Development of a protocol for the use of feeding conjugated linoleic acid (CLA) in lactating gilts

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Executive Summary

In the lactating sow, feeding CLA in late gestation and/or lactation has been shown to alter the fatty acid profiles of colostrum and milk (Bontempo et al., 2004; Cordero et al., 2011; Peng et al., 2010) and improve progeny growth performance (Bee, 2000a, 2000b; Cordero et al., 2011; Corino et al., 2009). Additionally, piglets born to sows fed CLA also showed improved circulatory IgG concentrations (Bontempo et al., 2004; Corino et al., 2009; Wu et al., 2015) suggesting an immunomodulatory effect. Gilt progeny are born lighter (Hendrix et al., 1978; Miller et al., 2012b) and may be compromised in terms of protective immunity (Inoue et al., 1980; Klobasa et al., 1986; Miller et al., 2012b) compared to progeny born to multiparous sows. It has also been shown in previous research from our group, that feeding CLA to primiparous sows in lactation may improve liveborn pre-weaning survival rates (Craig et al., 2019). However, this earlier work is yet to determine the best inclusion rate to achieve these improvements in performance, without incurring unnecessary feed costs, as conjugated linoleic acid (CLA) feed additives are often quite expensive.

Therefore, the current project aimed to determine the best level of CLA inclusion in a top dress for primiparous sows, in order to achieve the best commercial outcomes from this feeding strategy. It also aimed to determine the best time for feeding in late gestation and/or lactation to achieve these improvements. This project involved two commercial experiments, the first investigating 3 different daily intakes of CLA isomers in primiparous sows and the impacts on colostrum and milk IgG, piglet serum IgG and serum energetic profiles (total triglycerides and glucose). The second experiment took the best CLA inclusion rate/feeding level from Experiment 1 and investigated several feeding times around farrowing and lactation to determine the optimal timing of this feeding strategy in a commercial operation.

In Experiment 1, *n* = 145 primiparous sows were fed 1 of 4 different diets, including 0% (control diet) or three different levels from 0.2-1.25% total CLA isomers, fed a top dress on the morning feed from entry to the farrowing house (111 ± 0.2 d of gestation) until weaning (fed for a total of 30.6 ± 0.3 days). Sow weight and P2 backfat was collected at entry to the farrowing house and at weaning. All piglets were individually tagged and weighed at birth and were weighed individually again at day 21 of lactation. A subset of piglets (2 per litter, 1 male and 1 female) were bled and serum was frozen at -20°C until further analysis. Piglet serum samples were analysed for energetic components such as non-esterified fatty acids (NEFA), glucose, triglycerides, and for total IgG to help to confirm the biological basis for any improvements seen in survival and growth in CLA fed piglets, as this additive is thought to increase their viability and energy levels at birth, encouraging them to suckle colostrum, improving acquisition of maternal immunity (Bontempo et al., 2004; Cordero et al., 2011; Corino et al., 2009). Results were analysed using the MIXED procedure in SPSS (version 25; IBM, Armonk NY, USA), and mortality results were further analysed using X^2.

Numerically, gilts fed 0.2% CLA had the highest number of piglets born alive (BA) and the lowest number of stillbirths (SB) per litter compared to the control and other CLA diets, although these results were not significant (*P* = 0.11 and *P* = 0.18, respectively). Total born (TB) was numerically highest in the 0.2% CLA group and higher in the other CLA groups compared to the control group (*P* = 0.41), which could not have been controlled for in this study. Born alive was still highest in the 0.2% CLA group when corrected for TB as a covariate in the model (*P* = 0.23) and overall the gilts fed CLA had higher number of piglets born alive than the control gilts (10.9-11.3 vs. 10.6, respectively). Numerically, the 0.2% CLA group had the lowest pre-foster mortality, and all CLA groups had lower mortality rates before fostering than the control group (3.8-6.3 vs. 7.1%, respectively). However, this

trend was the opposite for post-foster mortality (6.7-9.7 vs. 6.2, respectively). There were little differences in colostrum Brix% or piglet serum IgG, glucose, or triglyceride concentrations between dietary treatment groups. Wean to remate interval (WRI) was 2 days shorter in gilts fed 0.2% CLA than control gilts; but this effect was not significant (P = 0.60). There were no differences between treatments in terms of subsequent reproductive performance.

Although most results were not significant, numerically the gilts fed the CLA diets seemed to perform better than the control gilts around farrowing. This may have been impacted by the unexpected result of higher TB and BA in the CLA groups compared to the control, which we aimed to confirm in the second experiment. It was therefore concluded from these results that 0.2% total CLA isomers was the best inclusion rate to use for Experiment 2.

From Experiment 2, n = 144 primiparous sows were fed a CLA top dress equivalent to 0.2% CLA isomer inclusion (determined from Experiment 1) in their daily feed ration. Sows received CLA for 1 of 3 different feeding durations – 1 (treatment B) or 2 weeks before farrowing (treatment C) only or 1 week before farrowing and throughout lactation (treatment D), compared to a control group that received no CLA supplementation (treatment A). Sows and piglet performance throughout lactation was measured to determine the optimal timing of CLA supplementation to improve piglet growth and survival. Results were analysed using the MIXED procedure in SPSS, and mortality results were further analysed using X^2.

In this experiment, all dietary treatment groups had a similar number of pigs BA, TB, and a similar BA% (of TB; all $P \geq 0.10$). Sows in CLA treatment groups tended to lose less body weight in lactation (at entry to farrowing house until weaning) than control sows (P = 0.089). Sows in treatment B tended to have a higher average daily feed intake (ADFI) in lactation compared to control sows (P = 0.063). Litter weights at birth were similar between dietary treatments ($P \geq 0.10$). At day 19 of lactation, litters from sows in treatment B tended to be heavier than control litters (P = 0.077) and were significantly higher than treatment C litters (P = 0.049). They also had more pigs in the litter at day 19 than control sows (P = 0.031) and treatment C sows (P = 0.094), and litter weight gain from fostering to day 19 was highest for treatment B litters ($P < 0.10$). Treatment B sows weaned the most piglets ($P < 0.10$) and this was reflected in the X^2 analysis of post-foster mortality, where treatment B sows had the lowest mortality in this period (P = 0.019). These results suggested that feeding CLA had the biggest benefit when supplemented in the last week prior to farrowing, and no additional benefit was gained from feeding for two weeks prior, or feeding throughout lactation.

From the results obtained from these 2 experiments, it was determined that the ideal feeding strategy for a CLA-based top-dress in primiparous sow diets is 0.2% total CLA isomers, fed one week prior to farrowing. From Experiment 2 it seems that feeding CLA throughout lactation does not further improve their reproductive performance, or the pre-weaning performance of their progeny. Using these results, we have developed a best practice guideline for producers to follow in order to get the most of out of CLA supplements in their gestation/lactation diets for gilts.

Table of Contents

List of Tables

List of Figures

1. Background to Research

In the lactating sow, CLA has been shown to alter the fatty acid profiles of colostrum and milk (Bontempo et al., 2004; Cordero et al., 2011; Peng et al., 2010) and improve progeny growth performance (Bee, 2000a, 2000b; Cordero et al., 2011; Corino et al., 2009). Additionally, piglets born to sows fed CLA also showed improved circulating immunoglobulin concentrations (Bontempo et al., 2004; Corino et al., 2009; Wu et al., 2015) suggesting an immunomodulatory effect. Gilt progeny are born lighter (Hendrix et al., 1978; Miller et al., 2012b) and may be compromised in terms of protective immunity (Inoue et al., 1980; Klobasa et al., 1986; Miller et al., 2012a) compared to progeny born to multiparous sows. Gilt progeny have also been shown to fail to reach first mating in the breeding herd, most likely due to their lighter birth weights and growth rates (Craig et al., 2017a). However, once these animals are mated once, their reproductive performance and longevity does not differ from that of mated sow progeny. Therefore, improving growth and survival pre-weaning of gilt progeny via these feeding strategies may improve their reproductive success as breeding gilts themselves, therefore increasing genetic gain (gilt progeny are the 'next generation') and reproductive outcomes in the breeding herd.

In a previous project with APL (2014/621), we looked at using feeding strategies such as feeding CLA in late gestation/lactation on improving the pre-weaning performance of gilt progeny. Feeding a commercial CLA product (20% CLA isomers) at a 25 g/kg inclusion showed some benefits to gilt (and sow) progeny, increasing serum IgG concentrations in progeny when fed from approx. d107 of gestation until weaning and reducing pre-weaning mortality (Craig et al., 2019). The optimum timing of CLA supplementation and inclusion rate to improve survival of gilt progeny is yet to be determined, which was the focus of the current project.

The current project addresses APLs priority "Revisiting technologies available to the Australian pork industry to improve production efficiencies". Considering the growth, health and survival of gilt progeny is a substantial contributor to overall yearly targets, and so finding potential solutions, such as CLA supplementation, which focuses on these pigs in particular, could help improve overall farm productivity (i.e. increased weaned pigs/sow/year).

2. Objectives of the Research Project

We aimed to produce a recommended inclusion rate and feeding regime of CLA for gilts during gestation and/or lactation for producers to follow from the results of this project. It was hypothesised that overall herd efficiency could be greatly improved following this guideline, as gilts and their progeny make up a substantial percentage of the herd. Overall herd health could also be improved, reducing the need for expensive and time-consuming health regimes, whilst improving growth performance of the herd and increasing the size and volume of animals to market.

Success of the current project would result in a protocol for feeding CLA to gilts in late gestation/lactation that producers could adopt to improve the performance and survival of their gilt progeny. If feeding CLA to gilts could improve the early immunity and survival of their progeny, increase their growth rates and improve the quality of their colostrum and milk, farmers could implement these strategies on farm to increase their production and improve piglet welfare. Furthermore, being able to determine where the greatest benefit of feeding CLA lies would help us to carry out and better refine a cost-benefit analysis.

Therefore, our objectives for the current project were:

1. Determine the optimal feeding level of CLA in late gestation and/or lactation to gilts in order to improve the survival and growth of their progeny.
2. Determining the optimal time period for feeding CLA to late gestating/lactating gilts in order to improve the survival and growth of their progeny.
3. Provide producers with a guideline for feeding CLA to gestating/lactating gilts in order to improve production outcomes.

3. Introductory Technical Information

In a previous project with APL (2014/621), we looked at using feeding strategies such as feeding CLA in late gestation/lactation on improving the pre-weaning performance of gilt progeny. This additive was used on the basis that it has been shown to improve circulating IgG levels in piglets when fed to the dam in late gestation and/or lactation (Bontempo et al., 2004; Corino et al., 2009; Wu et al., 2015), may alter the fatty acid and IgG content of colostrum (Bontempo et al., 2004; Cordero et al., 2011; Peng et al., 2010), and has been shown to improve progeny growth rates (Bee, 2000a, 2000b).

In our previous experiment, feeding a commercial CLA product (20% CLA isomers) at a 25 g/kg inclusion showed some benefits to gilt (and sow) progeny, increasing serum IgG concentrations in progeny when fed from approx. d107 of gestation until weaning and reducing pre-weaning mortality (Craig et al., 2017b). Therefore, the current project sought to follow on from this and find the optimal inclusion rate and feeding duration for CLA in primiparous sow diets in order to improve their progeny performance.

Furthermore, in previous experiments we used a powdered CLA product (Lutrell® Pure; BASF Nutrition, Germany) mixed in with the final feed, whereas in the current project we sought to use a CLA oil (Lodestar CLA50®; Berg + Schmidt, Germany), that is formulated to protect the oil from oxidation and prolong shelf life for storage in silos, making the product more stable in pig feeds and therefore optimising pig performance.

4. Research Methodology

All experimental procedures were approved by the Rivalea (Australia) Animal Care and Ethics Committee under protocol numbers 18R069C (Experiment 1) and 19R037C (Experiment 2) in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

4.1 Experiment 1: Determining the ideal inclusion rate of CLA

4.1.1 Experimental Design

A total of *n* = 145 gestating primiparous sows (Primegro™ Genetics, Corowa NSW, Australia) were allocated to 1 of 4 experimental dietary treatments based on their body weight and backfat P2 thickness at entry to the farrowing house (*n* = 36 per treatment, *n* = 37 for treatment C). Treatments were based on a total daily CLA inclusion, descriptions for which for both Experiments 1 and 2 are shown in Table 1.1 and are further explained in section 4.1.2. Conjugated linoleic acid (CLA) was provided as a top dress on top of the morning feed and fed from entry to the farrowing house (111 ± 0.2 d of gestation) to weaning (24.4 ± 0.2 d of lactation).

