USING DIETARY BETAINE SUPPLEMENTATION TO ALLEVIATE SUMMER INFERTILITY AND IMPROVE LITTER SIZE

Report prepared for the

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Executive Summary

Betaine, along with Folic Acid (FA) and Vitamin B12 (B12), is a major dietary source of the methyl groups required for methionine formation and subsequent DNA methylation. Embryonic and foetal demand for methyl groups is exceedingly high, and in addition to impairing embryonic cell function and proliferation, inadequate supplies of methyl groups and B-complex vitamins result in the accumulation of homocysteine. Homocysteine is a known teratogen, and is associated with embryonic abnormality and loss. In previous, CRC supported studies, we have demonstrated improved reproductive performance in response to betaine supplementation of summer gestation diets, and increased ovulation rate in summer-mated gilts receiving betaine prior to mating (CRC Final Report 2D-110). It is also evident from the literature that metabolic demand for folate and vitamin B12 increase dramatically during the first 30 – 60 days of geatation. Although, folate supplementation during gestation can improve litter size, the response has been variable, with inadequate vitamin B12 suggested as the limiting the response to additional folate. In light of these findings, the current project consisted of three commercial studies designed to determine whether supplementing the diets of breeding gilts and sows with betaine and / or folate plus vitamin B12 would improve reproductive performance.

In summary, the current data demonstrate that betaine supplementation (7.5 to 9.0 g/sow/day) during gestation can improve reproductive performance; reducing the proportion of sows returning to oestrus between days 15 and 30 post-insemination, and increasing litter size in higher parity sows (parity 4 plus). Further, the addition of 2 g / kg of betaine to the pre-mating diets of gilts reared outdoors during summer / autumn increased daily liveweight gain by 70 g / day ($P < 0.05$) and decreased the interval from breeding herd entry to first insemination from 18.7 ± 0.71 to 17.5 ± 0.47 days ($P = 0.2$). Regardless of treatment there was a significant ($P < 0.05$) negative correlation ($R = 0.35$) between gilt liveweight at entry into the breeding herd and interval to first insemination.

The aim of the final study was to determine whether supplementing gestation diets with betaine and / or folate + vitamin B12 would improve reproductive performance. A total of 1079 sows (parities 2 – 9 at mating) received a standard gestation diet supplemented with either nothing (control); 3 g / kg betaine; 20 mg / kg folic acid plus 150 µg/kg vitamin B12; or 3 g / kg betaine plus 20 mg / kg folic acid plus 150 µg/kg vitamin B12. To summarise, betaine supplementation increased litter size in higher (parity 4 plus) sows ($P < 0.05$), whilst the addition of folic acid and Vitamin B12 decreased incidences of early (< day 35) pregnancy failure by 4% ($P < 0.05$) and increased the litter size of parity 2 and 3 sows ($P < 0.05$). Folic acid and Vitamin B12 supplementation decreased ($P<0.001$) plasma homocysteine by 2.2 and 2.8 µM, respectively, on days 3 and 107 of gestation, whilst betaine supplementation decreased ($P<0.05$) plasma homocysteine on day 3 only. We have previously demonstrated a relationship between elevated plasma homocysteine in early gestation and incidences of early pregnancy failure, suggesting a reduction in homocysteine may have partially responsible for the observed improvement in pregnancy outcomes.

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1. Introduction

Betaine, a widely available dietary supplement, acts as a potent organic osmolyte, and has the potential to increase thermotolerance and 'protect' the pig from the negative effects of heat stress. Exposure to high ambient temperatures during the pre- and peri- implantation period impairs embryo development, inhibits maternal recognition of pregnancy and reduces embryo and foetal survival. In a recently completed CRC funded study (2D-114), we demonstrated that supplementing the gestation diets of sows mated in summer (January 2008) tended to reduce the incidence of late pregnancy loss by 2% (4 versus 6%; $P < 0.1$), and increased total litter size and born alive by 0.5 pigs ($P < 0.05$). Importantly, the beneficial effects of supplementary betaine during gestation were most evident in older parity sows (parity 3 plus), where total litter size increased by 1.5 piglets ($P < 0.01$) compared to control fed sows of the same parity. In addition, we also demonstrated in a small study at Roseworthy that betaine supplementation of gilt diets prior to, and during, boar exposure, reduced the interval to puberty ($P<0.05$), and alleviated the heat induced reduction in ovulation rate of gilts.

Betaine, along with Folic Acid (FA) and Vitamin B12 (B12), is a major dietary source of the methyl groups required for methionine formation and subsequent DNA methylation. Embryonic and foetal demand for methyl groups is exceedingly high, and in addition to impairing embryonic cell function and proliferation, inadequate supplies of methyl groups and B-complex vitamins result in the accumulation of homocysteine. High homocysteine levels impair embryo development, and low betaine levels are associated with abnormal pregnancies in humans. Requirements for methyl groups reflect the physiological maturity of the sow as well as the number of developing embryos present in the uterus. In light of the promising results of our previous trials, this study had three primary objectives, which were addressed in three separate trials. Study one determined whether betaine supplementation of gestation diets during summer and winter reduced pregnancy losses and increased litter size. The aim of Study three was to determine whether supplementing gestation diets during winter with betaine and / or Folic acid and Vitamin B12, increases litter size and reduces pregnancy loss in older parity sows. The aim of the third study was to determine whether betaine supplementation during the rearing, pre-mating and gestation periods of gilts mated during summer would improve the timing of the pubertal response to boar stimulation, and improve pregnancy outcomes.

2. Methodology

Study 1: Betaine supplementation of summer gestation diets: effects on pregnancy rates and litter size

Animals, housing and feeding

This study was conducted on two commercial farms (Farm A and Farm B), with 798 and 842 sows used on farms A and B respectively. Sows were selected from those mated between the 12th December 2008 and 13th February 2009 (Farm A) and the 3rd January 2009 and 10th March 2009 (Farm B). Based on date of first insemination, sows were allocated to receive either a standard gestation diet (Control; n = 401 on farm A and 420 on farm B) or a betaine supplemented diet (Betaine: n = 397 on farm A and 424 on farm B). Sows on Farm A were multiparous as were sows allocated to treatment on Farm B, but these were much lower parity. Sows on farm A were housed individually for the first 35 days of gestation, and then in groups of 12 – 20 until farrowing shed entry. Sows on farm B were housed individually from mating through to farrowing shed entry. e specifications of the diets used are detailed in Table 1, and all sows were fed at the same level during gestation (Table 2). The betaine diet was first fed from day 3 of gestation. On farm A, betaine inclusion rate was altered during gestation to ensure a daily intake of between 7.5 and 9.0 g / sow / day. On farm B, betaine was included at a set rate of 3 g / kg of feed throughout gestation, equating to a daily intake of 7.5 to 9.0 g / sow / day (Table 2).

