THE EFFECT OF CONJUGATED LINOLEIC ACID (CLA) ON SOW AND LITTER IMMUNE STATUS AND PERFORMANCE

by

Robert E. Patterson

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

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FOREWORD

This thesis was prepared following a manuscript format. There are two manuscripts corresponding to two chapters. Both manuscripts have been formatted to meet the Guidelines for the Journal of Animal Science manuscript preparation.

ABSTRACT

Sub-optimal transfer of passive immunity from sows to piglets contributes to poor litter health and performance. In addition, intestinal disease caused by enterotoxigenic *Escherichia coli* (ETEC) which may lead to post-weaning diarrhea (PWD) can be hastened when passive immune protection is limiting. Typically, sub-therapeutic levels of antibiotics are added to piglet diets to quell the deleterious effects of PWD. However given the current trend of withdrawing in-feed antibiotics there is a need to investigate alternative strategies for bolstering passive immunity. Because of the known immune stimulating properties of conjugated linoleic acid (CLA), addition into feed has been proposed as a means of boosting the immune status of sows and piglets. With this in mind, experiments were designed to evaluate the effects of supplemental CLA on sow and litter performance and immune stimulation as well as post-weaning immune status of piglets through the use of an oral *E. coli* $K88^+$ challenge model. In the first study, fourteen Cotswold sows were allotted to completely random repeated measures design. Treatments corresponded to dietary CLA (0% or 2%) and sow maturity (Immature = 0 or 1 parity; Mature = 2 or greater parities) forming the following 2 x 2 factorial arrangement: 1) 0%-I, n = 3; 2) 0%-M, n = 4; 3) 2%-I, n = 3; 4) 2%-M, n = 4. Treatment diets were provided as gestation rations from d 85 to d 112 and for 4 d post-weaning and as lactation rations from d 112 of gestation until weaning. Sow body weight, back fat depth, condition score and feed intake were recorded and blood samples collected on d 85, 105 and 112 of gestation, d 1, 3 and 17 of lactation and 4 d post-weaning. Piglet blood samples were also collected via jugular vein puncture on d 3 and 17 of nursing.

Blood samples were analyzed for fatty acid profiles, plasma urea nitrogen and immunoglobulin concentrations. Dietary CLA-supplementation had no effect on sow body weight during gestation or lactation ($P > 0.10$). However, sows consuming CLA-supplemented diets had less back fat than control sows during gestation ($P = 0.05$) but not lactation ($P = 0.15$). Immature sows consuming CLA-supplemented diets tended ($P = 0.09$) to lose less back fat than control sows during lactation. Dietary CLA had no effect on calculated whole body lipid percentage ($P > 0.10$) but increased whole body protein percentages during gestation ($P = 0.05$). Piglets nursing sows consuming CLA-supplemented diets were lighter than piglets nursing control sows ($P = 0.06$). However, dietary CLA had no affect on total litter weight weaned ($P > 0.10$). Dietary CLA had no effect on the concentrations of immunoglobulins A and G of sows and piglets ($P > 0.10$). In experiment number two, 78 piglets weaned at 17 ± 1 d of age from sows in experiment number one were randomly assigned to a CRD based on the assigned nursery diet (ND; 0 % or 2 % CLA) or their dam's lactation ration (LR; 0 % or 2 % CLA) forming the following 2 x 2 factorial arrangement of treatments: 1) 0%-0%, (0% LR : 0% ND), n = 6; 2) 0%-2% (0% LR : 2% ND), n = 6; 3) 2%-0% (2% LR : 0% ND), n = 8 ; 4) 2%-2% (2% LR: 2% ND), n = 7. At 28 ± 2 d of age all piglets received an oral *E. coli* $K88^+$ challenge (6 ml containing 10^9 cfu/ml ETEC $K88^+$) and were subsequently monitored for scour development and general morbidity and mortality. One piglet per pen was randomly selected for blood and tissue collection at 36 ± 2 d of age. Overall growth performance was not affected by dietary CLA ($P > 0.10$). Piglets weaned from CLA supplemented sows had less severe scours ($P < 0.05$) and elevated IgA ($P < 0.001$) and IgG titers ($P = 0.03$). These piglets also had reduced ($P = 0.04$) intestinal inflammation compared to

piglets weaned from control sows suggesting CLA facilitated improved gut health. Thus, the addition of CLA into gestating and lactating sow diets is a potential means of boosting passive immune transfer to piglets which in turn can protect against post-weaning intestinal disease.

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LIST OF ABBREVIATIONS

ADFI	Average daily feed intake
ADG	Average daily gain
BW	Body weight
CCAC	Canadian Council on Animal Care
CD	Crypt depth
cfu	Colony forming unit
CLA	Conjugated linoleic acid
CP	Crude protein
d	Day
DE	Digestible energy
ELISA	Enzyme linked immuno-sorbent assay
ETEC	Enterotoxigenic *E. coli*
FA	Fatty acid
FAME	Fatty acid methyl ester
GC	Gas Chromatogram
G:F	Gain to feed ratio
IgA	Immunoglobulin A
IgG	Immunoglobulin G
LA	Linoleic acid
LAB	Lactic acid bacteria
MUFA	Monounsaturated fatty acid
N	Nitrogen

PCR	Polymerase chain reaction
PUFA	Polyunsaturated fatty acid
PUN	Plasma urea nitrogen
PWD	Post-weaning diarrhea
SFA	Saturated fatty acid
VFA	Volatile fatty acid
VH	Villous height
wk	Week

CHAPTER 1

GENERAL INTRODUCTION

The health promoting effects of CLA have been examined extensively. From reducing tumor formation and arterial hardening to stimulating the immune system, CLA is becoming an increasingly important player within the biological sciences.

The name CLA refers to a group of isomers of octadecanoic acid (linoleic acid) which have in common a conjugated double bond arrangement with double bonded carbon pairs being separated from each other by one single bonded carbon pair, with double bonds in either the *cis* or *trans* formation (Banni et al., 2004). In theory, many isomers could potentially exist, however, only two predominate naturally, these being the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers (Belury, 2002). Other isomers have been detected but only at trace levels when compared to these two.

In nature, CLA can be found occurring in the food products derived from ruminant animals, with meat and dairy products serving as principle sources within human diets. Concentrations of CLA within these products are not constant and vary depending on species, dietary components, age, breed, season and physical environment (McGuire and McGuire, 1999). For example, it has been shown that milk CLA concentrations can be increased through dietary fortification of linoleic acid (Pariza et al., 2001). It has also been shown that CLA concentrations are affected by the microbial environment within the rumen (Jahreis et al., 1999). Commercial blends and purified isomers of CLA are also available which have been synthetically manufactured (Reaney et al., 1999).

Aside from common associations as an anti-carcinogen, anti-diabetic and anti-

obesity agent, CLA has also been shown to possess potent immune stimulating properties (Belury, 2002). So far, studies examining this property in humans have been inconclusive (Kelly and Erickson, 2003). However, in multiple animal models CLA has been shown to have broad immune modulating effects (O'Shea et al., 2004). For example, CLA has been shown to abate catabolism normally associated with lipo-polysaccharide injection in chickens, mice and pigs (Miller et al., 1994; Bassaganya-Riera et al., 2004; Graves et al., 2005). In addition, CLA has been shown to modulate T-cell sub-populations (Bassaganya-Riera et al., 2001) and increase B-cell activity (Sugano et al., 1998).

It is well known that piglets are born with poorly developed, naive immune systems and are almost exclusively reliant on their mothers for disease defense (Rooke and Bland, 2002). This so called passive immunity is conferred by the production of colostrum and milk by the sow and subsequent consumption by the piglet. In actuality, the relative abundance of immune compounds such as immune cells, immunoglobulins, cytokines and other immuno-peptides combined with the total amount of colostrum consumed will determine the extent of immune stimulation in any given piglet (Le Dividich et al., 2005). Boosting either the amount of colostrum and milk ingested and/or the concentration of immune compounds may in turn have positive impacts on piglet performance and overall health.

Arguably, the development of early weaning resulting in greater number of litters and piglets weaned annually has been one of the most substantial successes in modern swine management (Blank et al., 2001; Hollis and Curtis, 2001). However, coupled with this success has been the challenge of minimizing the growth lag typically experienced by

piglets during the initial days after weaning. Multiple explanations have been offered as to why this growth depression occurs, including social, environmental and disease stressors along with the transition from highly digestible and palatable milk diet rich in immune compounds onto dry feed (Blank et al., 2001; Nyachoti et al., 2006). This dietary transition is further complicated by the fact that the digestive capacity of piglets is limited until several weeks of age, making the dietary utilization of nursery diets even more difficult (Gu and Li, 2003).

In addition to the challenges early weaning systems have placed on post-weaning piglet management are the nutritional and physiological demands placed on sows, particularly at first and second parity. In pigs as in other mammals, successful reproduction is a correlate of good body condition. Because initial breeding occurs when animals are not fully mature, often adequate lipid and protein reserves typically have not been accumulated (Slevin and Wiseman, 2003). The nutritional demands of suckling piglets require sows to mobilize large amounts of body stores for conversion into colostrum and milk. Sub-adequate reserves or excessive expenditures could result in insufficient or poor quality milk production, which in turn could lead to poor piglet performance, health and development.

Because CLA has been shown to modulate both nutrient partitioning (Ostrowska et al., 1999) and immune system functioning (Corino et al., 2002), it has been proposed as a novel dietary agent in gestating and lactating sow rations. Previous work has shown that supplemental CLA can lead to isomer enrichment within colostrum and milk and may lead to a more vigorous immune system (Bee, 2000a; Bontempo et al., 2004). However, it has yet to be established how piglets weaned from CLA supplemented sows,

will respond to an enteric antigen common to nursery barns, or how the effects of CLA on whole body nutrient partitioning are established in gestating and lactating sows.

For this research, it was hypothesized that dietary provision of CLA to gestating and lactating sows would increase the transfer of passive immunity to suckling piglets while positively affecting sow performance. It was also hypothesized that these benefits would be most apparent in immature sows receiving dietary CLA supplementation. To this end, the objectives of the research contained within this thesis were to: 1) Monitor changes in sow body composition from mid-gestation through to post-weaning rebreeding while assessing changes in circulating plasma fatty acid composition and serum immunoglobulin concentrations for both sows and piglets and 2) Determine whether enhanced passive immune transfer could extend into the nursery period, allowing for greater disease resistance for piglets weaned from CLA supplemented sows.

CHAPTER 2

LITERATURE REVIEW

The health benefits associated with conjugated linoleic acid (CLA) are numerous and span many species models. To date, CLA has been shown to have potential benefits in the treatment of cancer (Belury, 2002a; Lee et al., 2005), atherosclerosis (McLeod et al., 2004), diabetes (Belury, 2002a), and immune related conditions (Hontecillas et al, 2002; O'Shea et al., 2004) within rodent, livestock and human models (Sugano et al., 1998; Bassaganya-Riera et al., 2002). CLA has also been shown to affect nutrient repartitioning via reductions in adipose stores (Bee, 2001). However, inconsistencies exist within the current body of CLA research (Kelly and Erickson, 2003). The focus of this literature review will be to highlight key research areas where CLA has been investigated, establish areas of swine production where dietary CLA supplementation may be most effective, summarize the development of passive immune transfer between sows and piglets while outlining important events in enteric pathology common to new-born piglets and finally to present potential mechanisms by which CLA may bring about these physiological benefits.

CLA Synthesis

Biological Production

Conjugated linoleic acid is a family of octadecadienoic polyunsaturated fatty acids (PUFA) whose isomers are characterized by various geometrical and positional diene double bonds (Banni et al., 2004) and which occur naturally in ruminant meat and dairy products. Of the multiple isomers, the most common naturally occurring is cis-9,

trans-11 (c9-t11), accounting for more than 80% and 75% of CLA in milk fat and beef fat, respectively (McGuire and McGuire, 1999). The biologically active trans-10, cis-12 (t10-c12) isomer occurs in lower concentrations naturally. However, all constituent isomers vary with environment, diet, species and genetics (Jahreis et al., 1999).

Production of CLA is primarily due to incomplete biohydrogenation of dietary fatty acids. Linoleic (18:2, n-6) and linolenic (18:3, n-3) acids serve as precursors of c9-t11 CLA and c9-t11 conjugated linolenic acid, with both being biohydrogenated by rumen bacteria into vaccenic acid (18:1, t11) which can be directly absorbed or further metabolized by rumen microbes (Pariza et al., 2001). Absorbed vaccenic acid is converted to c9-t11 CLA by Δ-9 steroyl CoA desaturase within mammary tissue prior to secretion into milk (Mosely et al., 2006).

Excess dietary fat inhibits the process of biohydrogenation. When this occurs, linoleic acid can be converted directly into c9-t11 CLA via isomerization by rumen bacteria and is subsequently absorbed and accumulates within muscle tissues (Pariza et al., 2001). The formation of t10-c12 CLA is also largely due to isomerization of linoleic or linolenic acids. It has been suggested that since mammals lack the Δ-12 desaturase enzyme, microbial isomerases convert dietary linoleic acid directly to CLA, serving as the principle route of t10-c12 generation (Pariza et al., 2001).

CLA has also been shown to occur naturally in non-ruminant species. Some commencal enteric microorganisms such as *Lactobacillus sp* and *Bifidobacterium sp* are able to produce CLA directly from linoleic acid via isomerization (Ogawa et al., 2005). However, since absorption of long-chain fatty acids occurs in the upper gut of monogastric species, CLA produced in the large intestine would contribute little to

circulating concentrations. Finally, Tsuzuki et al (2006) recently showed that production of CLA in monogastrics may be independent of microbial metabolism. In this study, it was shown that c9-t11 CLA was produced by Δ13-desaturation of conjugated linolenic acid by visceral organs.

Commercial Production

The majority of research conducted on CLA utilizes synthetic preparations which generally contain approximately equal proportions of c9-t11 and t10-c12 isomers along with other isomers with less biological activity (Pariza et al., 2001). Typically, vegetable oils with high concentrations of linoleic acid such as corn, safflower and soybean oils are used for CLA production. In these matrices, linoleic acid must first be converted from triglyceride to free fatty acid form before CLA production can begin. High temperature hydrolysis combined with an acid catalyst is one method of producing free fatty acids from glycerides. However, alkali hydrolysis at moderate temperatures results in purer fatty acid yields, since unlike acid hydrolysis, the product is formed by an irreversible reaction (Reaney et al., 1999). Free linoleic acid is then converted to CLA in a reaction vessel by alkali isomerization.

