2C-125: *Lauric acid, a potentially new feed additive for the Australian pork industry*

Report prepared for the Co-operative Research Centre for High Integrity Australian Pork

By

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Executive Summary

The ability to manipulate key production variables and disease expression, e.g., ileitis caused by *Lawsonia intracellularis*, by dietary means whilst simultaneously improving gastrointestinal health outcomes is a desirable outcome for the Australian pork industry. Control of feed intake at certain times of the year, e.g., during autumn, could be of benefit to (some) producers to control carcass composition. Nutrient specifications of diets are manipulated with season to take into account the changes in feed intake but autumn is still a real issue for high P2 backfat levels, hence in this regard dietary manipulation to cap intakes is an area of interest. Previous research by Pluske *et al*. (2015) showed that feeding lauric acid (LA; dodecanoic acid; $C_{12}H_{24}O_2$) at 25 g/kg or 50 g/kg (2.5 or 5%) to late-finishing pigs decreased voluntary feed intake by 10.1% and 9.0%, respectively, and improved FCR by 4.9% and 1.7%, respectively, relative to a control diet. Pluske (2016) conducted further commercial-scale studies and noted a reduction in feed intake in late-finishing pigs only fed 25 g/kg LA, without any change in daily gain. During this study it was noted that some pigs showed ill-thrift and (or) were scouring. Subsequently, it was recognised that none of the pigs fed the LA diets showed ill-thrift and (or) were scouring, suggesting that feeding LA had bactericidal/bacteriostatic effects. Moreover, monolaurin (MLA; $C_{15}H_{30}O_4$), the monoester (monoglyceride) formed from LA following lipase action, is purportedly more biologically active than LA in killing viruses and bacteria.

Collectively, the authors are unaware of any other studies being conducted examining the potential for LA or MLA in diets for late-finishing pigs to enhance production and possess likely modulation of some bacterial populations. Therefore, the overall aim of the studies described in this report was to examine the effects of feeding increasing levels of lauric acid (LA; added at 0, 5, 10, 15, 20, 25 g/kg; Experiment 1) or monolaurin (MLA; the monoglyceride formed from LA; MLA added at 0, 5, 10 or 20 g/kg; Experiment II), relative to LA added at 20 g/kg, on aspects of production, meat quality and bacteriological indices (specifically Gram-positive bacteria) in late finishing pigs. Experiment III additionally examined whether LA (20 g/kg inclusion rate) can have positive health outcomes on finishing pigs infected with *Lawsonia intracellularis* versus a diet without added LA.

The **main outcomes** of this project can be summarized as follows:

- Experiment I:
    - Pigs fed diet LA 20 grew faster than pigs fed LA 25 and the Control pigs (p<0.05) in the first 7 days after experimental diets commenced, but there were no other differences in ADG in that period or at any other time interval in the experiment. Pigs fed diets LA 5, LA 10, LA 15 and LA 20 ate more feed (p<0.05) during days 14-21 and in the overall period, days 7-28, than pigs fed LA 25 or the Control diet. Pigs fed diets LA 15 and LA 20 were more efficient (p<0.05) at converting feed to bodyweight gain during days 7-14 of the experiment.
    - Modelling the LA inclusion rate (second order polynomial) based on the significant production data demonstrated (a) for ADG d 7-14, maximum ADG occurred at an inclusion rate of LA of 11.8 g/kg (~1.2%), and (b) for ADFI d 7-28, maximum ADFI occurred at an inclusion rate of LA of 13.4 g/kg (~1.3%).

- Experiment II:
    - There were no treatment x sex interactions for any measurements in the study, although males were heavier throughout the experiment (p<0.001) and showed a tendency to grow faster than females (1.12 vs 1.08 kg/pig/day, p=0.08). Male pigs ate more feed than female pigs throughout the treatment period (2.85 vs 2.55 kg/pig/day, p<0.001), and overall, males had a higher FCR than females throughout the treatment period (2.65 vs 2.36, p<0.001).
    - At day 26, after 14 days of feeding, there was a significant Linear effect of feeding MLA (p=0.035) on final BW. However, there was no difference (contrast; p>0.05) in BW between feeding LA or the average of the MLA treatments.
    - In the overall feeding period from d 12-26, pigs fed diets MLA10 and MLA20 grew faster than pigs fed MLA5 (p<0.05), but these were not statistically different to pigs fed the Control diet or LA. There were no differences (p>0.05) in ADFI between treatments during the experimental period. However, for FCR d 12-19 (i.e., the first week of feeding the experimental diets), pigs fed MLA5 converted feed less efficiently (p<0.05) than pigs fed all other diets, and for the overall period d 12-26, pigs fed diets LA and MLA5 converted feed less efficiently (p<0.05) than pigs fed all other diets.
    - Modelling the MLA inclusion rate (second order polynomial) based on the significant production data demonstrated that for (a) ADG d 12-26, maximum ADG (1.12 kg/day) occurred at an inclusion rate of MLA of 20 g/kg (2%), and (b) FCR d 12-26, minimum FCR occurred also at an inclusion rate of 20 g/kg (2.4).

- Experiment III:
    - Feed supplemented with 20 g/kg (2%) LA did not significantly reduce the severity of ileitis in finisher pigs (measured as numbers of *Lawsonia intracellularis* shed in faeces, serum antibody concentrations, diarrhoea severity and production losses). Nevertheless, there were strong trends for Control pigs to shed higher *Lawsonia intracellularis* numbers than LA-fed pigs at days 7 (p=0.063) and 14 (p=0.064) post-infection, and there was a significant interaction (p=0.018) between treatment and time with higher *Lawsonia intracellularis* numbers excreted by Control pigs at day 7 post-infection.
    - Feeding LA significantly reduced the variation in feed intake between pigs early in infection and also the feed:gain ratio in mid infection.

Both Experiments I and II showed no deleterious effects of LA or MLA on aspects of meat quality and carcass characteristics, nor were any statistical differences detected in the proportion of Gram-positive bacteria or selected families between treatments. However, there was a clear linear reduction in the proportion of the family *Streptococcaceae* in the faeces of pigs fed different levels of LA in the late-finishing period in Experiment I.

Collectively, these data suggest that there is potential for the use of lauric acid or monolaurin in diets for late-finishing pigs, both from production and bacterial/health-related perspectives. Optimum cut-off inclusion rates of LA or MLA using statistical modelling showed that ~12-20 g/kg (1.2-2%) of product had some beneficial impacts on some production variables, although the timing and the

magnitude of the responses differed between LA and MLA. Feeding LA modified some specific bacterial populations in the faeces of potential interest, but this requires further exploration.

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1. Introduction

The ability to manipulate key production variables such as feed intake by dietary means whilst simultaneously improving gastrointestinal health outcomes is a desirable outcome for the Australian pork industry. Control of voluntary feed intake at certain times of the year, e.g., during autumn, could be of benefit to (some) producers to control carcass composition. Production performance during the finisher period can vary considerably due to the effects of climatic conditions on feed intake, with the extra feed consumed above requirements being deposited as fat that subsequently can result in significant price penalties. Nutrient specifications of diets are manipulated with season to consider the changes in feed intake but autumn is still a real issue for high P2 backfat levels, hence in this regard dietary manipulation to cap intakes is an area of significant interest.

Evidence presented by Pluske *et al*. (2015) and Pluske (2016) showed that feeding lauric acid (LA; dodecanoic acid; $C_{12}H_{24}O_2$) at levels from 25-50 g/kg to late-finishing pigs decreased voluntary feed intake by 10.1% and 9.0%, respectively. Carcass weight and P2 backfat were not affected. The inclusion of LA also improved FCR by 4.9% and 1.7%, respectively, relative to a control diet. Pluske (2016) conducted follow-up commercial-scale studies and noted a reduction in feed intake in late-finishing pigs only fed 25 g/kg LA, without any change in daily gain. During the course of this study it was noted that some pigs showed ill-thrift and (or) were scouring. Faecal swabs were collected and shown by PCR to be positive for *Lawsonia intracellularis*, with Dr Susan Dawson (Portec Veterinary Services) saying, "I can confidently say that this pig and the others that scoured (or didn't gain weight), were affected with proliferative haemorrhagic enteritis (PHE) (form of ileitis in older pigs)". None of the pigs fed the LA diets showed ill-thrift and (or) were scouring, suggesting that feeding LA had bactericidal/bacteriostatic effects that in turn, might have improved FCR (even though voluntary feed intake was reduced 9-10%). A preliminary cost-benefit analysis on the production data alone showed that, relative to the control, feeding 25 g/kg of LA was still more expensive even with the significant reduction in feed intake, therefore lower inclusion levels of LA warrant further investigation. Indeed, the lack of any ill-thrift or scouring in pigs only fed LA, indicative of reduced *Lawsonia intracellularis* colonization/proliferation in the GI tract, suggests that LA might have direct bactericidal/bacteriostatic properties and (or) modulate the bacterial composition of the GI tract.

Lauric acid most likely exerts its effects on voluntary feed intake through reduced motility (peristalsis) thereby increasing transit time through the GI tract, and (or) through increased production and (or) secretion of hormones (e.g., CCK, PYY) known to have counter-effects against feed intake (Little *et al.*, 2005; Black *et al.*, 2009). In regard to antiviral and antibacterial activity, and comparatively speaking, LA has a greater antiviral and antibacterial activity than other medium-chain triglycerides such as caprylic acid (C8), capric acid (C10), or myristic acid (C14) (Lieberman *et al.*, 2006), which may (in part) explain the results seen by Pluske *et al*. (2015) for Lawsonia. Nevertheless, monolaurin ($C_{15}H_{30}O_4$), the monoester (monoglyceride) formed from LA following lipase action, is more biologically active than LA in killing viruses and bacteria, and research has suggested that monolaurin exerts viricidal and bactericidal effects by solubilizing the lipids and phospholipids in the envelope of the pathogen causing the disintegration of its envelope (Lieberman *et al.*, 2006). Due to the chemical characteristics of alpha-monolaurin, the molecule is pH independent and will not dissociate in the intestinal tract (pH around 6-6.5).

Moreover, alpha-monolaurin can be absorbed into the lymphatic system and bloodstream. We were only able to find one report regarding the use of monolaurin in pigs; Black *et al*. (2015) fed 20 g/kg monolaurin to weaner pigs and noticed a reduction in FCR relative to a diet containing an antibiotic, but this was not statistically significant. The authors are unaware of any studies being conducted examining the potential for monolaurin in diets for finishing pigs to enhance production and possess likely pathogen control/"gut health" properties.

In general terms, LA and (or) monolaurin are more effective against Gram-positive bacteria than Gram-negative bacteria. This is because Gram-positive bacteria contain a thick mesh cell wall lattice made of sugars and amino acids, with the potentially problematic bacteria in this category being the families of *Streptococcus*, *Staphylococcus*, *Corynebacterium*, *Listeria*, *Bacillus*, and *Clostridium* (Lieberman *et al*., 2006). *Streptococcus suis* is an important cause of a wide variety of infections in pigs, including meningitis, pneumonia, septicaemia and arthritis, with *S. suis* serotype 2 the most prevalent type isolated from diseased pigs (Su *et al*., 2008). In the young pig, *S. suis* predominates in the stomach samples of weaned piglets with high population levels and is also dominant in the jejunum and ileum digesta after weaning, demonstrating the post-weaning dominance of this pathogen (Su *et al*., 2008). *Streptococcus suis* is also a zoonotic agent causing severe infections to people in close contact with infected pigs or pork-derived products. Studies suggest that the gastrointestinal tract may be one of the main niches for the pathogenic *S. suis* in pigs, and that its excretion with the faeces may provide an important source for human infection. Furthermore, antibiotic resistance is now firmly a "front and centre" issue for the pig industry and pig-derived MRSA (methicillin-resistant *Staphylococcus aureus*) infections of humans has been documented (e.g., Lewis *et al*., 2008). Intervention strategies to reduce the shedding and load of gram-positive bacteria such as MR *S. aureus* with LA or monolaurin deserves further attention.

Ileitis is an economically important disease causing reduced growth, poor feed efficiency, diarrhoea and increased medication or vaccination costs. Lauric acid is known to possess bactericidal activity against Gram-positive bacteria by solubilizing the lipids in the pathogen cell membrane and disintegrating it. However, a serendipitous finding during a LA trial in finisher pigs (Pluske *et al*., 2015) suggested that LA might also be effective in reducing clinical signs of ileitis, as well as decreasing voluntary feed intake in late finishing pigs. An experimental challenge trial was therefore undertaken ad the final experiment in this project to investigate the potential antibacterial properties of LA against *Lawsonia intracellularis*.

2. Methodology

Three experiments were conducted in this project. The overall aim of these studies was to examine the effects of feeding increasing levels of lauric acid (LA; Experiment 1) or monolaurin (MLA, the monoglyceride formed from LA; Experiment II), relative to LA, on aspects of production (especially feed intake), meat quality and faecal bacteriological indices in late finishing pigs. Furthermore, Experiment III examined whether LA could have positive health outcomes on finishing pigs infected with *Lawsonia intracellularis*.