Table 1.1 *Description of dietary treatments for Experiments 1 and 2.*

	Experiment 1	Experiment 2
A (Control)	0% CLA inclusion (no top dress added)	0% CLA inclusion (no top dress added)
B	0.2% total CLA isomer inclusion	0.2% total CLA isomer inclusion, provided 1 week prior to farrowing only
C	0.8% total CLA isomer inclusion	0.2% total CLA isomer inclusion, provided 2 weeks prior to farrowing only
D	1.25% total CLA isomer inclusion	0.2% total CLA isomer inclusion, provided 1 week prior to farrowing to weaning

Gilt weights and P2 backfat thickness were recorded at entry to the farrowing house and again at weaning. At farrowing, number of piglets born alive, stillborn, mummified, and total born were recorded for each gilt. Piglets were individually weighed and tagged at birth. Minimal fostering was carried out as per commercial practices on this farm, with fostering occurring within treatment where possible. All gilt feed intakes were recorded from entry to the farrowing house until weaning, and all gilt and piglet mortalities, removals, and medications were recorded. All piglets were individually weighed again at day 21 of lactation.

A colostrum sample was obtained at farrowing from a subsample of gilts (*n* = 26) where the start of farrowing could be observed, within 1 h of birth of the first piglet. This sample was analysed for Brix% using a Brix refractometer (Shoof International Pty., Tullamarine Vic, Australia) as a measure of total protein, and hence IgG content (Balzani et al., 2016; Hasan et al., 2016). The remaining sample was stored at -20°C until further analysis. A milk sample was collected from each gilt at day 21 of lactation, following intravulval injection of 10 IU of oxytocin (Ilium Syntocin; Troy Laboratories Pty Ltd, Glendenning NSW). Up to 5 mL of colostrum and milk was collected from each sow where possible.

Colostrum and milk were pooled from the first 3 anterior teats on each side of the udder and stored at -20°C until further analysis.

A blood sample was collected by jugular venepuncture into a Vacutainer® tube containing no anticoagulants or clot activators (BD; Franklin Lakes NJ, USA) from 1 male and 1 female piglet from each litter during the first 24 h of life from each litter. A second blood sample was collected from these pigs at 21 days of age. Serum was extracted by centrifuging each blood sample at 2,000-3,000 x g for 10 minutes at 4°C, aliquoting into separate vials and freezing at -20°C until further analysis.

Subsequent reproductive performance data was collected for all sows after weaning, including wean to remate interval (WRI), subsequent gestation length (GL), farrowing rate (FR), number of piglets born alive (BA) and number of piglets weaned (#W) in the next farrowing.

4.1.2 Diets and Feeding

The composition of the top dress diet for Experiment 1 is shown in Table 1.2. Conjugated linoleic acid oil (LodeStar CLA-50®; Berg + Schmidt, Germany) was added to the top dress diet at a rate of 6%, replacing tallow. This oil product contained 58% CLA isomers (*cis*-9 *trans*-11 and *trans*-10 *cis*-12).

Top dress was given as a proportion of the estimated daily ration, which was standard from start of top dress provision (before farrowing) up until 3 days after farrowing, and estimated from previous daily feed intake from day 4 after farrowing onwards, as sows were given *ad libitum* access to feed intake from this day throughout the rest of lactation. It was first estimated that sows would consume 6 kg of feed per day when provided feed *ad libitum*, based on previous work (Craig et al., 2019). However, this was monitored closely and re-evaluated weekly if required. Sows were fed 2.5 kg from entry to the farrowing house up until farrowing, and then on a 'step up' feed program from the day after farrowing (day 1) until 3 days after farrowing (day 3), with 2.5 kg fed on day 1, 3 kg fed on day 2, 4 kg on day 3, and *ad libitum* thereafter. A 'top dress calculator' was used to estimate the amount of the daily feed ration required to be replaced by the top dress, see Figure 1.1.

Table 1.2 *Macro-composition of diets used for Experiments 1 and 2.*

	Diet	
Ingredient (%)	***Lactation Diet***	***Top Dress***
Rolled wheat	*56.8*	*56.8*
Barley	*10.0*	*10.0*
Canola meal (38% CP)	*10.0*	*10.0*
Mill mix	*6.7*	*6.7*
Meat meal (60% CP)	*3.3*	*3.3*
Soybean meal (46% CP)	*2.5*	*2.5*
Tallow	*6*	*-*
CLA oil[1]	*-*	*6*
Limestone	*1.0*	*1.0*

[1]LodeStar CLA-50® Oil containing 58% CLA isomers *cis*-9 *trans*-11 CLA and *trans*-10 *cis*-12 CLA (Berg+Schmidt; Hamburg, Germany).

A) Experiment 1 **Top dress CLA incl% 0.06**

	Inclusion	ADFI (kg/d) 2.5	3	4	5	6	7	8
Nil (A)	0	kg/d top dress						
Low (B)	0.002	0.083	0.100	0.133	0.167	0.200	0.233	0.267
Med (C)	0.008	0.333	0.400	0.533	0.667	0.800	0.933	1.067
High (D)	0.0125	0.521	0.625	0.833	1.042	1.250	1.458	1.667

Adjust Lodestar amount per day/week of lactation depending on average feed intake

B) Experiment 2 **Top dress CLA incl% 0.06**

	Inclusion	ADFI (kg/d) 2.5	3	4	5	6	7	8
CON	0	kg/d top dress						
CLA	0.002	0.083	0.100	0.133	0.167	0.200	0.233	0.267

Figure 1.1 *'Top dress calculator' used for determining the amount of daily top dress to include in the sow rations for Experiments 1 (A) and 2 (B).*

4.1.3 Gilt and Progeny Management

All animals were managed under commercial conditions at Rivalea's Module 1 Research and Innovation facility in Corowa, NSW. The experiment ran from May 2019 until December 2019 when subsequent farrowing data was collected. Gilts received their first mating when they reached the desired weight range indicated on the Allometric Growth Tape for Gilts (University of Alberta, Edmonton, Canada), most being mated on their second or third oestrus. Gilts were housed in pens of up to 40 pigs per pen fitted with an electronic sow feeder (ESF) for the entirety of gestation until being loaded into the farrowing house at approximately 111 days of gestation. Gilts were housed in conventional farrowing crates, fitted with drinkers for the sow and piglets, a heat lamp, and a creep mat. Minimal fostering was carried out, within treatment where possible, and sows and piglets were managed under normal commercial conditions. Piglets were processed at 3 days of age, where their tails were docked with a cauterising iron, and they were given 2 mL of supplemental iron.

4.1.4 Laboratory Analyses

Piglet serum was analysed for total glucose and triglycerides via colorimetric methods using the Infinity™ Glucose Oxidase and Trigycerides Reagents (Thermo Fisher; Waltham MA, USA) and a chemistry calibrator with reference values of 8.20 mmol/L for glucose and 0.73 mmol/L for triglycerides (Pointe Scientific; Canton MI, USA). Assays were carried out according to the manufacturers' instructions, with samples assayed in triplicate. The inter- and intra-assay CV were 6.31% and 5.24%, respectively, for the glucose assay; and 4.53% and 4.27%, respectively, for the triglycerides assay. Serum of piglets at birth was also analysed for total IgG concentration using a commercial ELISA kit (Bethyl Laboratories, Montgomery TX, USA) according to the manufacturer's instructions, with standard curves assayed in duplicate and samples assayed in singlicate.

4.1.5 Statistical Analysis

Performance data were analysed as a linear mixed model using the MIXED procedure of SPSS (IBM SPSS Statistics version 25; Armonk, NY). For all sow performance parameters, the sow or litter was used as the experimental unit, with dietary treatment as a fixed factor and replicate as the blocking factor. Shed (nested within replicate) was tested as a random factor and pre-farrow feed days (number of days the sow received the experimental diet prior to farrowing), total feed days (number of days the sow received the experimental diet throughout gestation and lactation), lactation length, total born and litter size after fostering all tested as covariates as appropriate. Individual piglet weight at birth was used to calculate a within-litter coefficient of variation (CV) for birth weight, which was analysed on a per litter basis. Additionally, a number of planned *post hoc* comparisons were made between the control and all three pooled CLA treatments, between 0.2% CLA and the pooled results of 0.8% and 1.25% CLA treatments, and between 0.8% and 1.25% CLA treatments, using SPSS syntax, with no adjustment for multiple comparisons. The CLA inclusion was also modelled as a covariate in the model to investigate linear, quadratic, and cubic responses to CLA inclusion level in all of the continuous response variables.

For individually weighed piglets, birth weight categories were set up based on quartile values. All piglets born alive were assigned either as very light (< 1.11 kg; n = 380), light (1.11 to 1.30 kg; n = 396), medium (1.31 to 1.48 kg; n = 366), and heavy (> 1.48 kg; n = 386). Birth weight categories were analysed using X^2 analysis. Piglet mortality data was analysed on a per litter basis as a categorical variable in a linear mixed model, and mortality data based on individual data from tagged piglets was also analysed using X^2 analysis.

4.2 *Experiment 2: Determining the ideal time to feed CLA*

4.2.1 *Experimental Design*

A total of 144 primiparous sows (*n* = 36 per treatment) were used in Experiment 2 (Primegro™ Genetics, Corowa NSW, Australia), over 2 time replicates from May to July 2020. Sows were allocated to 1 of 4 treatments, based on their estimated due date (calculated as 116 days from first mating). Timing of CLA top dress supplementation was different for each treatment (see Table 1.1). Sows in Treatment C were fed on the floor for approximately one week before entering the farrowing house in individual mating stations in the boar shed. This was due to commercial constraints that did not allow them to enter the farrowing house any earlier than one week before their due date to farrow.

At entry to the farrowing house (108.3 ± 0.2 days of gestation), all primiparous sows (including Treatment C) were weighed and had P2 backfat measured (6.5 mm down the midline, at the head of the last rib). At farrowing, number of piglets born alive, stillborn, and mummified were recorded for each litter, and total born was calculated by addition of these. Litters were weighed within 24 hours of birth, before fostering, including both piglets born alive and piglets born dead. Any piglets that were fostered after this time were also weighed, to get a total litter weight after fostering for each litter. Minimal fostering was carried out as per commercial practices on this farm, with fostering occurring within treatment where possible. For the sows where the start of farrowing could be observed (*n* = 20), a few drops of colostrum were collected from the first 3 anterior pairs of teats on the udder and Brix% was calculated using a Brix refractometer (Shoof International Pty., Tullamarine Vic, Australia) as a measure of colostrum protein (Balzani et al., 2016; Hasan et al., 2016). Colostrum was collected within 1 hour of birth of the first-born piglet.

Daily sow feed intakes were recorded on a daily basis throughout lactation in order to calculate ADFI for each sow. All mortalities, removals, and litter medications were recorded throughout the experimental period. Litters were then weighed again at 19 days of lactation, in order to estimate litter weight change and hence milk production in lactation. All primiparous sows and their litters were weaned at 27.1 ± 0.2 days of lactation, and sow liveweight and P2 was recorded on day of weaning. Unfortunately, subsequent reproductive performance data were unable to be collected for Experiment 2 at the time of writing, due to time constraints. However, these data will be available from November 2020 onwards, and can be presented to the reader upon request.

4.2.2 *Diets and Feeding*

The composition of the top dress diet for Experiment 2 is shown in Table 1.2. From the results from Experiment 1 it was decided that the CLA inclusion for diets in Experiment 2 would be 0.2% CLA (B diet from Experiment 1). Sows were fed 2.5 kg/d up until farrowing, then *ad libitum* for the entirety of lactation, different to the 'step up' program that was used in the R&I facility for Experiment 1. The date of farrowing recorded for the experiment was recorded the next morning after sows had finished farrowing; however, some sows (*n* = 16 for the first replicate, *n* = 17 for the second replicate) were given *ad libitum* feed on the day that farrowing started if observed by farrowing house staff. Hence, lactation feed intake for these sows included the feed intake for the actual day of farrowing. Sows would have received top dress on that day before farrowing date was recorded, if in treatments B-D.