Table 1 Specifications of diets fed during gestation on Farms A and B

	Inclusion rate	
	Farm A	Farm B
Digestible energy (MJ/kg)	13.02	13.0
Crude Protein (%)	15.07	14.95
Fat (%)	4.41	3.59
Crude Fibre (%)	4.80	5.05
Lysine (%)	0.67	0.64
Methionine (%)	0.25	0.22
Choline (mg / kg)	1335.23	-

Table 2 Daily feed intake and betaine intake during gestation for Control and Betaine sows on Farms A and B.

Period of gestation	Daily feed intake (kg/sow)	Betaine inclusion rate (g/kg feed)	Daily Betaine intake (g/sow)	Daily Energy intake (MJ/DE)	Daily lysine intake (g/day)
Farm A					
Days 3 – 42	2.2	4	8.8	28.6	14.7
Day 43 – 84	2.5	3	7.5	32.6	16.8
Day 85 - Farrowing	3.0	3	9.0	39.1	20.1
Farm B					
Days 1 – 42	2.5	3	7.5	32.5	16.0
Days 43 – 84	2.7	3	8.1	35.1	17.3
Days 85 – 112	3.0	3	9.0	39.0	19.2

Animal measurements and reproductive parameters

On farm A, sows returning to oestrus between days 18 and 23 after first artificial insemination were identified and recorded as regular returns. On farm B, sows returning to oestrus were detected using fenceline boar contact between days 15 and 30 post-insemination. Transcutaneous ultrasound was performed on approximately day 35 on both farms to determine pregnancy status, with not-pregnant sows checked a week later to confirm pregnancy status on Farm B, the number of pregnant and non-pregnant sows was recorded. At farrowing, the total number of piglets born was recorded. The number of piglets born alive, stillborn and mummified were also recorded. The total number of piglets born, born alive, stillborn and mummified was also recorded per sow mated.

For a subset of litters on Farm A (n = 47 Control and 43 Betaine litters), liveborn piglets were weighed individually within 24 hours of farrowing. In addition, blood samples were collected from a subset of sows (n = 20 sows / treatment) on days 3, 31, 59, 91 and 108 of gestation (where day 0 = first 24 hours post first artificial insemination). Blood samples were assayed for homocysteine concentrations.

Statistical analysis

Values in the text are expressed as Mean ± S.E.M. A general analysis of variance model, with block built in, was used to study the effects of dietary treatment and sow parity on all variates. Between treatment differences were examined using least significant difference. Treatment effects on pregnancy rates were examined using a chi-squared test. All analyses, except for chi-squared analyses, were performed using Genstat, eighth edition (Committee of the Statistics Department, Rothamsted Experimental Station, Harpenden).

Study 2: Effect of betaine, folic acid and Vitamin B12 supplementation during gestation on pregnancy outcomes of older parity sows

Animals, housing and feeding

The study was conducted on a commercial piggery at Corowa, NSW (Rivalea Australia Pty Ltd). A total of 1079 multiparous Large White / Landrace (parity 4.4 ± 0.1 at mating (range: 2– 9)) gestating sows were used in this study over five weeks of allocation (approximately 220 sows per week). Sows had a weaning to oestrus interval (WOI) of 5.2 ± 0.1 days, were artificially inseminated (AI) at their first observed postweaning oestrus (d 0) and 24 h later between the 27th March and 5th May 2010, and weaned 9.4 ± 2.2 piglets from the lactation prior to AI .

The experimental design was a 2 x 2 x 2 factorial, incorporating two levels of betaine supplementation (0 versus 3 g added betaine/ kg feed (0BET and SBET, respectively)), two levels of Folic acid plus vitamin B12 supplementation (0 versus 20 mg/kg Folate and 150 µg / kg vitamin B12 added as a supplement (0FOLB12 and SFOLB12, respectively), and two parity groups (parities 2 and 3 versus parity 4 and greater). The base diet contained 5 mg/kg folate and 20 μg/kg vitamin B12 in the complete diet. Diets were fed from d 1 post AI until d 112 of gestation. The specifications of the diets used are detailed in Table 1. All sows were fed a base diet (0 supplementation) at the same level during gestation: 2.5 kg / d; 2.7 kg / d and 3.0 kg / d on d 1 to 42, d 43 to 84 and d 85 to 112 of gestation, respectively. Sows were housed individually in stalls from d 1 gestation until entry to the farrowing shed on day 112 of gestation. Sow stalls were in rows of 55 with a feed and water trough common within the row. Once a day at 0700 h each row was fed their base diet by hand using a calibrated feed scoop followed by a 200 g top dress of their respective treatment diet. Treatments were allocated as a row to avoid diet contamination between sows on different treatments. Sows were detected for oestrus by a detection boar in the aisle way providing fenceline contact at 18 – 24 days after mating. At d 30, sows were detected for pregnancy by real time ultrasound fitted with a 5 MHz transabdominal sector probe (Agri-Scan A-7, ECM, Angoulême, France). To confirm the diagnosis, sows that were tested to have had a failed pregnancy were re-tested the following week. Sows that returned to oestrus or had a failed pregnancy confirmed by ultrasound were removed from their stall and recorded. Sows that were removed for sudden death or poor health were also recorded.

On d 112 of gestation sows were walked to an adjoining farrowing house. All sows were fed a common lactation diet (14.9 MJ DE/kg, 9 g total lysine/kg, 185 g crude protein/kg) 3 kg once a day at 0700 h until day 2 post-parturition. Sows were then offered the lactation diet three times a day or to appetite until weaning.