Experiment I

Effects of increasing levels of added lauric acid on production indices, meat quality and carcass characteristics, MRSA levels and selected bacterial populations in late-finishing Improvac-treated pigs

A randomized complete block design experiment comprising six dietary treatments (0, 5, 10, 15, 20 and 25 g/kg LA), with pens as the experimental unit, was conducted using late-finishing male (Improvac treated) pigs sourced from a commercial supplier. Pigs were given their second Improvac injection approximately 3 weeks before slaughter. A total of 430 pigs (~ 68 kg at entry) sourced in two batches was used with 10 replicate pens per treatment and 7 pigs/pen (10 spares). Upon arrival, pigs were weighed and allocated to treatment according to location in the building and bodyweight (BW), to achieve a similar commencing BW and standard deviation in each treatment. Pigs were acclimatized (control diet) for 7 days and then fed the treatment diets *ad libitum* for 28 days (≈ 113 kg at exit). Unfortunately, in batch 2, the LA diets ran out before the end of the 4-week feeding period and measurements had to be concluded one week earlier, when pigs weighed 103.8 kg LW. Data for batch 2 have therefore been removed from the analysis for the final week. The performance of pigs [average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ration (FCR)] was recorded. At about 110 kg and 4 weeks after the start of feeding, pigs were transported to Linley Valley Pork (Wooroloo, WA) for slaughter according to normal commercial practices. Diets were formulated [Dr Jae Kim (DAFWA) and Christine Sydenham (Weston Milling Animal Nutrition)] (Appendix 1) and manufactured as pellets by Wesfeeds (Bentley, WA) (Appendix I).

On day 0, 3 pigs/pen were selected for post-slaughter meat sampling (for objective pork quality measurement). Faecal and nasal swab samples (2 pigs/pen) were taken at the same time points for PCR identification of Lawsonia and the presence or absence of MRSA (via nasal swabs). The MRSA analysis (conducted under the direction of Dr Sam Abraham, Murdoch University) was achieved using a CultureSwab™ sterile pouch (opened from the package) with the cap twisted to remove it from the transport tube, and the swab then gently inserted 3-4 cm into the naris. The swab was gently rotated against the anterior nasal mucosa for 3 seconds and then using the same swab, was repeated for the other naris. The swab was then placed back into the transport tube for subsequent processing at Murdoch University using established methods (Abraham, *personal communication*).

Faeces were collected (pooled sample per pen) following 2 weeks of feeding the experimental diets and frozen for subsequent next generation sequencing (NGS) analysis of bacteria. Briefly, DNA was extracted using a Magmax multi-sample DNA extraction kit Isolation kit (Life Technologies) according to the manufacturer's instructions. The 16S RNA amplification was performed using the primers 16Sf

(TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and 16sr (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC), targeting the V3-V4 hypervariable region and containing Illumina adapter overhangs. Library preparation was performed using Nextera XT reagents according to the manufacturer's protocols and sequenced on an Illumina Miseq platform using a MiSeq V3 2 x 300 flowcell. De-multiplexed paired-end reads were imported into the Quantitative Insights into Microbial Ecology platform (QIIME 2) using the fastq manifest protocol. Read trimming, primer removal, denoising, read merging and amplicon sequence variant (ASV) clustering were performed using the DADA2 plugin pipeline. Taxonomy was assigned using the QIIME 2 feature classifier with the Greengenes v13.8 99% OTU classification.

Twenty-four hours after slaughter the colour [Surface lightness (L*), redness (a*), and yellowness (b*)] of the M. *obliquus internus abdominus* (belly muscle used to visually evaluate pork colour in the Singaporean market) was measured using a Minolta Chromameter CR-400 (Minolta, Osaka, Japan). Samples, ~500 g section of the *Longissimus thoracis et lumborum* muscle, were removed from the left-hand side of carcasses from selected pigs. For determination of pH and colour a 2-cm thick steak was cut from the sample and after a 30-minute bloom time colour (L*, a* and b*) was measured with a Minolta Chromameter CR-400 using D65 illumination, a 2° standard observer, and an 8-mm aperture in the measuring head, standardised to a white tile. The muscle pH was measured using a portable pH/temperature meter (Cyberscan pH 300, Eutech Instruments, Singapore) fitted with a polypropylene spear-type gel electrode (Ionode IJ44, Ionode Pty Ltd, Brisbane, QLD) and a temperature probe. Standard carcass characteristics assessed were P2 (65 mm from the midline), hot carcass weight, chilled carcass weight (after 24 h) and chiller loss (%).

Crude fat (% DM) and fatty-acid methyl esters' analyses (proportions and concentrations) were conducted at the NSW Government Department of Primary Industries (Wagga Wagga Agricultural Institute, NSW) by the hexane Soxtec extraction method (PS-042) and gas chromatography (LMOP 2-1122), respectively. Other analyses were conducted at INVIVO Labs Vietnam using AOAC or AAS08 approved methods.

Statistical analyses

Linear mixed-model methodology was used to fit a statistical model to each variable. All models were fitted using the statistical software package *asreml* (Butler, 2009) within the R (R Core Team 2018) computing environment. The initial statistical model for traits measured in the interval day 7 to day 28 was used as follows (after Wilkinson and Rogers, 1973),

trait ~ 1 + Trt + **Batch** + **Batch.Block** + **units,**

where 1 is the overall mean, terms in bold font are considered random terms and associated with each of these terms is a variance parameter, and Trt is treatment. The term **units** is associated with the residual variance and is not explicitly fitted in the call to *asreml*. The preferred method for estimating variance parameters is residual (or restricted) maximum likelihood (REML) (Patterson and Thompson, 1971).

For performance traits measured in the interval day 28 to day 35, the initial statistical model is written as,

trait ~ 1 + Trt + **Block** + **units**.

For meat quality traits, the initial statistical model was written as,

trait ~ 1 + Trt + **Block** + **Pen** + **units**.

The initial statistical model for both performance and meat quality traits showed a significant (p<0.05) effect of bodyweight (BW; 'BW at day 7') and ADG after the 7-day adaptation period ('Adaptation period'), hence these terms were only included in the model used for the prediction of diet treatment means (and standard errors and least significant differences) when statistically significant at p<0.05. Technical details associated with prediction in linear mixed models can be found in Welham *et al*. (2004). Where significant, the initial statistical model for a trait was modelled as a second or third order polynomial to establish the optimum dose of LA. Statistical significance was accepted at p<0.05.

Experiment II

Effects of increasing levels of added monolaurin and lauric acid on production indices, meat quality and carcass characteristics, MRSA levels and selected bacterial populations in late-finishing female and Improvac-treated pigs

A randomized complete block design experiment comprising five dietary treatments), with pens as the experimental unit, was conducted using late-finishing female pigs and male (Improvac treated) pigs sourced from a commercial supplier. The experimental design consisted of the optimum level of LA determined in Experiment 1 (20 g/kg), and was compared against a Control diet and three levels of monolaurin (5 g/kg, 10 g/kg, 20 g/kg; described as MLA 5, MLA10 and MLA20, respectively). A total of 360 pigs (≈ 58 kg at entry) (including 12 spares) sourced in two batches was used with 12 replicate pens per treatment (6 pens for female pigs and 6 pens for Improvac male pigs; pigs were given their second Improvac injection approximately 3 weeks before slaughter) and 6 pigs/pen. Upon arrival, pigs were weighed and allocated to treatment according to location in the building and bodyweight (BW), to achieve a similar commencing BW and standard deviation in each treatment. Pigs were acclimatized (Control diet) for 12 days and then fed the treatment diets *ad libitum* for 14 days (≈ 105-110 kg at exit). The performance of pigs [average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ration (FCR)] was recorded. At about 110 kg and 14 days after the start of feeding, pigs were transported to Linley Valley Pork (Wooroloo, WA) for slaughter according to normal commercial practices.

This differed to the original experimental design, i.e., feeding for 3 weeks, because an insufficient quantity of glycerol monolaurate (monolaurin; MLA) was sent (despite it being ordered) resulting in there only being enough to manufacture enough feed to offer to pigs for the 2 weeks. The MLA was also sent in solid (i.e., solid fat) form rather than powdered form, which was originally anticipated, meaning that it needed to be melted down (i.e., like tallow) before it could be incorporated onto the pellets. This could not be done at the commercial feed mill and had to be done at Medina Research Station; essentially, the liquefied fat was coated onto the pellets in a vertical mixer. Diets were formulated [Dr Jae Kim (DAFWA) and Christine Sydenham (Weston Milling Animal Nutrition)] (Appendix 1) and manufactured as pellets by Wesfeeds (Bentley, WA) (Appendix II).

On day 0, 3 pigs/pen were selected for post-slaughter meat sampling (for objective pork quality measurement). Faecal and nasal swab samples (2 pigs/pen) were taken at the same time points for PCR identification of Lawsonia and the presence or absence of MRSA (via nasal swabs). The MRSA analysis (conducted under the direction of Dr Sam Abraham, Murdoch University) was achieved using a CultureSwab™ sterile pouch (opened from the package) with the cap twisted to remove it from the transport tube, and the swab then gently inserted 3-4 cm into the naris. The swab was gently rotated against the anterior nasal mucosa for 3 seconds and then using the same swab, was repeated for the other naris. The swab was then placed back into the transport tube for subsequent processing at Murdoch University using established methods (Abraham, *personal communication*).

Faeces were collected (pooled sample per pen) following 2 weeks of feeding the experimental diets and frozen for subsequent next generation sequencing (NGS) analysis of bacteria. Briefly, DNA was extracted using a Magmax multi-sample DNA extraction kit Isolation kit (Life Technologies) according to the manufacturer's instructions. The 16S RNA amplification was performed using the primers 16Sf (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and 16sr (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC), targeting the V3-V4 hypervariable region and containing Illumina adapter overhangs. Library preparation was performed using Nextera XT reagents according to the manufacturer's protocols and sequenced on an Illumina Miseq platform using a MiSeq V3 2 x 300 flowcell. De-multiplexed paired-end reads were imported into the Quantitative Insights into Microbial Ecology platform (QIIME 2) using the fastq manifest protocol. Read trimming, primer removal, denoising, read merging and amplicon sequence variant (ASV) clustering were performed using the DADA2 plugin pipeline. Taxonomy was assigned using the QIIME 2 feature classifier with the Greengenes v13.8 99% OTU classification.

Twenty-four hours after slaughter the colour [Surface lightness (L*), redness (a*), and yellowness (b*)] of the M. *obliquus internus abdominus* (belly muscle used to visually evaluate pork colour in the Singaporean market) was measured using a Minolta Chromameter CR-400 (Minolta, Osaka, Japan). Samples, ~500 g section of the *Longissimus thoracis et lumborum* muscle, were removed from the left-hand side of carcasses from selected pigs. For determination of pH and colour a 2-cm thick steak was cut from the sample and after a 30-minute bloom time colour (L*, a* and b*) was measured with a Minolta Chromameter CR-400 using D65 illumination, a 2° standard observer, and an 8-mm aperture in the measuring head, standardised to a white tile. The muscle pH was measured using a portable pH/temperature meter (Cyberscan pH 300, Eutech Instruments, Singapore) fitted with a polypropylene spear-type gel electrode (Ionode IJ44, Ionode Pty Ltd, Brisbane, QLD) and a temperature probe. Standard carcass characteristics assessed were P2 (65 mm from the midline), hot carcass weight, chilled carcass weight (after 24 h) and chiller loss (%).

Diet analyses

Analysis of the diets used in Experiments I and II was conducted at INVIVO Labs (Vietnam) or Agrifood Technology (Bibra Lake, Western Australia) using AOAC or AAS08 approved methods (Appendices III and IV, respectively). Crude fat (% DM) and fatty-acid methyl esters' analyses (proportions and concentrations) were conducted at the NSW Government Department of Primary Industries (Wagga Wagga Agricultural Institute, NSW) by the hexane Soxtec extraction method (PS-042) and

gas chromatography (LMOP 2-1122), respectively (Appendices V and VI, respectively).

Statistical analyses
Linear mixed-model methodology was used to fit a statistical model to each variable. All models were fitted using the statistical software package *asreml* (Butler, 2009) within the R (R Core Team 2018) computing environment. The initial statistical model for traits measured in the interval day 12 to day 26 was used as follows (after Wilkinson and Rogers, 1973),

Batch \ Block \ Pen, i.e., pens within replicate blocks within batches.

The blocking structure for meat quality traits (with the exception of CCW and Chill Loss Percentage) was used as follows:

Batch \ Block \ Pen \ ID, i.e., animals within pens within replicate blocks within batches.

The initial statistical model for traits measured in the interval day 12 to day 26 was written as follows (after Wilkinson and Rogers, 1973):

trait 1 + Trt + **Batch** + **Batch.Block** + **units**,

where 1 is the overall mean. Terms in bold font are considered random terms and associated with each of these terms is a variance parameter. The term units is associated with the residual variance and is not explicitly fitted in the call to asreml. The preferred method for estimating variance parameters is residual (or restricted) maximum likelihood (REML) (Patterson and Thompson, 1971).

For meat quality traits (with the exception of CCW and Chill Loss Percentage) an initial statistical model was written as:

trait 1 + Trt + Batch + Batch.Block + Pen + units,

where pens are individually numbered and not numbered as pens within blocks. For the meat quality traits CCW and Chill Loss Percentage the random effects are reduced to Block + Pen + units and Trt is a factor with 2 levels; M0 and LA.

The initial statistical model for both performance and meat quality traits showed a significant (p<0.05) effect of BW at day 12 ('BW at day 12'), hence this term was considered as a covariate where appropriate (after Urquhart, 1982). Bodyweight at day 12 was only included in the model used for the prediction of diet treatment means (and standard errors and least significant differences) when statistically significant at p<0.05. Technical details associated with prediction in linear mixed models can be found in Welham *et al*. (2004). Where significant, the initial statistical model for a trait was modelled as a second or third order polynomial to establish the optimum dose of LA. Statistical significance was accepted at p<0.05.

Experiment III

Can lauric acid reduce* Lawsonia intracellularis *colonization and shedding in experimentally-infected pigs?