4.2.3 *Gilt and Progeny Management*

All animals were housed at Rivalea's Module 2 farm under commercial conditions. Gilts received their first mating when they reached the desired weight range indicated on the Allometric Growth Tape for Gilts (University of Alberta, Edmonton, Canada), most being mated on their second or third oestrus. From mating, gilts were housed in dynamic groups of gestating gilts at different stages of gestation (n ~ 200 per pen), in a large pen fitted with ESFs. At 101.8 ± 0.4 days of gestation, primiparous sows in treatment C were removed from the gestation pens using an automatic sorting system at the ESF that allowed sows at a certain gestation day to be sorted out into a separate area. At 108.3 ± 0.2 days of gestation, the remaining A, B, and D sows were sorted out and loaded into the farrowing house. As with Experiment 1, gilts were housed in conventional farrowing crates, fitted with drinkers for the sow and piglets, a heat lamp, and a creep mat. Minimal fostering was carried out, within treatment where possible, and sows and piglets were managed under normal commercial conditions. Piglets were processed at 3 days of age, where their tails were docked with a cauterising iron and they were given 2 mL of supplemental iron. Each time replicate was located in a separate farrowing house with similar dimensions and pen types.

4.2.4 *Statistical Analysis*

Performance data were analysed as a linear mixed model using the MIXED procedure of SPSS (IBM SPSS Statistics version 25; Armonk, NY). For all sow performance parameters, the sow or litter was used as the experimental unit, with dietary treatment as a fixed factor and replicate as the blocking factor. Sow body weight and/or P2 backfat level at entry to the farrowing house, number of piglets BA, TB, and number of piglets post-foster were tested as covariates as appropriate. Comparisons were made between individual treatment means using the Fisher's LSD test (with no adjustment for multiple comparisons). A similar *post-hoc* analysis of between treatment comparisons was done as was performed for Experiment 1. The three comparisons made were A vs. B, C, and D (Control vs. all CLA treatments), B vs. C and D, and C vs. D. Mortality results were analysed both as a continuous variable (percentage pre- and post-foster mortality on a per litter basis) using the MIXED procedure, and as a binary variable using chi-square (X^2).

5. Results

5.1 Experiment 1: Determining the ideal inclusion rate of CLA

Three sows farrowed on the day they entered the farrowing house, before receiving any of the experimental diet. These sows were not included in the analysis for BA, SB, TB, litter weight or average piglet weight at birth, nor were their piglets included in the individual analysis of birth weight.

One sow in the first replicate only gave birth to a single stillborn piglet and was therefore removed from the experiment at farrowing. Five sows from replicate 2 suffered from agalactia and had unthrifty litters during lactation and therefore piglets were removed, and another sow from the first replicate was removed from the experiment for management reasons 2 days into lactation (moved to a larger farrowing crate). In addition, 2 sows from the second replicate died suddenly from undiagnosed causes in mid-lactation. Performance data from these sows and their piglets were included in the analysis up until the point that they were removed from the experiment or died. During lactation, the board between an experimental pen and a non-experimental pen fell down and piglets from the two litters were mixed. Data from these experimental pens (*n* = 24) were not included in the analysis after this point, but data can be made available upon request. Piglet data at day 21 was not recorded for another 5 sows in the second replicate as piglets were weaned before they could be weighed; however, BW and P2 backfat measurements were obtained for these sows at weaning.

5.1.1 Gilt Reproductive Performance in Lactation

Gilts were fed CLA for 5.1 (± 0.2) days before farrowing, totalling 30.6 (± 0.3) days of CLA supplementation throughout the experimental period. Gilt body weight and P2 backfat (BW_E and $P2_E$) were numerically similar between all dietary treatments at entry and at weaning (Table 1.3) but gilts fed 1.25% CLA lost significantly more body weight in lactation than control gilts ($P < 0.05$), although the overall dietary effect was not significant ($P = 0.15$). Numerically, all gilts on CLA diets lost more weight and P2 backfat in lactation (from farrowing house entry to weaning) than control gilts (Table 1.3). In the planned contrasts, loss of body weight in lactation was significantly higher ($P = 0.050$) in the pooled CLA group compared to the control group (Table 1.6).

Gilts on the 0.2% CLA diet had a significantly ($P < 0.05$) higher number of piglets BA than the control gilts that did not receive CLA, although the main diet effect was not significant ($P = 0.11$; Table 1.3). However, numerically, gilts on the control (no CLA) diet had a lower number of piglets born (total born; TB) than gilts on the CLA diets (Table 1.3). Nonetheless, when correcting for total born as a covariate, gilts on the CLA diets had a numerically ($P = 0.23$) higher number of piglets born alive than the control gilts (10.9-11.3 piglets vs. 10.6 piglets, respectively). When BA as a percentage of TB was analysed as a continuous variable itself, despite no overall effect of dietary treatment ($P = 0.21$), control gilts had a lower BA percentage (86.3 ± 1.9%) than those on the CLA treatments (91.8 ± 1.9%, 90.0 ± 1.8%, and 90.0 ± 1.9%, with increasing CLA inclusion, respectively). Similarly, within the planned contrasts, BA was significantly higher in the CLA groups than the control group ($P = 0.036$; Table 1.6) and there were significant linear, quadratic, and cubic responses in BA to CLA inclusion ($P < 0.05$; Table 1.6). This was also reflected in linear ($P = 0.020$), quadratic ($P = 0.028$), and cubic ($P = 0.035$) responses in litter weight at birth, and also after fostering ($P < 0.10$), as well as in litter size after fostering ($P < 0.05$; Table 1.6).

There was no difference in average daily feed intake (ADFI) between dietary treatments ($P = 0.43$; Table 1.3) confirming that all gilts within dietary treatment would have consumed a similar volume of CLA isomers, as the top dress was given as a proportion of estimated daily feed intake.

5.1.2 Gilt Progeny Performance in Lactation

At birth, litters from gilts fed 0.2% CLA were significantly heavier ($P < 0.05$) than litters from control gilts, with litters from other CLA treatment gilts intermediate (Table 1.4), mostly as a result of the higher litter sizes in the CLA groups. Average piglet birth weight ($P = 0.97$) and within-litter birth weight coefficient of variation (CV; $P = 0.99$) of live born piglets were similar between treatments. Average piglet birth weight and within-litter CV were not significant ($P \geq 0.10$) when adjusted for total born. There was no difference between dietary treatments in proportion of piglets born <1.10 kg ($P = 0.84$) or piglets born >1.48 kg ($P = 0.78$; data not shown).

At day 21 of lactation, litter weight ($P = 0.68$), litter size ($P = 0.58$), average piglet weight ($P = 0.73$) and within-litter CV ($P = 0.20$) were similar between all treatments (Table 1.4). The changes in litter weight and individual piglet weight from birth to day 21 were also similar between treatments ($P \geq 0.10$), even when adjusting for litter or piglet weight after fostering (data not shown).

When litter mortality was analysed as a continuous variable, pre-fostering ($P = 0.48$) and post-fostering ($P = 0.44$) litter mortality rates were similar between treatments (Table 1.4). A similar result was yielded when data was analysed using chi-square analysis (Table 1.5). However, numerically, all CLA treatments had lower pre-fostering mortality rates than the control treatment (Table 1.4). The 0.2% CLA treatment tended to have the lowest mortality rates of liveborn tagged piglets from day 0 to day 3 ($P = 0.085$; Table 1.5).

Table 1.3 *Effect of conjugated linoleic acid (CLA) inclusion in the late gestation and lactating diet on primiparous sow reproductive performance obtained from the linear mixed model analysis (results presented as mean ± SE).*

		Diet[1]				**P-value**
Parameter[2]	**n**	**0% CLA**	**0.2% CLA**	**0.8% CLA**	**1.25% CLA**	**Diet**
Farrowing performance						
GL (days)	145	115.9 ± 0.2	116.2 ± 0.2	116.1 ± 0.1	116.1 ± 0.1	0.80
BA	145	10.2 ± 0.4^{a}	11.7 ± 0.4^{b}	10.9 ± 0.4^{ab}	11.1 ± 0.4^{ab}	0.11
SB	145	1.3 ± 0.2^{a}	0.6 ± 0.2^{b}	1.0 ± 0.2^{ab}	1.1 ± 0.2^{ab}	0.18
TB	145	11.6 ± 0.5	12.7 ± 0.5	12.2 ± 0.5	12.5 ± 0.5	0.41
Lactation performance						
BW_E (kg)	145	197.1 ± 2.6	197.1 ± 2.6	196.0 ± 2.6	196.9 ± 2.6	0.99
BW_W (kg)	137	188.0 ± 2.9	183.6 ± 2.8	183.3 ± 2.8	182.2 ± 2.9	0.51
ΔBW (kg)[3]	137	-9.9 ± 1.7^{a}	-13.7 ± 1.7^{ab}	-12.8 ± 1.7^{ab}	-15.4 ± 1.7^{b}	0.15
$P2_E$ (mm)	145	18.8 ± 0.6	18.8 ± 0.6	18.5 ± 0.6	18.7 ± 0.6	0.99
$P2_W$ (mm)	137	17.3 ± 0.6	16.3 ± 0.6	16.8 ± 0.6	16.8 ± 0.6	0.64
ΔP2 (mm)	137	-1.6 ± 0.4	-2.5 ± 0.4	-1.9 ± 0.4	-2.0 ± 0.4	0.44
ADFI (kg/day)[3]	136	6.56 ± 0.14	6.61 ± 0.13	6.32 ± 0.13	6.50 ± 0.14	0.43

[abcd] Different superscripts within rows denote a significant pairwise difference (*P* < 0.05; Fisher's LSD test) between diet means.

[1] CLA fed as a top-dress, with a daily amount given such that the final CLA percentage reported corresponds to the total CLA isomers consumed daily per primiparous sow as a proportion of total feed consumed. See text for further details.

[2] ADFI = average daily feed intake; BA = piglets born alive; ΔBW = change in sow body weight form entry to the farrowing house until weaning; ΔP2 = change in P2 from entry to the farrowing house until weaning; FR = farrowing rate; GL = gestation length; $P2_E$ = sow P2 backfat at entry to farrowing house; $P2_W$ = sow P2 backfat at weaning; SB = number of stillborn piglets; BW_E = sow body weight at entry to farrowing house; BW_W = sow body weight at weaning; TB = total born.

[3] Adjusted for number of days experimental diets fed before farrowing (pre-farrow feed days), included as a covariate in the model.

***Table 1.4** Effect of conjugated linoleic acid (CLA) inclusion in the late gestation and lactating diet on primiparous sow litter performance obtained from the linear mixed model analysis.*

		Diet[1]				P-value
Parameter[2]	**n**	**0% CLA**	**0.2% CLA**	**0.8% CLA**	**1.25% CLA**	**Diet**
At birth						
Litter WT (kg)	144	12.8 ± 0.6[a]	14.8 ± 0.6[b]	13.8 ± 0.6[ab]	14.2 ± 0.6[ab]	0.12
Average piglet WT (kg)	144	1.28 ± 0.04	1.28 ± 0.04	1.29 ± 0.04	1.30 ± 0.04	0.97
Within-litter CV (%)	142	16.8 ± 0.9	16.8 ± 0.9	16.6 ± 0.9	16.5 ± 0.9	0.99
After fostering						
Litter WT (kg)	143	12.8 ± 0.6	14.3 ± 0.5	13.5 ± 0.5	14.1 ± 0.6	0.22
Litter size (n)	145	9.7 ± 0.4[a]	11.0 ± 0.4[b]	10.3 ± 0.4[ab]	10.6 ± 0.4[ab]	0.073
Average piglet WT (kg)	143	1.33 ± 0.03	1.31 ± 0.03	1.32 ± 0.03	1.33 ± 0.03	0.98
Day 21 of lactation						
Litter WT (kg)	132	49.2 ± 2.1	51.3 ± 1.9	50.1 ± 2.0	52.6 ± 2.1	0.68
Litter size (n)	132	9.1 ± 0.4	9.8 ± 0.3	9.5 ± 0.4	9.7 ± 0.4	0.58
Average piglet WT (kg)	131	5.52 ± 0.15	5.26 ± 0.14	5.30 ± 0.14	5.45 ± 0.15	0.73
Within-litter CV (%)	126	17.2 ± 1.3	19.3 ± 1.1	20.1 ± 1.2	17.1 ± 1.2	0.20
ΔLW (kg)	130	35.9 ± 1.8	37.0 ± 1.6	36.1 ± 1.7	38.1 ± 1.8	0.81
ΔPW (kg)	130	4.10 ± 0.13	3.95 ± 0.12	3.99 ± 0.12	4.08 ± 0.13	0.81
Mortality (%)[3]						
Pre-foster mortality	142	7.1 ± 1.6	6.3 ± 1.6	6.6 ± 1.6	3.8 ± 1.6	0.48
Foster to weaning	132	6.2 ± 1.7	6.7 ± 1.6	9.7 ± 1.6	8.0 ± 1.7	0.44

[abcd] Different superscripts within rows denote a significant pairwise difference (*P* < 0.05; Fisher's LSD test) between diet means.