Table 3 Specifications of diets fed during gestation

	Control	Betaine	Folate + Vitamin B12	Betaine + Folate + Vitamin B12
Digestible energy (MJ/kg)	13.0	13.0	13.0	13.0
Crude Protein (%)	14.95	14.95	14.95	14.95
Fat (%)	3.59	3.59	3.59	3.59
Crude Fibre (%)	5.06	5.06	5.06	5.06
Lysine (%)	0.64	0.64	0.64	0.64
Methionine (%)	0.22	0.22	0.22	0.22
Betaine (g / kg)	-	3	-	3
Folate (mg / kg)	-	-	20	20

Vitamin B12(µg / kg)	-	-	150	150
Chemical analysis: betaine content (g/kg)	2.5	5.1		5.5

Reproductive measures

The number of sows returning to oestrus three weeks after AI (returns), experiencing early (< day 30 post-AI) or late (≥ day 30 post-AI) pregnancy failure and maintaining their pregnancy to parturition were recorded, as was the number of sows removed for non-reproductive reasons. At farrowing, the total number of piglets born, as well as the number of piglets born alive and dead (stillborns) and the number of mummified foetuses was recorded. For a subset of randomly selected litters (n = 50 litters per treatment), live born piglets were weighed individually and collated as a litter weight at birth within 24 hours of farrowing.

Blood sampling, hormone analysis and blood metabolites

Preprandial blood samples were collected from a subset of sows (n = 20 sows / treatment) on days 3, 30 and 107 of gestation (where day 0 = first 24 hours post first artificial insemination). Blood samples were collected by jugular venipuncture into 9 ml Lithium Chloride-coated collection tubes (Vacuette®, Griener Labortechnik, Austria). Blood samples were maintained on ice, and within an hour of collection were centrifuged for fifteen minutes at 2000g. Plasma was stored in 2 ml tubes at $-20^{o}C$ for later analysis.

Samples were assayed for homocysteine (HCY) concentrations. Plasma HCY was assayed in 25ul of sample by enzyme immunoassay using reagents obtained from BioRad, according to the manufacturer's instructions. Briefly in this assay, protein-bound HCY is reduced to free HCY, and is enzymatically converted to S-adenosyl-L-homocysteine (SAH) in a separate procedure prior to the immunoassay.

Statistical analysis

Values in the text are expressed as Mean ± S.E.M. A general analysis of variance model, with block built in and piglets weaned and WOI included in the model as co-variates, was used to study the effects of dietary treatment and sow parity group on all variates. Between treatment differences were examined using least significant difference. Unless interactions between treatments were observed, the main effect of treatment only is presented. Where there were interactions between the main effects, data on individual treatments have been shown.

Plasma HCY concentrations were analysed using a linear mixed model, in which sow was defined as the experimental unit and the fixed model included block, parity at mating and day of sample collection. Treatment effects on pregnancy rates were examined using a chi-squared test. All analyses, except for chi-squared analyses, were performed using Genstat, eighth edition (Committee of the Statistics Department, Rothamsted Experimental Station, Harpenden).

Study 3: Betaine supplementation of gilt diets during summer: effects on puberty attainment and pregnancy outcomes

A total of 485 gilts were used in this study, which ran from the 24th January 2010 to 20th October 2010. From arrival on farm at a liveweight (LW) of 108.9 ± 0.54 kg, gilts were allocated to receive either a standard gilt developer diet (Control; n = 143 gilts) or a standard gilt developer diet supplemented with 2 g / kg Betaine (Betaine; n = 340 gilts). Gilts were housed outside until 140.6 ± 0.40 kg, at which point they were moved to indoor group pens (8 gilts / pen) and commenced daily boar exposure until puberty attainment. Gilts were artificially inseminated at their first observed oestrus, and housed in groups until entry into the farrowing shed. Following their second insemination, Betaine gilts were randomly allocated to receive either a standard gestation diet (BetCont; n = 171 gilt) or the standard gestation diet supplemented with 3 g / kg betaine (BetBet; n = 169 gilts) through to farrowing shed entry on approximately day 110 of gestation. Consequently the three individual treatments were as follows: Control, BetCont and BetBet.

Gilts were weighed at start of dietary treatment (i.e. arrival on farm), at selection for entry into the breeding herd and again at first insemination. Transcutaneous ultrasound was performed on approximately day 24 of gestation to determine pregnancy status, the number of pregnant and non-pregnant sows was recorded. At farrowing, the total number of piglets born was recorded. The number of piglets born alive, stillborn and mummified were also recorded.

Statistical analysis

Values in the text are expressed as Mean ± S.E.M. A general analysis of variance model, with block built in, was used to study the effects of dietary treatment on all variates. Gilt liveweight at artificial insemination and average daily liveweight gain from selection to artificial insemination were included in the model to determine treatment effects on litter size. Between treatment differences were examined using least significant difference. Treatment effects on pregnancy rates were examined using a chi-squared test. All analyses, except for chi-squared analyses, were performed using Genstat, eighth edition (Committee of the Statistics Department, Rothamsted Experimental Station, Harpenden).

3. Outcomes

Study 1: Betaine supplementation of summer gestation diets: effects on pregnancy rates and litter size

Reproductive parameters

Farm A

There was no effect of treatment (Betaine versus Control) on total born, born alive, stillborns or mummies (Table 4). There was no effect of treatment (Betaine versus Control) on the proportion of pigs returning to oestrus (0.03 versus 0.03), not pregnant at 35 days (0.14 versus 0.10) or removed for non-reproductive reasons (0.02 versus 0.01). Total litter weight at birth was also unaffected by treatment: 15.5 ± 0.41 and 15.7 ± 0.40 kg for Control and Betaine, respectively.