A total of 24 male weaner pigs were selected from a high-health-status farm and maintained on medicated feed from 4 to 16 weeks of age to ensure they remained naïve to *L. intracellularis*. Blood and faecal samples collected during this period demonstrated freedom from *L. intracellularis* infection. Three pigs were sent to abattoir prior to the LA treatment commencing; two due to lameness, and one with a rectal prolapse.

At 16 weeks of age, the remaining finisher pigs were allocated to two treatment groups of (a) 11 pigs treated with LA (included at 20 g/kg) and (b) 10 pigs without dietary LA (Control). Diets fed to pigs were the same as per Experiment I, with the exception of a slight reduction in the LA content. There was no significant weight difference between these groups. All medication was removed from diets, and pigs were fed LA or Control diets from 4 days prior to oral challenge with 10^9 *L. intracellularis* to 21 days post-challenge. Individual faecal samples were collected at day 0, 7, 11, 14, 18 and 21 days post-challenge and the number of *L. intracellularis* per gram of faeces was enumerated by the Lawsonia qPCR (Collins and Barchia, 2014). Lawsonia numbers were $\log_{10}$ transformed to ensure normal distribution and differences between treatments were compared by repeated measures analysis and Student's *t*-test.

Faecal consistency scores were recorded daily and summed over each 7-day period and compared using an unbalanced ANOVA. Pigs were weighed weekly and feed intake was recorded over 3 days per week for one week pre-challenge and each week post-challenge. Differences in production measures were analysed by an unbalanced ANOVA with starting weight at 16 weeks as a covariate. Serum collected at 21 days post-challenge was tested for antibodies to Lawsonia using the Svanova Ileitis blocking ELISA and concentration expressed as percent inhibition. Bartlett's test for homogeneity was used to test whether variances in production and disease parameters were significantly different for the two treatment groups.

3. Outcomes

Experiment I

Diet fatty acid methyl ester analysis (Appendix II) showed a linear increase in the C12 (dodecanoic acid) proportion and concentration in accordance with added LA to the diet. Nasal swabs taken from focus pigs before and after feeding of the test diets for the presence of MRSA (methicillin-resistant *Staphylococcus aureus*) showed that none were detected at either time point, signifying the lack of this bacterium in this population of pigs. Similarly, no diarrhoea (ileitis) was observed and hence no samples were collected for *Lawsonia* presence or quantification.

Performance traits

For pig bodyweight (BW), there were significant polynomial effects ($p<0.05$) of feeding LA on BW at days 14, 21, 28 and 35 of the experiment, with the optimum dosage of LA being 11.9 g/kg, 10.8 g/kg, 12.3 g/kg and 13.1 g/kg for an average pig BW of 84.4 kg, 94.4 kg, 104.1 kg and 114.6 kg, respectively.

Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) conditional F-statistics and associated *p*-values for the effects of Treatment, bodyweight (BW) at day 7 (commencement of feeding experimental diets), and the adaptation period (i.e., pen ADG for the period where pigs were fed a common Control diet for the first 7 days after entry), are shown in Table 1.1. Treatment diet means, their standard errors, and the least significant difference (LSD) for performance traits are shown in Table 1.2.

Pigs fed diet LA 20 grew faster than pigs fed LA 25 and the Control pigs ($p<0.05$) in the first 7 days after experimental diets commenced, but there were no other differences in ADG in that period or at any other time interval in the experiment. Pigs fed diets LA 5, LA 10, LA 15 and LA 20 ate more feed ($p<0.05$) during days 14-21 and in the overall period, days 7-28, than pigs fed LA 25 or the Control diet. Pigs fed diets LA 15 and LA 20 were more efficient ($p<0.05$) at converting feed to bodyweight gain during days 7-14 of the experiment (Table 1.2).

Modelling the LA inclusion rate as a second order polynomial based on the significant production data demonstrated that (a) for ADG d 7-14, maximum ADG occurred at an inclusion rate of LA of 11.8 g/kg (~1.2%) and (b) for ADFI d 7-28, maximum ADFI occurred at an inclusion rate of LA of 13.4 g/kg (~1.3%) (Figures 1.1 and 1.2, respectively). Based on the second order polynomial statistical model, pigs fed diets LA 10 and LA 15 grew faster ($p<0.05$) in d 7-14 than pigs fed the Control diet or diet LA 25, but there was no difference between pigs fed LA 5, LA 10, LA 15 and LA 20 in ADG. For ADFI and based on the second order polynomial statistical model, pigs fed diets LA 5, LA 10, LA 15 and LA 20 all ate more feed ($p<0.05$) between d 7-28 than Control-fed pigs, with pigs fed the Control diet and LA 25 ate a similar amount.

Table 1.1 F-statistics (F. con) and associated *p* values for average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR), for the effects of Treatment, bodyweight (BW) at day 7 (as a covariate; commencement of feeding experimental diets), and the adaptation period (as a covariate; pigs were fed common Control diet for the first 7 days after entry), in Experiment I.

	Treatment		BW at day 7		Adaptation period	
Trait	F. con	p value	F. con	p value	F. con	p value
ADG						
d 7-14	2.97	0.022	12.36	<0.001	5.39	0.024
d 14-21	0.54	0.749	1.47	0.231	0.56	0.459
d 21-28	1.24	0.306	1.94	0.170	0.37	0.548
d 7-28	1.54	0.197	0.87	0.355	0.07	0.800
d 7-35A	1.59	0.212	2.86	0.104	0.32	0.578
ADFI						
d 7-14	1.54	0.195	35.05	<0.001	5.43	0.024
d 14-21	3.31	0.012	11.74	0.001	1.52	0.223
d 21-28	2.85	0.024	8.00	0.007	0.36	0.550
d 7-28	4.10	0.003	20.39	<0.001	0.51	0.480
d 7-35A	2.13	0.099	6.29	0.020	0.33	0.571
FCR						
d 7-14	3.42	0.010	5.12	0.028	0.81	0.371
d 14-21	1.88	0.116	0.90	0.348	2.20	0.144
d 21-28	2.63	0.035	10.84	0.002	0.12	0.726
d 7-28	1.53	0.197	17.79	<0.001	0.12	0.729
d 7-35A	1.54	0.219	2.54	0.125	0.02	0.892

[A] For Batch 1 only

Table 1.2 Predicted treatment values, standard errors (in parentheses) and the least significant difference (LSD; 5% level) for average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) in Experiment I.

	Treatments						
Item	Control	LA 5	LA 10	LA 15	LA 20	LA 25	LSD (5%)
ADG, kg							
d 7-14	1.13 (0.041)	1.18 (0.041)	1.15 (0.041)	1.20 (0.041)	1.24 (0.041)	1.06 (0.041)	0.106
d 14-21	1.38 (0.086)	1.43 (0.086)	1.43 (0.086)	1.38 (0.086)	1.39 (0.086)	1.32 (0.086)	0.157
d 21-28	1.38 (0.08)	1.47 (0.077)	1.32 (0.077)	1.47 (0.076)	1.25 (0.076)	1.39(0.076)	0.228
d 7-28	1.23 (0.039)	1.36 (0.039)	1.30 (0.039)	1.35 (0.039)	1.30 (0.039)	1.25 (0.039)	0.132
d 7-35A	1.26 (0.039)	1.37 (0.04)	1.35 (0.04)	1.37 (0.039)	1.38 (0.039)	1.28 (0.039)	0.134
ADFI, kg							
d 7-14	2.34 (0.073)	2.43 (0.074)	2.42 (0.074)	2.34 (0.073)	2.30 (0.073)	2.22 (0.073)	0.217
d 14-21	2.58 (0.099)	2.93 (0.092)	2.86 (0.092)	2.95 (0.095)	2.98 (0.091)	2.75 (0.091)	0.227
d 21-28	3.28 (0.104)	3.80 (0.010)	3.52 (0.099)	3.63 (0.098)	3.64 (0.098)	3.51 (0.098)	0.294
d 7-28	2.66 (0.075)	3.05 (0.063)	2.93 (0.063)	3.00 (0.066)	2.97 (0.063)	2.83 (0.063)	0.188
d 7-35A	2.97 (0.104)	3.34 (0.105)	3.33 (0.105)	3.24 (0.104)	3.40 (0.104)	3.24 (0.105)	0.480
FCR, kg:kg							
d 7-14	2.09 (0.085)	2.08 (0.085)	2.10 (0.086)	1.96 (0.085)	1.87 (0.085)	2.10 (0.085)	0.149
d 14-21	1.87 (0.100)	2.07 (0.092)	2.03 (0.092)	2.16 (0.096)	2.17 (0.092)	2.12 (0.092)	0.306
d 21-28	2.45 (0.164)	2.66 (0.158)	2.67 (0.158)	2.48 (0.157)	2.65 (0.156)	2.56 (0.157)	0.406
d 7-28	2.14 (0.054)	2.25 (0.046)	2.25 (0.046)	2.20 (0.048)	2.31 (0.045)	2.25 (0.045)	0.175
d 7-35A	2.35 (0.050)	2.44 (0.051)	2.46 (0.051)	2.38 (0.050)	2.48 (0.051)	2.52 (0.051)	0.179

[A] For Batch 1 only

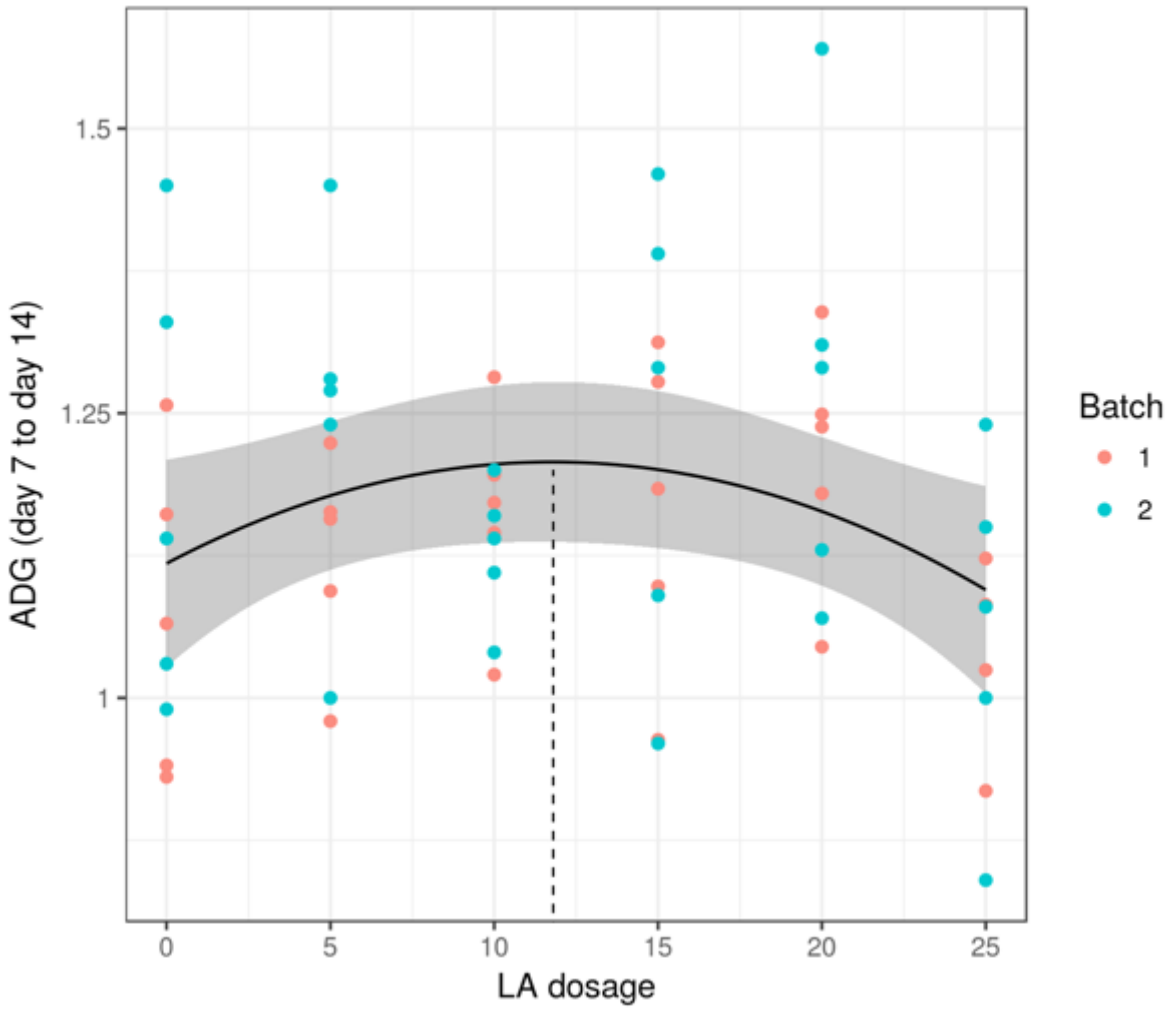

Figure 1.1 The fitted second-order polynomial for ADG d 7-14 for all pigs used in the experiment, with an approximate 95% confidence band. Maximum ADG is estimated to occur at a LA dosage of **11.8 g/kg**. Fitted equation (with standard errors) for the first (dose) and second (dose^2) order polynomial terms is 1.12 (0.046) + 0.015 (0.0076) – 0.0006 (0.0003) (p<0.05).