[1] CLA fed as a top-dress, with a daily amount given such that the final CLA percentage reported corresponds to the total CLA consumed daily per primiparous sow as a proportion of total feed consumed. See text for further details.

[2] ΔLW = change in litter weight from day of birth (after fostering) until day 21 of lactation; ΔPW = change in average piglet weight from day of birth (after fostering) until day 21 of lactation; CV = within litter co-efficient of variation of birth weight; WT = weight.

[3] Liveborn litter mortality analysed as a continuous variable (as a proportion of piglets present in the litter at birth or at fostering). Foster to weaning mortality includes all piglets that were tagged at birth.

Table 1.5 *Effect of conjugated linoleic acid (CLA) inclusion in the late gestation and lactating diet on primiparous sow litter mortality obtained from the X^2 analysis.*

		Diet[1]					
Parameter	**n**	**0% CLA**	**0.2% CLA**	**0.8% CLA**	**1.25% CLA**	**X^2**	**P-value**
Mortality (%)							
Pre-foster[2]	1555	7.6	7.1	6.5	4.4	3.83	0.28
Post-foster[2]	1505	6.0	7.1	9.0	6.8	2.54	0.47
Day 0 to 3[3]	1489	5.3	3.8	7.6	4.2	6.63	0.085
Day 4 to wean[3]	1412	7.7	7.8	7.0	8.0	0.27	0.97
Total[3]	1489	11.1	11.1	11.1	10.8	0.02	1.00

[1] CLA fed as a top-dress, with a daily amount given such that the final CLA percentage reported corresponds to the total CLA consumed daily per primiparous sow as a proportion of total feed consumed. See text for further details.

[2] Pre- and post-foster mortality calculated for all experimental piglets. Pre-foster includes piglets that were born but not tagged as they died before fostering, and this is calculated as a proportion of total piglets born alive. Post-foster includes piglets that survived to fostering, were tagged and fostered onto an on trial sow, calculated as a proportion of total piglets on experimental litters after fostering was carried out. Piglets could have been fostered on from another sow that was not originally part of the study.

[3] Mortality proportions from day 0 to 3, day 4 to wean, and total mortality here are from piglets that were tagged individually (excluding piglets that died before fostering), as a proportion of all tagged piglets. Mortality day 4 to wean is presented as a proportion of piglets alive on day 4.

Table 1.6 *Effect of conjugated linoleic acid (CLA) inclusion (planned contrasts and linear and polynomial effects) in the late gestation and lactating diet on primiparous sow reproductive performance obtained from the linear mixed model analysis (results presented as mean ± SE).*

		Contrast P-value			P-value[4]		
Parameter[1]	**n**	**A v. BCD**[2,3]	**B v. CD**	**C v. D**	**Linear**	**Quadratic**	**Cubic**
Farrowing performance							
GL (days)	145	NS	NS	NS	NS	NS	NS
TB	145	NS	NS	NS	NS	NS	NS
BA	145	0.036	NS	NS	0.015	0.022	0.030
SB	145	NS	NS	NS	0.034	0.050	0.074
Lactation performance							
BW_E (kg)	145	NS	NS	NS	NS	NS	NS
BW_W (kg)	137	NS	NS	NS	NS	NS	NS
ΔBW (kg)[5]	137	0.05	NS	NS	NS	NS	NS
$P2_E$ (mm)	145	NS	NS	NS	NS	NS	NS
$P2_W$ (mm)	137	NS	NS	NS	NS	NS	NS
ΔP2 (mm)	137	NS	NS	NS	NS	NS	NS
ADFI (kg/day)[5]	136	NS	NS	NS	NS	NS	NS
Litter performance at birth							
Litter WT (kg)	144	0.032	NS	NS	0.020	0.028	0.035
Ave piglet WT (kg)	144	NS	NS	NS	NS	-	-
Within-litter CV (%)	142	NS	NS	NS	NS	NS	NS
After fostering							
Litter WT (kg)	143	0.078	NS	NS	0.054	0.057	0.061
Litter size (n)	145	0.024	NS	NS	0.011	0.014	0.018
Ave piglet WT (kg)	143	NS	NS	NS	NS	-	-
Day 21 of lactation							
Litter WT (kg)	132	NS	NS	NS	NS	NS	NS
Litter size (n)	132	NS	NS	NS	NS	NS	NS
Ave piglet WT (kg)	131	NS	NS	NS	NS	NS	NS
Within-litter CV (%)	126	NS	NS	0.084	NS	NS	NS
ΔLW (kg)	130	NS	NS	NS	NS	NS	NS
ΔPW (kg)	130	NS	NS	NS	NS	NS	NS
Mortality (%)[6]							
Pre-foster	142	NS	NS	NS	NS	NS	NS
Foster to weaning	132	NS	NS	NS	NS	NS	NS
Subsequent gestation (second parity)							
WRI (d)	134	NS	NS	NS	NS	NS	NS
Gestation length (d)	118	NS	NS	NS	NS	NS	NS
TB	118	NS	NS	NS	NS	NS	NS
BA	118	NS	NS	NS	NS	NS	NS
#W	118	NS	NS	NS	NS	NS	NS

[1] ADFI = average daily feed intake; BA = piglets born alive; ΔBW = change in sow body weight form entry to the farrowing house until weaning; ΔLW = change in litter weight from day of birth (after fostering) until day 21 of lactation; ΔP2 = change in P2 from entry to the farrowing house until weaning; ΔPW = change in average piglet weight from day of birth (after fostering) until day 21 of lactation; CV = within litter co-efficient of variation of birth weight; FR = farrowing rate; GL = gestation length; $P2_E$ = sow P2 backfat at entry to farrowing house; $P2_W$ = sow P2 backfat at weaning; SB = number of stillborn piglets; BW_E = sow body weight at entry to farrowing

house; BW_W = sow body weight at weaning; TB = total born; WRI = wean to remate interval; WT = weight; #W = number of piglets weaned.

[2] Treatments are: A – 0% CLA (control); B – 0.2% CLA; C – 0.8% CLA; and, D – 1.25% CLA.

[3] CLA fed as a top-dress, with a daily amount given such that the final CLA percentage reported corresponds to the total CLA isomers consumed daily per primiparous sow as a proportion of total feed consumed. See text for further details.

[4] Where no P-value appears, adding higher order polynomials to the model did not improve the model and therefore only the first order polynomial was included.

[5] Adjusted for number of days experimental diets fed before farrowing (pre-farrow feed days), included as a covariate in the model.

[6] Liveborn litter mortality analysed as a continuous variable (as a proportion of piglets present in the litter at birth or at fostering).

5.1.3 Colostrum, Milk, and Piglet Serum IgG

There were no significant differences (*P* ≥ 0.10) in Brix% of colostrum or milk between the four dietary treatments (Table 1.7). Unfortunately, inaccurate dilution of standards during the serum IgG ELISA resulted in very high inter-plate variation (inter-assay CV 54%; calculated from standard curves), with the last plate assayed indicating that the standard samples may have been contaminated or not properly mixed during dilution. Therefore, the results from this ELISA plate were not included in the analysis, and ELISA plate was fitted in the model as a blocking factor. Without this plate included, the inter-assay CV was still quite high (17%), and results should be interpreted with caution. Nonetheless, there was no significant difference (*P* = 0.51) in serum IgG concentration between any of the dietary treatment groups (Table 1.7). Concentrations were numerically lower in the CLA groups compared to the control; however, serum IgG concentration increased with increasing dietary CLA inclusion within the CLA groups.

Table 1.7 *Effects of conjugated linoleic acid (CLA) inclusion rate in the late gestation and lactating diet on colostrum and milk Brix%, recorded using the Brix refractometer, and piglet serum IgG concentrations (results from the mixed models analysis presented as mean ± SE).*

		Diet[1]				**P-value**
Parameter	**n**	**0% CLA**	**0.2% CLA**	**0.8% CLA**	**1.25% CLA**	**Diet**
Brix%						
Colostrum (d 0)	26	28.1 ± 0.8	27.0 ± 1.1	28.0 ± 1.2	26.2 ± 0.8	0.33
Milk (d 21)	83	14.1 ± 0.3	14.5 ± 0.2	14.8 ± 0.2	14.3 ± 0.2	0.40
Serum IgG (mmol)						
Birth (within 24 h)	214	22.3 ± 3.4	16.7 ± 4.1	18.9 ± 4.0	20.9 ± 4.0	0.51

[1] CLA fed as a top-dress, with a daily amount given such that the final CLA percentage reported corresponds to the total CLA isomers consumed daily per primiparous sow as a proportion of total feed consumed. See text for further details.

5.1.4 Gilt Progeny Serum Energy Profile

Within the cohort of piglets blood sampled for their serum energy profile, there was no difference between individual birth weight (*P* = 0.89) or weight at day 21 (*P* = 0.83; Table 1.8). There was a significant diet x sex interaction (*P* = 0.027) for birth weight in this cohort, as female pigs selected for blood sampling in the control group were lighter than the males in this group, whereas the opposite was true for the 0.2% and 1.25% CLA groups (data not shown).

Piglets born to gilts fed 0.2% CLA had the numerically highest concentrations of triglycerides and glucose at birth (*P* = 0.48 and *P* = 0.85, respectively) compared to the other dietary treatments (Table 1.8). At day 21, control piglets had the highest serum triglycerides concentration, which was significantly higher (*P* < 0.05) than those from the 1.25% CLA treatment (Table 1.8). There was no significant difference in serum glucose concentrations (*P* = 0.83) between dietary treatments at this age, and males (6.15 ± 0.08 mmol) had significantly higher levels than females (5.93 ± 0.08 mmol; *P* = 0.021).

Table 1.8 *Effects of conjugated linoleic acid (CLA) inclusion rate in the late gestation and lactating diet on piglet serum triglycerides (TAG) and glucose (GLUC) concentration within 24 hours of birth and at day 21 of age, obtained from the linear mixed model analysis (results presented as mean ± SE).*

		Diet[1]				P-value		
Parameter[2]	**n**	**0% CLA**	**0.2% CLA**	**0.8% CLA**	**1.25% CLA**	**Diet (D)**	**Sex (S)**	**D*S**
Day 0 (within 24 h of birth)								
BWT (kg)	267	1.37 ± 0.03	1.34 ± 0.03	1.37 ± 0.03	1.38 ± 0.03	0.89	NS	0.027
TAG (mmol)	233	0.80 ± 0.06	0.92 ± 0.06	0.80 ± 0.06	0.82 ± 0.06	0.48	NS	NS
GLUC (mmol)	223	5.36 ± 0.16	5.51 ± 0.16	5.31 ± 0.15	5.39 ± 0.16	0.85	NS	NS
Day 21								
BWT (kg)	243	5.43 ± 0.17	5.37 ± 0.17	5.50 ± 0.16	5.53 ± 0.17	0.92	NS	NS
TAG (mmol)	178	0.75 ± 0.02[a]	0.74 ± 0.02[ab]	0.71 ± 0.02[ab]	0.70 ± 0.02[b]	0.074	NS	NS
GLUC (mmol)	177	5.99 ± 0.13	6.00 ± 0.13	6.04 ± 0.12	6.14 ± 0.13	0.83	0.021	0.075

[abcd] Different superscripts denote significant ($P < 0.05$) differences between individual treatment means.