Farm B

There was no effect of treatment (Betaine versus Control) on total born, born alive, stillborns or mummies (Table 4). There was no effect of treatment (Control versus Betaine) on the proportion of not pregnant pigs (0.08 versus 0.08) or the proportion of pigs removed for non-reproductive reasons (0.03 versus 0.05). **However, a significantly lower ($P < 0.05$) proportion of Betaine compared to Control sows returned to oestrus post-insemination: 0.04 versus 0.07.**

Table 4 Effect of gestation diet (Control versus Betaine) on total litter size, born alive, stillborns and mummies on Farms A and B

	Farm A		Farm B	
	Control (n = 331)	Betaine (n=339)	Control (n = 338)	Betaine (n = 350)
Parity at AI	4.3 ± 0.07	4.3 ± 0.07	1.9 ± 0.08	1.9 ± 0.08
Weaning-oestrus interval			6.1 ± 0.45	5.8 ± 0.43
Total born	11.8 ± 0.17	11.9 ± 0.17	11.8 ± 0.15	11.8 ± 0.14
Born alive	10.6 ± 0.16	10.5 ± 0.16	11.3 ± 0.14	11.2 ± 0.13
Stillborns	1.25 ± 0.09	1.36 ± 0.08	0.37 ± 0.05	0.67 ± 0.05
Mummies	0.15 ± 0.03*	0.09 ± 0.03*	0.16 ± 0.03	0.19 ± 0.03

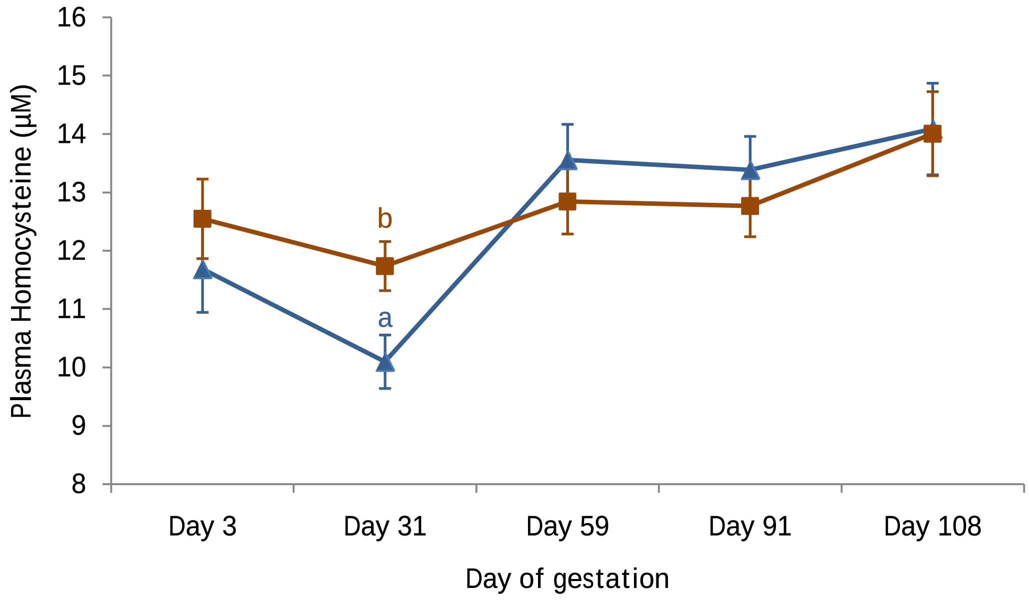

Figure 1 Homocysteine concentrations in plasma collected from Betaine (▲) and Control (■) sows (n = 20 sows / treatment) on days 3, 31, 59, 91 and 108 of gestation; [ab] within day indicate significant difference (P < 0.05)

Study 2: Effect of betaine, folic acid and Vitamin B12 supplementation during gestation on pregnancy outcomes of older parity sows

Reproductive measures

There was a significant interaction between parity at mating and the addition of Betaine to the gestation diet on litter size (Table 6), **with parity 4 plus sows supplemented with Betaine giving birth to significantly more piglets,** both total and born alive, than Control parity 4 plus sows (11.6 ± 0.18 versus 11.1 ± 0.19 and 10.6 ± 0.17 versus 10.1 ± 0.18, respectively). In contrast, **folic acid plus vitamin B12 supplemented Parity 2 + 3 sows gave birth to more piglets (total born) than folic acid plus vitamin B12 supplemented parity 4 plus sows (12.3 ± 0.30 versus 11.7 ± 0.34, respectively).** There was a significant effect of sow parity at mating (parities 2 and 3 versus parity 4 and greater) on the total number of piglets born (12.0 ± 0.2 versus 11.3 ± 0.1) and the number of piglets born alive (11.2 ± 0.2 versus 10.3 ± 0.1), but not the number of stillborn piglets (0.8 ± 0.1 and 0.9 ± 0.1) or mummified fetuses (0.07 ± 0.02 and 0.08 ± 0.01).

The addition of Folic acid plus vitamin B12 to the gestation diet significantly reduced the incidence of early pregnancy failure compared to Control sows (Table 6). However, there was no effect of treatment (Betaine versus Control) on incidences of early pregnancy failure (Table 6). There were no effects of Folic acid plus vitamin B12 or Betaine on the proportion of sows returning to oestrus post insemination, exhibiting late pregnancy loss, or failing to farrow for non-reproductive reasons (Table 6).

There was no effect of treatment (betaine versus no betaine) on piglet birth weight (1.48 ± 0.02 versus 1.51 ± 0.02 kg) or variation in piglet birth weight (0.20 ± 0.01versus 0.20 ± 0.01). Equally piglet birth weight and variation in birth weight were similar for Folic acid Plus Vitamin B12 supplemented and unsupplemented sows (1.49 ± 0.02 versus 1.51 ± 0.02 kg and 0.20 ± 0.01versus 0.20 ± 0.01). However, piglets born to older parity sows (parity 4 plus) were 80 grams lighter ($P < 0.05$) at birth than those born to younger (parity 2 and 3) sows (1.56 ± 0.03 versus 1.48 ± 0.02 kg). Similarly, the coefficient of variation within litters was significantly higher for litters farrowed by older (parity 4 plus) compared to younger (parity 2 and 3) sows: 0.21 ± 0.01 versus 0.18 ± 0.01).