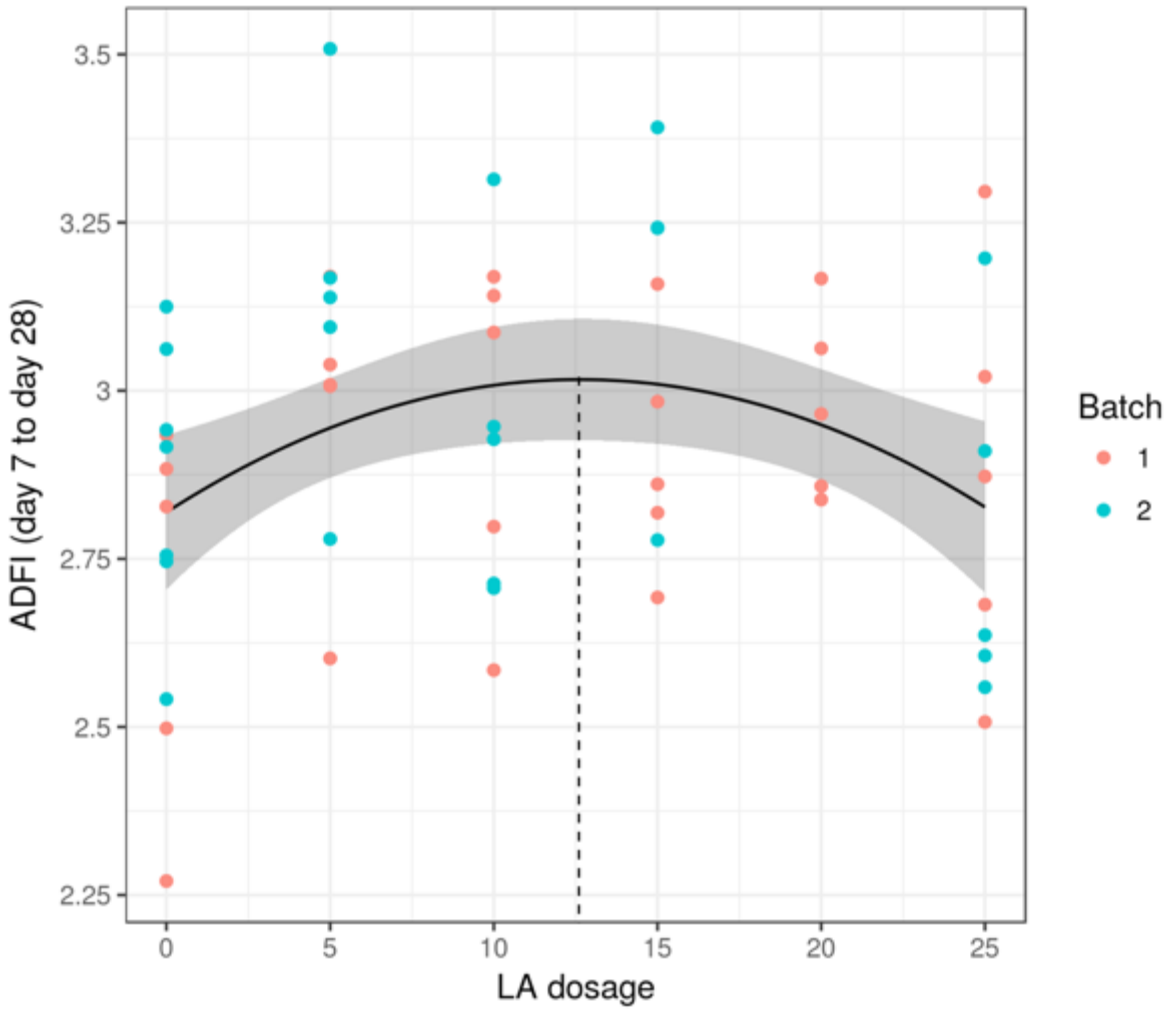

Figure 1.2 The fitted second-order polynomial for ADFI d 7-28 for all pigs used in the experiment, with an approximate 95% confidence band. Maximum ADG is estimated to occur at a LA dosage of **13.4 g/kg**. Fitted equation (with standard errors) for the first (dose) and second (dose^2) order polynomial terms is 2.75 (0.069) + 0.042 (0.0123) – 0.0016 (0.0004) (p<0.05).

Meat quality traits

Meat quality traits assessed on pigs demonstrated that there were no statistically significant effects for any meat quality traits, at either the 5% or 10% level (Table 1.3). Data for carcass characteristics and meat quality traits are presented in Table 1.4.

Table 1.3 F-statistics and associated *p* values for the effects of Treatment, bodyweight (BW) at day 7 (commencement of feeding experimental diets), and the adaptation period (pigs were fed common Control diet for the first 7 days after entry), on meat quality traits. Meat quality data could only be measured on pigs in the first batch.

P2, mm	1.69	0.182	0.17	0.684	4.93	**0.037**
HCW, kg	1.22	0.336	117.6	**<0.001**	0.12	0.729
CCW, kg	1.25	0.322	113	**<0.001**	0.03	0.865
Chill loss %	0.38	0.858	0.07	0.793	0.90	0.353
pH	1.01	0.434	0.00	0.971	1.30	0.266
Colour	0.09	0.994	0.39	0.540	0.97	0.336
L*	1.85	0.147	2.92	0.102	0.20	0.661
a*	0.33	0.897	0.01	0.922	1.88	0.172
b*	0.12	0.985	0.63	0.436	0.04	0.849

P2: backfat measured at P2 position; HCW: hot carcass weight; CCW: chilled carcass weight; Chill loss%: loss of carcass weight measured after entry to chiller; pH: pH assessed 24 h after slaughter; Colour: surface lightness, L*; redness, a*; yellowness, b*.

Table 1.4 Carcass characteristics and meat quality data from Experiment 1. No traits were significantly different at $p<0.05$.

P2, mm	12.0	13.2	13.8	13.5	13.2	12.7	1.96
HCW, kg	75.1	76.1	75.6	76.1	75.7	74.09	2.67
CCW, kg	72.9	74.2	73.6	74.1	73.8	72.2	2.70
Chill loss %	2.76	2.68	2.54	2.71	2.65	2.51	0.573
pH	5.47	5.42	5.47	5.44	5.46	5.46	0.073
Colour							
L*							
a*	14.0	14.2	14.2	14.2	13.8	14.0	1.12
b*	8.0	8.2	8.0	8.0	8.0	7.9	1.08

Bacterial populations

There were no statistical differences (data analysed using Stata SE v.13 binomial logistic regression functions) in the proportion of Gram-positive, Gram-negative, or the ratio of Gram-positive:Gram-negative organisms, in the faeces of pigs sampled in the late-finishing period (Figures 1.3 to 1.5). Even though not statistically different, there was a clear (linear) reduction in the proportion of the family *Streptococcaceae* in the faeces of pigs fed different levels of LA in the late-finishing period (Figure 1.6). Conversely, there appeared to be a (linear) increase in the proportion of the family *Enterobacteriaceae* in the faeces of pigs fed different levels of LA in the late-finishing period (Figure 1.7).

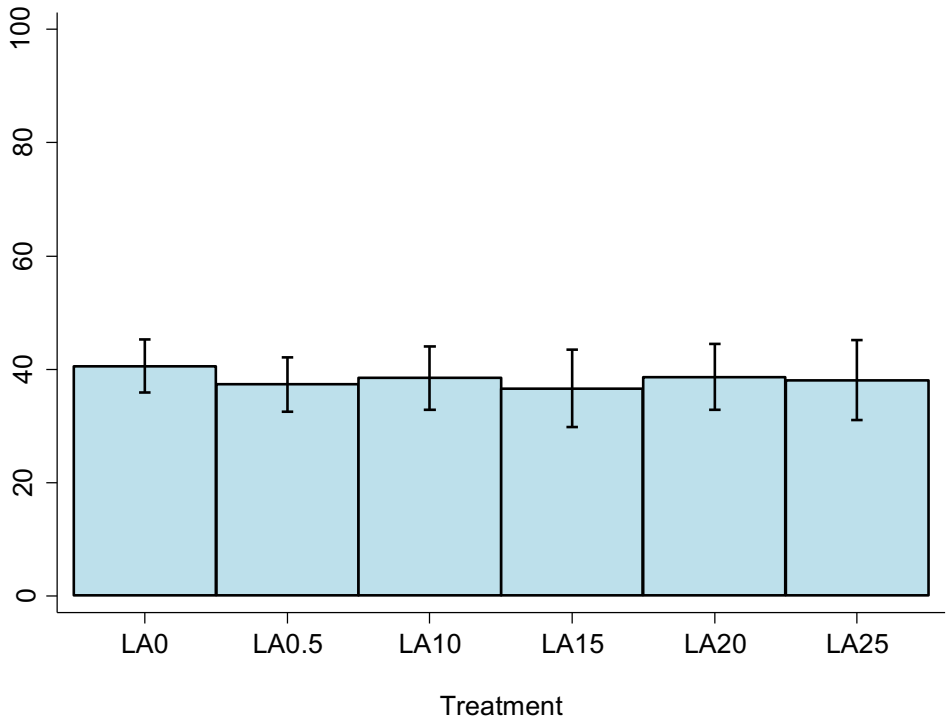

Figure 1.3 The proportion of Gram-positive organisms in the faeces of pigs fed different levels of LA in the late-finishing period.

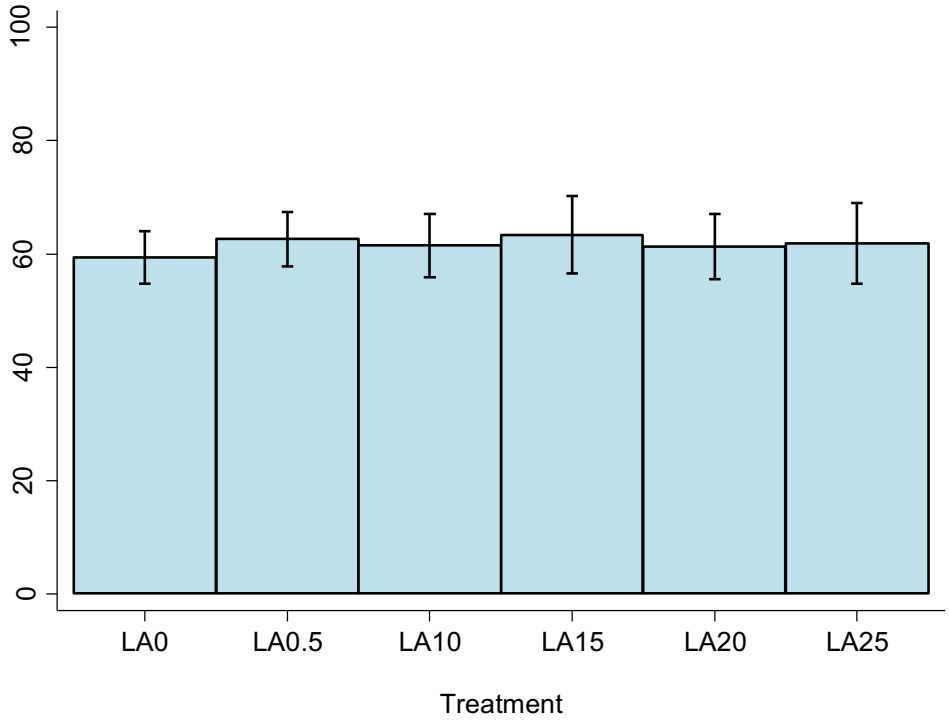

Figure 1.4 The proportion of Gram-negative organisms in the faeces of pigs fed different levels of LA in the late-finishing period.

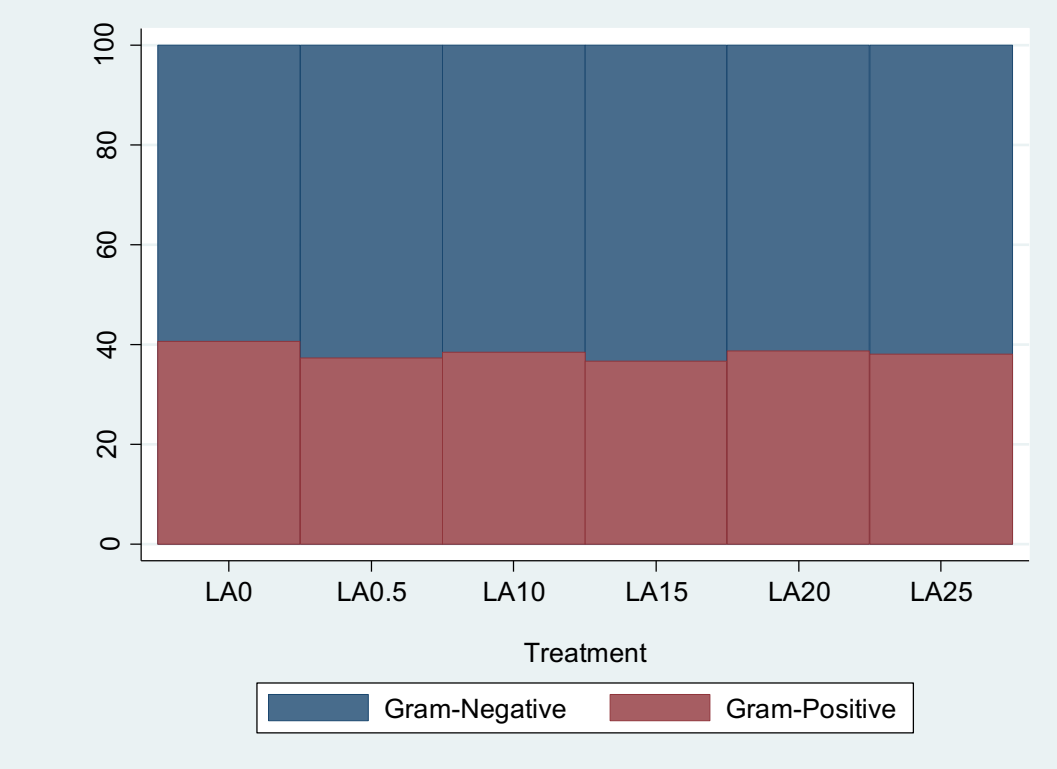

Figure 1.5 The ratio of Gram-negative to Gram-positive organisms in the faeces of pigs fed different levels of LA in the late-finishing period.