[1] CLA fed as a top-dress, with a daily amount given such that the final CLA percentage reported corresponds to the total CLA isomers consumed daily per primiparous sow as a proportion of total feed consumed. See text for further details.

[2] BWT = piglet body weight (this cohort only).

5.1.5 Subsequent Reproductive Performance

There was no significant difference in WRI between gilts on the control diets or on the CLA diets (Table 1.9). However, numerically this was 2 days shorter for gilts on the 0.2% CLA diet compared to the control diet (8 vs. 6 days, respectively). This was largely due to a number of gilts (n = 21) that failed to return to oestrus in the first 7 days after weaning and therefore had a prolonged WRI (which was further prolonged in control gilts; 5 gilts averaging 27.6 days). If these WRIs were removed from the analysis there was no significant or numeric difference in WRI between the dietary treatments ($P \geq 0.10$). There was no significant difference in farrowing rate ($P = 0.72$) between the four groups; however, farrowing rate was highest in the 1.25% CLA group and lowest in the 0.2% CLA group (Table 1.9).

Table 1.9 *Effects of conjugated linoleic acid (CLA) inclusion rate in the late gestation and lactating diet on sow reproductive performance in parity 2 obtained from the linear mixed model analysis (results presented as mean ± SE).*

		Diet[1]				P-value
Parameter[2]	**n**	**0% CLA**	**0.2% CLA**	**0.8% CLA**	**1.25% CLA**	**Diet**
WRI (days)	134	8.23 ± 1.26	6.38 ± 1.19	6.79 ± 1.22	8.25 ± 1.26	0.60
WRI > 7 days (%)[3]	134	15.6	11.1	17.7	18.8	0.83
Farrowing rate (%)[3]	136	87.9	83.3	82.4	90.9	0.72
Gestation length (d)	117	115.7 ± 0.3	116.0 ± 0.3	116.1 ± 0.3	116.6 ± 0.3	0.18
TB	118	12.1 ± 0.5	12.8 ± 0.5	13.3 ± 0.5	12.6 ± 0.5	0.37
BA	118	11.4 ± 0.5	12.0 ± 0.4	12.3 ± 0.5	11.6 ± 0.4	0.52
#W	118	10.1 ± 0.4	10.3 ± 0.4	10.2 ± 0.4	10.6 ± 0.4	0.87

[1] CLA fed as a top-dress, with a daily amount given such that the final CLA percentage reported corresponds to the total CLA isomers consumed daily per primiparous sow as a proportion of total feed consumed. See text for further details.

[2] BA = number of piglets born alive; TB = total piglets born; #W = number of piglets weaned; WRI = wean to remate interval.

[3] Proportion of WRI > 7 days and farrowing rate analysed by chi-square.

Number of total born (TB) piglets in the subsequent farrowing was not significantly different (P = 0.37) between the four dietary treatment groups; however, the gilts previously on the CLA diets showed a higher TB than those that had not previously received CLA (Table 1.9). These results were similar for BA and number of piglets weaned in the subsequent lactation; with no significant difference (P = 0.52 and P = 0.87, respectively) between dietary treatments, but the highest values in the CLA treatments when compared to the control (Table 1.9).

5.2 *Experiment 2: Determining the ideal time to feed CLA*

One sow allocated to the control treatment did not farrow soon after her due date, therefore she was removed from the experiment and no data for this sow was included in the analysis (*n* = 35 for treatment A).

In the first replicate, one sow was moved between pens in the farrowing house as her original pen was broken and she was able to escape. All data for this sow was kept in the analysis. Another sow had her piglets removed after farrowing, as she harmed four of her newborn piglets. All farrowing data before fostering was included for this sow. One sow in each replicate was moved between pens as a hose to the drinker nipple broke off and flooded each pen, and these sows remained on trial. In the second replicate, one sow died of unknown causes two days after farrowing, and therefore only included in the farrowing performance data. Finally, two sows were removed during lactation (on days 9 and 17) as they were removed from the herd for feet and leg problems. These sows were included in the farrowing data, only. For four sows, the stillborn piglets were not weighed, and therefore no litter birth weight data was included in the analysis for these sows.

5.2.1 Gilt Reproductive Performance in Lactation

The number of days between entry to the farrowing house and farrowing was similar between dietary treatments ($P = 0.70$; Table 1.10). Gestation length was similar between dietary treatments ($P = 0.20$; Table 1.10), as was number of piglets born alive ($P = 0.37$), stillborn ($P = 0.34$), and total born ($P = 0.34$; Table 1.10). Similarly, BA as a percentage of TB was not significantly different between dietary treatments ($P = 0.50$). From the *post hoc* hypothesis testing, it was revealed that sows in treatments C and D had significantly longer gestation lengths than sows in treatment B ($P = 0.074$; Table 1.11).

At entry to the farrowing house, there was no significant effect of treatment overall ($P = 0.11$); however, sows in treatment C were significantly heavier ($P < 0.05$) than those in treatment B and treatment D (Table 1.10). There was also a significant effect of replicate on sow body weight at entry to the farrowing house ($P = 0.011$), where sows in the second replicate (196.7 ± 2.1 kg) were heavier than those in the first replicate (189.1 ± 2.1 kg). Sow P2 tended to be different between dietary treatments ($P = 0.071$; Table 1.10), with sows in treatments B and C having a significantly lower P2 backfat than those in treatment D ($P < 0.05$), but all were similar to that of the control sows ($P \geq 0.10$).

Unfortunately, only *n* = 1 sow on the C diet (second replicate) was observed farrowing and able to get a Brix% reading from the refractometer (26%). The results reported here describe the linear mixed models results with this value left in (and hence high standard errors for this treatment are reported). There was no difference in Brix% of colostrum between dietary treatments ($P = 0.91$) or replicates ($P = 0.53$). Numerically, Brix% was highest in treatment B (28.3 ± 1.2%) and D (28.5 ± 2.2%) sows compared to control (A) sows (28.1 ± 1.2%). Treatment C sows hence gave the lowest LS mean from this analysis (25.4 ± 3.9%), but there was no replication within this treatment group.

All sows were a similar weight at weaning ($P = 0.72$; Table 1.10) and there was no longer a difference between replicates ($P = 0.14$). Overall, sows in the C treatment lost the most body weight during lactation (from entry to the farrowing house until weaning), and control sows (A) lost the least ($P = 0.015$; Table 1.10). From the *post hoc* analysis, sows on the CLA diets (B, C, and D) tended to lose more weight in lactation (from entry to the farrowing house until weaning) than control (A) sows (*P*

= 0.089; Table 1.11). There was a significant effect of replicate on sow body weight loss (*P* < 0.001), where sows lost more weight in the second replicate (-13.5 ± 1.5 kg) than they did in the first (-1.5 ± 1.4 kg). Sow P2 backfat at weaning was similar between treatments (*P* = 0.39; Table 1.10), and sows in the first replicate had a higher P2 backfat than those in the second replicate (16.3 ± 0.5 mm vs. 14.7 ± 0.5 mm, respectively; *P* = 0.030). Sows in all dietary treatments had a similar change in P2 backfat during lactation (*P* = 0.36; Table 1.10); however, sows in the first replicate lost less P2 backfat than those in the second replicate (-0.01 ± 0.35 mm vs. -1.81 ± 0.36 mm, respectively; *P* < 0.001).

The overall dietary treatment effect on ADFI was not significant (*P* = 0.14). However, from the pairwise comparisons between treatment means, sows in treatment B tended to eat more (*P* = 0.063) than those in the control group (A) and ate significantly more than those in treatment D (*P* = 0.032). From the *post hoc* analysis it seemed that most of the main effect of dietary treatment on ADFI was due to the trend in treatment B sows eating more than sows in treatments C and D pooled together (*P* = 0.054), but there was no difference in CLA sows (B, C, and D) compared to the control (A) sows (*P* ≥ 0.10; Table 1.11). Sows ate significantly more (*P* < 0.001) in the second replicate (6.95 ± 0.09 kg/d) than the first (6.06 ± 0.09 kg/d).

The proportion of sows medicated in each dietary treatment group was similar (*P* = 0.34; Table 1.10). Numerically, a lower proportion of sows in treatment C were medicated (13.9%) in comparison to sows in treatments A (28.6%), B (22.2%), and D (30.6%).

Table 1.10 *Effect of timing of conjugated linoleic acid (CLA) inclusion in the late gestation and/or lactating diet on primiparous sow reproductive performance obtained from the linear mixed model analysis (results presented as mean ± SE).*

		Diet[1,2]				**P-value**
Parameter[3]	**n**	**A**	**B**	**C**	**D**	**Diet**
Feed days (days)						
Entry to farrowing	143	7.6 ± 0.5	7.1 ± 0.5	7.8 ± 0.5	7.1 ± 0.5	0.70
Total on CLA	139	0.0 ± 0.4[a]	7.1 ± 0.3[b]	14.3 ± 0.3[c]	34.5 ± 0.3[d]	< 0.001
GL	143	115.9 ± 0.2	115.3 ± 0.2	116.0 ± 0.2	115.7 ± 0.2	0.20
Farrowing performance						
BA	143	11.0 ± 0.5	11.5 ± 0.5	12.1 ± 0.5	11.6 ± 0.5	0.37
SB	143	0.68 ± 0.15	0.36 ± 0.15	0.50 ± 0.15	0.69 ± 0.15	0.34
TB	143	11.8 ± 0.5	11.9 ± 0.5	12.9 ± 0.5	12.4 ± 0.5	0.34
BA/TB (%)	143	94.2 ± 1.3	96.1 ± 1.3	94.2 ± 1.3	93.3 ± 1.3	0.50
Lactation performance						
BW_E (kg)	143	193.0 ± 3.0[ab]	189.9 ± 2.9[a]	198.8 ± 2.9[b]	189.8 ± 2.9[a]	0.11
BW_W (kg)	140	188.3 ± 3.2	184.0 ± 3.1	184.5 ± 3.2	183.8 ± 3.2	0.72
ΔBW (kg)	140	-4.5 ± 2.1[a]	-5.9 ± 2.0[a]	-13.2 ± 2.0[b]	-6.5 ± 2.0[a]	0.015
$P2_E$ (mm)	143	17.0 ± 0.7[ab]	15.7 ± 0.6[a]	15.5 ± 0.6[a]	17.6 ± 0.6[b]	0.071
$P2_W$ (mm)	140	15.8 ± 0.7	14.6 ± 0.7	15.4 ± 0.7	16.4 ± 0.7	0.39
ΔP2 (mm)	140	-1.2 ± 0.5	-1.1 ± 0.5	-0.1 ± 0.5	-1.2 ± 0.5	0.36
Lactation ADFI (kg/day)	139	6.40 ± 0.13[ab]	6.73 ± 0.12[a]	6.53 ± 0.12[ab]	6.36 ± 0.12[b]	0.14
Medicated (%)[4]	143	28.6	22.2	13.9	30.6	0.34

[abcd] Different superscripts within rows denote a significant pairwise difference (P < 0.05; Fisher's LSD test) between diet means.

[1] CLA fed as a top-dress, with a daily amount given such that the final CLA percentage reported corresponds to the total CLA isomers consumed daily per primiparous sow as a proportion of total feed consumed. See text for further details.

[2] Treatments are: A – 0% CLA (control); B – 0.2% CLA fed 7 days before farrowing (on average) until farrowing; C – 0.2% CLA fed 14 days before farrowing (on average) until farrowing; and, D – 0.2% CLA fed 7 days before farrowing (on average) until weaning.