Table 5 Effect of Folate + Vitamin B12 (FB12), Betaine (BET) supplementation during gestation and sow parity at mating (PAR) on total litter size, born alive, still born and mummified fetuses

FB12	Yes				No				SEM[A]	Significance[B]						
BET	No		Yes		No		Yes									
PAR	2 + 3	4 +	2 +3	4 +	2 + 3	4 +	2 + 3	4 +		F	B	P	F.P	B.P	F.B	F.B.P
TB	12.3	11.0	12.4	11.4	12.0	11.1	11.3	11.7	0.36	0.83	0.23	<0.01	<0.05	<0.01	0.68	<0.05
BA	11.5	10.1	11.3	10.3	11.2	10.0	10.7	108	0.34	0.98	0.24	<0.05	<0.05	<0.01	0.52	<0.05
SB	0.72	0.80	1.09	1.00	0.77	1.00	0.52	0.82	0.15	0.60	0.73	0.26	0.41	0.68	0.12	0.12
Mum	0.09	0.07	0.08	0.07	0.05	0.08	0.03	0.08	0.03	0.97	0.70	0.62	0.59	0.93	0.99	0.77

[A]standard error of mean for Folate plus B12 x Betaine x Parity

[B] Folate (F); Betaine (B); Parity (P)

Table 6 Effect of Betaine and Folate + Vitamin B12 supplementation during gestation on the proportion of sows returning to oestrus post-insemination, experiencing early (< day 30 post-AI) or late (≥ day 30 post-AI) pregnancy loss, or culled for non-reproductive reasons.

	Betaine		Folate / vitamin B12	
	Res[1]	Supp[1]	Res	Supp
Returns to oestrus	0.01	0.01	< 0.01	0.01
Early (< day 30 post-AI) pregnancy loss[A]	0.05	0.05	0.07[b]	0.03[a]
Late (≥ day 30 post-AI) pregnancy loss	< 0.01	< 0.01	< 0.01	< 0.01
Culled for non-reproductive reasons[B]	0.07	0.05	0.06	0.05
Proportion of sows farrowing	0.87	0.90	0.86	0.92

Means within a row, and main effect, followed by different letters for each variable differ significantly (at $P < 0.05$).

[A] excludes sows exhibiting a regular return to oestrus; [B] includes sows culled for structural and health reasons. [1] Res (residual levels in diet); Supp (supplementary)

Plasma homocysteine

The effects of adding betaine or folic acid plus vitamin B12 to gestation diets on plasma HCY concentrations are presented in **Error! Reference source not found.**. **Overall, HCY concentrations were decreased (P < 0.001) by 1.7 uM in response to folic acid plus vitamin B12 supplementation, but were unaffected by the addition of betaine to the diet. Day of gestation significantly affected plasma HCY, with concentrations significantly higher (P < 0.001) on days 3 and 107 compared to day 30 of gestation: 14.6 ± 0.27 and 14.0 ± 0.27 versus 12.8 ± 0.28 uM, respectively.** There was a significant interaction between day of gestation and the effect of diet. **HCY concentrations were 2.2 and 2.8 uM lower (P < 0.001) on days 3 and 107 of gestation, respectively, for folic acid plus vitamin B12 supplemented compared to unsupplemented sows, but similar on day 30 (Error! Reference source not found.).** Similarly, betaine supplementation decreased (P < 0.05) HCY concentrations by 1.2 uM on day 3 of gestation, but did not affect HCY concentrations on days 30 and 107 of gestation (**Error! Reference source not found.**).

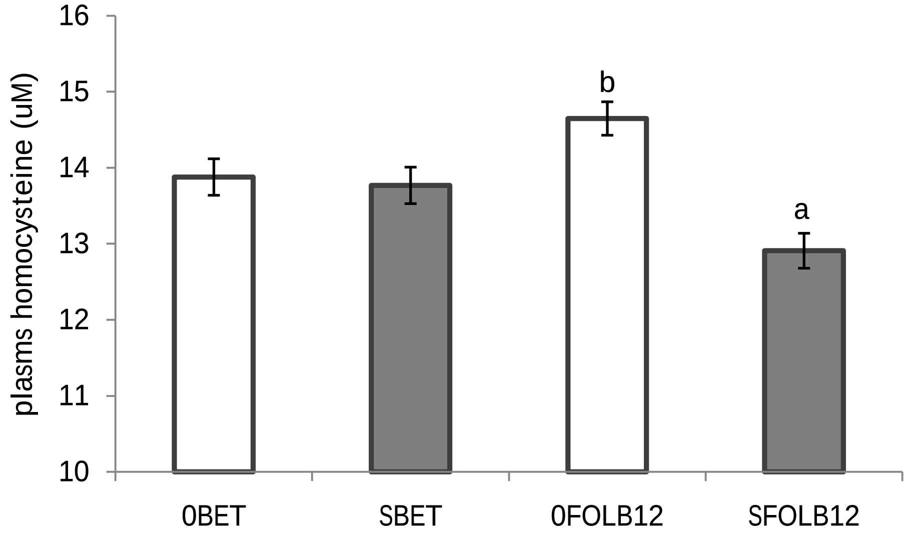

Figure 2. Main effects of gestation diet (0 g / kg versus 3 g / kg betaine; 0BET versus SBET) and (0 versus 20 mg/kg folate and 150 µg / kg vitamin B12; 0FOLB12 versus SFOLB12) on plasma homocysteine concentration. Different superscripts, within main effect, indicate significant difference (P < 0.001).

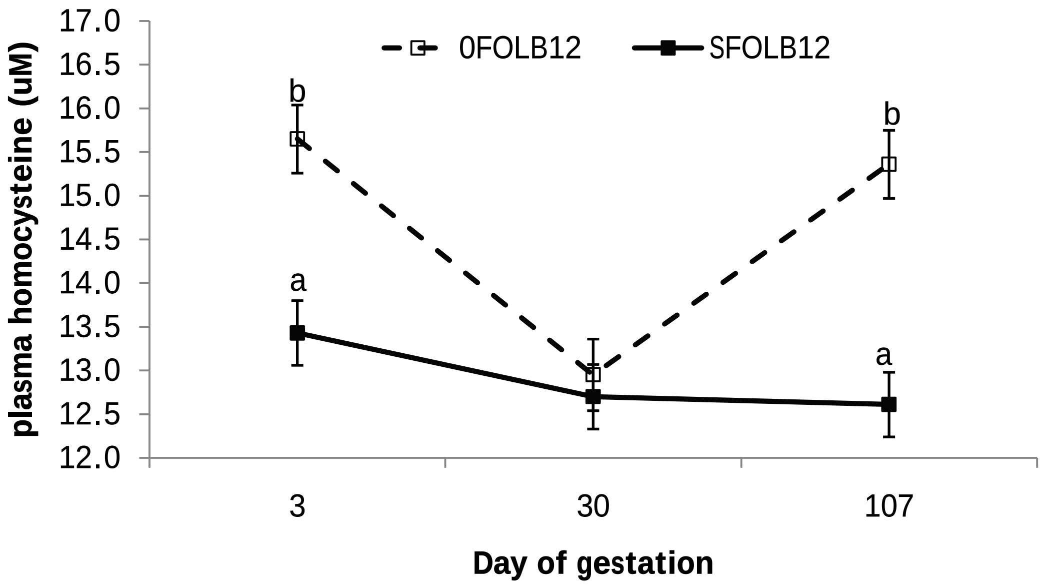

Figure 3 Effects of gestation diet (0 versus 20 mg/kg folate and 150 µg / kg vitamin B12; 0FOLB12 versus SFOLB12) on plasma concentrations of homocysteine. For the same day of gestation different superscripts differ at P < 0.001.