Proportion as a % of total

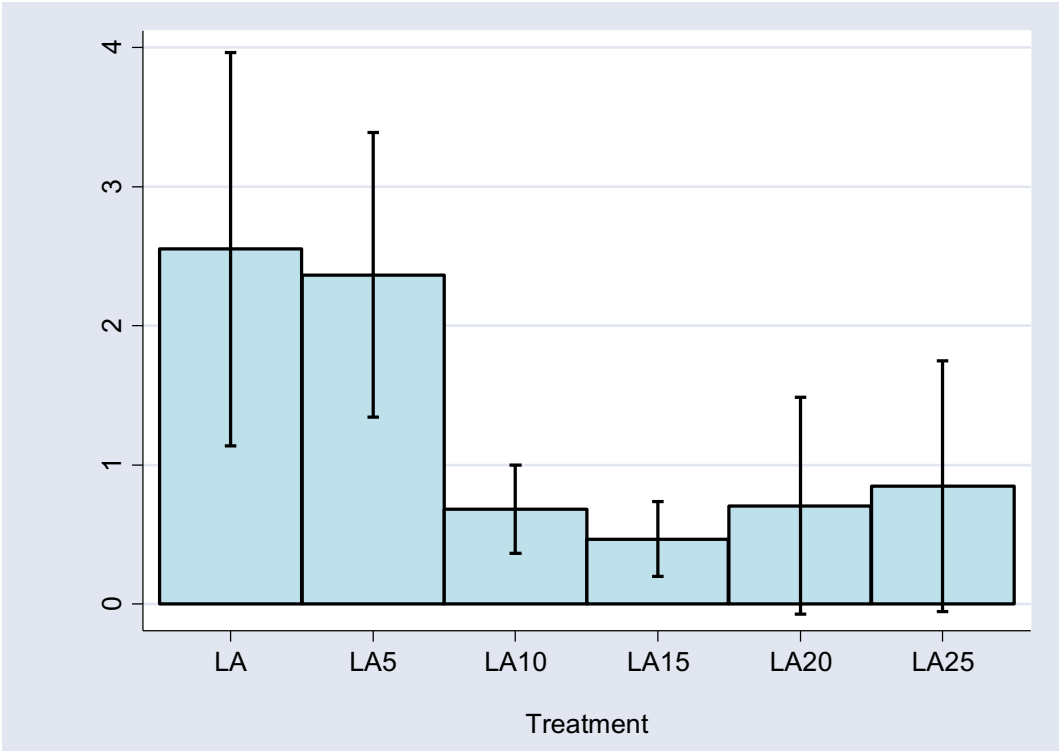

Figure 1.6. The proportion of the family *Streptococcaceae* in the faeces of pigs fed different levels of LA in the late-finishing period.

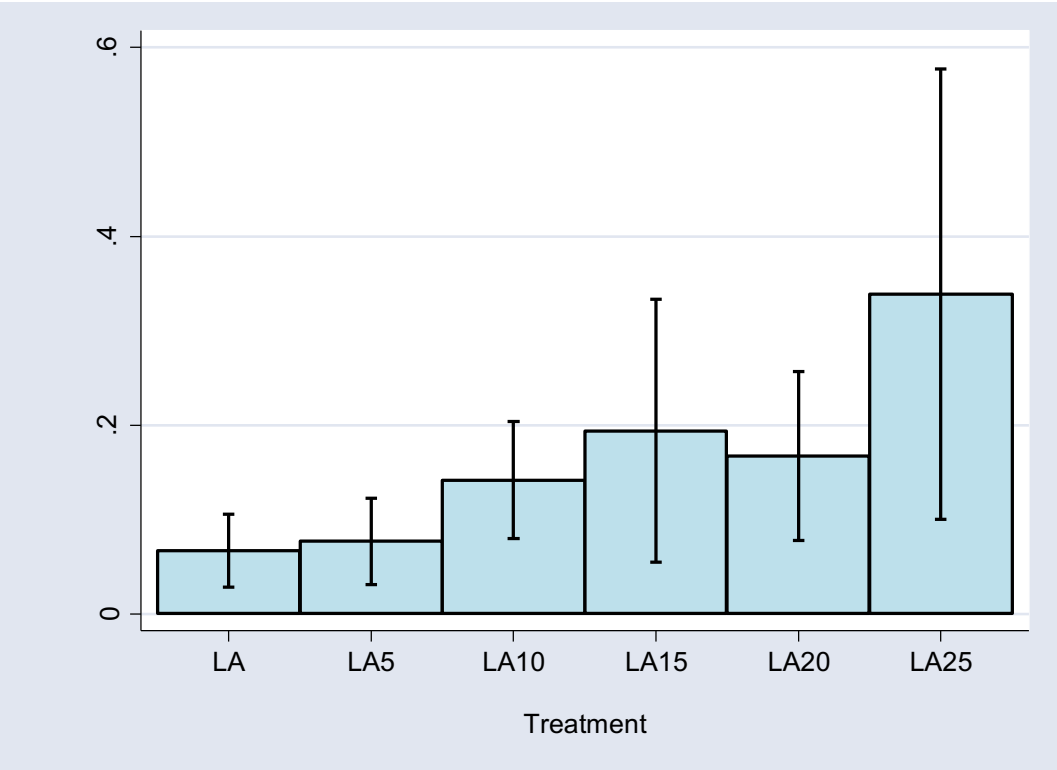

Figure 1.7. The proportion of the family *Enterobacteriaceae* in the faeces of pigs fed different levels of LA in the late-finishing period.

Experiment II

Diet fatty acid methyl ester analysis (Appendix VI) showed a linear increase in the C12 (dodecanoic acid) proportion and concentration in accordance with added LA to the diet. Nasal swabs taken from focus pigs before and after feeding of the test diets for the presence of MRSA (methicillin-resistant *Staphylococcus aureus*) showed that none were detected at either time point, signifying the lack of this bacterium in this population of pigs. Similarly, no diarrhoea (ileitis) was observed and hence no samples were collected for *Lawsonia* presence or quantification.

For pig bodyweight (BW), there was no overall treatment effect at day 19 of the study, i.e., 7 days after commencement of feeding the experimental diets, but at day 26, BW was different (p=0.044) between treatments [90.2, 90.3, 89.5, 90.6 and 90.8 kg (LSD 0.035), for Control, LA, MLA5, MLA10 and MLA20, respectively]. Fitting a polynomial model showed a significant Linear effect of feeding MLA (p=0.035) on final BW. However, there was no difference (contrast; p>0.05) in BW between feeding LA or the average of the MLA treatments.

Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) conditional F-statistics and associated p-values for the effects of Treatment and bodyweight (BW) at day 12 (commencement of feeding experimental diets) are shown in Table 2.1. Treatment diet means, their standard errors, and the least significant difference (LSD) for performance traits are shown in Table 2.2.

In the overall period from d 12-26, pigs fed diet MLA10 and MLA20 grew faster than pigs fed MLA5 (p<0.05), but these were not statistically different to pigs fed the Control diet or LA. There were no differences (p>0.05) in ADFI between treatments during the experimental period. However, for FCR d 12-19 (i.e., the first week of feeding the experimental diets), pigs fed MLA5 converted feed less efficiently (p<0.05) than pigs fed all other diets, and for the overall period d 12-26, pigs fed diets LA and MLA5 converted feed less efficiently (p<0.05) than pigs fed all other diets (Table 2.2).

There were no treatment x sex interactions for any measurements. Despite females having a higher BW at the time of selection on farm (d 0, p<0.001), males were heavier throughout the experiment on d 12, 19 and 26 (p<0.001). Female pigs grew faster than males from d 12 to 19 (1.04 vs 0.97 kg/pig/day, p=0.05), but males grew faster than females from d 19 to 26 (1.27 vs 1.12 kg/pig/day, p<0.001). Overall (d 12-26) there was a tendency for males to grow faster than females (1.12 vs 1.08 kg/pig/day, p=0.08). Male pigs ate more feed than female pigs throughout the treatment period (2.85 vs 2.55 kg/pig/day, p<0.001), and overall, males had a higher FCR than females throughout the treatment period (2.65 vs 2.36, p<0.001).

Table 2.1 F-statistics (F. con) and associated p values for average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR), for the effects of Treatment and bodyweight (BW) at day 12 (covariate; commencement of feeding experimental diets) in Experiment II.

	Treatment		BW at day 12	
Trait	F. con	p-value	F. con	p-value
ADG				
d 12-19	1.60	0.189	6.53	<0.013
d 19-26	0.63	0.646	29.0	<0.001
d 12-26	2.63	0.045	4.88	0.040
ADFI				
d 12-19	1.35	0.262	5.36	0.024
d 19-26	2.14	0.089	14.35	0.001
d 12-26	1.68	0.169	26.09	<0.001
FCR				
d 12-19	3.72	0.010	35.63	<0.001
d 19-26	1.44	0.232	0.00	0.972
d 12-26	6.05	<0.001	0.34	0.563

Table 2.2 least significant difference (LSD; 5% level) for average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) in Experiment II.

	Diet					
	Control	LA[A]	MLA5	MLA10	MLA20	LSD (5%)
ADG, kg						
d 0-12	1.38 (0.189)	1.42 (0.189)	1.39 (0.189)	1.31 (0.190)	1.35 (0.189)	0.071
d 12-19	1.04 (0.082)	1.03 (0.082)	0.92 (0.082)	1.00 (0.083)	1.01 (0.082)	0.147
d 19-26	1.17 (0.152)	1.22 (0.152)	1.23 (0.152)	1.17 (0.152)	1.25 (0.152)	0.156
d 12-26	1.07 (0.038)	1.08 (0.038)	1.03 (0.038)	1.10 (0.038)	1.12 (0.038)	0.060
ADFI, kg						
d 12-19	2.54 (0.064)	2.66 (0.063)	2.61 (0.063)	2.48 (0.065)	2.60 (0.063)	0.223
d 19-26	2.73 (0.302)	3.01 (0.302)	2.88 (0.302)	2.69 (0.303)	2.78 (0.302)	0.189
d 12-26	2.63 (0.170)	2.84 (0.171)	2.75 (0.170)	2.58 (0.171)	2.69 (0.170)	0.172
FCR, kg:kg						
d 12-19	2.48 (0.252)	2.62 (0.252)	2.88 (0.252)	2.49 (0.253)	2.63 (0.252)	0.231
d 19-26	2.34 (0.079)	2.50 (0.079)	2.38 (0.079)	2.31 (0.081)	2.25 (0.079)	0.279
d 12-26	2.45 (0.143)	2.61 (0.143)	2.67 (0.143)	2.37 (0.144)	2.41 (0.143)	0.146

[A]LA: Lauric acid (20 g/kg).

Modelling the MLA inclusion rate as a second order polynomial based on the significant production data demonstrated that for (a) ADG d 12-26, maximum ADG (1.12 kg/day; SE 0.033) occurred at an inclusion rate of MLA of 20 g/kg, and (b) FCR d 12-26, minimum FCR occurred also at an inclusion rate of 20 g/kg (2.4; SE 0.115) (Figures 2.1 and 2.2, respectively).

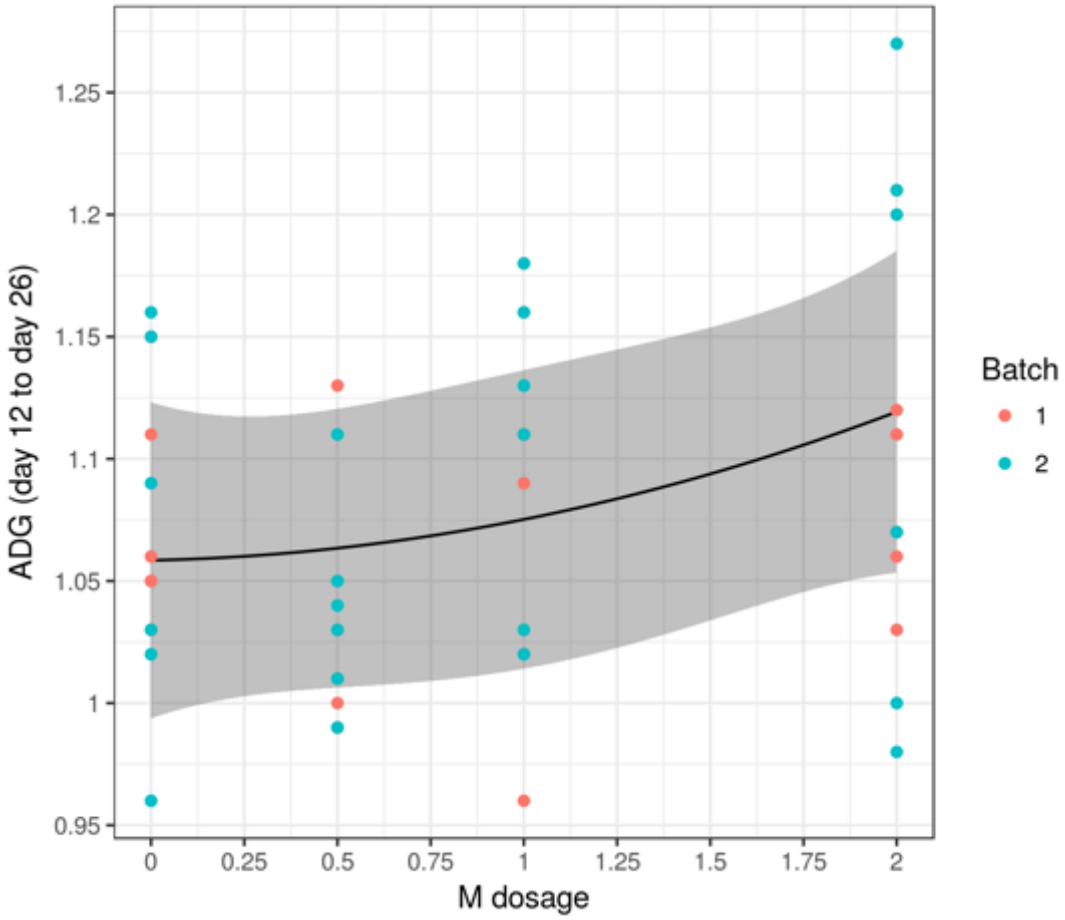

Figure 2.1. The fitted second-order polynomial for ADG d 12-26 for all pigs used in the experiment, with an approximate 95% confidence band. Maximum ADG is estimated to occur at a MLA dosage of **20 g/kg (2%)**. Fitted equation (with standard errors) for the first (dose) and second (dose^2) order polynomial terms is 2.51 (0.114) + 0.013 (0.140) – 0.033 (0.065) (p<0.05).