[3] ADFI = average daily feed intake; BA = piglets born alive; ΔBW = change in sow body weight form entry to the farrowing house until weaning; ΔP2 = change in P2 from entry to the farrowing house until weaning; GL = gestation length; $P2_E$ = sow P2 backfat at entry to farrowing house; $P2_W$ = sow P2 backfat at weaning; SB = number of stillborn piglets; BW_E = sow body weight at entry to farrowing house; BW_W = sow body weight at weaning; TB = total born.

[4] Number of primiparous sows medicated during the course of the experiment for farrowing difficulties, litter scours, etc. As a proportion of total sows, analysed by X^2 (X^2 = 3.35).

Table 1.11 *Effect of timing of conjugated linoleic acid (CLA) inclusion (planned contrasts) in the late gestation and/or lactating diet on primiparous sow reproductive performance obtained from the linear mixed model analysis (results presented as mean ± SE).*

		Contrast P-value		
Parameter[1]	**n**	**A v. BCD**[2,3]	**B v. CD**	**C v. D**
Farrowing performance				
GL (days)	143	NS	0.074	NS
TB	143	NS	NS	NS
BA	143	NS	NS	NS
SB	143	NS	NS	NS
Lactation performance				
BW_E (kg)	143	NS	NS	0.032
BW_W (kg)	140	NS	NS	NS
ΔBW (kg)	140	0.089	NS	0.021
$P2_E$ (mm)	143	NS	NS	0.025
$P2_W$ (mm)	140	NS	NS	NS
ΔP2 (mm)	140	NS	NS	NS
ADFI (kg/day)	139	NS	0.054	NS
Litter performance at birth				
Liveborn litter WT (kg)	142	NS	NS	NS
Ave liveborn piglet WT (kg)	141	NS	NS	NS
Total litter WT (kg)	142	NS	NS	NS
Ave piglet WT – all pigs (kg)	142	NS	NS	NS
After fostering				
Litter WT (kg)	141	NS	NS	NS
Litter size (n)	141	NS	NS	NS
Ave piglet WT (kg)	141	NS	NS	NS
Day 19 of lactation				
Litter WT (kg)	114	NS	0.042	NS
Litter size (n)	114	NS	0.065	NS
Ave piglet WT (kg)	114	NS	NS	NS
ΔLW (kg)	113	NS	0.021	NS
Piglet ADG (g/day)	113	NS	0.067	NS
Mortality (%)[4]				
Pre-foster	142	NS	NS	NS
Foster to weaning	130	NS	0.048	NS

[1] ADFI = average daily feed intake; BA = piglets born alive; ΔBW = change in sow body weight form entry to the farrowing house until weaning; ΔLW = change in litter weight from day of birth (after fostering) until day 19 of lactation; ΔP2 = change in P2 from entry to the farrowing house until weaning; ΔPW = change in average piglet weight from day of birth (after fostering) until day 19 of lactation; GL = gestation length; $P2_E$ = sow P2 backfat at entry to farrowing house; $P2_W$ = sow P2 backfat at weaning; SB = number of stillborn piglets; BW_E = sow body weight at entry to farrowing house; BW_W = sow body weight at weaning; TB = total born; WT = weight; #W = number of piglets weaned.

[2] Treatments are: A – 0% CLA (control); B – 0.2% CLA fed 7 days before farrowing (on average) until farrowing; C – 0.2% CLA fed 14 days before farrowing (on average) until farrowing; and, D – 0.2% CLA fed 7 days before farrowing (on average) until weaning.

[3] CLA fed as a top-dress, with a daily amount given such that the final CLA percentage reported was 0.2% and corresponds to the total CLA isomers consumed daily per primiparous sow as a proportion of total feed consumed. See text for further details.

[4] Liveborn litter mortality analysed as a continuous variable (as a proportion of piglets present in the litter at birth or at fostering).

5.2.2 Gilt Progeny Performance in Lactation

Both total liveborn litter weight (*P* = 0.53) and average liveborn piglet weight (*P* = 0.97) were similar between all treatment groups (Table 1.12). Similarly, when controlling for BA as a covariate, liveborn litter weight was similar (*P* = 1.00) between treatment groups, as was average liveborn piglet weight at birth (*P* = 0.99; data not shown). A similar result was obtained with total litter weight at birth (including the weight of stillborn piglets; Table 1.12). Controlling for total born gave a similar outcome (*P* = 1.00) in the case of total litter weight at birth (data not shown) and did not improve the overall model. The average piglet weight of all pigs born was not significantly different (*P* = 1.00: Table 1.12) between treatment groups, and similarly when corrected for TB as a covariate (*P* = 0.91; data not shown). After fostering, average piglet weight (*P* = 0.81), total litter weight (*P* = 0.93), and litter number (*P* = 0.59) were all similar between treatment groups (Table 1.12), even when correcting for relevant covariates (data not shown). For all birth weight variables and variables after fostering, controlling for sow body weight or P2 backfat at entry to the farrowing house as a covariate did not improve the model and showed similar between-treatment results (data not shown).

At day 19 of lactation, litters from treatment B sows were significantly heavier than litters from sows in treatment C (*P* = 0.049) and tended to be heavier than litters from treatment A sows (*P* = 0.077); however, the overall treatment effect was not significant (*P* = 0.19; Table 1.12). When litter weight at day 19 was corrected for sow body weight at entry to the farrowing house (treatment B sows were lightest; Table 1.10) this result became more significant (*P* = 0.085) and litters from sows in treatment B were significantly heavier than those from treatment A (43.7 vs. 36.6 ± 2.5 kg, respectively; *P* = 0.049) and treatment C (35.3 ± 2.2 kg; *P* = 0.015) and tended to be heavier than those from treatment D (37.9 ± 2.3 kg; *P* = 0.092). From the *post hoc* analysis it was seen that sows in treatment B had a significantly higher litter weight at day 19 than those from treatments C and D combined (*P* = 0.042), and this was even more significant when correcting for sow body weight at entry to the farrowing house (*P* = 0.019; Table 1.11). Correcting day 19 litter weight for litter number at day 19 resulted in the best model for this variable, and treatment differences were no longer significant (*P* = 0.40; data not shown).

Litter number at day 19 was not significantly different between dietary treatment (*P* = 0.16; Table 1.12); however, litters from treatment B sows had significantly more piglets at day 19 than those in treatment A (*P* = 0.031), and tended to have more piglets than treatment C sows (*P* = 0.094). Litter number at day 19 was not significantly impacted by sow body weight or P2 backfat at entry to the farrowing house (*P* ≥ 0.10) and adding these into the model as covariates did not improve model fit. However, the model was improved by adjusting for litter number post foster (treatment *P* = 0.055). In this adjusted model, litter number at day 19 was significantly higher for treatment B sows than treatment A sows (*P* = 0.010) and treatment C sows (*P* = 0.028) and tended to be higher than for treatment D sows (P = 0.068; data not shown). Average piglet weight at day 19 was similar between treatments (*P* = 0.41; Table 1.12), even when correcting for average piglet weight after fostering (*P* = 0.39; data not shown). Correcting for sow body weight at entry or litter number at day 19 did not improve the model for average piglet weight at day 19.

The overall dietary treatment effect on litter weight gain in lactation (from after fostering [day 1] to day 19) was not significant (*P* = 0.11; Table 1.12); however, between treatment comparisons revealed that litters from treatment B sows gained significantly more weight than those from treatment C (*P* = 0.027; Table 1.12), and tended to gain significantly more weight than A (*P* = 0.053) and D litters (*P* = 0.060). Correcting for sow body weight and P2 backfat at entry and litter or average piglet weight post-foster did not improve any of this model. Piglet ADG (calculated as [average piglet weight at day 19 – average piglet weight after fostering]/19) was similar between dietary treatments (*P* = 0.39; Table 1.12); however, tended to be greater in piglets from treatment B sows compared to treatment C sows (*P* = 0.098). When correcting this model for sow body weight at entry to the farrowing house (sow P2 backfat did not improve the model and was left out), ADG for piglets from treatment B sows was significantly higher than those from treatment C sows (*P* = 0.028), although the overall treatment effect remained non-significant in this model (163 ± 9, 171 ± 9, 145 ± 8, and 159 ± 8 g/d for treatment A, B, C, and D sows, respectively; *P* = 0.15). From the *post hoc* hypothesis tests, ADG of piglets from treatment B sows tended to be higher than those of C and D sows combined (*P* = 0.067), and total change in litter weight in lactation was significantly higher in these B sow litters, compared to C and D litters (*P* = 0.021; Table 1.11).

Number of pigs weaned was similar between all dietary treatments (*P* = 0.38; data not shown). However, when adjusting for number of piglets in the litter after fostering as a covariate, sows in treatment B had a significantly higher (*P* = 0.029) number of pigs weaned than those in the control (A) treatment (9.7 ± 0.3 vs. 8.7 ± 0.4 pigs, respectively) and sows in treatment D (8.8 ± 0.4 pigs; *P* = 0.041). Sows in treatment B also tended to wean more piglets than that of sows in treatment C (9.0 ± 0.3 pigs; *P* = 0.093) in the corrected model, and the overall treatment effect almost tended towards significance (*P* = 0.10). Number of piglets weaned was similar (*P* ≥ 0.10) for treatment C sows compared to the control, and in treatment D sows compared to the control.

There was no significant difference in litter pre-foster mortality rate between dietary treatments (*P* = 70; Table 1.12). Overall, there was no significant difference in post-foster mortality between dietary treatments (*P* = 0.25); however, sows in treatment B tended to have lower post-foster mortality rates within their litters than treatment C sows (*P* = 0.055), but both were similar to those of control (A) sows (*P* ≥ 0.10). From the chi-squared analysis, there was no difference in pre-foster mortality rates between dietary treatments (*P* = 0.68; Table 1.13). There was a significant difference in total post-foster mortality between dietary treatments (*P* = 0.019), with this mortality rate lower in treatment B litters and highest in treatments C and D litters (Table 1.13). This effect was seen mostly when broken down into mortality from day 4 to weaning (*P* = 0.041) and was reflected in total mortality as well (*P* = 0.035). There was no difference in mortality rate between dietary treatments from day 0 to day 3 of lactation from this analysis (*P* = 0.77; Table 1.13).

Table 1.12 *Effect of timing of conjugated linoleic acid (CLA) inclusion in the late gestation and/or lactating diet on primiparous sow litter performance obtained from the linear mixed model analysis.*

		Diet[1,2]				P-value
Parameter[3]	**n**	**A**	**B**	**C**	**D**	**Diet**
Litter performance at birth						
Liveborn litter WT (kg)	142	14.0 ± 0.6	14.5 ± 0.6	15.3 ± 0.6	14.8 ± 0.6	0.53
Ave liveborn piglet WT (kg)	141	1.29 ± 0.03	1.27 ± 0.03	1.26 ± 0.03	1.28 ± 0.03	0.97
Total litter WT (kg)[4]	142	14.7 ± 0.6	14.8 ± 0.6	15.8 ± 0.6	15.4 ± 0.6	0.54
Ave piglet WT – all pigs (kg)[4]	142	1.26 ± 0.03	1.26 ± 0.03	1.26 ± 0.03	1.27 ± 0.03	1.00
After fostering						
Litter WT (kg)	141	14.2 ± 0.5	14.0 ± 0.5	14.4 ± 0.5	14.4 ± 0.5	0.92
Litter size (n)	141	11.1 ± 0.3	10.8 ± 0.3	11.2 ± 0.3	10.9 ± 0.3	0.59
Ave piglet WT (kg)	141	1.29 ± 0.03	1.30 ± 0.03	1.28 ± 0.03	1.32 ± 0.03	0.81
Day 19 of lactation						
Litter WT (kg)	114	36.5 ± 2.6[ab]	43.0 ± 2.6[a]	36.2 ± 2.2[b]	37.2 ± 2.4[ab]	0.19
Litter size (n)	114	8.2 ± 0.4[a]	9.6 ± 0.4[b]	8.6 ± 0.4[ab]	8.6 ± 0.4[ab]	0.16
Ave piglet WT (kg)	114	4.38 ± 0.18	4.50 ± 0.18	4.10 ± 0.16	4.30 ± 0.17	0.41
ΔLW (kg)	113	22.3 ± 2.5[ab]	29.1 ± 2.5[a]	21.9 ± 2.1[b]	22.8 ± 2.2[ab]	0.11
Piglet ADG (g/day)	113	162 ± 9	169 ± 9	148 ± 8	156 ± 8	0.39
Mortality (%)[5]						
Pre-foster	142	5.0 ± 1.7	7.5 ± 1.6	7.3 ± 1.6	7.0 ± 1.6	0.70
Foster to weaning	130	15.2 ± 3.0	11.2 ± 2.7	18.7 ± 2.8	17.0 ± 2.7	0.25

[abcd] Different superscripts within rows denote a significant pairwise difference (*P* < 0.05; Fisher's LSD test) between diet means.