CLA can also be produced through the use of fermentation technology. As mentioned above, lactic acid bacteria have the ability to produce CLA via isomerization of linoleic acid. Large scale commercial production of CLA by way of microbial fermentation and enzymatic conversion via isolated isomerases is currently being investigated as alternatives to alkali isomerization. This is due to the high purity yields that have been obtained to date, without the use of potentially hazardous chemicals (Lin

et al., 2003; Pariza et al., 2001).

CLA in Health and Disease

Body Composition

The ability of CLA to modulate body condition has been demonstrated in multiple experimental animal models. Chief among these is the effect CLA has been shown to have on reducing adipose tissue accretion. In growing pigs, Ostrowska et al. (1999) reported that CLA linearly reduced whole carcass percent fat while increasing percent lean tissue, results similar to findings reported elsewhere (Dugan et al., 1997; Thiel-Cooper et al., 2001). However, Bee (2001) reported that although CLA modulated the fatty acid isomer constituents within adipose tissue, dietary supplementation had no significant ability to reduce total carcass fat percentage in the sow. This apparent variability was explained by Kelly and Erickson (2003) to be due to age differences (e.g. nursery vs. grower vs. finisher), plus the concentration and isomer composition of the CLA source used and inconsistencies with techniques used to measure body fat composition. In terms of isomer effects, it should be noted that t10-c12 has been shown to be the most potent modulator of adiposity (Belury, 2002a).

In humans, the actions of CLA in relation to body composition are less clear. Reviews by both Belury (2002a) and Kelly and Erickson (2003) discuss studies that showed that mixed isomer CLA supplementation at various dietary concentrations reduced adipose tissue accumulation or had no effect on body condition. These observations are supported by the work of Desroches et al. (2005) who observed no metabolic changes in obese men provided CLA supplemented diets. These authors

concluded that a paucity of data exists and conclusions made regarding the *in vivo* effects of CLA supplementation to humans would currently be premature.

Type-2 Diabetes

Literature surrounding the effects of CLA on the metabolic dysfunction characteristic of type-2 diabetes is mixed. Animal models have been able to show that CLA has promise in combating the onset of type-2 diabetes while human trials have yet to reach such a consensus. *In vivo* rodent studies have shown that CLA can reduce fasting insulin, leptin, glucose, triglycerides, and free fatty acid concentrations (Aminot-Gilchrist and Anderson, 2004; Taylor and Zahradka, 2004). However, as previously mentioned the therapeutic benefits of CLA have yet to be clearly shown in clinical trials. Moloney et al. (2004) reported conflicting results, with mixed isomer supplementation improving cholesterol metabolism while concurrently increasing fasting glucose levels and reducing insulin sensitivity. Furthermore, when diets were supplemented with purified c9-t11 isomers, insulin resistance was increased in obese men (Riserus et al., 2004). It is thus appears that, similar to body compositional changes, t10-c12 may be more helpful in ameliorating diabetic symptoms as seen in studies comparing purified to mixed isomer supplemented diets. Whether or not these benefits can be attributed to the ability of CLA to repartition dietary nutrients or to other mechanisms has yet to be clearly demonstrated.

Cancer

Since the initial observation that CLA could prevent the formation of tumors, numerous studies and reviews have offered similar conclusions (Ha et al., 1987; Lee et al., 2005; MacDonald, 2000; Pariza et al., 2000). From these, it seems that CLA can act at multiple points in tumor development. Three major stages of development where CLA appears to have noticeable effects as outlined by Lee et al. (2005) are initiation, promotion and progression. Here, initiation refers to DNA damage leading to mutagenesis; promotion refers to the expansion of an actively dividing mass of cancerous cells; and progression refers to the stage at which the growing tumor becomes able to spread and become increasingly malignant. However, due to the broad nature of cancer pathology combined with the wide-ranging activity spectrum of CLA, the means by which CLA facilitates prevention has yet to be determined.

One hypothesis surrounding the ability of CLA to prevent tumor development rests in its multiple biological activities. CLA has been shown to be a potent antioxidant and able to scavenge multiple free radical species (Yu, 2001; Yu et al., 2002b). If CLA can reduce the detrimental activities associated with free radical formation, DNA damage and subsequent mutagenesis could be avoided. CLA has also been shown to have anti-inflammatory properties, particularly in affecting the synthesis of prostaglandin E_2 (PGE_2; Yu et al., 2002a). PGE_2 has been associated with tumor development, although a direct mechanism has yet to be determined (Cunningham et al., 1997; Lee et al., 2005). Runaway cell growth due to a lack of apoptosis is characteristic of malignant tumors. CLA has been shown to facilitate apoptosis in some cell types which may be how it contributes to reducing tumor progression (Lee et al., 2005).

Immune Function

Some of the most promising evidence surrounding the efficacy of CLA has focused on immune modulation within animal models. In chickens, CLA has been shown to reduce growth depression and muscle catabolism associated with immune stimulation (Cook et al., 1993). In rats and pigs, dietary CLA supplementation has been associated with increased immunoglobulin production (Sugano et al., 1998; Yamasaki et al, 2000a; Bontempo et al., 2004). CLA has also been shown to affect T-cell subpopulations with a greater ratio of CD8 (natural killer cells) to CD4 (cytotoxic cells) being observed in supplemented animals (Bassaganya-Reira et al., 2001). Cytokines associated with immune functioning have also been shown to be affected by CLA supplementation. In multiple tissue types, CLA supplementation has been associated with reduced expression of proinflammatory cytokines tumor necrosis factor alpha (TNF-α) and interferon gamma (INF-γ; Yu et al., 2001; Hontecillas et al., 2002). Within the livestock industry, immune stimulation is a known contributor to depressed growth performance. With numerous studies demonstrating that CLA can stimulate immune function without reducing growth performance there is thus great potential for CLA within animal production science.

CLA and the Pregnant Sow - Reproductive Performance

Lipid metabolism is extremely dynamic in the pregnant sow. Modern production systems have focused on maximizing litter size and piglet output and as a result the nutritional demands on the sow have become considerable (Knol, 2003). Large litters require the dam to produce large quantities of milk to meet the rapidly growing piglets' nutritional demands. Growing piglets have high energy requirements and fat represents a

major energy source within milk. This means that lactating sows must have adequate fat reserves to ensure the energy needs of the suckling piglets are met. However, adequate fat reserves tend to be seen in older sows, since early pregnancies tend to occur while animals are still undergoing growth and development (Slevin and Wiseman, 2003). Thus, first and second parity sows represent an opportunity where nutritional management may be able to improve sow and litter performance. Consequently, CLA may represent a novel supplement to take advantage of this opportunity.

Fatty acids play an important role in reproductive function and performance. Linoleic acid is the precursor to arachidonic acid, which through several enzymatic reactions yield prostaglandins. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and PGE_2 both contribute to the signaling, maintenance and termination of pregnancy (Ziecik, 2002). Here, $PGF_{2\alpha}$ is luteolytic participating in normal cycling while it has been proposed that PGE_2 may act to counter-balance the actions of $PGF_{2\alpha}$ with a critical ratio being needed in order for pregnancy to be maintained (Ziecik, 2002). As a result, changes in dietary fatty acids, such as incorporation of CLA, may affect the production of these compounds, possibly leading to important implications during pregnancy.

For breeding sows, successful pregnancy is a function of proper nutrition and body condition. Commercial feeding programs focus on minimizing the occurrence of under or over conditioned animals because these tend to have poor performance. An intricate system of hormonal signals exist which relays information regarding body condition to the central nervous system and the rest of the reproductive axis. Hormones such as insulin, insulin like growth factor-1 (IGF-1) and leptin have all been shown to be related to the hypothalamic-pituitary gonadal axis in pigs (Barb et al., 2001a). In general,

these hormones act permissively, allowing normal release of gonadotropins during optimal conditions and preventing release during times of under or over nourishment (Barb et al., 2001a; Smith et al., 2002).

In the pig, a strong link exists between adipose reserves and reproductive competency. As outlined previously, CLA has been shown to have numerous physiological and nutritional modulating properties including the ability to influence circulating hormones such as leptin, insulin, and IGF-1 (Bontempo et al., 2004). Of these, leptin would appear most sensitive to dietary CLA supplementation because of its known link with adiposity and reproductive competency (Barb, 2001b).

Leptin receptors and receptor mRNAs have been found along the reproductive neuroendocrine axis of multiple species including pigs (Henry et al., 1999; Iqbal et al., 2000; Lin et al., 2001; Spicer, 2001). In the pig, it appears that leptin is most noticeable at the hypothalamus and pituitary in affecting gonadotropin releasing hormone (GnRH) and leutinizing hormone (LH) release (Barb et al., 2001b). It has been suggested that leptin may indirectly influence the release of these hormones by interacting with other neuroendocrine compounds such as pro-opiomelanocortin (POMC), and melanocortin stimulating hormone (MSH) which are synthesized in proximity to GnRH neuron centers (Smith et al., 2002). In addition to POMC neurons, neuropeptide-Y (NPY) and Agouti related peptide (AgRP) neurons are in proximity to GnRH centers and the expression of these neurotransmitters in the pig have been shown to be affected by both lipid status and circulating leptin levels (Barb et al., 2001b; Smith et al., 2002; Bajari et al., 2004).

Few studies have focused on the effects of CLA supplementation on circulating leptin levels. In rats, one week of CLA supplementation significantly reduced circulating leptin levels (Yamasaki et al., 2000b). However, after 10, 18 and 28 days of CLA supplementation to lactating sows, leptin was significantly elevated while insulin and IGF-1 remained unaffected (Bontempo et al., 2004). Interpreting these differences becomes difficult due to the paucity of data surrounding dietary CLA and leptin. If CLA is able to increase circulating leptin in lean gilts or sows, one could speculate that dietary supplementation may improve reproductive capacity during first and second parities and may also boost reproductive performance in these animals.

CLA and Passive Immune Transfer

Pre-weaning mortality is a substantial economic concern for swine farmers. According to Edwards (2002), upwards of 20% of piglet mortality in the UK can be accounted for by low viability (3%), starvation (15%) and scours (3%), which are predominantly rooted in inadequate colostrum intake or passive immunity failure. In light of these data, the increasing trend for large scale production units and a growing trend to restrict the use of in-feed antibiotics have accentuated the need to improve passive immune transfer (McGlone and Johnson, 2003).

Piglets are born in a "hypo-immune" state because transfer of immunoglobulins to the fetus is impeded by the nature of the porcine placenta (Pabst and Rothkotter, 1999). New-born un-suckled piglets have been observed with circulating immunoglobulins presumed to be non-functional due to their molecular structure (Rooke and Bland, 2002). Thus, in the first days of life, maternally derived immunoglobulins present in colostrum

and milk serve to protect the immune-challenged piglet.

The new-born piglet must consume colostrum within approximately the first 12 – 24 hours of life in order for maximal survival (Le Dividich et al., 2005). It is during these first hours that large macro-molecules such as IgG in the colostrum are able to cross the gastro-intestinal tract (GIT) mucosa. After this time, cell populations within the GIT undergo maturational shifts known as gut closure and no-longer absorb such compounds (Rooke and Bland, 2002). Since 7 to 10 days are required until non-maternal immunoglobulins can be detected in the serum of piglets, ingestion of colostrum and milk during the first week of life is imperative to the ability of piglets to fend off pathogens and resist disease (Drew and Owen, 1988).

The types of antigens that piglets are exposed to change during the first week of life. As a result, the categories of immunoglobulins, growth factors, lymphocytes, and biologically active peptides within colostrum and milk that are responsible for fighting these antigens change accordingly. The primary colostral immune compound is IgG, which is transferred from the sow plasma by specific mammary receptors (Huang et al., 1992). The new-born piglet requires rapid intestinal development to support growth while having the simultaneous ability to fend off a broad variety of pathogens, both of which are accomplished by the wide immunological properties of IgG (Drew and Owen, 1988). By 7 to 10 days after birth and until weaning, piglet B-cells are beginning to be able to synthesize endogenous immunoglobulins. It is during this time that piglets face a greater risk for developing mucosal infections, such as from enterotoxigenic *E. coli* (ETEC). There is thus a concomitant increase in IgA within the sow's milk from approximately 5 to 7 days until weaning during which point IgG concentrations begin to

decline (Le Dividich et al., 2005). As opposed to IgG which is predominantly a systemic antibody, IgA is primarily associated and secreted by the immune cells associated with mucosal tissues (O'Shea et al., 2004). Here, mucosal IgA provide protection by preventing the adherence of pathogenic bacteria in a process known as immune exclusion (Mestecky et al., 1999; Gaskins, 2006).

It is currently unclear as to the carry-over duration of passive immune transfer after weaning. Studies have shown that a positive relationship exists between post-weaning survival and serum immunoglobulin titers at the time of weaning (Le Dividich et al., 2005). Nutritional agents that increase immunoglobulin production, such as CLA, could serve to increase piglet immunity during suckling and potentially after weaning as well. Bontempo et al. (2004) showed no effect of sow milk CLA on piglet IgG levels 15 days post-weaning, however, these sows only received CLA for 8 days prior to farrowing, which may not have been enough time for CLA to affect sow immunoglobulin production. In the same study, piglets receiving CLA via suckling and which were given CLA supplemented nursery diets only showed elevated IgG titers after 25 days post-weaning or approximately 52 days of age. These data are similar to another study which found that piglets consuming post-weaning diets supplemented with CLA experienced immune stimulation after 26 days or 42 days of age (Bassaganya-Riera et al., 2001). Further studies investigating the effects of CLA supplementation throughout gestation and lactation on piglet immune function would be warranted to establish if immune stimulation can be observed in piglets within approximately 3 weeks post-weaning.