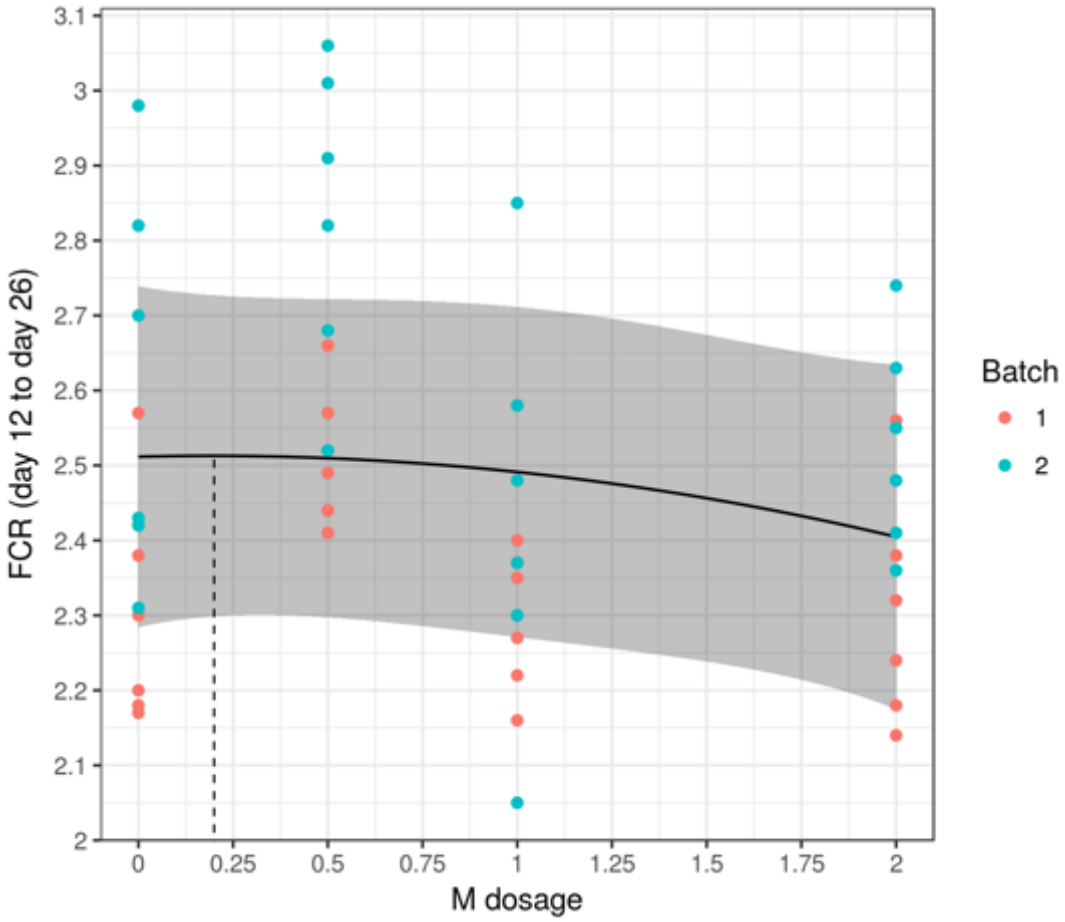

Figure 2.2. The fitted second-order polynomial for FCR d 12-26 for all pigs used in the experiment, with an approximate 95% confidence band. Minimum FCR is estimated to occur at a MLA dosage of **20 g/kg (2%)**. Fitted equation (with standard errors) for the first (dose) and second (dose^2) order polynomial terms is 1.06 (0.032) + 0.003 (0.052) + 0.014 (0.024) (p<0.05).

Meat quality traits and carcass measurements

There were no statistical differences in any of the meat quality or carcass measurements between treatments, although there was a trend (p=0.057) for pH to be lower in pigs fed MLA20 relative to pigs fed all other treatments (5.36 *vs* an average of 5.57; LSD = 0.129) (Table 2.4).

Table 2.3 Conditional F-statistics and associated *p* values for the effects of Treatment and bodyweight (BW) at day 12 (covariate; commencement of feeding experimental diets) on carcass characteristics and meat quality traits in Experiment II.

P2, mm	0.77	0.553	0.11	0.750
HCW, kg	1.42	0.230	1.42	0.235
CCW, kg	1.71	0.196	0.96	0.330
Chill loss %	0.90	0.374	0.00	0.983
pH				
Colour				
L*	1.55	0.188	2.11	0.156
a*	0.49	0.745	0.93	0.341
b*	0.65	0.628	2.49	0.195

P2: backfat measured at P2 position; HCW: hot carcass weight; CCW: chilled carcass weight; Chill loss%: loss of carcass weight measured after entry to chiller; pH: pH assessed 24 h after slaughter; Colour: surface lightness, L*; redness, a*; yellowness, b*.

Table 2.4 Carcass characteristics and meat quality data from Experiment II.

P2, mm	9.9	10.1	10.2	9.5	10.4	1.29
HCW, kg	64.4	65.2	64.9	62.6	65.0	2.70
CCW, kg	64.1	65.3	65.2	64.9	65.2	3.14
Chill loss %	3.0	2.9	2.7	2.8	2.9	1.10
pH	5.57	5.58	5.55	5.58	5.51	0.041
Colour						
L*						
a*	14.4	14.5	14.3	14.7	14.8	0.91
b*	8.4	8.5	8.5	8.8	8.9	0.73

Bacterial populations

There were no statistical differences (data analysed using Stata SE v.13 binomial logistic regression functions) in the proportion of Gram-positive, Gram-negative, or the ratio of Gram-positive:Gram-negative organisms, in the faeces of pigs sampled in the late-finishing period (Figures 2.3 to 2.5). There were no major changes in the proportion of selected families of bacteria associated with feeding MLA to pigs.

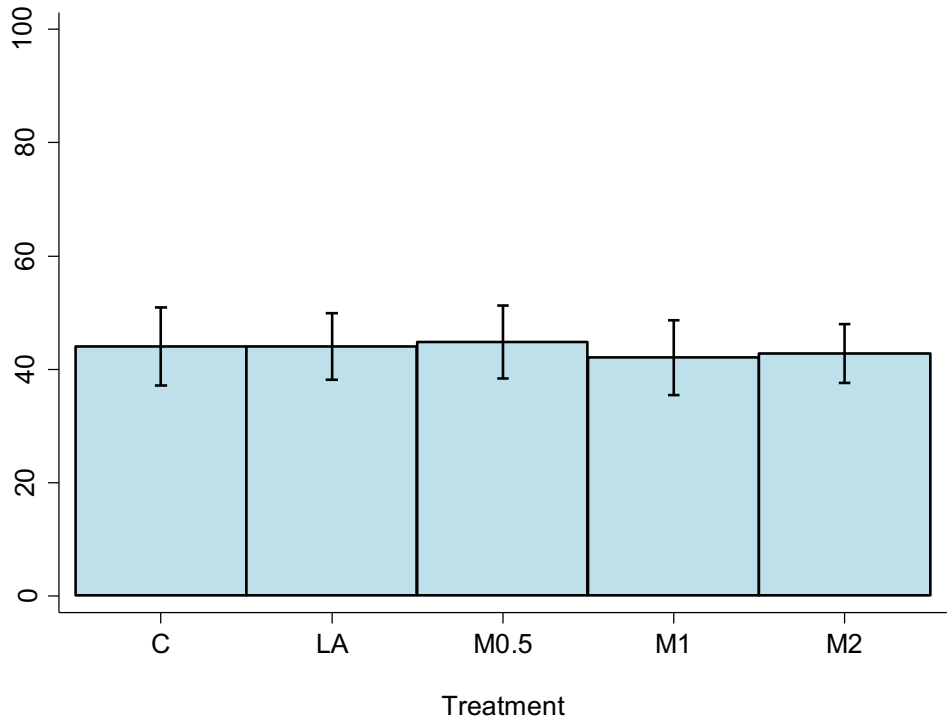

Figure 2.3 The proportion of Gram-positive organisms in the faeces of pigs fed LA or different levels of MLA in the late-finishing period.

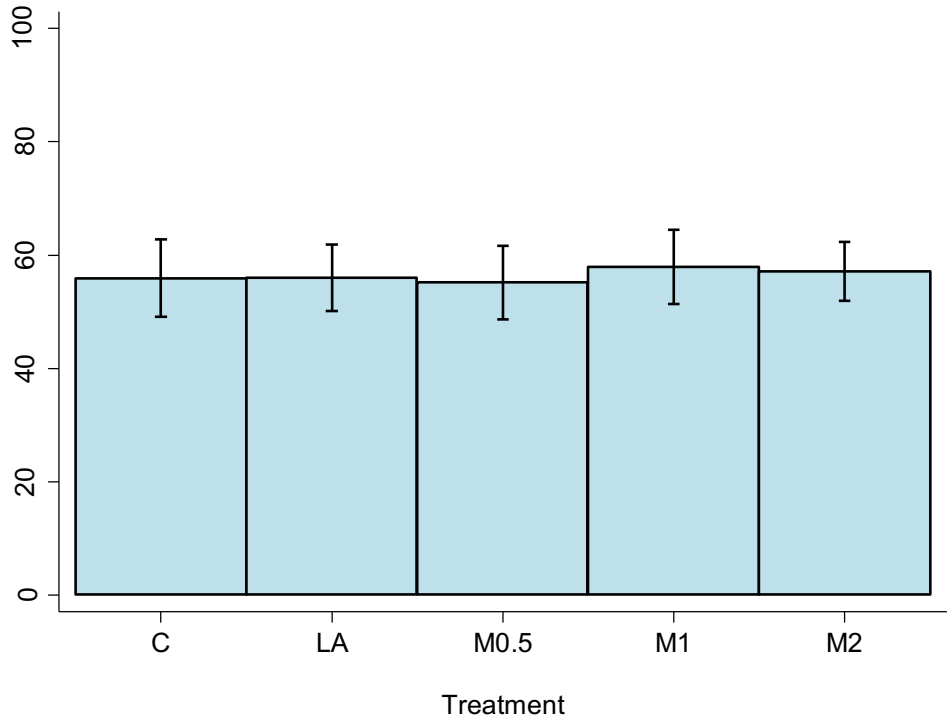

Figure 2.4 The proportion of Gram-negative organisms in the faeces of pigs fed LA or different levels of MLA in the late-finishing period.

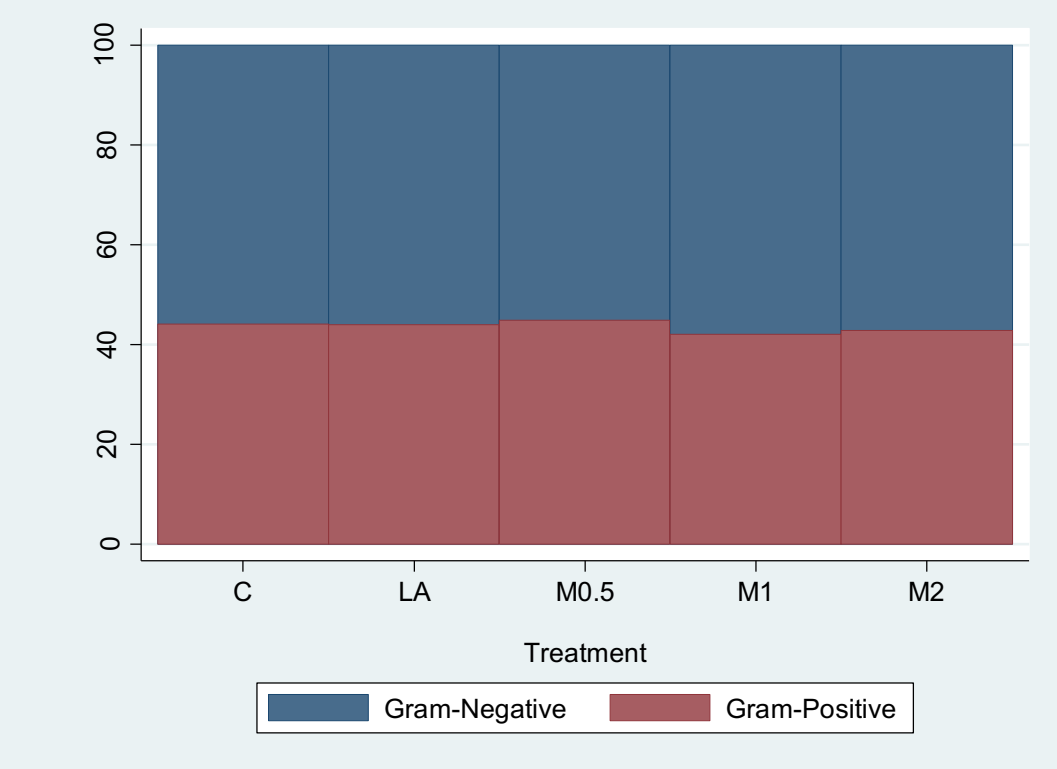

Figure 2.6 The ratio of Gram-negative to Gram-positive organisms in the faeces of pigs fed different levels of LA in the late-finishing period.