[1] CLA fed as a top-dress, with a daily amount given such that the final CLA percentage reported corresponds to the total CLA isomers consumed daily per primiparous sow as a proportion of total feed consumed. See text for further details.

[2] Treatments are: A – 0% CLA (control); B – 0.2% CLA fed 7 days before farrowing (on average) until farrowing; C – 0.2% CLA fed 14 days before farrowing (on average) until farrowing; and, D – 0.2% CLA fed 7 days before farrowing (on average) until weaning.

[3] ΔLW = change in litter weight from day of birth (after fostering) until day 21 of lactation; ΔPW = change in average piglet weight from day of birth (after fostering) until day 19 of lactation; CV = within litter co-efficient of variation of birth weight; WT = weight.

[4] Excluding mummified piglets.

[5] Liveborn litter mortality analysed as a continuous variable (as a proportion of piglets present in the litter at birth or at fostering). Foster to weaning mortality includes all piglets that were tagged at birth.

Table 1.13 *Effect of conjugated linoleic acid (CLA) inclusion in the late gestation and lactating diet on primiparous sow litter mortality obtained from the X^2 analysis.*

		Diet[1,2]					
Parameter	**n**	**A**	**B**	**C**	**D**	**X^2**	**P-value**
Mortality (%)							
Pre-foster[3]	142	6.5	7.8	8.5	6.7	1.53	0.68
Post-foster[3]	127	13.2	10.9	17.6	17.4	9.97	0.019
Day 0 to 3[4]	142	11.1	10.7	10.0	12.3	1.14	0.77
Day 4 to wean[4]	130	14.8	8.9	15.0	12.0	8.24	0.041
Total[4]	142	17.6	16.5	23.1	22.4	8.61	0.035

[1] CLA fed as a top-dress, with a daily amount given such that the final CLA percentage was 0.2% corresponds to the total CLA consumed daily per primiparous sow as a proportion of total feed consumed. See text for further details.

[2] Treatments are: A – 0% CLA (control); B – 0.2% CLA fed 7 days before farrowing (on average) until farrowing; C – 0.2% CLA fed 14 days before farrowing (on average) until farrowing; and, D – 0.2% CLA fed 7 days before farrowing (on average) until weaning.

[3] Pre- and post-foster mortality calculated for all experimental piglets. Pre-foster includes piglets that were born but not tagged as they died before fostering, and this is calculated as a proportion of total piglets born alive. Post-foster includes piglets that survived to fostering, were tagged and fostered onto an on trial sow, calculated as a proportion of total piglets on experimental litters after fostering was carried out. Piglets could have been fostered on from another sow that was not originally part of the study.

[4] Mortality proportions from day 0 to 3 and total mortality here are presented as a proportion of all piglets born alive. Mortality day 4 to wean is presented as a proportion of piglets alive on day 4.

6. Discussion

The results from the current project suggest that CLA only needs to be fed at a small inclusion (~0.2% total isomers) and for a small number of days before farrowing (1 week) to have the best impact on the lactation performance of primiparous sows and their progeny. Although results were variable, Experiment 1 showed that 0.2% CLA inclusion in the pre-farrowing and lactation diet, in the form of a top dress, gave the best performance results, and no further improvement was seen by feeding in excess of this inclusion. Most differences were numerical differences and may be required to be reaffirmed with a larger number of sows in future studies. This may also have been further impacted by the higher number of total piglets born in CLA treatment sows compared to control sows in this experiment. However, 0.2% was chosen as the best level of CLA from this experiment for inclusion in the diets of sows in Experiment 2, as not only was lactation performance enhanced, but this inclusion was the most economical (see Section 7 of this report for further details on the cost benefit analysis). It was then shown from the results of Experiment 2 that it is not necessary to feed CLA to primiparous sows beyond farrowing, and supplementation in just the last week before farrowing can result in the best lactation performance, reducing piglet mortality and improving litter weight gain to 19 days of age (and most likely to weaning).

The design of Experiment 1 was based on a previous experiment by our group (Craig et al., 2019) that showed that feeding CLA before farrowing (at an inclusion of 0.5%, fed for 12-13 days before farrowing in this experiment) up until weaning could improve pre-weaning survival of both gilt and sow progeny. In that study, TB was similar between the 0.5% CLA (13.1) and the control treatment not supplemented with CLA (13.4), but BA was numerically lower in the 0.5% CLA sows (11.6) compared to control sows (12.6), and this was due to significantly more SB piglets in the 0.5% CLA treatment (1.2 vs. 0.5, respectively). The opposite was true in Experiment 1 of the current project, where it appeared that feeding a CLA top dress to gilts may improve the proportion of piglets born alive (although this finding was not statistically significant). This result from Experiment 1 may have been an artefact of the unintentional inclusion of sows with a higher TB in the CLA treatments compared to the control treatments, which may explain the discrepancies between this study and that of Craig et al. (2019), and this finding was not replicated in Experiment 2. Nonetheless, in Experiment 2, the proportion of piglets BA in the litter (BA%) was highest in treatment B sows where CLA was fed for 1 week before farrowing only. The number of piglets BA was higher when their dams were supplemented with approximately 1.2% CLA isomers in a study by Barowicz et al. (2002); however, this has not been seen in more recent studies involving CLA supplementation (Cordero et al., 2011; Corino et al., 2009).

Nonetheless, this represents a universal difficulty when utilising primiparous sows in pre-farrow feeding experiments, as there is no way of predicting the number of TB piglets to balance between treatments at the commencement of the experiment. This can be predicted to a certain degree for multiparous sows based on her TB in previous farrowings; however, this luxury cannot be afforded to primiparous sows. This could be somewhat avoided by performing an ultrasound to count the number of embryos in late gestation before allocating to treatment but is seldom carried out as this approach is labour intensive and would still present an opportunity for operator error/inaccuracy. However, an approach such as this may be implemented in future studies to control for TB.

It is important to note that in Experiment 1, gilts were randomly allocated to their experimental diets to control for their weight and P2 at entry to the farrowing house, and therefore unsurprisingly all gilts were a similar weight and P2 backfat at entry to the farrowing house. It is therefore interesting that control gilts had a numerically lower number of piglets born (and therefore a lower litter weight at birth) than gilts on the CLA diets, and the average weight of piglets born alive was similar between all treatments. This may suggest that piglets from gilts on the control diet may have been heavier at day 110 of gestation (or similar in total litter weight to the CLA litters, hence the similar gilt body weights), but ended up being a similar weight as the piglets from gilts on CLA diets at birth. Therefore, CLA may act to improve the growth and development of piglets in late gestation, which would be in agreement with the results of Corino et al. (2009), who found feeding CLA in place of soybean oil to gestating sows significantly increased piglet birth weights. Unfortunately, this could not be confirmed by measures in the current study and requires further investigation. Weight of gilts at entry to the farrowing house could rely on a number of other factors other than the weight of the litter they are carrying (placental weight, fluid retention, etc.) and further studies may look to investigate the impact of feeding CLA on foetal development of gilt progeny in late gestation. Primiparous sows supplemented CLA in Experiment 1 had a higher litter weight at birth, seemingly due to having more piglets surviving the farrowing process but adjusting the model for BA in this experiment gave a similar result. It was unfortunate that stillborn piglets were unable to be weighed in Experiment 1, and piglets were unable to be individually weighed at birth in Experiment 2, as this may have told us more about how CLA may impact the development of piglets *in utero*.

Individual piglet weights were taken in Experiment 1 to examine differences in variation within litters, as CLA has been shown to improve piglet birth weights (Corino et al., 2009) and reduce the proportion of piglets that are light (<1 kg) at birth (Bontempo et al., 2004). It was thought that this may result in a benefit for only light born gilt progeny, or all gilt progeny as they are born lighter than their sow progeny counterparts. However, there was no difference between the dietary treatments in Experiment 1 in terms of proportion of piglets in these low birth weight groups. Unfortunately, individual birth weights were not measured in Experiment 2 and therefore within-litter birth weight variation (CV) could not be calculated from this data. It would be of interest to further investigate this concept in future studies.

In the current study we did not see CLA supplementation improve piglet serum IgG concentrations soon after birth as we did in our previous study (Craig et al., 2019), and as has been shown in studies from other research groups (Bontempo et al., 2004; Corino et al., 2009; Wu et al., 2015). Serum IgG concentration seemed to increase linearly with increasing CLA inclusion in the diet, but regardless, all values were lower than that of the control piglets. Issues with dilution of standards and ELISA protocols may have introduced unwanted variation in the results of the current study. Another factor impacting this finding may be the timing of CLA supplementation, as diets were fed from day 107 of gestation in Craig et al. (2019); whereas in Experiment 1, due to the commercial setting determining timing of entry into the farrowing house, CLA supplementation started from day 111 of gestation, and unfortunately we could not quantify serum IgG concentration of piglets in Experiment 2. Coupled with the unforeseen differences in total piglets born between the treatment groups and the fact that no difference in Brix% of colostrum was seen between dietary treatments in either experiment or in colostrum IgG concentration in our previous study (Craig et al., 2019), these results require further investigation, as previous work suggests that CLA supplementation may result in improvements in colostrum immunoglobulins and hence piglet immunity before weaning (Bontempo et al., 2004; Corino et al., 2009; Wu et al., 2015).

It would also be of interest to further investigate the physiological reason for any improvements in piglet growth when dams are supplemented with CLA before farrowing, as it seems from previous studies that feeding CLA to the dam may improve piglet energy stores, in circulation and in the muscle (Bee, 2000a,b; Park et al., 2005; Patterson et al., 2008; Poulos et al., 2004). However, from the results of Experiment 1 it seemed that CLA supplementation did not improve piglet glucose or triglyceride concentrations soon after birth or in late lactation. The total non-esterified fatty acid content (NEFA) was unfortunately not able to be determined in the serum samples obtained from piglets in Experiment 1 of the current project due to travel and visitor restrictions imposed by the COVID-19 situation in Australia at the time; however, samples have been stored and may be further analysed in the future to further explore these mechanisms. Similarly, we were unable to measure total fat in colostrum and milk in Experiment 1 due to COVID-19 travel restrictions at the time. It would be of interest to further explore the impact of dietary CLA in gilt diets on the total fat content of milk, although it seems from Craig et al. (2019) that CLA supplementation does not improve colostrum or milk fat content. Previous studies suggest that CLA supplementation may actually reduce the total fat content of colostrum and milk (Cordero et al., 2011; Harrell et al., 2000; Poulos et al., 2004; Wu et al., 2015), and that the real benefit may lie in alteration of fatty acid profile of these secretions (Bee, 2000a,b; Bontempo et al., 2004; Cordero et al., 2011; Peng et al., 2010).