CLA and Weaned Piglets

The ability of CLA to enhance immunity in piglets is important within the pork industry since it is well documented that nursery mortality, presumably due to insufficient immunoglobulin transfer from sow to piglet via colostrum and milk represents an economic liability to producers. Rooke and Bland et al. (2002) have shown that nutritional intervention is a practical means of increasing colostrum immunoglobulin concentrations. In addition, it has been shown that inclusion of CLA into the nursery piglet diet increases the ability of piglets to respond to an antigen challenge while protecting against catabolic effects commonly observed with immune system stimulation (Bassaganya-Riera et al., 2001, 2004; Weber et al., 2001).

Numerous studies have demonstrated that CLA has an immunomodulatory capacity. Corino et al. (2002) demonstrated that serum IgG concentrations can be increased when 45 day old piglets are provided with CLA for 28-days, while supplementation for 26 days was sufficient to modulate T-cell subpopulations and increase white blood cell counts (Bassaganya-Riera et al., 2001). Additionally, Bontempo et al. (2004) observed enhanced IgG concentrations in colostrum and serum of sows fed CLA supplemented diets. Serum IgG titers in piglets suckling these sows were also elevated compared to controls, indicating an ability of CLA to promote passive immunity transfer between dam and offspring. These data provide evidence that the immune-enhancing capability of CLA may extend to the pre-weaned and newly weaned piglet with potential to benefit producers by reducing post-weaning morbidity and mortality.

Post-Weaning Diarrhea

Post-weaning diarrhea due to enteric *E. coli* infection is a leading cause of piglet mortality worldwide and is generally accepted to be accented by the stress of weaning, lack of antibodies present in sow's milk and changes in dietary regimes (Cardinal et al., 2006). The adoption of early weaning by producers has been attributed to a worldwide increase in *E. coli* related diarrhea infections that are typically associated with $K88^+$ (F4) *E. coli* (Fairbrother et al., 2005). The $K88^+$ serotype along with others such as F18 are typically described as enterotoxigenic *E. coli* (ETEC) due to their shared characteristic of expressing adhesins allowing for adhesion to the intestinal wall, along with the secretion of toxins responsible for diarrhea (Fairbrother et al., 2005).

The $K88^+$ and F18 designations given to these serotypes are based on characteristic fimbriae expressed on the cell surface of these bacterial types. Fimbriae act as cellular appendages assisting bacteria in substrate attachment. Four variants of $K88^+$ fimbria have been identified each having a conserved "a" region and variable b, c and d regions (Fang et al., 2000). Genes responsible for encoding these fimbriae are located on plasmids and the expression of these genes has been shown to be affected by environmental factors such as temperature, with expression proceeding at 37°C and ceasing at 18°C, an attribute beneficial when the organism is in an ideal environment such as the GI tract.

Following ingestion of either $K88^+$ or F18 ETEC, colonization of the small intestine can proceed rapidly when conditions are favorable and if supported by interactions between fimbriae and intestinal epithelial receptors (Francis et al., 1998). The degree of proliferation along the small intestine determines the level of pathology

observed. Fimbriae are able to bind to specific receptors associated with intestinal cell membranes or aspecifically to the intestinal mucosal layer and typically populate the small intestine from the proximal jejunum to the distal ileum (Fairbrother et al., 2005).

Diarrhea associated with ETEC infection is brought about through the production and subsequent release of enterotoxins by colonizing bacteria. In the case of $K88^+$, the most commonly associated enterotoxin is heat-liable toxin (LT). The LT is an 84 kDa hexameric protein with an enzymatically active A subunit which binds its receptor via five B subunits (de Haan and Hirst, 2004; Nagy and Fekete, 2005). Both subunits are synthesized in the cytoplasm, transported to and assembled in the periplasm and stored until released (Nagy and Fekete, 2005).

The LT binds with the greatest affinity to GM1 gangliosides, which are galactose rich glycosphingolipids located in the outer leaflet of the plasma membrane of many cell types including enterocytes (Angstrom et al., 1994; de Haan and Hirst, 2004). The GM1 ganglioside has been shown to be associated with the detergent insoluble glycosphinglolipid-rich membrane domains which are important constituents of lipid rafts and therefore play a role in cell signaling. Binding to GM1 initiates endocytotic internalization of LT followed by delivery of the endosome bound toxin to the Golgi network where it undergoes retrograde trafficking to the endoplasmic reticulum (ER) (de Haan and Hirst, 2004).

Full toxicity is only achieved when the A subunit is cleaved from its B-subunits (de Haan and Hirst, 2004). Thus, after retrograde transport through the Golgi network the disulphide bond connecting the A and B portions of LT is cleaved enzymatically via protein-disulphide isomerase (PDI; Orlandi, 1997). The A subunit, bound to PDI is then

transported to the Sec61p channel of the ER, a pore protein that plays a central role in the translocation of proteins from the ER into the cytosol (Lencer et al., 1999). During this migration, the A-PDI complex associates with Ero1, an oxidase enzyme which participates in the release of the A unit from PDI and once released from PDI, the A unit is chaperoned into the cytosol via Sec61p (de Haan and Hirst, 2004).

Watery diarrhea and intestinal fluid accumulation characteristic of ETEC infection is initiated with the arrival of free LT A-subunit into the cytoplasm. Interaction between A-subunit and G-protein leads to the activation of adenylate cyclase resulting in elevated intracellular concentrations of cyclic adenosine monophosphate (cAMP; de Haan and Hirst, 2004; Fairbrother et al., 2005). The cAMP serves as a signal intermediary for multiple cellular processes and in the case of enterocytes may include: (a) activation of protein kinase A (PKA) that in turn phosphorylates and activates chloride (Cl^-) channels increasing secretion of Cl^- (Nataro and Kaper, 1998), (b) increasing Cl^- secretion via interaction with the cystic fibrosis transmembrane conductance regulator (CFTR; Thiagarajah and Verkman, 2003), (c) induction of phospholipase-A2 (PLA-2) synthesis leading to liberation of arachidonic acid and subsequent production of E-series prostaglandins (PGE) which may increase Cl^- secretion (Nataro and Kaper, 1998) and (d) secretion of 5-hydroxytryptamine (5-HT, serotonin), a smooth muscle contractor that may also play a role in prostaglandin synthesis and Cl^- secretion (Turvill et al., 1998). Thus, the accumulation of Cl^- into the intestinal lumen coupled with reduced absorption of sodium ions creates a hypertonic environment leading to osmotic movement of water into the intestinal lumen resulting in severe diarrhea,

morbidity and mortality (Andren and Persson, 1983; de Haan and Hirst, 2004; Fairbrother et al., 2005).

Potential Mechanisms of Action

A consensus has not yet been reached as to the means by which CLA exerts its biological effects. Some have suggested that CLA follows a metabolic pathway which results in a 20 carbon structure which is incorporated into plasma membranes and is able to compete with arachadonic acid and subsequently affects eicosanoid production (Belury, 2002a). Others have hypothesized that CLA primarily exerts its function at the cellular or molecular level, acting as a ligand for receptors associated with various inter- and intra-cellular functions (Moya-Camarena et al., 1999).

In addition to mechanistic actions on hormone production and cellular receptor interactions, CLA may exert effects by alternative means. As summarized before, CLA has antioxidant properties, beneficial in attenuating mutagenesis and tumor onset. However, variability between isomers and actions on different radical species is still under debate. CLA may also affect metabolic enzyme activity, specifically stearoyl-CoA desaturase (SCD), the enzyme responsible for the rate limiting step in fatty acid desaturation with implications relating to the ability of CLA to modulate body nutrient partitioning. In weaned pigs fed CLA diets, Smith et al. (2002) observed that desaturation activity within subcutaneous adipocytes was significantly lower than in those pigs fed diets supplemented with tallow and concluded that desaturase inhibition may be a means by which CLA reduces adiposity. However, the means by which CLA isomers cause this inhibition remains unknown.

Research focusing on the molecular activities of CLA is still in its infancy. However, the current literature suggests that the primary means of action are at the hormonal and intra-cellular receptor levels. This is because the most practical models explaining the multiple physiological effects of CLA are constructed when built in the context of hormonal, sub-cellular and genetic functioning.

Hormone Interactions - Eicosanoid Synthesis

Research has shown that dietary lipid intake can modify cell membrane PUFA isomer constituents. Thus, a proposed mechanism of CLA is via cell membrane incorporation and downstream alterations in eicosanoid production (Eberhart and Dubois, 1995).

Eicosanoids are hormones derived from long chain fatty acids and include prostaglandins, thromboxanes and leukotrienes (Eberhart and Dubois, 1995). Prostaglandins and thromboxanes are primarily proinflammatory exerting vasodilatory and vascular permeability effects (Hadley, 2000). Leukotrienes are also involved in vascular permeability but generally have vasoconstriction properties (Hadley, 2000). Arachidonic acid is a 20 carbon fatty acid with 4 double bonds of the n-6 series, serves as the parent compound for the majority of eicosanoids (Belury, 2002a). Although small amounts of arachidonic acid are obtained via food stuffs, the major source of AA is through *de novo* synthesis via the metabolism of dietary linoleic acid (18:2, n-6, LA). Belury (2002a) proposed the pathway shown in figure 1 as a possible route by which LA is converted to arachidonic acid.

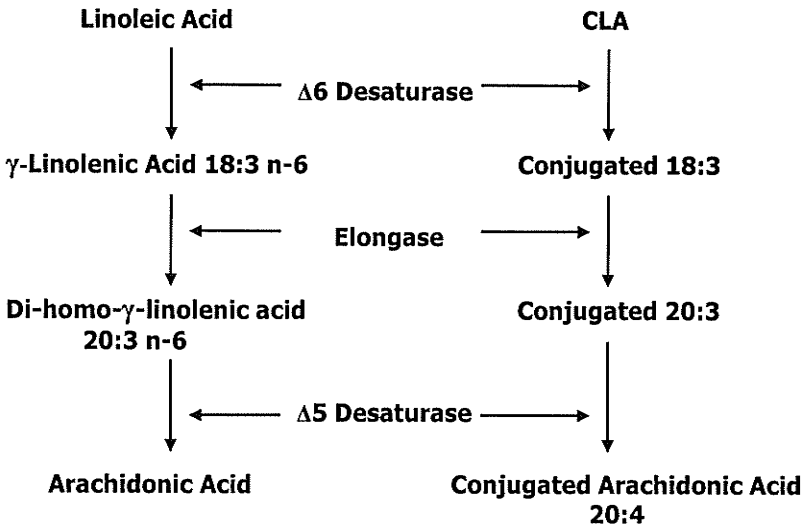

Figure 1. Metabolic pathway leading to the formation of arachidonic and conjugated arachidonic acid from LA and CLA as proposed by Belury (2002a).

Cyclooxygenase (COX) and lipoxygenase (LOX), both located in the ER, are rate limiting steps for the synthesis of prostaglandins, thromboxanes and leukotrienes, respectively (Lee and Katayama, 1985). As in figure 2, upon release of bio-accumulated arachidonic acid from the plasma membrane by phospholipase A_2 (PLA_2), available arachidonic acid is converted into prostaglandins and thromboxanes by two principal COX isoforms (COX-1 expressed constitutively and inducible COX-2) and into leukotrienes by LOX (Eberhart and Dubois, 1995). Selectively blocking or providing alternative substrate for these enzymes can manipulate the physiological effects associated with their downstream compounds. For example, non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin act by irreversibly acetlyating one serine residue on both COX isoforms which changes the shape of the enzyme and prevents access to arachidonic acid and subsequently reduces prostaglandin synthesis (Lee and Katayama, 1985). Thus, aspirin reduces inflammation, blood platelet agglutination and

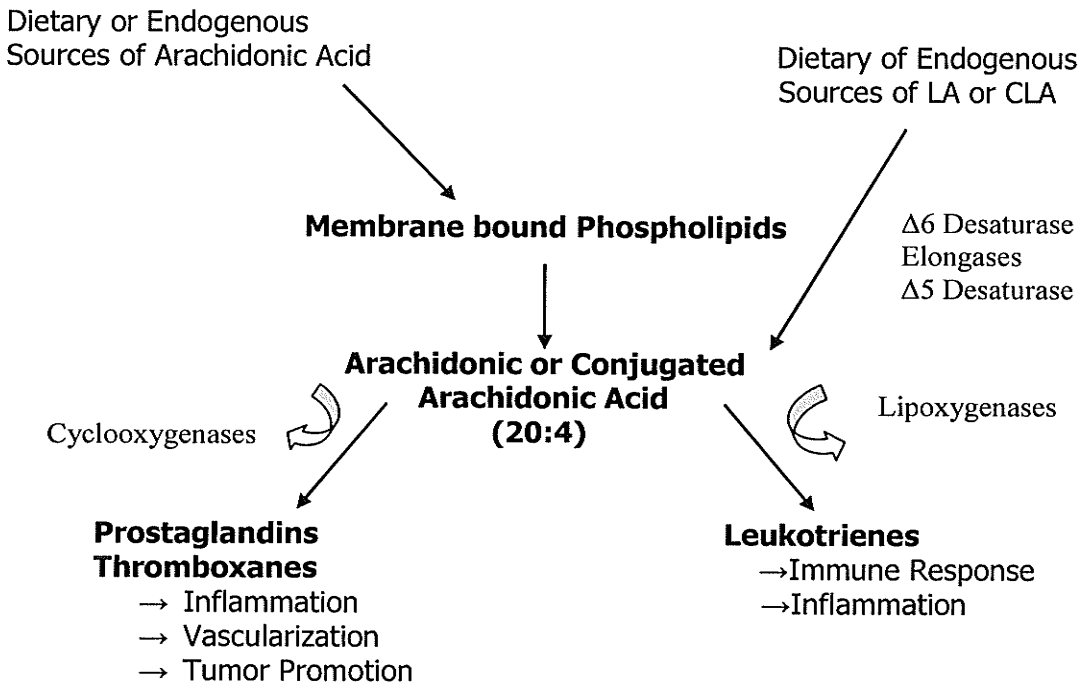

Figure 2. Outline of eicosanoid synthesis from dietary or endogenous arachidonic acid and/or from dietary LA or CLA as proposed by Belury (2002a).

insult injury associated edema associated with PGH_2, PGI_2, TXA_2 respectively (Lee and Katayama, 1985). Other NSAIDs such as Vioxx specifically inhibit COX-2 reducing pain and inflammation without deleterious side-effects associated with prolonged aspirin consumption (e.g. gastrointestinal bleeding and/or gastric ulceration due to reduced PGE synthesis). However, COX-2 blockers have recently been linked with heightened risks of heart attacks and many have been withdrawn from public markets.