Experiment III

All pigs were naïve to Lawsonia before challenge, but began continuously shedding Lawsonia in their faeces from 7 days to 21 days post-challenge. Analysis of treatment differences by Student's T test suggested that there was no significant difference in numbers of Lawsonia shed between treatments at any time point, although there was a strong trend for Control pigs to shed higher Lawsonia numbers than LA-fed pigs at days 7 (p=0.063) and 14 (p=0.064), respectively (Table 3.1).

Table 3.1. Mean number of Lawsonia excreted per gram of faeces (log_{10} transformed) in Lawsonia-challenged pigs treated with LA or untreated Controls (Student's *t* test).

	Day 7	Day 11	Day 14	Day 18	Day 21
Lauric Acid	6.15	6.62	7.67	7.93	7.12
Control	6.93	7.04	8.14	7.59	6.49
p value	0.063	0.131	0.064	0.20	0.11

Lawsonia numbers differed over time (p<0.001) with the peak in infection occurring between 14 and 18 days post-challenge. As previous numbers of Lawsonia could influence subsequent Lawsonia numbers, the data were also analysed by repeated measures ANOVA. The interaction between treatment and time was also significant with higher Lawsonia numbers excreted by Control pigs at day 7 (p=0.018).

Clinical signs of the haemorrhagic form of ileitis were only found in three Control pigs and two LA pigs. Pigs had blood in their faeces, but only one Control pig passed tarry black faeces characteristic of the haemorrhagic form of ileitis. Two control pigs had to be treated with Lincomix on days 14 and 15 post-challenge due to the severity of blood loss in their faeces. The reduction in Lawsonia numbers (Log_{10} transformed) from day 14 to 22 was greater (p=0.012) for the two treated pigs (3.18) compared to their eight untreated cohorts (1.27), suggesting that Lincomix effectively reduced faecal shedding of Lawsonia. The antibiotic treatment of these two Control pigs reduced the mean severity of disease in Control pigs in the last week of the trial. However, if left untreated, these pigs may have died.

There was no significant difference in weekly weight gains (Table 3.2), feed intake (Table 3.3) or feed to gain (Table 3.4) between treatments, although LA pigs tended to grow less than Control pigs in the 4th week of the trial, between days 18 and 22 post-challenge (p=0.090).

Serum IgG antibodies to Lawsonia were not significantly different between treatments at Day 21 post-challenge (Table 3.5). Faecal consistency was scored daily as: 1 = normal; 2 = solid faeces but with some blood; 3 = semi-solid faeces with blood; 4 = liquid bloody faeces. Faecal consistency was not different (P=0.22) between treatments over the whole 3-week trial or over any single week (Table 5).

Table 3.2. Mean weekly weight gain (kg) of finisher pigs challenged with Lawsonia and either treated with Lauric acid (LA) or untreated (Control).

	Weight gain (kg) Week 1	Weight gain (kg) Week 2	Weight gain (kg) Week 3	Weight gain (kg) (4 d of Week 4)
Lauric acid	12.63	11.66	9.482	4.269
Control	12.12	12.00	8.450	6.604
p value	0.483	0.630	0.511	0.090

Table 3.3. Mean weekly feed intake (kg) of finisher pigs challenged with Lawsonia and either treated with Lauric acid (LA) or untreated (Control).

	Feed Intake (kg) Week 1	Feed Intake (kg) Week 2	Feed Intake (kg) Week 3	Feed Intake (kg) (4 d of Week 4)
Lauric Acid	27.51	30.27	29.70	25.62
Control	31.14	31.73	27.64	26.81
p value	0.284	0.407	0.459	0.702

Table 3.4. Mean weekly feed to gain ratio of finisher pigs challenged with Lawsonia and either treated with Lauric acid (LA) or untreated (Control).

	Feed:Gain week 1	Feed:Gain week 2	Feed:Gain week 3	Feed:Gain Week 4
LA	2.149	2.641	3.529	2.904
Control	2.608	2.637	4.738	2.946
p value	0.136	0.979	0.455	0.969

Table 3.5. Weekly mean faecal consistency score and serum IgG antibody concentration (expressed as percent inhibition (PI) in Svanova blocking ELISA).

	Faecal consistency score			Antibody concentration (PI)
	Day 0 to 7	Day 7 to 14	Day 14 to 21	Day 21
LA	7.0	7.5	8.3	93.3%
Control	7.0	7.0	7.5	94.7%
p value	ND	0.140	0.322	0.130

There was no overall effect of feeding LA on mean weight gain (p=0.623), feed intake (p=0.538) or feed:gain (p=0.867) over the whole trial period. Control of ileitis is often measured as reduced variation in production measures as ileitis is a common cause of increased variation within batches of pigs. In this regard, the variation in feed intake was greater (p=0.020) in Control compared with LA pigs between day 4 to 11 (Table 3.6). Variation in feed:gain was also greater (p<0.001) in Control

compared with LA pigs between day 11 to 18. Conversely, variation in feed:gain was significantly greater in LA pigs compared with Control pigs between day 18 and 22 (Table 3.6), coinciding with the delay of 4 days in peak excretion of Lawsonia in LA pigs compared with Control pigs (Table 3.1).

The variation in Lawsonia numbers (Log_{10} transformed) showed a strong trend (*p*=0.051) to be greater in Control pigs compared with LA pigs at Day 11 (Table 3.6). However, there were no significant differences in variation in weight gains between treatments over any week of the trial.

Table 3.6. Significant variation between treatments in fed intake, feed to gain ratio and number of Lawsonia excreted in finisher pigs challenged with Lawsonia and either treated with Lauric acid (LA) or untreated (Control).

	Variation in feed intake Day 4 to 11	**Variation in feed:gain Day 11 to 18**	**Variation in feed:gain Day 18 to 22**	**Variation in Lawsonia numbers at Day 11**
LA	6.03	2.2	8.793	0.160
Control	30.08	21.92	1.728	0.609
P value	0.020	<0.001	0.022	0.051

4. Discussion and Application of Research

Data from Experiments I and II demonstrated some positive performance effects of feeding either LA or MLA to late-finishing male (Improvac-treated) and female pigs. Polynomial regression analyses (based on statistically significant differences in performance traits) indicated in Experiment I that feeding approximately 12-13.5 g/kg of LA (~1.2 to 1.4% of the diet) in substitution for tallow generated positive responses in LW and ADG, especially in the initial feeding period, and ADFI, although there were no beneficial effects of FCR. In Experiment II when pigs were fed either 5, 10 or 20 g/kg MLA, again in substitution for tallow, the polynomial regression analyses indicated beneficial responses in terms of both ADG and FCR over the 14-day feeding period with an optimum inclusion level of 20 g/kg (2%) showing the most promising results. In this regard, the authors could not find any published work examining the effects of feeding either LA or MLA on production responses in late-finishing pigs to support or refute these findings. Most research using C12 fatty acids or medium-chain triglycerides concerns 'gut-health' and (or) antimicrobial and anti-inflammatory properties of these compounds (e.g., Zentek *et al.*, 2011; Liu, 2015) rather than production responses.

Medium-chain triglycerides (MCT) are used directly for the acyl-modification (activation) of ghrelin within the GIT, and this could have caused beneficial effects on production. Ghrelin is primarily produced by endocrine cells of the gastric mucosa for secretion into the circulation. Studies have identified multiple physiological functions for ghrelin, including growth hormone (GH) release, appetite stimulation, cellular proliferation, apoptosis inhibition, and regulation of lipid metabolism and tissue fat distribution in muscle (Nishi *et al.*, 2005). Furthermore, exogenous ghrelin administration for 5 days to 18 day-old weaned pigs increased their weight gain (Salfen *et al.*, 2004). In 60 kg pigs fed 6% MCT [a preparation of a refined form of coconut oil containing octanoic acid (C8) (65–75 %), decanoic acid (C10) (25–35 %), and hexanoic acid (C6) (<1 %] for 3 weeks, Miller *et al*. (2016) found that the MCT treated pigs had a higher plasma concentration of ghrelin compared to the control pigs, although plasma concentrations of growth hormone and weight were not affected. Even though C12 LA was not fed in the study by Miller *et al*. (2016), it can be speculated that as LA is typically a (major) component of coconut oil, then similar effects related to ghrelin might have occurred in the current studies.

These data are seemingly in contrast to the preliminary work of Pluske *et al*. (2015) and Pluske (2016) who showed that feeding LA at 25 g/kg or 50 g/kg (2% or 5%) to late-finishing female pigs decreased voluntary feed intake by 10.1% and 9.0%, respectively, and improved FCR by 4.9% and 1.7%, respectively, relative to a control diet. Pigs in the study of Pluske *et al*. (2015) were different to those used in the current experiments as they were females, they were lighter at slaughter, they were housed individually, and the LA was added 'on-top' rather than being incorporated into the diet, and either (or all) of these factors might have contributed to the differences seen. The use of a larger number of pigs housed in pens and fed commercially-formulated diets as in Experiments I and II is likely a more realistic assessment of the effects of LA and MLA. Nevertheless, the work from Pluske (2016) examining feeding 25 g/kg or 50 g/kg LA was conducted at the same experimental site with pigs from the same supplier and under similar housing and penning conditions and showed a reduction in feed intake at 25 g/kg LA compared to the Controls, which was not replicated in the current experiment because the opposite occurred. This is hard to explain, however, differences in the timing of the

experiments throughout the year might have contributed to the differences, as well as a difference in the supplier of the LA between studies. Moreover, there was some anecdotal evidence from the Farm Manager that feed wastage was higher in the LA-fortified diets; indeed and in contrast to the previous studies, the FCR values (Table 1.2) for most of the LA-fed diets were higher (albeit not always statistically) than the Control diet, which again is very hard to explain and makes no physiological sense.

Another difference between this study and that of Pluske (2016) was the use of Improvac-treated male pigs in Experiment I (in the current study), compared to females by Pluske (2016). Improvac-treated males increase their feed intake after the second injection (e.g., Dunshea et al., 2001). In Experiment II of the current study where females were used, male pigs ate more feed than female pigs throughout the treatment period (2.85 vs 2.55 kg/pig/day, $p<0.001$), and overall, males had a higher FCR than females throughout the treatment period (2.65 vs 2.36, $p<0.001$).

Both Experiments I and II demonstrated no adverse effects of feeding either LA or MLA at different levels on a suite of meat quality measurements and carcass characteristics, in agreement with data from Pluske (2016), although the trend for a decline in pH in pigs fed MLA at 2% compared to pigs fed all other treatments (5.36 *vs* an average of 5.57; LSD = 0.129) suggests that the meat had a decreased water-holding capacity and likely a lighter colour, and hence feeding this higher level of MLA should be avoided from this perspective. Aspects of post-slaughter food safety, e.g., microbial contamination, were not examined in these studies, but interestingly and in broilers, Zeiger *et al*. (2017) showed that lauric acid (5% fat-enriched diet with palm kernel fatty acids including high levels of lauric acid; 42–53%) reduced the numbers of *Campylobacter coli* in meat. No effects of feeding either LA or MLA were seen with the proportion of *Campylobacteraceae* in the faeces of pigs in the current experiments.

Both Experiments I and II also showed no reduction in Gram-positive organisms in the faeces, although in Experiment I, there was a clear linear decline in the proportion of *Streptococcaceae* in the faeces; the high intra-treatment variability prevented any statistical detection of differences. Generally, LA and (or) MLA are more effective against Gram-positive bacteria than Gram-negative bacteria (Lieberman *et al*., 2006). It is probable that sampling from faeces rather than in parts of the GIT *in situ*, e.g., stomach, small intestine, thwarted statistical differences being detected. Furthermore, a greater number of samples and the use of individual pigs rather than a pooled pen sample might also have detected differences. Nevertheless, *Streptococcus suis* is an important cause of a wide variety of infections in pigs, including meningitis, pneumonia, septicaemia and arthritis, with *S. suis* serotype 2 the most prevalent type isolated from diseased pigs (Su *et al*., 2008). In the young pig, *S. suis* predominates in the stomach samples of weaned piglets with high population levels and is also dominant in the jejunum and ileum digesta after weaning, demonstrating the post-weaning dominance of this pathogen (Su *et al*., 2008). *Streptococcus suis* is also a zoonotic agent causing severe infections to people in close contact with infected pigs or pork-derived products.