Our previous study suggested that supplementing sows with CLA in late gestation and/or lactation could improve pre-weaning survival rates of gilt (and sow) progeny (Craig et al., 2019), and this was further demonstrated in this project, albeit at different times in lactation between the two experiments (early life vs. later in lactation). Experiment 1 results suggest that feeding CLA to primiparous sows could reduce the proportion of piglets that die before fostering (within 24 hours of birth), although there was no impact on the proportion of piglets born light when feeding CLA, and this effect was not seen again in Experiment 2. Krogh et al. (2012) found that sows (both primiparous and multiparous) supplemented CLA (equivalent to ~ 0.6-0.7% CLA isomers) from day 108 until weaning actually had a higher litter mortality rate in the first 7 days after farrowing than control sows. One possible reason for this discrepancy could be that the CLA levels used by Krogh et al. (2012) were too high, and that our Experiment 1 results may suggest that 0.2% maximises the chance of piglet survival at birth. The fact that there was no difference seen in Experiment 2 in pre-foster mortality between sows in treatment A and D suggests that this finding and the mechanisms behind it require further investigation. Experiment 2 results suggested that feeding 0.2% CLA before farrowing only could improve post-foster mortality rates, and this is in agreement with Barowicz et al. (2002) and Hadaš et al. (2013). However, Barowicz et al. (2002) found that feeding ~1.2% CLA isomers from day 90 of gestation until farrowing (> 2 weeks before farrowing) to sows also improved pre-weaning survival of their progeny, in contrast to our Experiment 2 where post-foster mortality was actually highest in the treatment C litters. Similarly, Hadaš et al. (2013) fed CLA from day 108 of gestation until weaning (fed as Lutalin® from BASF, Germany), and found pre-weaning survival was improved, but this was not replicated in our treatment D litters from Experiment 2. These differences may be explained by the sow parities used in each study, and unfortunately parities used were not reported in the studies of Barowicz et al. (2002) or Hadaš et al. (2013). The mechanisms for improved pre-weaning piglet survival rates when supplementing CLA for different periods and at different inclusion rates deserves further investigation.

Previous studies have shown that supplementing CLA either before farrowing and/or throughout lactation can improve milk production (Krogh et al., 2012) and piglet growth performance in lactation (Bee, 2000a, 2000b; Cordero et al., 2011; Corino et al., 2009). From the results of Experiment 1, it seemed that litter growth performance in lactation was not significantly improved by CLA inclusion in the diet. While gilts on the CLA diets lost more body weight in lactation (from entry to the farrowing house to weaning), it seems that this did not translate into improved growth performance of the litter from birth to 21 days of age. This higher loss of body weight may be more attributable to the higher litter size at birth of gilts in the CLA groups. However, there was a numerically higher loss of P2 backfat in CLA gilts in lactation which is in agreement with the findings of Cordero et al. (2011), and requires further investigation. Therefore, we expected that in Experiment 2, the optimum feeding duration for CLA may be up until the point of farrowing and feeding beyond this may not result in significant improvements in lactation performance. This was confirmed in Experiment 2, as treatment B sows that were fed for only 1 week prior to farrowing and not during lactation out-performed sows in the other CLA treatments in terms of piglet growth from birth to 19 days of age. Treatment B sows also had the highest ADFI in lactation in Experiment 2. Furthermore, treatment D sows generally performed the worst out of the three treatments (fed CLA before and after farrowing), and in some cases did not show improvements in lactation performance compared to control sows. This is somewhat in agreement with Park et al. (2005), who fed CLA in gestation for differing times (15-75 days post-mating until weaning) and found that the longer that the CLA was supplemented in gestation, the lighter the piglets were at weaning.

Results from Experiment 2 suggest that CLA may be most valuable when feeding for 1 week prior to farrowing, rather than throughout the whole of lactation as first thought. Furthermore, it seemed that feeding CLA for more than 1 week before farrowing negated any positive effects of feeding CLA for the last week only. It must be acknowledged that this finding may have been impacted by the fact that primiparous sows in this treatment were housed in gestation stalls for 1 week before being transferred into the farrowing house, in order to ensure they received the correct diet (this was not able to happen in the group gestation housing). This may have influenced their behaviour and/or performance in lactation as these sows were confined for an extra week (Barnett et al., 2001; Jarvis et al., 2006) and were moved in late gestation one more time than sows in other treatments, and the stress of both events could impact their stress response and hence their overall performance (Tilbrook et al., 2018). Loading sows into a farrowing house more than 1 week in advance of their due date is not currently commercial practice, and it would be difficult to administer a top dress any earlier than this in most commercial systems. Furthermore, due to the study design and labour constraints in Experiment 2, sows in treatment C were not weighed at the start of the CLA supplementation period and were randomly selected for the study based on their farrowing due date. Hence, we were not able to allocate to treatment evenly based on sow weight at the start of the experiment, which could have confounded this treatment and impacted sow and/or litter performance.

The design of studies looking at incorporating CLA into a gestating/lactating sow diet need to be carefully written and interpreted. Most of the current literature report the proportion of CLA product used in the final feed product, but some fail to report the proportion of CLA isomers in these products themselves, hence making it difficult for the reader to interpret the exact concentration of CLA in the final feed consumed by the sow. The CLA inclusion rates stated throughout this paper are the inclusion of total CLA isomers. In our previous study (Craig et al., 2019; from APL project 2014/461), 25 g/kg of a commercial 20% CLA product (Lutrell® Pure; BASF, Ludwigshafen, Germany) was used. i.e. 5 g/kg of total CLA isomers in the daily ration. For the experiments in the current project, we used a different 58% CLA product (LodeStar CLA50; Berg+Scmidt, Hamburg, Germany) at levels to ensure the inclusion of CLA isomers were at the appropriate rates. In Experiment 1, we used 1.2 mL/kg or ~1.2 g/kg (approx. 2 L/tonne of Lodestar CLA product) as recommended by the manufacturer, 5 g/kg (8 L/tonne; similar to Craig et al., 2019), and 7.5 g/kg (12.5 L/tonne; to determine whether a higher rate of CLA has additional benefits to survival and/or pre-weaning performance). These levels were also based on those used in previous studies in breeding sows (Bontempo et al., 2004, 3 g/kg total CLA isomers; Cordero et al., 2011, 6 g/kg total CLA isomers; Corino et al., 2009, 3 g/kg total CLA isomers). Producers must consider carefully the CLA product used in their diets to maximise performance, especially considering fatty acids such as CLA may be volatile and this can impact their stability in pig diets. Products such as LodeStar CLA50 are ideal, as their protectant formula can increase feed 'shelf-life' in storage on farm.

It is important to continue the work on feeding CLA commercially to confirm that CLA may improve the pre-natal development of gilt progeny in late gestation and their survival chance and growth performance throughout lactation. From both experiments in this project it seems that the reproductive response of primiparous sows to CLA supplementation in late gestation and/or lactation is largely variable. There seemed to be a number of numerical improvements in performance when primiparous sows were supplemented with CLA in both experiments, but it was difficult to control for factors other than dietary CLA supplementation that may have influenced the study, such as number of piglets *in utero* at the beginning of the supplementation period in Experiment 1. Further studies in this area must consider a larger sample size to assess the impact of CLA supplementation on reproductive performance of primiparous (and multiparous) sows. It is recommended that further work be done to maximise the potential for CLA to improve piglet immunity and survival rate soon after birth, and to determine the mechanisms by which CLA or it's metabolites may be transferred to the foetus *in utero* or through colostrum or milk. If supplemental CLA in primiparous sow diets could improve gilt progeny immunity and vigour at birth, and this feeding strategy was implemented on a commercial level, overall herd efficiency would be greatly improved as gilts and their progeny make up a substantial percentage of the Australian sow herd (Craig et al., 2017a). A greater retention of breeding females from gilt litters would contribute to an improvement in HFC and advanced genetic progress. Overall herd health would also be improved, reducing the need for antibiotics, whilst improving growth performance of the herd and increasing the size and volume of animals to market.

In conclusion, this project has shown that feeding CLA to primiparous sows in late gestation and/or lactation can have positive benefits on the performance and survival of gilt progeny. Feeding CLA as a top dress to primiparous sows in the week before farrowing (i.e. at entry to the farrowing house) is an economically feasible way to improve the lactation performance of sows and the survivability of their progeny soon after birth; however, further studies in this area are warranted.

7. Implications & Recommendations

The outcomes of this project have shown that CLA can be included in primiparous sow diets before farrowing using a top dress to improve progeny survival around birth and increase sow and litter performance in lactation. This additive can be included in late gestation diets for primiparous sows quite cheaply (30 cents per litter per lactation), as 0.2% total CLA isomers fed only 7 days before farrowing is enough to see substantial improvements in performance.

7.1 Cost Benefit Analysis

At a current cost of approximately $11/L, the approximate additional costs per gilt on each CLA supplemented diet from Experiment 1 (per litter per lactation) were:

- 0.2% CLA isomers - $3.08 *(1.8 c/kg of feed; $11/L = 2.2 c/kg of feed minus saving of 0.4 c/kg on tallow)*
- 0.8% CLA isomers - $11.29 *(6.6 c/kg of feed)*
- 1.25% CLA isomers - $19.24 per sow *(11.25 c/kg of feed)*

These figures were calculated on the basis of sows being fed 2.5 kg/day for 6 days before farrowing, and 6.5 kg/day for 24 days of lactation.

In Experiment 2, the additional costs of the experimental diets including 0.2% CLA isomers (per litter per lactation) were:

- 1 week pre-farrow - $0.30 *(1.8 c/kg of feed)*
- 2 weeks pre-farrow - $0.60
- Pre-farrow (7 days) + lactation (27 days) - $3.43

These figures were calculated on the basis of sows being fed 2.5 kg/day pre-farrowing and 6.4 kg/day during lactation.

If pre-weaning mortality of gilt progeny can be reduced by 1.1% by feeding CLA, as shown in Experiment 2 with feeding CLA for 7 days before farrowing, this would bring in approximately $3.34 additional revenue per litter in a commercial system such as that at Rivalea (PigEV; Hermesch et al., 2012, 2013). In addition, the added 6.8 kg increase in litter weight gain from birth until day 19 of lactation, resulting in an improvement of 7 g/day ADG per piglet in these 19 days (or, say, in weaning weight) could further increase this to $19.55 additional revenue per litter. There is also potential for improvements in post-weaning performance and survival, which could result in improved health status, feed efficiency, a reduction in days to market, etc., which requires further investigation. This largely outweighs the additional costs of the CLA top dress, even if fed at the highest inclusion level used in Experiment 1. There is an additional labour cost with feeding a top dress, but this is minimal as the top dress amount given per day is low.

The two experiments in this project have shown that feeding CLA to primiparous sows in a top dress can improve litter growth in lactation and piglet survival to weaning. This positive outcome can be achieved by feeding a top dress for one week before farrowing only, costing only an additional 30 cents (AUD) per primiparous sow, the benefits of which largely outweigh the costs of the feed additive.

8. Intellectual Property

Published, widely disseminated and promoted, and/or training and extension provided.

9. Technical Summary

We have developed a best practice guideline for producers to feed a CLA top dress product to their gestating and lactating gilts based on the results from these two experiments.

Guidelines for Producers

→ A CLA supplement should be provided as a top dress for primiparous sows in late gestation leading up until farrowing;

→ We suggest including CLA at a rate of 0.2% CLA isomers – e.g. if your CLA additive is 50% CLA isomers, and you include in your top dress diet at a rate of 6%, then sows would receive a top dress of 83 g on top of a daily ration of 2.5 kg (producers can use the Top Dress Calculator from Figure 1);

→ We suggest feeding CLA top dress for the last week before farrowing (at entry into the farrowing house) to maximise performance of primiparous sows and their progeny in lactation;

→ Take care to store the top dress product properly and make in small batches so that fats do not oxidise and become rancid – this can be avoided by using a protected CLA product, such as Lodestar CLA50 from Berg+Schmidt (Germany);

→ It is not recommended to feed CLA in excess of 0.2-0.8% of the daily ration, or for longer than 1 week prior to farrowing.

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11. Publications Arising

None to date.

Results from these experiments are currently being prepared in manuscripts for submission to Livestock Science (previously Animal), with the option to publish 1 or 2 one-page papers for APSA in 2021, depending on timeline of publication.

Working titles for these manuscripts are:

1) Craig JR, Cadogan DJ, Brewster CJ, Henman DJ, Walker J, Wilkinson A, Smits RJ, and Dunshea FR (unpublished) *Determining the optimal inclusion rate of conjugated linoleic acid (CLA) in the lactation diet of primiparous sows to enhance the pre-weaning performance and survival of gilt progeny.*

2) Craig JR, Cadogan DJ, Brewster CJ, Gardiner N, Hollier JC, and Dunshea FR authors (unpublished) *Determining the optimal timing of supplementation of conjugated linoleic acid (CLA) in primiparous sow diets to enhance gilt progeny performance in lactation.*

We will seek approval from APL for disclosure before submitting these manuscripts.