M., Zhang L., Zhong X., Wang C., Wang T. (2019). Effect of curcumin on growth performance, inflammation, insulin level, and lipid metabolism in weaned piglets with IUGR. *Animals* 9, p. 1098.
Oksbjerg N., Gondret F., Vestergaard M. (2004). Basic principles of muscle development and growth in meat-producing mammals as affected by the insulin-like growth factor (IGF) system. *Domestic Animal Endocrinology* 27, p. 219-40.
Pearce S.C., Gabler N.K., Ross J.W., Escobar J., Patience J.F., Rhoads R.P., Baumgard L.H. (2013). The effects of heat stress and plane of nutrition on metabolism in growing pigs. *Journal of Animal Science* 91, p. 2108-2118.
Quiniou N., Noblet J. (1999). Influence of high ambient temperatures on performance of multiparous lactating sows. *Journal of Animal Science* 77, p. 2124-34.
Ramis G., N. B. Evangelista J., Quereda J., Pallarés F.J., M. de la Fuente J., Munoz Luna A. (2011). Use of betaine in gilts and sows during lactation: Effects on milk quality, reproductive parameters, and piglet performance. *Journal of Swine Health and Production* 19, p. 226-232.

Sales J. (2011). A meta-analysis of the effects of dietary betaine supplementation on finishing performance and carcass characteristics of pigs. *Animal Feed Science and Technology* 165, p. 68-78.

Schoknecht P.A., Ebner S., Skottner A., Burrin D.G., Davis T.A., Ellis K., Pond W.G. (1997). Exogenous insulin-like growth factor-I increases weight gain in intrauterine growth-retarded neonatal pigs. *Pediatric Research* 42, p. 201-207.

Schrama J.W., Heetkamp M.J.W., Simmins P.H., Gerrits W.J.J. (2003). Dietary betaine supplementation affects energy metabolism of pigs. *Journal of Animal Science* 81, p. 1202-1209.

Slifierz M.J., Friendship R., de Lange C.F.M., Rudar M., Farzan A. (2013). An epidemiological investigation into the association between biomarkers and growth performance in nursery pigs. *BMC Veterinary Research* 9, p. 247-247.

van Wettere W.H.E.J., Herde P., Hughes P.E. (2012). Supplementing sow gestation diets with betaine during summer increases litter size of sows with greater numbers of parities. *Animal Reproduction Science* 132, p. 44-49.

van Wettere W.H.E.J., Smits R.J., Hughes P.E., Hughes P.E. (2013). Methyl donor supplementation of gestating sow diets improves pregnancy outcomes and litter size. *Animal Production Science* 53, p. 1-7.

Wan X., Wang S., Xu J., Zhuang L., Xing K., Zhang M., Zhu X., Wang L., Gao P., Xi Q., Sun J., Zhang Y., Li T., Shu G., Jiang Q. (2017). Dietary protein-induced hepatic IGF-1 secretion mediated by PPAR γ activation. *PLoS ONE* 12, p. e0173174.

11. Publications Arising

Research article:

- Effects of duration of betaine supplementation on growth performance and blood IGF-1 in light and normal weight weaner pigs under commercial conditions (accepted by Animal Production Science)

Conference abstract:

- Betaine supplementation during summer lactation did not affect subsequent oestrus-to-ovulation interval of sows that exhibited a normal weaning-to-oestrus interval (submitted to Nutrition Society of Australia 2020 conference)