Eicosanoid production can also be influenced by dietary PUFA intake. Omega-3 PUFA such as α-linolenic acid (18:3), eicosapentaenoic acid (EPA, 20:5) and docosahexanoic acid (DHA, 22:6) have all been shown to exert physiological effects through competition with arachidonic acid (Stulnig and Zeyda, 2004). Research has shown that these fatty acids (especially EPA) will act as substrate competitors with

arachidonic acid for COX and LOX. Serhan et al. (2000) showed EPA incubated with human peripheral mononuclear (PMN) cells *in vitro* produced 18R-hydroxy-eicosapentaenoic acid (18R-HEPE) and 15R-HEPE which were further metabolized into leukotrienes with oxygen inserted at carbon 5 (i.e. 5-series), differing from arachidonic acid metabolism which yields 15-hydroperoxyarachidonic acid and 4-series leukotrienes. These 5-series leukotrienes were shown to be less potent activators of micro-inflamation in comparison to traditional 4-series members.

CLA has been shown to follow a similar metabolic route as LA, utilizing the same Δ6-desaturase, elongase, Δ5-desaturase enzyme combination (Banni, 2002; Banni et al, 2004). It is, thus, conceivable that the resulting 20:4 fatty acid metabolite would be a potential substrate for the COX or LOX enzymes. Numerous *in vivo* and *in vitro* studies have shown that CLA reduces PGE_2 production in multiple tissues including placenta and uterus (Harris et al., 2001), bone (Li and Watkins, 1998) and liver (Turek et al., 1998) among others. This suggests that CLA has the ability to manipulate prostaglandin synthesis by either competing with arachidonic acid as COX substrate or by inhibiting the actions of COX all together. In fact, Bassaganya-Riera et al. (2002) reported that interaction of COX isoforms and 20:4 CLA metabolites may produce a novel series of prostaglandins and leukotrienes (potentially similar to those observed by Serhan et al. (2000) which may have different biological activities compared to traditional series.

If in fact consumption of CLA leads to changes in eicosanoid subsets, the reproductive and immune systems would most likely be affected (Rotondo and Davidson, 2002). As mentioned previously, PGE_2 and $PGF_{2\alpha}$ play critical roles during pregnancy and during normal cycling in the sow. If one or both of these series had altered activity

levels, reproductive performance may be affected. Similarly, the inflammatory and immune-related activities of eicosanoids will be affected by variations in activity. However, further research is justified since the outcome of eicosanoid modification through dietary augmentation within these systems has yet to be defined.

CLA and PPAR Interaction

Peroxisome proliferator-activated receptors (PPARs) are high affinity nuclear receptors which act as inter-cellular lipid sensors (Belury, 2002a). To date, PPARs have been associated with a diverse array of physiological processes including lipid metabolism, energy balance, thermogenesis, glucose metabolism, carcinogenesis and immune functioning (O'Shea et al., 2004; Feige et al., 2006). Four isotypes have currently been identified, PPAR-α and PPAR-β are very similar in their expression patterns both being found in lipid rich tissues such as adipose, liver, kidney, heart and intestine. The other two, PPAR-γ and PPAR-δ, have been isolated from adipocytes where they have been associated with cellular differentiation, as well as from lymphoid and immune cells where a clear action pathway has yet to be determined.

From a dietary standpoint, it appears that PPARs function to link dietary lipids with various genes possessing peroxisome proliferator response elements (PPRE) therefore allowing for adaptation to dietary fat content and type (Jump and Clarke, 1999). CLA are powerful modulators of PPARs (Belury et al., 2002; Bassaganya-Riera et al., 2004), and changes in expression profiles associated with PPAR activation are thought to be one potential means by which CLA exerts physiological effects.

Consumption of high fat diets is a known risk factor for the development of

obesity and type-2 diabetes and it has been suggested that one means by which CLA may combat these conditions is via activation of PPARs. Indeed, reviews by Taylor and Zahradka (2004) and Belury (2002a) have detailed the ability of CLA to activate PPAR-α and subsequently restore insulin sensitivity and reduced body weight. It has been shown that activation of PPAR-α is associated with increased expression of fatty acid binding protein (FABP), acyl Co-A oxidase (ACO) and cytochrome P450 4A1 within hepatic tissue (Peters et al., 2001; Barish, 2006). It has also been demonstrated that insulin sensitizing drugs (i.e. thiazolidinediones) target PPAR-γ (Barish, 2006), highlighting the potential role of co-activation as a means of CLA's action. However, it is unclear how changes in these expression products affect the pathology associated with obesity and type-2 diabetes and whether other as of yet unidentified expression products play an indirect or direct role in the ability of CLA to combat symptoms of obesity and insulin resistance (Peters et al., 2001; Taylor and Zahradka, 2004).

CLA has been implicated in reduction of inflammation within a range of animal models and a growing literature of work has begun to link activation of PPARs with this stimulation. For example, Yu et al. (2002a) showed that CLA decreased expression of pro-inflammatory cytokines TNF-α, interleukin-1β and interleukin-6 within mouse macrophages and linked these reductions with PPAR-γ activation. Additionally, PPAR-γ was shown to be activated by CLA within pig colonic cells experimentally infected with *Brachyspira hyodysenteriae* and that induction was associated with reduced colonic inflammation and reduced expression of INF-γ and interleukin-10 (Hontecillas et al., 2002). These data suggest that the expression products of PPAR activation may serve as molecular linkages for dietary CLA, potentially abating inflammation associated with

immune activation by reducing the expression of pro-inflammatory cytokines. Indeed, in an experimental model of colitis, CLA up-regulated the expression of PPAR-γ and PPAR-δ with concurrent activation resulting in reduced expression of TNF-α and reduced colonic epithelial inflammation (Bassaganya-Riera et al., 2004).

The fact that CLA has been shown to have agonistic effects on PPARs merits further research. CLA has been repeatedly shown to activate PPAR within adipocytes, circulating immune cells and colonic epithelial and immune cells of pigs and other animal models (Moya-Camarena et al., 1999; Yu et al., 2002a; Bassaganya-Riera et al., 2004, 2006). The activities of the expression products of PPAR activation though have not clearly been established. In terms of intestinal infection and inflammation, it seems that PPARs reduce pro-inflammatory cytokines within the lower gut. However, there is little to no data describing any similar anti-inflammatory relationship between CLA and PPAR activation within the small intestine, where a high infection risk exists within newly-weaned piglets. There is also limited data on how the expression products of PPAR activation affect immune cell sub-populations which may potentially have an impact on B-cells and thus immunoglobulin production presumably within the sow and piglet. Although evidence exists that CLA mediates biological effects through PPAR activation, a clear pathway must still be established through future research.

Conclusions

From this review it can be seen that dietary CLA has broad biological effects across multiple species models. Currently, many clear cause and effect results from dietary CLA supplementation have been shown. However, the exact route by which

these effects arise, whether at the cellular, molecular or genetic level, have yet to be defined. Additionally, the fact that different isomers have been shown to have different effects and that animal studies tend to incorporate mixed isomers, may contribute to the diverse biological effects of CLA that exists in the current literature. Research is beginning to become focused on single isomer supplementation however this can be expensive, especially in large animal models such as the pig.

In the sow and piglet, the most promising effects of dietary CLA relate to nutrient repartitioning and the ability to stimulate the immune system. Modern production techniques have increased the demand for sows to produce larger litters with rapidly growing piglets, which has increased the demand on producers to meet the nutritional and physiological needs of these animals. If CLA can be shown to reduce the stress being placed on these animals by either improving nutrient retention capacity of pregnant and lactating sows and/or improving the immune status of sows and subsequent immune transfer to piglets, inclusion of CLA into sow and piglet diets could be a benefit to swine producers.

CHAPTER 3

MANUSCRIPT ONE

Potential of dietary conjugated linoleic acid (CLA) when provided to sows during gestation and lactation to improve sow and piglet performance and immune status

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ABSTRACT

Fourteen Cotswold sows were assigned according to parity (I=Immature or M=Mature) or diet (0% or 2% CLA) to the following dietary treatments: 1) 0%-I, n = 3; 2) 0%-M, n=3; 3) 2%-I, n=4; 4) 2%-M, n=4. Diets were fed as gestation rations from d 85 through d 112 of pregnancy and for 4 d post-weaning and as lactation rations from d 112 of gestation until piglets were weaned at 17 ± 1 d of age. Sow BW, back fat depth, condition score and feed intake were recorded and plasma and serum samples collected on d 85, 105 and 112 of gestation and d 1, 3 and 17 of lactation plus 4 d post-weaning. Piglet BW was recorded on d 1, 3 and 17 of age and plasma and serum collected on d 3 and 17. Plasma samples were analyzed for fatty acid profiles and urea nitrogen concentration and serum samples for IgA and IgG titers. Sow BW and back fat depth were greater in mature sows during gestation and lactation compared to immature sows ($P < 0.05$). Dietary CLA-supplementation had no effect on sow BW during gestation or lactation ($P > 0.10$). Sows receiving CLA-supplemented diets had less back fat than control sows during gestation ($P = 0.05$) but not during lactation ($P = 0.15$). From d 1 of lactation until 4 d post-weaning, immature sows consuming CLA-supplemented diets tended to lose less back fat than immature sows consuming control diets ($P = 0.09$). Average daily feed intake was greater in mature sows than immature sows ($P < 0.001$), but did not differ between dietary treatments ($P > 0.10$). Piglets born to sows receiving CLA-supplemented diets were lighter than piglets born to sows receiving control diets ($P = 0.007$), but total litter wt at weaning was not affected by dietary CLA-supplementation ($P > 0.10$). Mature sows had greater whole body lipid and protein percentages compared to immature sows during gestation and lactation ($P < 0.05$). Dietary CLA-

supplementation increased sow whole body protein percentage during gestation but not lactation ($P = 0.05$). Additionally, dietary CLA had no effect on sow whole body lipid during gestation or lactation ($P > 0.10$). Maturity and dietary CLA-supplementation did not affect sow IgA or IgG concentrations during gestation or lactation ($P > 0.10$). Piglet IgA and IgG concentrations were not affected by dietary CLA-supplementation or by sow maturity ($P > 0.10$). Piglets nursing CLA-supplemented sows had greater PUN concentrations compared to piglets nursing control sows at d 3 and 17 of age. CLA isomers were detectable in sows receiving supplemented diets by d 105 of gestation and were detected in colostrum samples taken from sows receiving CLA-supplemented diets. Although supplementation of sow diets with CLA did not improve litter performance or immune status, when provided to immature sows, dietary CLA may diminish deleterious body condition loses associated with lactation.

Key Words: CLA, litter health, passive immunity, piglets, sows

INTRODUCTION

Increased disease prevalence and general mortality and morbidity in nursery piglets has been attributed to increased sub-optimal transfer of passive immunity from sow to piglet (Drew and Owen, 1988). In a recent survey conducted in the UK, it was suggested that upwards of 20% of piglet mortality could be attributed to poor colostrum intake and incomplete passive immune transfer (Edwards, 2002). In the pig, the physical structure of the placenta prevents the transfer of maternal antibodies to the fetus *in utero* (Pabst and Rothkotter, 1999) and the new born piglet is therefore dependent on colostral

immunoglobulins for survival during the first days of life (Rooke and Bland, 2002). Due to the potential economic impact failed passive immune transfer has on producers, it seems pertinent that means of bolstering this process be explored.

Dietary intervention via conjugated linoleic acid (CLA) supplementation may be a novel means of reinforcing passive immune transfer between sows and piglets. Conjugated linoleic acid is a group of positional and geometric isomers of linoleic acid (Banni, 2002) with proven health benefits to both humans and livestock (Belury, 2002). The ability of CLA to stimulate immune function has been well documented in weaned pigs (Bassaganya-Riera et al., 2001; Corino et al., 2002). In the sow, dietary addition of CLA resulted in isomer enrichment within colostrum and milk (Bee, 2000) as well as increased IgG within these same matrices (Bontempo et al., 2004). However, the current literature regarding the ability of CLA to augment immunity within gestating and lactating sows and their litters is limited.

The objectives of this study were to evaluate the time course of any immunological and physiological changes associated with CLA-supplementation during the last trimester of gestation and throughout lactation in gilts and sows and to determine if these changes were associated with enhanced sow and litter performance and immune status.

MATERIALS AND METHODS

Animals and Diets

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee (Protocol #F05-015) and pigs were cared for

Table 1. Composition and calculated nutrients of sow gestation and lactation diets

	Gestation		Lactation	
Ingredient, % CLA, %:	0	2	0	2
Barley	60.75	60.75	29.3	29.3
Wheat	---	---	33.53	33.53
Wheat shorts	23.0	23.0	11.2	11.2
Canola meal	5.00	5.00	4.50	4.50
Soybean meal, 48%	4.50	4.50	14.9	14.9
Canola oil	3.20	---	3.20	---
CLA-60[1]	---	3.20	---	3.20
Biophos	1.40	1.40	1.20	1.20
Limestone (eggshell)	1.65	1.65	1.50	1.50
Salt	0.25	0.25	0.25	0.25
Vitamin-mineral premix[2]	0.15	0.15	0.15	0.15
Lys HCl	0.08	0.08	0.25	0.25
Alimet-88 (88% Met)	0.02	0.02	0.02	0.02
Total	100.0	100.0	100.0	100.0
Calculated nutrient content				
DE, kcal/kg	3130	3130	3290	3290
CP, %	14.51	14.51	18.33	18.33
Lys, %	0.58	0.58	0.89	0.89
Met, %	0.23	0.23	0.26	0.26
Cys, %	0.26	0.26	0.29	0.29
Met & Cys, %	0.48	0.48	0.55	0.55
Ca, %	1.00	1.00	0.93	0.93
Available P, %	0.33	0.33	0.36	0.36
Total P, %	0.80	0.80	0.73	0.73
Ca : P	1.26	1.26	1.27	1.27

[1] CLA-60 containing 28% c9-t11 and 28% t10-c12 isomers at guaranteed minimums

[2] Provided per kilogram of complete diet: Vit A, 12 KIU; Vit D_3, 1600.5 IU; Vit E, 75 IU; Vit B_{12}, 30 µg; Vit K, 3 mg; Niacin, 40 IU; Biotin, 0.4 mg, Folacin, 2 mg; Riboflavin 7.5 mg; Thiamine 2 mg; Pantothenic acid, 22 mg; Pyridoxin 2 mg; Ethoxyquin 0.0015 %; Mn, 40 mg; Zn, 150 mg; Fe, 100 mg; Cu, 25.01 mg; Se, 0.30 mg; I, 0.50 mg

according to the guidelines of the Canadian Council on Animal Care (CCAC, 1993). All diets were formulated to meet or exceed the specific nutrient requirements of each animal category in accordance with NRC (1998) recommendations (Table 1).