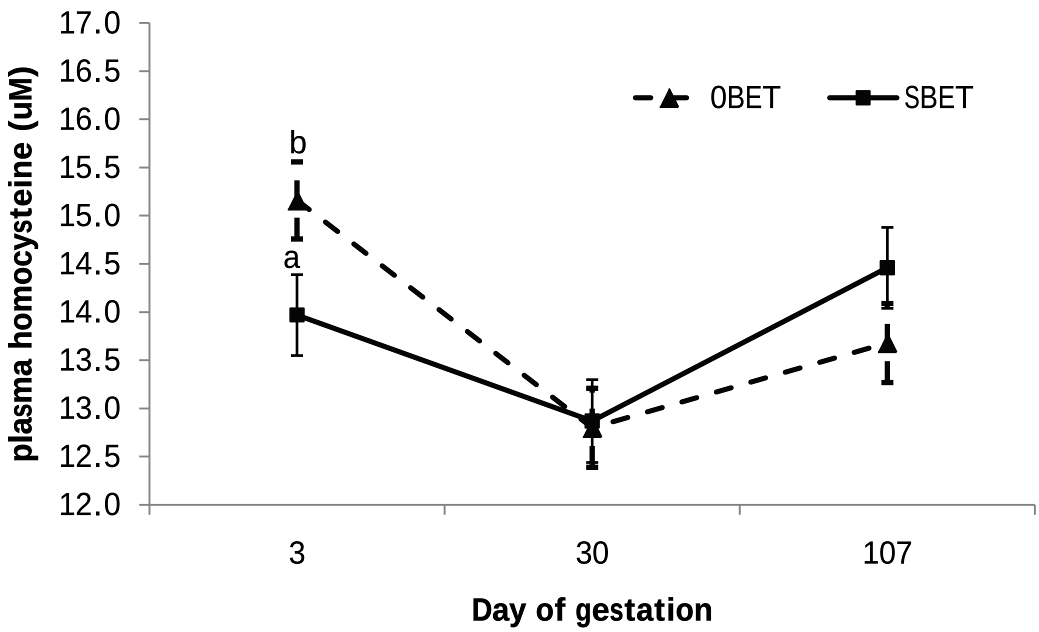

Figure 4 Effects of gestation diet (0 g / kg versus 3 g / kg betaine; 0BET versus SBET) on plasma concentrations of homocysteine. For the same day of gestation different superscripts differ at P < 0.001).

Study 3: Betaine supplementation of gilt diets during summer: effects on puberty attainment and pregnancy outcomes

There was no effect of Betaine supplementation, either before or after mating, on any aspect of litter size (Table 7). However, betaine supplementation prior to insemination, specifically from selection until artificial insemination, increased ($P < 0.05$) gilt growth rate by 70 g per day (Table 7). Betaine supplementation during the rearing phase resulted in a decrease ($P = 0.2$) in the interval from entry to the breeding shed and artificial insemination: 17.5 ± 0.47 versus 18.7 ± 0.71 days for Betaine and Control gilts. **The proportion of gilts not-pregnant on day 24 post-insemination was affected by treatment ($P < 0.05$), with a significantly higher proportion of Betaine-Control gilts not-pregnant compared to Betaine-Betaine gilts (Table 7).**

Regardless of treatment, there was a significant ($P < 0.05$) negative correlation ($r = 0.35$) between gilt liveweight at entry into the breeding herd and interval to first insemination.

Table 7 Effect of pre- and post-artificial insemination (AI) diets on the average daily weigh gain (ADG) and litter size of replacement gilts

	Control	Betaine-Control	Betaine-Betaine
Number of sows	138	160	167
ADG: selection to AI	0.76 ± 0.02[a]	0.83 ± 0.02[b]	0.83 ± 0.02[b]
Total litter size	10.6 ± 0.25	10.9 ± 0.24	11.0 ± 0.23
Born Alive	9.9 ± 0.25	10.2 ± 0.24	10.4 ± 0.23
Born Dead	0.66 ± 0.10	0.79 ± 0.09	0.71 ± 0.09
Not pregnant gilts (%)	0.04[ab]	0.06[b]	0.01[a]

Table 8 Effect of two sow parity groupings (parity 2 & 3 versus parity 4 plus) on total litter size on Farms A and B and studies 1 and 2.

Farm	Study	Total Litter size		Number of sows	
		Sow parity		Sow parity	
		2 and 3	4 plus	2 and 3	4 plus
Farm A	One	11.8 ± 0.19	11.9 ± 0.16	258	401
Farm B	One	12.2 ± 0.15	11.9 ± 0.32	335	72
Farm B	Two	12.0 ± 0.22[b]	11.3 ± 0.13[a]	227	678

[ab] different superscripts within row indicate significant differences; $P < 0.05$

4. Application of Research

Effects of betaine on sow fertility

Overall, it is evident from the series of studies reported here that supplementing gestating sow diets with betaine (7.5 – 9.0 g / day) can improve reproductive performance. Specifically, betaine supplementation reduced the proportion of sows returning to oestrus post-insemination in study one and increased the total number of piglets born to older (parity 4 plus) sows in study two. However, it is worth noting that beneficial effects appear limited to older parity sows. Equally, the increase in litter size of betaine supplemented, older parity sows appears limited to instances when litter size is lower than would be expected, namely when a decrease in litter size is observed with increasing parity. It is probably worth noting, that on Farm B (study 1) the effects of Betaine on litter size in older parity sows are unlikely to have been identified due to the low number of animals included in the trial. The observed improvement in the litter sizes of older parity sows in response to gestational betaine supplementation supports the previous findings of 2D-110.