We also examined nasal swabs for the presence of MRSA (methicillin-resistant *Staphylococcus aureus*) but were unable to detect this bacterium in any of the pigs from both experiments. Pig-derived MRSA infections of humans has been documented (e.g., Lewis *et al*., 2008), and intervention strategies to reduce the

shedding and load of Gram-positive bacteria such as with LA or MLA was an objective of these experiments.

In Experiment III, which was a controlled *Lawsonia intracellularis* challenge study, feed supplemented with 20 g/kg LA (2% LA) did not significantly (p<0.05) reduce the severity of ileitis in finisher pigs, measured as numbers of Lawsonia shed in faeces, serum antibody concentrations, diarrhoea severity and production losses. Nevertheless, there were strong trends for Control pigs to shed higher Lawsonia numbers than LA-fed pigs at days 7 (p=0.063) and 14 (p=0.064) post-infection, and there was a significant interaction (p=0.018) between treatment and time with higher Lawsonia numbers excreted by Control pigs at day 7 post-infection. Use of a greater number of pigs in this experiment would likely have caused these indices to become statistically significant (p<0.05), however, a *Lawsonia intracellularis* challenge study is difficult to undertake and expensive to run, hence in this light, the data achieved in this experiment are promising.

Furthermore, LA reduced the variation in feed intake between pigs early in infection and also the feed:gain ratio in mid infection. Variation in growth and feed efficiency in pigs reduces production efficiency by increasing the proportion of 'tail-ender' pigs or by increasing the number of days to slaughter (Payne *et al.*, 1999). Variation in slaughter weights and delayed emptying of sheds in all-in-all-out (AIAO) production systems also reduces profitability by increasing housing costs per pig (Magowan *et al.*, 2007). Simulation modelling of variation in pig weights at slaughter revealed that a 10% reduction in weight variance led to an increase in return above the feed cost of US$0.78 per pig (DiPietre and Adam, 2012). Therefore, any treatment that reduces variation in weight gain or feed efficiency should lead to increased production efficiency and increased profitability. Both Enterisol ileitis vaccination and medication with antibiotics have been shown previously to reduce variation in pig weights (Pollock and Marr, 2010; Miljkovic *et al.*, 2012; Marsteller *et al.*, 2001).

A cost-benefit analysis is recommended on a commercial scale to determine if the reduction of variation in production makes LA a useful dietary control strategy for ileitis, perhaps in conjunction with a standard vaccine strategy.

5. Conclusions

The major findings from Experiments I and II in this project suggest that there is potential for the use of lauric acid or monolaurin in diets for late-finishing pigs to improve some production outcomes. Optimum inclusion rates of LA or MLA using statistical polynomial modelling showed that ~12-20 g/kg (1.2-2%) of product had some beneficial impacts on some production variables, although the timing and the magnitude of the responses differed between LA and MLA. Feeding LA but not MLA modified some specific bacterial populations in the faeces of potential interest, but this requires further exploration.

Experiment III, using a *Lawsonia intracellularis* challenge model, demonstrated that feed not supplemented with 20 g/kg LA showed strong statistical trends to shed higher *Lawsonia intracellularis* numbers than LA-fed pigs at days 7 and 14 post-infection, and there was a significant interaction between treatment and time with higher *Lawsonia intracellularis* numbers excreted by Control pigs at day 7 post-

infection. Feeding LA significantly reduced the variation in feed intake between pigs early in infection and also the feed:gain ratio in mid infection.

6. Limitations/Risks

The greatest limitations to the implementation of using lauric acid or monolaurin in diets for finishing pigs are price of the product, given that these were products used for human applications, feed manufacturing issues associated with inclusion of the products in diets, and the inconsistency of effects seen between this study and that reported previously (Pluske, 2016). Feed manufacturing issues were especially salient for MLA, which came in a solid form and required heating before incorporation onto the pellets. Whilst not insurmountable, extra consideration must be given before their use in a practical setting. Moreover, a margin over feed cost (MOFC) exercise should therefore be conducted using cheaper-sourced products.

7. Recommendations

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

- Conduct (larger) field-scale studies using lauric acid or MLA sourced from cheaper suppliers, to confirm or refute the production outcomes observed in the current studies. Such studies should focus specifically on the final 2-3 weeks of the finishing period.
- Conduct a MOFC exercise as part of these studies.
- Consider examining the possible beneficial effects of feeding LA or MLA to younger pigs, e.g., in the post-weaning period.
- Explore further some of the possible beneficial bacterial modifications observed with feeding higher levels of LA, e.g., with the *Streptococcaceae* numbers.
- Conduct a larger-scale *Lawsonia intracellularis* challenge study, or apply a dietary intervention in situation with a known, diagnosed ileitis outbreak.

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10. Appendices

Appendix 1: Formulation and calculated analysis of diets (g/kg as fed) used in Experiment I.

Ingredient	**Control**	**LA 5 g/kg**	**LA 10 g/kg**	**LA 15 g/kg**	**LA 20 g/kg**	**LA 25 g/kg**
Barley	477.3	474.2	471.1	468	464.9	420.5
Wheat	150	150	150	150	150	150
Mill-run	127.1	130.5	134	137.5	140.9	150
Lupins	20	20	20	20	20	66.8
Canola meal	150	150	150	150	150	150
Soybean meal	28.0	27.7	27.4	27.2	26.9	15
Tallow	27.3	22.2	17.1	12	6.9	-
Tallow press	-	-	-	-	-	2.8
Lauric acid	-	5	10	15	20	25
Lysine	3.6	3.6	3.6	3.6	3.6	3.4
Methionine	0.47	0.47	0.47	0.47	0.47	0.46
Threonine	1.3	1.3	1.3	1.3	1.3	1.2
BJ-Pig Grower Plus[A]	20	20	20	20	20	20
Choline chloride	0.3	0.3	0.3	0.3	0.3	0.3
Limestone	6.1	6.1	6.1	6.2	6.2	6
Dicalcium phosphate	4.6	4.6	4.5	4.5	4.5	4.6
Salt	2	2	2	2	2	2
Calculated analysis						
DE (MJ/kg)	13.6	13.6	13.6	13.6	13.6	13.6
Crude protein	156	156	156	156	156	161
Crude fat	56	56	56	56	56	58.9
Crude fibre	60.2	60.4	60.5	60.6	60.8	67
SID Lysine (%)	0.82	0.82	0.82	0.82	0.82	0.82
SID Lysine:DE	0.06	0.06	0.06	0.06	0.06	0.06
Av. P (%)	0.25	0.25	0.25	0.25	0.25	0.25
Ca (%)	0.5	0.5	0.5	0.5	0.5	0.5

[A]Provided the following nutrients (per kg of air-dried diet): vitamins: A, 7000 IU; D3, 1400 IU; E, 20 mg; K, 1 mg; thiamine, 1 mg; riboflavin, 3 mg; pyridoxine, 1.5 mg; cyanocobalamin, 15 ¤g; calcium pantothenate, 10.7 mg; folic acid, 0.2 mg; niacin, 12 mg; biotin, 30 ¤g. Minerals: Co, 0.2 mg (as cobalt sulfate); Cu, 10 mg (as copper sulfate); iodine, 0.5 mg (as potassium iodine); iron, 60 mg (as ferrous sulfate); Mn, 40 mg (as manganous oxide); Se, 0.3 mg (as sodium selenite); Zn, 100 mg (as zinc oxide); BJ Grower 1, BioJohn Pty Ltd., WA, Australia.

Appendix II: Formulation and calculated analysis of diets (g/kg as fed) used in Experiment II.

Ingredient	**Control**	**LA 20 g/kg**	**MLA 5 g/kg**	**MLA 10 g/kg**	**MLA 20 g/kg**
Barley	477.3	464.9	500	488.2	502.7
Wheat	150	150	150	150	150
Mill-run	127.1	140.9	101.7	100	100
Lupins	20	20	20	20	20
Canola meal	150	150	150	149.7	103.2
Soybean meal	28.0	26.9	30	44.5	73.3
Tallow	27.3	6.9	22.8	17.9	10.7
Lauric acid	-	20	-	-	-
Monolaurin (MLA)	-	-	5	10	20
Lysine	3.6	3.6	3.6	3.2	2.9
Methionine	0.47	0.47	0.47	0.35	0.49
Threonine	1.3	1.3	1.3	1.1	1.1
BJ-Pig Grower Plus[A]	20	20	20	20	20
Choline chloride	0.3	0.3	0.3	0.3	0.42
Limestone	6.1	6.2	6.0	5.9	6.0
Dicalcium phosphate	4.6	4.5	4.8	4.7	5.1
Salt	2	2	2	2	2
Calculated analysis					
DE (MJ/kg)	13.6	13.6	13.6	13.6	13.6
Crude protein	156	156	155	160	160
Crude fat	56	56	56	56	56
Crude fibre	60.2	60.8	59.2	59.0	55.3
SID Lysine (%)	0.82	0.82	0.82	0.82	0.82
SID Lysine:DE	0.06	0.06	0.06	0.06	0.06
Av. P (%)	0.25	0.25	0.25	0.25	0.25
Ca (%)	0.5	0.5	0.5	0.5	0.5

[A]Provided the following nutrients (per kg of air-dried diet): vitamins: A, 7000 IU; D3, 1400 IU; E, 20 mg; K, 1 mg; thiamine, 1 mg; riboflavin, 3 mg; pyridoxine, 1.5 mg; cyanocobalamin, 15 ¤g; calcium pantothenate, 10.7 mg; folic acid, 0.2 mg; niacin, 12 mg; biotin, 30 ¤g. Minerals: Co, 0.2 mg (as cobalt sulfate); Cu, 10 mg (as copper sulfate); iodine, 0.5 mg (as potassium iodine); iron, 60 mg (as ferrous sulfate); Mn, 40 mg (as manganous oxide); Se, 0.3 mg (as sodium selenite); Zn, 100 mg (as zinc oxide); BJ Grower 1, BioJohn Pty Ltd., WA, Australia.

Appendix III: Determined analysis of diets (g/kg as-fed) used in Experiment I.

Item	**Control**	**LA 5 g/kg**	**LA 10 g/kg**	**LA 15 g/kg**	**LA 20 g/kg**	**LA 25 g/kg**
GE (MJ/kg)	15.5	15.5	15.4	15.5	15.4	15.4
Crude protein	152	158	157	153	150	152
Crude fat	52	54	51	52	49	50
Crude fibre	62	53	53	55	54	64
Ash	38	39	38	37	36	37
P	4.8	5.2	5.0	5.0	4.8	4.7
Ca	5.0	6.2	5.5	5.1	4.7	5.0

Appendix IV: Determined analysis of diets (g/kg as fed) used in Experiment II.

Item	**Control**	**LA 20 g/kg**	**MLA 5 g/kg**	**MLA 10 g/kg**	**MLA 20 g/kg**
DE (MJ/kg)[A]	13.1	12.9	13.4	13.3	13.2
NFE[B]	583	575	587	592	598
Crude protein	146	149	151	149	143
Crude fat	52	49	59	55	52
Crude fibre	57	46	44	48	49
Ash	40	42	44	37	40
P	5.3	5.6	5.2	5.7	5.5
Ca	5.7	6.9	5.7	7.6	6.7
Zn	2	6.2	1.4	1.9	1.8

[A] Derived from calculated ME/0.96.
[B]NFE: nitrogen-free extract.

Appendix V: Determined crude fat and fatty acid methyl esters' analysis of diets used in Experiment I (g/kg as-fed unless otherwise stated). Analyses conducted at NSW DPI, Wagga Wagga, NSW.

Item	**Control**	**LA 5 g/kg**	**LA 10 g/kg**	**LA 15 g/kg**	**LA 20 g/kg**	**LA 25 g/kg**
Crude fat (%, DM)	5.45	5.7	5.55	5.55	5.45	5.75
C12:0:						
proportion						
Batch 1	0.14	8.9	17.4	24.5	32.5	38.3
Batch 2	0.16	8.6	17.9	25.4	-	37.6
concentration (g/kg DM)						
Batch 1	0.08	4.8	9.4	13.3	18.0	21.4
Batch 2	0.09	4.9	9.8	13.6	-	21.4
SFA:						
proportion						
Batch 1	29.5	34.5	39.5	43.8	48.8	51.4
Batch 2	30.1	35.6	40.2	44.6	-	51.1
concentration (g/kg DM)						
Batch 1	15.8	18.7	21.2	23.9	26.9	28.7
Batch 2	15.8	20.1	22.1	24.0	-	29.2
MUFA:						
proportion						
Batch 1	34.1	30.2	27.6	24.9	22.3	20.4
Batch 2	34.3	30.1	27.1	24.7	-	20.6
concentration (g/kg DM)						
Batch 1	18.3	16.4	14.8	13.6	12.3	11.4
Batch 2	18.0	16.9	14.9	13.3	-	11.8

Appendix VI: Determined crude fat and fatty acid methyl esters' analysis of diets used in Experiment II (g/kg as-fed unless otherwise stated). Analyses conducted at NSW DPI, Wagga Wagga, NSW.

Item	**Control**	**LA 2 g/kg**	**MLA 0.5 g/kg**	**MLA 1 g/kg**	**MLA 2 g/kg**
Crude fat (%, DM)	5.29	5.90	5.46	5.79	5.78
C12:0: proportion	0.10	14.8	3.8	7.4	12.9
concentration (g/kg DM)	0.05	9.4	13.3	18.0	21.4
SFA: proportion	27.3	44.8	33.8	36.8	42.7
concentration (g/kg DM)	12.9	23.5	16.5	19.0	22.0
MUFA: proportion	34.3	23.8	30.0	28.0	24.2
concentration (g/kg DM)	16.2	14.6	14.4	12.3	12.5