Using a completely randomized repeated measure design, fourteen Cotswold sows were randomly allotted to a 2 x 2 factorial arrangement of treatments. The four treatments were based on diet (0% or 2% CLA) and parity (I = Immature or M=Mature) and corresponded to the following: 1) 0%-I, n = 3; 2) 0%-M, n=3; 3) 2%-I, n=4; 4) 2%-M, n=4. Immature sows were zero or first parity animals, mature sows were at second parity or greater. Treatment diets were fed as gestation rations from d 85 through d 112 and for 4 d post-weaning, and as lactation rations from d 112 of gestation until weaning. Gestation rations were provided from weaning for an additional 4 d. Treatment diets were supplemented with either a commercial source of CLA (CLA-60, BASF Corp, Mississauga, ON, Canada) or canola oil (chosen so that diets would be balanced for linoleic acid; Table 2) and were formulated to be isoenergetic and isonitrogenous (Table 1). Sows were provided 1.5 kg gestation ration twice daily for a total of 3.0 kg per d. Lactation rations were provided similarly until farrowing subsequent to which rations were increased to full feed based on individual sow consumptions. Throughout the trial, feed intake was recorded daily and sows had free access to feed and water.

Sow BW, back fat depth at the P2 position via ultrasonograph, and condition scores were recorded on gestation d 85, 105, and 112 and lactation d 1, 3 and 17 plus 4 d post-weaning. Blood samples were collected on these same days into 10 ml vacutainers (BD, Mississauga, ON, Canada) via jugular puncture and were immediately placed on ice. Plasma was harvested from lithium heparin vacutainers via centrifugation at 2000

Table 2. Fatty acid composition of sow diets

	Gestation		Lactation	
CLA, %	0	2	0	2
Crude Fat	5.71	5.62	4.82	5.58
Fatty Acid	g/100g Total Fatty Acids[1]			
16:1	0.56	0.32	0.58	0.55
18:0	0.11	0.21	0.12	0.15
18:2	4.40	1.59	4.31	2.15
20:0	0.49	0.24	0.52	0.22
20:1	0.03	0.02	0.03	0.02
20:2	0.01	0.19	0.01	0.17
20:3	0.02	0.04	0.02	0.03
CLA				
c-9, *t*-11	nd	1.44	nd	0.75
t-10, *c*-12	nd	1.66	nd	0.79

[1] nd = not detected

rpm for 10 min. Serum was harvested from blood collected into coagulant free 10 ml vacutainers after being allowed to clot for 12 hr at -4°C followed by centrifugation at 2000 rpm for 10 min. Plasma and serum were stored at -20°C for subsequent analyses.

At farrowing, litter sizes were recorded including total numbers born, born alive and still born. Piglet weights were recorded at farrowing, on d 3 and at weaning (d 17 ± 1). Needle teeth were clipped on d 1 and tails docked and males castrated on d 3. Four piglets per litter were selected randomly on d 3 for blood collection via jugular puncture into 7 ml vacutainers (BD, Mississauga, ON, Canada). Plasma and serum were harvested similar to sow samples. Blood was collected from these same piglets again at weaning.

Chemical Analyses

Total lipids in plasma were extracted using 2:1 chloroform:methanol, based on a procedure modified from Folch et al. (1956). To each sample 100 μL of heptadecanoic acid (17:0; Sigma-Aldrich, Oakville, ON, Canada) was added to serve as an internal standard. Lipid extracts were methylated using 0.5 M methanolic acid and the reaction was carried out at 80°C for 1 h. Fatty acid methyl esters were determined using a Hewlett Packard (Hewlett Packard Canada, Mississauga, ON, Cananda) HP 5890A gas chromatogram equipped with a flame ionization detector and separated on a HP88 100 m x 0.25 mm x 0.2 μm fused-silica column (Agilent Technologies Inc., Mississauga, ON, Canada). Oven temperatures were as follows: initial temperature 70°C for 1 min; raised to 180°C at 8°C/min; raised to 195°C at 1°C/min and held for 10 min; raised to 220°C at 1.2°C/min and held for 5 min; total run time 65.58 min. The injection temperature was 220°C and the detection temperature was at 290°C. Individual isomers were identified

via comparisons to known standard retention times. Crude fat was determined via hexane extraction using a VELP SER 148 solvent extractor (VELP Scientifica, Plainview, NY).

Plasma samples were analyzed for urea N using a Nova Stat profile M blood gas and electrolyte analyzer (Nova Biomedical Corporation, Waltham, MA).

Serum immunoglobulin (Ig) titers were measured using a quantitative commercial ELISA kit (E100-104, Bethyl Laboratories Inc., Montgomery, TX). Both capture and detection antibodies were goat anti-pig IgA and IgG. Detection antibody was conjugated to horseradish peroxidase whose reaction with an alkaline phosphatase substrate formed a product after 5 min incubation at room temperature. Samples were read at 450 nm with a BIORAD 3550 Microplate Reader (BIORAD Laboratories, Hercules, CA).

Statistical Analyses and Calculations

All data was subjected to ANOVA as a completely randomized repeated measures design using the MIXED procedure and repeated statement of SAS (SAS Inst., Inc., Cary, NC). Treatments effects were analyzed in a factorial such that the main effects of diet (D), maturity (M) and period (P) and their two-way (D x M), (D x P), (M x P) and three-way (D x M x P) interactions could be identified. Because of the non-uniformly spaced sampling periods for sow gestation, lactation and piglet performance data, analyses were carried out using the unstructured co-variance option. Due to the unbalanced nature of the design, data were reported as least square means. Treatment means were considered to be trends when P-values < 0.10 were obtained and significant when P-values < 0.05 were obtained.

Whole body nutrient partitioning was determined according to the calculations of

Whittemore and Yang (1989). For each of whole body lipid and protein the following linear equation was applied: $Y = a + b_1(LW) + b_2(P2)$ where Y represented the nutrient fraction of interest, LW represented BW (kg) and P2 back fat depth (mm). The constants a, b_1 and b_2, differed for each nutrient fraction and were -20.4, 0.205 and 1.48 for whole body lipid and -2.31, 0.186 and -2.16 for whole body protein, respectively.

RESULTS

Sow and Piglet Performance

Sow performance data is shown in Table 3. As expected, mature sows were heavier and had greater back fat depths than immature sows throughout the gestation and lactation periods ($P < 0.05$). Mature sows also tended to lose less BW than immature sows from the beginning until the end of lactation ($P = 0.07$). Dietary CLA supplementation did not affect sow BW during gestation ($P = 0.11$) or lactation ($P = 0.27$). During gestation, sows consuming CLA-supplemented diets had less back fat than sows consuming control diets ($P = 0.05$). Although immature sows lost more back fat than mature sows from d 1 of lactation until 4 d post-weaning ($P = 0.01$), immature sows consuming CLA-supplemented diets tended to lose less back fat than immature sows consuming control diets ($P = 0.09$). Mature sows tended to have greater body condition scores during lactation versus immature sows ($P = 0.06$). However, body condition scores were not affected by diet, maturity, period or any of their interactions for the remainder of gestation and lactation (Appendix 4). As expected, mature sows had greater ADFI than immature sows ($P < 0.001$). However, sow ADFI was not affected by dietary CLA-supplementation ($P > 0.10$).

Table 3. Performance data for mature and immature sows fed control or CLA-supplemented diets during gestation and lactation[1]

Item	Treatment Group				SEM	P-Values[2]						
	0% CLA		2% CLA									
	Immature	Mature	Immature	Mature		D	M	D x M	P	D x P	M x P	D x M x P
Gestation												
BW, kg												
d 85	167.4	227.6	197.9	248.9	13.2	0.11	0.002	0.93	<0.001	0.49	0.81	0.43
d 105	186.2	237.1	203.8	259.0	13.8							
d 112	193.2	247.1	216.3	268.3	12.5							
Back fat, mm												
d 85	20.1	24.1	17.8	19.3	2.20	0.05	0.003	0.91	<0.001	0.49	0.65	0.35
d 105	18.1	25.1	16.9	19.1	2.38							
d 112	21.4	25.1	18.2	20.0	2.35							
Lactation												
BW, kg												
d 1	178.1	243.5	196.5	252.2	14.1	0.27	0.002	0.54	<0.001	0.44	0.07	0.26
d 3	173.5	241.4	192.7	250.0	14.4							
d 17	164.7	232.9	195.9	237.8	14.4							
4 d pw	152.8	215.6	186.2	223.7	14.7							
Back fat, mm												
d 1	17.3	23.9	18.8	20.4	2.74	0.15	0.003	0.61	<0.001	0.19	0.01	0.09
d 3	19.3	25.6	18.1	22.7	2.32							
d 17	13.1	22.9	15.3	20.6	2.46							
4 d pw	12.6	23.9	15.2	19.5	2.51							
ADFI, kg/d												
Gestation	2.81	3.27	2.69	3.23	0.06	0.34	<0.001	0.34	<0.001	0.15	0.85	0.91
Lactation	4.63	5.10	4.75	5.34	0.12							

[1] Values are LSmeans; SEM are averaged for each period; pw = post-weaning
[2] Main effects of diet (D), maturity (M), sampling period (P) or their interactions (D x M, D x P, M x P, D x M, x P)

Litter performance is shown in Table 4. Here it should be noted that two mature sows receiving control diets had unusually small litters (3 and 4 piglets respectively), effectively creating a significance artifact for dietary CLA ($P = 0.007$), maturity ($P = 0.02$) and their interactions with each other and period. Because of these abnormally small litter sizes, it is likely that maturity and diet had no effect on litter survival, or total litter size. Plus, dietary treatments were not started until last trimester, a point at which litter size should not be affected. Piglets born to mature sows were heavier than those born to immature sows on d 1, 3 and 17 of nursing ($P < 0.001$; Table 4). Piglets born to sows consuming CLA-supplemented diets tended to have lower BW than those from control sows ($P = 0.06$). However, total litter weight did not differ between CLA supplemented treatment groups ($P > 0.10$).

Whole Body Nutrient Partitioning

During gestation and lactation, mature sows had greater calculated whole body lipid and protein fractions compared to immature sows ($P < 0.05$; Table 5). Dietary CLA did not affect sow whole body lipid percentage during gestation or lactation ($P > 0.10$) but did increase whole body protein percentage during gestation ($P < 0.05$). From d 1 of lactation until 4 d post-weaning mature sows had a greater reduction of whole body protein than did immature sows ($P = 0.002$). Although immature sows receiving CLA-supplemented diets lost less whole body lipid and protein (15.3%, 4.0%) compared to control sows (26.2%, 13.0%), these differences were not significant ($P > 0.10$).

Table 4. Effects of dietary CLA-supplementation and sow parity on litter performance parameters[1]

	Treatment Group					P-Values[2]						
	0% CLA		2% CLA									
Item	Immature	Mature	Immature	Mature	SEM	D	M	D x M	P	D x P	M x P	D x M x P
Piglets / litter												
Born alive	9.67	4.33	13.5	10.3	1.07	0.007	0.02	0.09	<0.001	0.005	0.03	0.18
Stillborn	0.33	0.33	0.50	1.00	---							
Alive at d 3	9.67	4.33	10.3	10.0	1.30							
Alive at d 17	9.33	4.33	9.75	9.75	1.14							
Piglet BW, kg[3]												
d 1	1.53	1.95	1.14	1.66	0.12	0.06	<0.001	0.93	<0.001	0.95	0.04	0.38
d 3	1.97	2.61	1.69	2.23	0.12							
d 17	5.18	6.78	4.73	6.42	0.39							
Litter weight[4], kg	50.3	29.8	46.3	62.4	8.23	0.11	0.79	0.05	---	---	---	---

[1] Values are LSmeans; SEM are averaged per period; pw = post-weaning
[2] Main effects of diet (D), maturity (M), sampling period (P) or their interactions (D x M, D x P, M x P, D x M x P)
[3] d 1 = birth weight, d 17 = weaning weight
[4] Total weight of each litter at weaning

Table 5. Effects of dietary CLA-supplementation and parity on sow estimated whole body nutrient partitioning during gestation and lactation[1]

	Treatment Group					P-Values[2]						
	0% CLA		2% CLA									
Item	Immature	Mature	Immature	Mature	SEM	D	M	D x M	P	D x P	M x P	D x M x P
			Gestation									
Whole body lipid, %												
d 85	44.7	62.4	47.1	59.8	5.37	0.86	0.02	0.67	<0.001	0.63	0.25	0.61
d 105	44.6	65.4	46.4	61.0	5.44							
d 112	50.9	66.6	50.8	63.7	5.54							
Whole body protein, %												
d 85	25.5	35.3	31.1	40.4	2.20	0.05	0.003	0.91	<0.001	0.49	0.65	0.35
d 105	28.4	36.4	31.9	41.7	2.38							
d 112	29.0	37.4	34.0	42.8	2.35							
			Lactation									
Whole body lipid, %												
d 1	40.1	66.0	47.6	61.6	6.12	0.75	0.005	0.37	<0.001	0.48	0.82	0.26
d 3	43.7	67.0	45.8	64.3	6.07							
d 17	32.8	61.2	42.4	58.9	6.07							
4 d pw	29.6	59.2	40.3	54.4	6.07							
Whole body protein, %												
d 1	26.9	36.9	30.2	40.2	2.45	0.19	0.003	0.64	<0.001	0.46	0.002	0.14
d 3	25.8	37.1	29.6	39.2	2.44							
d 17	25.5	36.1	30.8	37.5	2.44							
4 d pw	23.4	32.6	29.0	35.1	2.44							

[1] Values are LSmeans; SEM are averaged per period; pw = post-weaning

[2] Main effects of diet (D), maturity (M), sampling period (P) or their interactions (D x M, D x P, M x P, D x M x P)

Sow Immunoglobulins

During gestation, sow IgA and IgG titers were not affected by diet or maturity ($P > 0.10$; Table 6). From d 85 to d 112 of gestation, serum IgA increased ($P < 0.001$) and IgG decreased ($P = 0.007$) within each treatment group. From d 85 to d 112 of gestation, serum IgA concentrations increased more in mature sows than in immature sows ($P = 0.02$). The average reduction in IgG concentrations from d 85 to d 112 of gestation within sows consuming CLA-supplemented diets was 17% compared to 27.9% for sows consuming control diets ($P > 0.10$). During lactation, IgA and IgG were not affected by dietary CLA-supplementation, maturity or period ($P > 0.05$). However, mature sows consuming CLA-supplemented diets had greater IgA titers at each sampling point of lactation ($P = 0.02$). Colostrum concentrations of IgA and IgG appeared to be unaffected by dietary CLA with an average of 4.42 g/L IgA and 6.35 g/L IgG for sows receiving control diets (n = 1) and an average of 4.14 g/L IgA and 5.59 g/L IgG for sows receiving CLA-supplemented diets (n = 5; Table 10). However, statistical analysis was not performed on colostrum data due to low sample numbers.