The failure of betaine to increase litter size on Farm A was surprising considering the positive response observed in the initial study conducted on that facility (Final Report 2D-110). However, it is possible this reflected differences in the feeding regimes used, specifically in the current study sows received an extra 300 g per day of feed (equating to an additional 3.9 MJ DE / day). Certainly studies in grower and finisher pigs indicate that dietary betaine reduces heat production and energy requirements for maintenance, effectively increasing energy retention (Schrama et al., 2003). While studies in feed restricted growing (36 – 64kg) pigs and gestating sows (Final report 2D-110) indicate that dietary betaine results in partitioning of nutrients into protein rather than fat deposition. It is, therefore, possible that the beneficial effects of betaine on litter size are only evident when energy and protein status are marginal. However, this does not explain why in study 2 betaine supplementation increased litter size in older (parity 4 plus) sows. Consistent with our previous study (Final Report 2D-110; Wettere et al., 2009), betaine supplementation increased litter size in older parity sows. Studies in humans report a reduction in betaine during early pregnancy and an inverse relationship with homocysteine (Velzing-aarts et al., 2005). The role of betaine as a methyl donor for homocystiene remethylation, at least in early gestation, is supported by the reduction in plasma homocysteine on day 3 of gestation. However, why the beneficial effects of betaine on prolificacy should be restricted to older parity sows is not entirely clear from the current data. Interestingly, dietary betaine supplementation increases basal growth hormone (GH) and GH pulse amplitude by 42% and 35% in growing pigs (Huang et al., 2006). Exogenous GH administration during early gestation increases placental nutrient transfer to the embryo, promoting the growth of smaller fetuses on day 28 of gestation (Rehfeldt et al., 2001) and overall fetal weight on day 50 of gestation (Gatford et al., 2009), as well as increasing embryo survival (Kelley et al., 1995). Further, Gatford et al. (2010) concluded that exogenous growth hormone exerts a greater effect in multiparous compared to nulliparous sows, with maternal demands for nutrients to support growth limiting placental response to GH. It could, therefore, be suggested that betaine induced increases in maternal GH secretion, or pattern of secretion, were responsible for the observed increase in litter size in older parity sows.

Interestingly, supplementing the diets of gilts with betaine during the rearing phase or gestation did not affect reproductive function. Although the current data is consistent with our previous report that betaine supplementation prior to mating improves ovulation rate but not embryo numbers (Final report 2D-110), it was unexpected that maintaining betaine though into gestation did not elicit an increase in litter size. Although there was a numerical improvement in litter size (0.3 – 0.4 piglets), this did not approach significance, possibly due to an insufficient sample size. It is important to note, that the proportion of not-pregnant animals was significantly higher when betaine was removed from the diet post-mating compared to when it was retained in the diet through till farrowing. Consistent with reports in growing pigs, betaine supplementation significantly increased the rate of liveweight gain prior to mating. It is, therefore, possible that removing betaine from the diet and the resultant alteration in the methionine cycle and energy partitioning may have negatively affected embryo development. Alterations in homocysteine levels, due to a reduction in dietary betaine ingestation, could possibly account for a reduction in embryo survival. Homocysteine is a known teratogen, with elevated levels associated with abnormal embryo development and death.

Effect of B-vitamins on sow fertility

Sow prolificacy, as measured by litter size at parturition, was improved when gestation diets were supplemented with the combination of folic acid plus vitamin B12 (B-vitamin supplement). To the best of our knowledge, this is the first time that supplementary folic acid together with vitamin B12 has been shown to increase litter size in sows and reduce incidences of early pregnancy failure. Consistent with previous studies (Matte et al., 2006) sow parity at mating affected the response to the B-vitamin and betaine supplement. Specifically, folic acid plus vitamin B12 increased litter size in early parity sows, while only older parity sows responded to betaine supplementation with increased litter size. Maternal homocysteine metabolism during gestation was also altered by B-vitamin and betaine supplementation, with plasma homocysteine reduced by 8% on day 3 of gestation in betaine supplemented sows, and by 18% and 17% on days 3 and 107 of gestation, respectively, in B-vitamin supplemented sows.

Gestation is characterised by a dramatic increase in metabolic demand for B-vitamins, as well as alterations in systemic homocysteine concentrations (reviewed by Holmes, 2003; Matte et al., 2006). Serum folates decline dramatically (33 – 42%) during the first 55 - 60 days of gestation (Matte et al., 1984; Matte et al., 1993; Harper et al., 1994) with substantial uterine absorption and transfer of folic acid and Vitamin B12 into the uterine lumen and developing fetuses occurring during the first 15 to 25 days of gestation (Vallet et al., 1999a; Guay et al., 2002). Homocysteine homeostasis is highly sensitive to changes in maternal B-vitamin status (Matte et al., 2006), and betaine and homocysteine are inversely related in pregnant women (Velzing-Aarts et al., 2005). Importantly, abnormally high concentrations of homocysteine in the blood are indicative of imbalances in the methylation pathway (Ikeda et al., 2010), and have been linked to pregnancy abnormalities and defective implantation in a number of species, including pigs, cattle and humans (Holmes, 2003; Di Simone et al., 2004; Matte et al., 2006; Ikeda et al., 2010). Consequently, systemic (plasma) levels of homocysteine were determined pre - (day 3) and post- (day 30) embryo implantation, and late (day 107) gestation as an indicator of treatment induced changes in the maternal and fetal methionine cycle. Consistent with previous studies (Guay et al., 2002a; Simard et al., 2007), supplementary folic acid plus vitamin B12 decreased plasma homocysteine by 14% on day 3 of gestation. Both folic acid and betaine donate monocarbon units for the remethylation of methionine from homocysteine (Matte et al., 2006; Solanky et al., 2010), and we have also demonstrated that betaine reduces systemic homocysteine in early gestation. Consequently the current data provides further evidence that methyl group availability appears to limit methionine remethylation during early gestation.