Piglet PUN and Immune Status

Piglets nursing sows receiving CLA-supplemented diets had greater PUN levels than piglets nursing sows receiving control diets ($P = 0.008$; Table 7). In addition, greater PUN levels were observed in piglets nursing mature sows compared to immature sows ($P = 0.002$). In each treatment group, PUN increased from d 3 to d 17 ($P < 0.001$), however, this increase was greatest in piglets consuming CLA-supplemented diets ($P = 0.001$).

Table 6. Effects of dietary CLA-supplementation and parity on sow serum immunoglobulins during gestation and lactation[1]

Item	Treatment Group				SEM	P-Values[2]						
	0% CLA		2% CLA			D	M	D x M	P	D x P	M x P	D x M x P
	Immature	Mature	Immature	Mature								
Gestation												
IgA, g/L												
d 85	1.09	0.88	0.85	0.63	0.20	0.56	0.90	0.97	<0.001	0.34	0.02	1.00
d 105	1.15	1.11	1.12	1.06	0.24							
d 112	1.22	1.42	1.16	1.35	0.18							
IgG, g/L												
d 85	6.75	6.35	6.65	6.35	0.14	0.90	0.76	0.50	0.007	0.89	0.13	0.67
d 105	6.39	5.99	6.42	6.02	0.30							
d 112	3.92	5.47	4.57	6.18	0.66							
Lactation												
IgA, g/L												
d 1	1.06	0.87	1.25	1.51	0.26	0.21	0.22	0.02	0.23	0.32	0.92	0.59
d 3	0.88	0.71	1.09	1.18	0.26							
d 17	1.25	0.52	0.88	1.30	0.25							
4 d pw	1.51	1.24	1.05	1.36	0.27							
IgG, g/L												
d 1	6.42	5.54	5.75	5.26	1.23	0.82	1.00	0.77	0.97	0.20	0.84	0.69
d 3	6.98	4.20	4.22	6.61	1.23							
d 17	4.73	5.92	5.46	5.14	1.17							
4 d pw	4.80	6.15	6.04	5.25	1.37							

[1] Values are LSmeans; SEM are averaged per period; pw = post-weaning

[2] Main effects of diet (D), maturity (M), sampling period (P) or their interactions (D x M, D x P, M x P, D x M x P)

Piglet serum IgA and IgG were not affected by dietary CLA-supplementation or maturity while nursing ($P > 0.10$; Table 7). However, piglets born to mature sows consuming CLA-supplemented diets tended to have greater IgA concentrations compared to piglets born to mature sows consuming control diets ($P = 0.06$). Piglet serum IgA concentrations were lower ($P < 0.001$) and IgG concentrations greater ($P < 0.002$) on 17 than on d 3 of nursing.

Fatty Acids

Compared to sows consuming control diets, sows receiving CLA-supplemented diets had detectable levels of CLA isomers by d 105 of gestation ($P < 0.05$; Table 8). By d 112 of gestation, both the *cis*-9, *trans*-11 ($P = 0.04$) and *trans*-10, *cis*-12 ($P = 0.10$) CLA isomers had increased in comparison to d 105. Circulating CLA isomer concentrations were not affected by maturity during gestation or lactation ($P > 0.10$). Trace levels (0.01 – 0.03 g/100g total fatty acids) of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers were detected in sows receiving control diets during lactation. However, the *cis*-9, *trans*-11 ($P = 0.06$) and *trans*-10, *cis*-12 ($P = 0.10$) tended to be elevated in sows consuming CLA-supplemented diets during lactation. Furthermore, the CLA isomers detected in sows consuming CLA-supplemented diets increased from d 1 of lactation until 4 d post-weaning ($P < 0.05$).

Piglets nursing CLA-supplemented sows had greater CLA isomer concentrations compared to piglets nursing control sows ($P < 0.01$; Table 9). Trace amounts (0.02 – 0.05 g/100g total fatty acids) of CLA isomers were detected in piglets suckling non-CLA supplemented sows on d 3 and d 17 of nursing. The concentration of *cis*-9, *trans*-11

Table 7. Effects of dietary CLA-supplementation and sow parity on litter immune and health status parameters[1]

	Treatment Group											
	0% CLA		2% CLA			P-Values[2]						
Item	Immature	Mature	Immature	Mature	SEM	D	M	D x M	P	D x P	M x P	D x M x P
Piglet PUN, mmol/L[3]												
d 3	2.41	3.25	2.69	2.98	0.21	0.008	0.002	0.02	<0.001	0.001	0.86	0.19
d17	2.22	3.47	3.85	3.82	0.25							
Piglet immunoglobulins, g/L												
IgA												
d 3	2.20	1.97	1.80	2.25	0.19	0.72	0.82	0.06	<0.001	0.80	0.37	0.13
d 17	0.23	0.13	0.19	0.15	0.02							
IgG												
d 3	33.0	16.7	26.5	24.4	5.20	0.76	0.28	0.38	0.002	0.58	0.14	0.28
d 17	37.2	37.7	34.1	34.6	4.98							

[1]Values are LSmeans; SEM are averaged per period; pw = post-weaning

[2] Main effects of diet (D), maturity (M), sampling period (P) or their interactions (D x M, D x P, M x P, D x M x P)

[3] Plasma urea nitrogen

Table 8. Effects of dietary CLA-supplementation and parity on sow plasma CLA isomer concentrations during gestation and lactation[1]

	Treatment Group											
	0% CLA		2% CLA			P-Values[2]						
Item	Immature	Mature	Immature	Mature	SEM	D	M	D x M	P	D x P	M x P	D x M x P
CLA isomer, g/100g			Gestation									
cis-9, *trans*-11												
d 85	nd	nd	nd	nd	----	0.05	0.57	0.57	0.04	0.04	0.50	0.50
d 105	nd	nd	0.10	0.06	0.11							
d 112	nd	nd	0.24	0.52	0.11							
trans-10, *cis*-12												
d 85	nd	nd	nd	nd	----	0.03	0.62	0.62	0.10	0.10	0.87	0.87
d 105	nd	nd	0.09	0.07	0.08							
d 112	nd	nd	0.33	0.20	0.09							
			Lactation									
cis-9, *trans*-11												
d 1	0.03	0.01	0.01	0.02	0.02	0.10	0.15	0.20	0.14	0.02	0.24	0.15
d 3	nd	nd	0.03	0.41	0.21							
d 17	0.01	nd	0.02	0.02	0.01							
4 d pw	0.01	0.02	0.10	0.52	0.14							
trans-10, *cis*-12												
d 1	0.01	0.02	0.03	0.01	0.05	0.06	0.11	0.36	0.15	0.01	0.69	0.32
d 3	nd	0.01	0.08	0.12	0.05							
d 17	nd	0.01	0.02	0.03	0.04							
4 d pw	0.01	0.03	0.16	0.20	0.05							

[1] Values are LSmeans; SEM are averaged per period; pw = post-weaning; nd = not detected

[2] Main effects of diet (D), maturity (M), sampling period (P) or their interactions (D x M, D x P, M x P, D x M x P)

Table 9. Effects of dietary CLA-supplementation and parity on piglet plasma CLA isomer concentrations[1]

	Treatment Group					P-Values[2]						
	0% CLA		2% CLA									
Item	Immature	Mature	Immature	Mature	SEM	D	M	D x M	P	D x P	M x P	D x M x P
CLA isomer, g/100g												
cis-9, *trans*-11												
d 3	0.04	0.03	0.49	0.54	0.24	0.001	0.72	0.66	0.67	0.67	0.59	0.52
d 17	0.02	0.05	0.53	0.27	0.09							
trans-10, *cis*-12												
d 3	0.04	0.03	0.49	0.48	0.11	<0.001	0.86	0.93	0.005	0.03	0.99	0.93
d 17	nd	nd	0.20	0.18	0.02							

[1]Values are LSmeans; SEM are averaged per period; pw = post-weaning

[2] Main effects of diet (D), maturity (M), sampling period (P) or their interactions (D x M, D x P, M x P, D x M x P)

isomers did not change between d 3 and d 17 of nursing ($P > 0.10$), however, the *trans*-10, *cis*-12 isomer decreased during this time period ($P = 0.005$).

Detectable levels of CLA isomers were only observed within the colostrum of sows receiving CLA-supplemented diets (n = 5; Table 10) with an average level of 5.95 g/100g of *cis*-9, *trans*-11 and 5.28 g/100g of *trans*-10, *cis*-12 being measured. While saturated isomers remained relatively constant between treatment (n = 5) and control (n = 1) sows, total mono and poly-unsaturated isomers appeared to be reduced in the colostrum of CLA-supplemented sows.

DISCUSSION

Animal performance

Previous research trials have examined the potential of dietary CLA to improve sow performance during gestation and lactation with varying results. As expected, mature sows were heavier than immature sows throughout the trial. The fact that dietary CLA-supplementation had no effect on sow BW from late gestation until weaning is similar to previous reports (Poulos et al., 2004; Park et al., 2005). In an earlier study where dietary CLA was included at 1% from the beginning of lactation until weaning, it was shown that sow back fat depth did not change (Harrell et al., 2002). Additionally, sow back fat depth was also not affected when dietary CLA was provided at 0.5% from d 40 and d 75 until weaning (Poulos et al., 2004). In the current study, however, sows consuming CLA-supplemented diets at 2% had less back fat than those consuming control diets from d 85 of gestation until 4 d post-weaning. These results are

Table 10. Immunoglobulin and fatty acid composition of sow colostrum[1]

Item	0% CLA (n = 1)	2% CLA (n = 5)
Immunoglobulin, g/L		
IgA	4.42	4.14
IgG	6.35	5.59
Fatty Acid	g/100g Total Fatty Acids	
14:0	1.70	1.69
16:0	18.1	20.2
18:0	32.9	22.2
20:0	0.02	0.08
22:0	0.35	0.15
16:1	3.72	6.69
18:1	3.29	2.12
20:1	5.33	2.12
18:2	23.3	17.7
20:2	0.44	0.29
22:2	0.18	0.08
18:3	0.22	0.23
20:3	0.38	0.27
22:3	0.08	0.08
20:4	0.58	0.70
22:4	0.21	0.14
20:5	0.14	0.15
22:5	0.56	0.43
Σ SFA	53.12	44.10
Σ MUFA	12.43	10.96
Σ PUFA	26.14	20.12
CLA		
c-9, *t*-11	nd	5.95
t-10, *c*-12	nd	5.28
16:1/16:0	0.21	0.33
18:1/18:0	0.10	0.10

[1] nd = not detected

similar to those of Park et al. (2005), who observed that sow back fat depths were reduced when dietary CLA was included at 1.5% from d 15 and d 74 of gestation until weaning. These results could imply that a minimum dietary inclusion level or duration may exist for dietary CLA to have an effect on back fat depth in the pregnant and lactating sow.

Limited work has examined the effect of dietary CLA on BW and back fat depth of immature sows. Since dietary CLA is typically associated with reduced adiposity (Ostrowska et al., 1999), it is interesting that from d 1 of lactation until 4 d post-weaning immature sows consuming CLA-supplemented diets tended to lose less back fat than immature sows consuming control diets. This apparent difference in the adipose associated activity of CLA between immature and mature animals was previously reported when gilts fed CLA-supplemented diets had no change in total carcass fat content (Ramsay et al., 2001). The authors suggested that CLA failed to reduce fat levels due to differences in metabolic activity between growing and finishing pigs. Alternatively, dietary fat intake and carcass fat level have been associated with the lipid reducing effects of CLA in the pig (Azain, 2004). Here it was reported that CLA will only reduce fat depots when sows have a P2 fat depth of approximately 23 mm and are fed diets containing approximately 2% fat (Dugan et al., 2001; Azain, 2004). Therefore, it is possible that dietary CLA could act to retain fat reserves in young animals that have yet to reach the minimum action level. To our knowledge, this is the first time CLA has been reported to have such an outcome within immature pregnant and lactating sows. This is significant in that energy reserves associated with adequate body condition which are required for sustaining large litters through the production of milk tend to be limited

in younger sows (Knol, 2003).

Previous studies have reported that dietary CLA does not increase sow ADFI, findings corroborated by the current study. For example, when dietary CLA was supplemented at 0.5% from 1 wk before lactation until weaning at 21 d, sow ADFI did not change between treatment groups (Bontempo et al., 2004). Further, Bee (2000a) provided CLA at 2% for the entirety of gestation plus 35 d of lactation and reported no effect on sow ADFI. That immature sows consuming CLA-supplemented diets lost less BW during lactation compared to sows consuming control diets while maintaining a similar ADFI implies improved feed efficiency, confirming previous reports (Dugan et al., 1997; Ostrowska et al., 1999).

Data surrounding the effects of dietary CLA on sow reproductive performance are variable. Harrell et al. (2002) and Poulos et al. (2004) reported no effect of dietary CLA on piglet and litter weights. In the current study, piglets born to CLA-supplemented sows were lighter than piglets from control sows, results similar to previous observations by Park et al. (2005) that showed increasing the duration of dietary CLA-supplementation further reduced piglet BW. Nevertheless, it is possible that piglets born to mature sows consuming control diets were larger due to a smaller number of piglets competing for nutrient resources. However, by 2 and 4 wk post-weaning piglet BW did not differ between CLA-supplemented and control piglets, results in line with those shown in Manuscript 2 (Chapter 4, Table 14, current thesis) where piglet BW did not differ between CLA treatment groups by 1.5 wk post-weaning. It is thus possible that nutrients required for piglet growth were limited while nursing, potentially by the nutrient repartitioning effects associated with CLA (Bontempo et al., 2004).

Nutrient Partitioning

The relationship between dietary CLA and nutrient repartitioning has been examined in growing and finishing pigs with mixed results (Ostrowska et al., 1999; Kelly and Erickson, 2003). The observation that whole body protein estimates were greater in sows consuming CLA-supplemented diets is in agreement with previous reports of dietary CLA promoting lean tissue accretion in growing pigs (Dugan et al., 1997; Ostrowska et al., 1999). However, the study of Ostrowska et al. (1999) indicated that CLA reduces adipose reserves within growing pigs potentially by reducing the amount of lipid accumulation within adipocytes or by increasing adipose tissue lipid oxidation (Azain, 2004). It is possible that whole body lipid estimates were not affected by dietary CLA-supplementation due to limitations in the formula used to make these estimates such as genetic bias and contribution of nutrient regimes. However, this cannot be confirmed since total carcass lipid measurements were not performed in the current study.

Fatty Acids

Inclusion of CLA into pregnant sow diets has been reported to affect lipid metabolism resulting in the re-distribution of constituent adipose and milk fatty acid isomers while increasing the presence of total CLA isomers (Bee, 2000a; Poulos et al., 2004). In addition to reducing whole body adiposity, dietary CLA has also been shown to reduce total colostrum and milk fat percentages (Harrell et al., 2002; Poulos et al., 2004). Furthermore, previous studies have reported that pigs fed diets containing CLA had increased total SFA and reduced total MUFA concentrations in milk and adipose tissue (Bee, 2000a; Bee, 2000b). The fact that sow plasma SFA and MUFA were

unaffected by dietary CLA-supplementation during gestation (Appendix 5) and that they varied during lactation (Appendix 6) could have been due to the transient nature and rapid uptake into metabolic tissues of the plasma lipids being measured (Bartley, 1989).

Limited colostrum sample numbers restricted statistical analyses. However, CLA isomers were detected in samples taken from animals receiving supplemented diets, which is in line with a previous report by Bontempo et al. (2004). Additionally, in comparison to control samples, the reduced proportion of unsaturated fatty acid isomers observed in CLA-supplemented sows is consistent with previous reports (Bee, 2000a). Although not directly measured, reduction in these unsaturated isomers could have been due to the inhibition of desaturase enzyme activity by the *trans*-10, *cis*-12 CLA isomer (Lee et al., 1998). This is because previous work has shown that CLA reduces the activity and expression of Δ-9 desaturase in hepatic and adipose tissues (Lee et al., 1998; Smith et al., 2002).

The fact that CLA isomers were identified in sows consuming non-CLA supplemented diets is not unique but does contradict previous findings (Bontempo et al., 2004). Chin et al. (1994) reported that rats fed diets high in linoleic acid (LA) had detectable levels of hepatic CLA after 2 wk of feeding and suggested CLA had been converted from LA by enzymatic isomerase activity of enteric bacteria. Thus, additional LA added to control rations for balancing purposes could have served as substrate for enteric bacteria, resulting in trace generation of CLA isomers detectable within sow and suckling piglet plasma.

Markers of immune modulation

Compared with other mammals, *in utero* immunoglobulin transfer to fetal piglets is restricted by the multi-layered structure of the sow placenta (Pabst and Rothkotter, 1999). As a result, piglets are born hypo-immune and are reliant upon immune-compounds contained within colostrum and milk such as immunoglobulins for disease protection until *de novo* synthesis of these compounds commences (Rook and Blande, 2002). This passive immune transfer is of economic importance since it has been shown that increased consumption of colostrum immune-compounds during the first hours of life corresponds to increased immune vigor during the post-weaning period (Rooke et al., 2003; Le Dividich et al., 2005).

In the present study, dietary CLA did not affect piglet immunoglobulin titers prior to weaning. Active immunity does not begin to develop in the piglet until several weeks of life (Bassaganya-Riera et al., 2001; Weber et al., 2001). In addition, 7 to 10 d is required for B-cell populations to begin synthesizing immunoglobulins in new-born piglets (Rook and Blande, 2002). In the sow, nearly all colostrum IgG and a large proportion of IgA are derived from serum, with the former being preferentially transported by specific mammary receptors (Bourne and Curtis, 1973; Huang et al., 1992). The numerically smaller serum immunoglobulin titers observed in CLA-supplemented sows on d 1 of lactation may reflect an enhanced capacity of these animals to mobilize serum immunoglobulins into colostrum for suckling piglets (Huang et al., 1992).

Piglet to sow serum immunoglobulin ratios may indicate that CLA-supplemented sows were better able to transfer immunoglobulins to their suckling piglets, a pattern also

reported by Bontempo et al. (2004). Although immunoglobulin production is highly variable between sows (Bontempo et al., 2004), greater transfer of immunoglobulins from CLA-supplemented sows to piglets could allow for greater immune vigor post-weaning.

In addition to being used as a marker of protein absorption and AA utilization, PUN has been used to indicate the extent of lean tissue catabolism and immune induction and acute phase protein synthesis in diseased piglets (Owusu-Asiedu et al., 2003). Little work had been done establishing normal PUN value in the suckling piglet. Elevated PUN levels could result from the metabolism of dietary AA which are not incorporated into muscle tissue. Alternatively, it is possible that elevated PUN values observed in piglets nursing CLA-supplemented sows may have been due to greater intestinal protein absorption (Bassaganya-Riera et al., 2001) brought about by the ability of CLA to improve gut structure (Hontecillas et al., 2002). This is possible since in Manuscript 2 (Chapter 4), piglets weaned from CLA-supplemented sows had reduced intestinal inflammation as well as elevated PUN concentrations.

The exact means by which CLA facilitates nutrient repartitioning and immune stimulation remain unknown. Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a nuclear receptor associated with numerous biological processes including influencing expression products associated with immune function and modulation plus fatty acid uptake, storage and metabolism (Gelman et al., 1999). In terms of immune stimulation, work conducted on immune challenged piglets provided with dietary CLA showed maximal PPAR-γ expression with parallel reductions in catabolism, intestinal inflammation and pro-inflammatory cytokine expression (Hontecillas et al., 2002; Bassaganya-Riera et al., 2004). Although CLA had no effect on sow and piglet

immunoglobulin production in the current study, it is possible that by activating PPAR-γ dietary CLA primed the immune systems of piglets nursing CLA-supplemented sows, which could account for the post-weaning immune stimulation observed in these piglets (Manuscript 2).

In the pig, PPAR-α and PPAR-γ are expressed in adipose tissue (Azain, 2004), and cell culture studies have shown that CLA increases PPAR-γ expression and adipocyte differentiation (Ding et al., 2000). Activation of PPAR-γ *in vivo* has also been associated with porcine adipocyte differentiation (Yu et al., 2006), however, data linking dietary fatty acids such as CLA with such an effect through the activation of PPAR-γ has yet to be established (Ding et al., 2003). It is thus conceivable that the nutrient repartitioning effects of CLA seen in the current study were mediated by PPAR activation.

The results of this study indicate that in terms of nutrient partitioning, supplementing immature sow diets with CLA may be a practical means of dampening body condition losses characteristic of early parity sows. From the standpoint of a producer, this could be beneficial in that immature sows consuming CLA-supplemented diets retained greater back fat depots and whole body protein estimates over the course of the experiment which could relate to improved reproductive performance and longevity. In addition, although dietary CLA had no effect on sow and piglet immunoglobulin concentrations, the transfer of immunoglobulins from sows to piglets may have been improved in piglets nursing CLA-supplemented sows, which could improve the capability of these piglets to combat infection post-weaning.

CHAPTER 4

MANUSCRIPT TWO

Response of piglets weaned from sows fed diets supplemented with conjugated linoleic acid (CLA) to an *Escherichia coli* K88^{+} oral challenge

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ABSTRACT

Seventy-eight Cotswold piglets weaned from sows receiving 0% or 2% CLA-supplemented rations from d 85 of gestation through lactation were allocated to nursery diets (ND) according to their dam's lactation ration (LR) as follows: 1) 0%-0% (0% CLA LR : 0% CLA ND); 2) 0%-2% (0% CLA LR : 2% CLA ND); 3) 2%-0% (2% CLA LR : 0% CLA ND); 4) 2%-2% (2% CLA LR: 2% CLA ND). At 28 ± 2 d of age all piglets received an oral *E. coli* $K88^+$ challenge and were subsequently monitored for scour development and overall health until 36 ± 2 d of age, after which blood and tissue samples were collected. Sow BW, back fat and feed intake were not affected by CLA-supplementation ($P > 0.05$). Similarly, there were no effects on piglet birth weight, BW or immunoglobulin synthesis on d 3 or d 17 of lactation ($P > 0.05$), with post-weaning growth performance remaining unaffected by CLA addition to ND ($P > 0.05$). The development of scours was less severe in piglets weaned from 2% CLA-supplemented sows at 8, 24, 48 and 56 h after *E. coli* $K88^+$ administration ($P < 0.05$). Intestinal coliform and lactic acid bacteria (LAB) populations post-challenge were not affected by CLA-supplementation. However, caecal ammonia-N was greatest in 0%-0% piglets compared to the remaining treatment groups and total volatile fatty acid production lowest in 0%-0% and 0%-2% piglets compared to 2%-0% and 2%-2% piglets. In addition, piglets weaned from 2% CLA-supplemented sows had increased ($P < 0.05$) serum immunoglobulin A and G levels and reduced ($P < 0.05$) intestinal mucosal inflammation compared to piglets weaned from control sows. Although there were no obvious additional health effects observed when CLA was provided in ND, supplementing sow rations with 2% CLA from mid-gestation through weaning appears to

have immune stimulating carry over effects post-weaning. Thus, supplementing sow rations with CLA may be a practical strategy for enhancing passive immune transfer and improving the immune status and overall gut health of nursery piglets.

Key words: CLA, ETEC, Passive Immunity, Piglets, Sows

INTRODUCTION

Sub-optimal transfer of passive immunity from sow to piglet has been associated with increased disease prevalence in nursery pigs (Drew and Owen, 1988). Of these diseases, post-weaning diarrhea caused by enterotoxigenic *E. coli* (ETEC) is most common. In a recent survey, it was shown that over 60% of nurseries reporting diarrhea outbreaks tested positive for *E. coli* $K88^+$ and that subsequent economic losses were considerable (Amezcua et al., 2002). When taken in combination with another report from the UK suggesting that upwards of 20% of piglet mortality could be attributed to poor colostrum intake and unsuccessful passive immunity (Edwards, 2002), it seems critical that any means of bolstering passive immune transfer be investigated.

A novel way by which passive immunity may be strengthened is through dietary supplementation with conjugated linoleic acid (CLA; Bontempo et al., 2004). Conjugated linoleic acid is a group of positional and geometric isomers of linoleic acid (Banni, 2002) with proven health benefits to both humans and livestock (Belury, 2002). The ability of CLA to stimulate immune function has been well documented in nursery pigs (Bontempo et al., 2004; Changhua et al., 2005). It has also been shown that dietary CLA supplementation during lactation results in colostrum and milk enrichment therefore allowing access of CLA isomers to the suckling piglet (Bee, 2000a). Although it has

been suggested that supplementing sow diets with CLA could lead to improved piglet immune status post-weaning (Bontempo et al., 2004), this theory has yet to be confirmed with the use of a disease model in the piglet. It thus follows that dietary CLA supplementation pre and post weaning may be a potential means of reducing the incidence of post-weaning diarrhea.

The objectives of this study were to evaluate early post-weaning performance, gut health and immune stimulation of piglets from sows provided CLA supplementation through gestation and lactation then receiving CLA in nursery diets immediately post-weaning and orally challenged with enterotoxigenic *E. coli* $K88^{+}$.

MATERIALS AND METHODS

Animals and Diets

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee (Protocol # F05-015) and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 1993). All diets were formulated to meet or exceed the nutrient recommendations for each animal category (NRC, 1998).