We have demonstrated, possibly for the first time, that homocysteine decreased during the first 30 days of gestation in unsupplemented sows, and then returned to peri-conception levels on day 107 of pregnancy. This decline in homocysteine was also observed in folic acid and vitamin B12 supplemented sows both in the current study and that of Simard et al., (2007), as well as betaine supplemented sows. Consequently, it appears to be a normal physiological event and unrelated to any deficiency in B-vitamins or availability of methyl-donors. In support of this, homocysteine declines in pregnant women, regardless of B-vitamin status, reaching minimum levels in the eighth week of pregnancy (Murphy et al., 2001). Pregnancy related changes in haemodilution and serum albumin are insufficient to account for this decline in homocysteine in either sows (Simard et al., 2007) or women (Murphy et al., 2001). Consequently, Holmes (2003) suggested that placental transfer of homocysteine, and incorporation into the fetal metabolic cycle and subsequent utilisation by the products of conception may explain this reduction in circulating homocysteine.

B-vitamin supplementation resulted in a 57% reduction in the proportion of mated sows losing their pregnancy prior to day 30 post-insemination, and an increase in the litter size of parity two and three sows by 5%. The influence of supplementary folic acid in gestation diets on litter size at parturition is equivocal, and despite similarities in folic acid inclusion rate, either a 10% increase (Lindemann and Kornegay, 1989; Thaler et al., 1989) or no change (Harper et al., 1994) in litter size has previously been reported. An increased litter size in response to folic acid supplementation in sows and gilts is most likely due to a reduction in embryo mortality prior to day 30 (Trembley et al., 1989). The decrease in embryo mortality has been associated with alterations in prostaglandin E_2 (PGE_2) metabolism coincident with, and after implantation (Giguere et al., 2000; Guay et al., 2004; Matte et al., 2006) as well as an increased rate of embryonic development prior to implantation (Matte et al., 1984; Duquette et al., 1997; Guay et al., 2002b). Certainly, alterations in PGE_2 metabolism, due to its role in maternal recognition of pregnancy, could explain the reduction in early pregnancy losses observed in the current study, and Lindemann and Kornegay (1989) also reported a reduction in the number of breedings per farrowing in folic acid supplemented sows. Equally, elevated homocysteine levels during early gestation have been associated with pregnancy failure (van Wettere et al., unpublished), suggesting a B-vitamin induced reduction in homocysteine may also have been responsible for the decrease in pregnancy losses.

An increased litter size in response to folic acid supplementation suggests that basal dietary levels are insufficient to optimise prolificacy. Litter size appears to only be increased when ovulation rates are high, such as in parity two or greater sows (Lindemann and Kornegay, 1989; Town et al., 2005) or flush fed gilts (Matte et al., 1994; Trembley et al., 1989). The majority of pre- and peri-implantation embryo loss is attributed to morphological and developmental diversity between litter mates, with less developed embryos failing to survive (reviewed by van Wettere et al., 2005). This naturally occurring developmental asynchrony reflects disparity in follicle development and oocyte maturity at ovulation, and is likely to increase with larger ovulation rates (Xie et al., 1990; van Wettere et al., 2005). Consequently, it is suggested that supplementary folic acid promotes the growth and maturation of less developed embryos, enabling them to successfully implant. Using similar levels of folic acid (15 mg/kg diet) and vitamin B12 (160 µg/kg) supplementation to the current study, Guay et al. (2002a), reported a 30% reduction in uterine flushing levels of homocysteine. Elevated intra-uterine homocysteine has been linked to trophoblast cell death and defective implantation in humans (Di Simone et al., 2003; Di Simone et al., 2004), as well as retarded pre-implantation development of bovine embryos (Ikeda et al., 2010). Folic acid is an integral co-factor for nucleic acid synthesis, and therefore cell division and proliferation, with large quantities required by the rapidly developing products of conception (Solanky et al., 2010). An inverse relationship between folates and homocysteine is evident in utero (Solanky et al., 2010), and folic acid promotes porcine blastocyst development and growth in vitro (Kim et al., 2009). An increase in intra-uterine concentrations of B-Vitamins, and thus availability for fetal and placental metabolism and development, and the resultant decline in local homocysteine, probably explains the improved reproductive performance of folic acid plus vitamin B12 supplemented sows.

Application of the research findings in the commercial world.

Opportunities uncovered by the research

Commercialization/Adoption Strategies

- Potential benefits to cost of production
- Ease of adoption by producers
- Impact of the research

5. Conclusion

In summary, the present data demonstrate that B-vitamin supplementation increased litter size in early parity sows and reduced incidences of early pregnancy across all parities studied. These findings extend those of previous studies investigating the effects of folic acid and vitamin B12 on embryonic development, by demonstrating that prolificacy of early parity sows is limited by inadequate B-vitamin levels. Further, our data provides additional, albeit indirect, support for the concept that vitamin B12 may limit response to folic acid in growing sows. In addition, we demonstrated that supplementary betaine increased the litter size of older parity sows. Together, these data indicate that maternal requirements for micronutrients to maximise reproductive function vary with age.

The current data also demonstrate that supplementing the diets of gilts prior to mating increases growth rate, but does not result in significant improvements in litter size. However, it appears that removing betaine from the diet at mating may exert a negative effect on pregnancy maintenance.

6. Limitations/Risks

The primary limitation to the current findings, are that betaine supplementation only appears to improve litter size in older parity sows, and the response varies between genotypes and years. The other major limitation is that removal of betaine from the diet at key points in the reproductive cycle (i.e. post-mating) may negatively affect pregnancy maintenance. The effects of adding B-Vitamins to gestation diets during summer should also be investigated at a commercial scale, with intensive studies required to understand the mechanism responsible for the improved litter size in early parity sows.

7. Recommendations

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

- Betaine should be added to the gestation diets of older parity sows at a dose of 7.6 – 9.0 kg / day
- If fed to gilts prior to mating, betaine should not be removed from the diet until further work is conducted to establish if there really is a negative effect on embryo development and pregnancy maintenance
- Additional studies should be conducted to
    - Confirm the observed improvement in pregnancy rates in response to Folate / vitamin B12 supplementation
    - Investigate whether Folate / B12 supplementation during summer improves pregnancy rates
    - Determine the involvement of elevated homocysteine during early gestation and incidence of pregnancy loss

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