Fourteen mixed parity Cotswold sows were randomly assigned to two treatment diets (0% CLA n = 6 or 2% CLA n = 8) beginning on d 85 of gestation. Diets were fed as gestation rations from d 85 through d 110 and as lactation rations from d 110 through to weaning. Treatment diets were supplemented with either a commercial source of CLA (CLA-60, BASF Corp, Mississauga, ON, Canada) or canola oil (chosen so that diets

Table 11. Composition and calculated nutrients of piglet diets

	Phase I		Phase II	
Ingredient, % CLA, %:	0	2	0	2
Corn	51.14	51.14	61.13	61.13
Soybean meal, 48 %	39.5	39.5	32.04	32.04
Oat groats	1.55	1.55	---	---
Dried whey	1.00	1.00	---	---
Soybean oil	3.20	---	3.20	---
CLA-60[1]	---	3.20	---	3.20
Biophos	1.25	1.25	1.00	1.00
Limestone	1.07	1.07	0.95	0.95
Salt	0.25	0.25	0.25	0.25
Vitamin-mineral premix[2]	1.00	1.00	1.00	1.00
Lys HCl	0.02	0.02	0.11	0.11
DL-Met	0.02	0.02	0.02	0.02
Chromic oxide	---	---	0.30	0.30
Total	100.0	100.0	100.0	100.0
Calculated nutrient content				
DE, Kcal/kg	3572	3292	3616	3336
CP, %	22.8	22.8	20.1	20.1
Lys, %	1.14	1.14	1.02	1.02
Met, %	0.32	0.32	0.29	0.29
Cys, %	0.31	0.31	0.28	0.28
Met & Cys, %	0.63	0.63	0.57	0.57
Ca, %	0.80	0.80	0.68	0.68
Available P, %	0.39	0.39	0.30	0.30
Total P, %	0.72	0.72	0.61	0.61
Ca : P	1.11	1.11	1.11	1.11

[1] CLA-60 containing 28% c9-t11 and 28% t10-c12 isomers as guaranteed minimums

[2] Provided per kilogram of complete diet: vitamin A, 8,225 IU; vitamin D3, 1,000 IU; vitamin E, 10.9 IU; vitamin B12, 0.115 mg; vitamin K, 1.1 mg; Niacin, 36.8 mg; Choline chlorie, 781.2 mg; Biotin, 0.25 mg, Folic acid, 0.75 mg, Mn (as MnO), 55 mg; Zn (as ZnO), 50 mg, Fe (as $FeSO_4 \cdot H_2O$), 80 mg, Cu (as CuO), 5 mg; Se (as $NaSeO_3$), 0.1 mg, I (as $Ca(IO_3)_2$), 0.28 mg

would be balanced for linoleic acid; Table 12) and were formulated to be isoenergetic and isonitrogenous (Table 1). Sows were provided 1.5 kg gestation ration twice daily for a total of 3.0 kg/d. Lactation rations were fed from d 112 of gestation at the same rate until farrowing subsequent to which rations were gradually increased to full-feed based on individual sow consumptions. Throughout the trial feed intake was recorded daily and sows had free access to water.

Sow BW and back-fat depth were recorded on gestation d 85 and 112 and lactation d 1 and 17. At farrowing, total number born, number born alive and number still born were recorded. Litter weights were recorded at farrowing, on d 3 and at weaning (d 17 ± 1). Needle teeth were clipped on d 1 and tails docked and males castrated on d 3.

At weaning, 39 piglets were randomly selected from each sow treatment group (total n = 78) and arranged in a 2 x 2 factorial based on lactation (L) and nursery diets (ND). The factors were: 1) 0:0 (0% CLA L: 0% CLA ND); 2) 0:2 (0% CLA L: 2% CLA ND); 3) 2:0 (2 % CLA L: 0 % CLA ND); 4) 2:2 (2 % CLA L: 2 % CLA ND). After balancing for BW, piglets were housed in mixed-sex groups of 3 per pen (1.2 m x 1.5 m, with plastic coated woven metal flooring) in an environmentally controlled room with an initial temperature of 31°C, which was decreased by 1.5°C each wk. Piglets from 0:0 and 2:0 treatment groups received diets formulated to meet or exceed NRC (1998) requirements without CLA supplementation. Piglets from 0:2 and 2:2 treatment groups received identical nursery diets supplemented with 2% CLA-60 (CLA-60, BASF Corp, Mississauga, ON, Canada). Piglets were given *ad libitum* access to feed for 3 wk in 2

Table 12. Fatty acid composition of sow and piglet diets[1]

	Gestation		Lactation		Phase I		Phase II	
CLA, %	0	2	0	2	0	2	0	2
Crude fat, %	5.71	5.62	4.82	5.58	4.97	5.66	5.12	5.31
Fatty acid	g/100g Total fatty acids							
16:1	0.56	0.32	0.58	0.55	0.70	0.48	0.64	0.48
18:0	0.11	0.21	0.12	0.15	0.21	0.15	0.19	0.17
18:2	4.40	1.59	4.31	2.15	4.39	2.27	4.17	2.64
20:0	0.49	0.24	0.52	0.22	0.32	0.23	0.28	0.23
20:1	0.03	0.02	0.03	0.02	0.02	0.01	0.01	0.02
20:2	0.01	0.19	0.01	0.17	0.002	0.18	0.003	0.18
20:3	0.02	0.04	0.02	0.03	0.02	0.03	0.02	0.02
CLA								
c-9, *t*-11	nd	1.44	nd	0.75	nd	0.78	nd	0.69
t-10, *c*-12	nd	1.66	nd	0.79	nd	0.83	nd	0.70

[1] Sow and piglet diets separated based on inclusion of 0% or 2% CLA, nd = not detected

phases (Phase I: d 17 to d 22; Phase II: d 23 to d 36; Table 11). Feed intake and BW were recorded weekly.

Bacterial Culture, Oral Challenge and Health Status

After 11 ± 2 d on the ND, all piglets were given an oral challenge with a strain of *E. coli* expressing the $K88^+$ (F4) fimbria obtained from the Animal Health Center, Veterinary Services Branch, Manitoba Agri-Food and Rural Initiatives (Winnipeg, MB, Canada). Cultures were grown over night in LB broth (BD Bioscience, Mississauga, ON, Canada) at 37°C from stock cultures stored at -20°C. DNA was extracted and polymerase chain reaction (PCR) applied to confirm expression of F4 fimbria and heat liable (LT) toxin. Briefly, DNA was extracted by centrifuging cell cultures at 3,000 rpm for 10 min (IEC CentraGP8, Needham, MA) and discarding supernatant. Cells were then transferred to sterile microcentrifuge tubes (Fisher, Fairlawn, NJ), washed with 400 µl of Tris EDTA buffer (TE), centrifuged and the supernatant discarded. Cells were re-suspended in 400 µl of TE buffer and heated at 95°C for 15 min to rupture the bacterial cell membrane. Into heated samples, 400 µl of phenol chloroform was added and vortexed thoroughly for 30 sec. The mixture was cooled to -70°C for 15 min and then centrifuged at 13,000 rpm (Microlite, Thermo IEC, Waltham, MA). After centrifugation, 300 µl of supernatant was collected into clean 1.5 ml microcentrifuge tubes. Phenol chloroform treatment was repeated by adding an equal volume of phenol chloroform to supernatant, vortexing and centrifugation as described above.

Polymerase chain reaction was performed using a thermocycler (Techne genius, Duxford, Cambridge, UK) with the following 36 cycle program: 94°C for 1 min, 72°C for

1 min plus 5 min at 68°C for final elongation. The following K88 primers designed in-house for this study: 5'-GCACATGCCTGGATGACTGGTG-3' forward, 5' CGTCCGCAGAAGTAACCCCACCT-3' reverse. The primers used for LT were: 5'-CCGTGCTGACTCTAGACCCCCA-3' forward, 5'-CCTGCTAATCTGTAACCATCCTCTGC-3' reverse according to Kotlowski et al. (2006). Amplification products were then electrophoresed with a 2% agarose gel and viewed following UV exposure. Samples were considered positive when a distinctive band was produced that corresponded to a migration pattern consistent with a standard DNA fragment.

At 28 ± 2 d of age each piglet was gavaged with 6 ml of cell suspension containing 10^9 cfu/ml of ETEC $K88^+$ from a syringe attached to a polyethylene tube to ensure ingestion of inoculum. Scour severities were monitored at 8, 24, 48 and 56 h post challenge based on the methods of Marquardt et al. (1999). Briefly, at each period scour severity scoring (0 = no scours, 1 = soft feces, 2 = mild diarrhea, 3 = severe diarrhea) was performed on each pen by trained personnel with no prior knowledge of the dietary treatments.

Digesta Collection and Microbial Enumeration

At 36 ± 2 d of age, 1 piglet per pen was randomly selected for blood and tissue collection. Each piglet was anesthetized with isoflorane until surgical depth was achieved. Blood was then collected via jugular puncture into 10 ml vacutainers (BD, Mississauga, ON, Canada) for serum and plasma, the latter containing lithium heparin. From each pig, a 5 cm segment of terminal ileum was collected and immediately placed

on ice for microbial enumeration. Piglets were then euthanized via intra-cardiac injection of sodium pentobarbital (110 mg/kg BW). Spleen, duodenum, jejunum and ileum were removed, flushed with 0.9% saline and blotted dry with adsorbent paper. Weights and lengths of small intestine were recorded and 5 cm segments of terminal ileum fixed in saline buffered formalin (Fisher Scientific Canada, Ottawa, ON, Canada) for histological evaluation. Fresh samples of caecal digesta were collected and frozen at -80°C until analyzed for indices of gut health.

Mucosal adhered LAB and total coliforms (TC) were enumerated from terminal ileum samples following a modified method of Krause et al. (1995). Each sample was cut longitudinally and flushed with distilled water to remove non-adhered material, and then the epithelial mucosa and sub-mucosa were removed from the gut wall using a blunt sterile knife handle. Scrapings were weighed and 1:10 serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) made with sterile peptone buffered water (BD Bioscience, Mississauga ON, Canada). Lactic acid bacteria were cultured in duplicate on sterile De Man, Rogosa, Sharpe (MRS) agar and TC on sterile LB agar (BD Bioscience, Mississauga ON, Canada) by dispensing 10-10 μL drops onto appropriate plates. Total coliform plates were incubated for 24 h and LAB for 48 h at 37°C. Morphologically distinct colonies were counted on each plate and the highest dilution used for calculating cfu/g.

Chemical Analyses

Total lipids in plasma were extracted using 2:1 chloroform:methanol, based on a procedure modified from Folch et al. (1956). To each sample 100 μL of heptadecanoic acid (17:0; Sigma-Aldrich, Oakville, ON, Canada) was added to serve as an internal

standard. Lipid extracts were methylated using 0.5 M methanolic acid and the reaction was carried out at 80°C for 1 h. Fatty acid methyl esters were determined using a Hewlett Packard (Hewlett Packard Canada, Mississauga, ON, Cananda) HP 5890A gas chromatogram equipped with a flame ionization detector and separated on a HP88 100 m x 0.25 mm x 0.2 μm fused-silica column (Agilent Technologies Inc., Mississauga, ON, Canada). Oven temperatures were as follows: initial temperature 70°C for 1 min; raised to 180°C at 8°C/min; raised to 195°C at 1°C/min and held for 10 min; raised to 220°C at 1.2°C/min and held for 5 min; total run time 65.58 min. The injection temperature was 220°C and the detection temperature was at 290°C. Individual isomers were identified via comparisons to known standard retention times. Crude fat was determined via hexane extraction using a VELP SER 148 solvent extractor (VELP Scientifica, Plainview, NY).

Plasma samples were analyzed for urea N using a Nova Stat profile M blood gas and electrolyte analyzer (Nova Biomedical Corporation, Waltham, MA). Volatile fatty acids (VFA) and ammonia-N were extracted at room temperature by adding 50 ml of 0.1N HCl to 5 g of digesta followed by continuous overnight (~16 h) shaking (Newbrunswick Scientific, Edison, NJ). After shaking, 10 ml of liquid was removed and frozen at -20°C until analyzed for ammonia-N. One ml of 25% metaphosphoric acid was added to remaining extracts, for VFA analysis, and mixed thoroughly prior to freezing.

Volatile fatty acid samples were prepared using a modified method of Nyachoti et al. (2006). Briefly, after thawing frozen extracts, 0.4 ml of NaOH and 0.64 ml of 0.3M oxalic acid was added to each sample, mixed thoroughly and then centrifuged for 20 min at 3,000 rpm. Approximately 2 ml of supernatant was then added to clean GC vials and analyzed using a Varian model 3400 gas chromatogram (Varian, Walnut Creek, CA)

equipped with a Carbopack B-DA 4% CARBOWAX 80/120 20 M column.

Ammonia-N samples were analyzed using the method of Novozamsky et al. (1974). Briefly, 1.5 ml of reagent one, composed of 100 ml alkaline phenolate, 200 ml 0.05% sodium nitroprusside and 10 ml 4% Na_2 EDTA plus 2.5 ml of reagent two, composed of 400 ml phosphate buffer and 100 ml 10% NaOCl were added to 50 μL of digesta extract and shielded from light to prevent UV interference. Following 30 min of incubation at room temperature, absorbencies were read at 630 nm and concentrations determined from a standard curve regression with a range of 2.5 to 20 mg/L.

Immunoglobulins A & G titer evaluation

Serum immunoglobulin (Ig) titers were measured using a quantitative commercial enzyme-linked immuno-sorbent assay (ELISA) kit (E100-104, Bethyl Laboratories Inc., Montgomery, TX). Both capture and detection antibodies were goat anti-pig IgA and IgG. Detection antibody was conjugated to horseradish peroxidase whose reaction with an alkaline phosphatase substrate formed a product after 5 min incubation at room temperature. Samples were read at 450 nm with a Bio-Rad 3550 Microplate Reader (Bio-Rad Laboratories, Hercules, CA).

Histology

Histological samples were prepared as per the procedures of Owusu-Asiedu et al (2003b). Briefly, 5 μm cross sections of paraffin embedded samples were stained with hematoxylin and eosin and mounted in duplicate. Villous height (VH) and crypt depth (CD) were measured on 10 well-oriented villi per sample where the crypt-villus junction

was readily distinguishable using a Nikon YS100 compound light microscope equipped with a Sony DSP 3CCD colour video camera. Similarly, mucosal thickness was calculated as the ratio of crypt depth to crypt width as per the method of Hontecillas et al. (2002). Images were captured and processed using Northern Eclipse Image Processing Software version 6.0 (Empix Imaging, Inc., Mississauga, ON, Canada).

Statistical Analysis

Both sow and piglet data was subjected to ANOVA as a completely randomized design using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). For nursery piglet data, pen was considered the experimental unit for all measured response criteria. Due to the unbalanced nature of the design, data were reported as least square means. Treatment means were separated using Tukey's test with P-values < 0.10 considered to be trends and P-values < 0.05 considered significant.

RESULTS

Sow and Piglet Performance

Table 13 shows performance responses of sows during gestation and lactation and of piglets until weaning. Conjugated linoleic acid supplementation had no effect ($P > 0.10$) on sow BW, back fat or ADFI. Sows receiving CLA supplemented diets had larger litters with more piglets born alive ($P = 0.01$) and tended ($P = 0.06$) to wean more piglets per litter compared to controls. However, it should be noted that within the control group, 2 sows had unusually small litters (3 and 4 piglets), the effect of which was to create a significant artifact for supplementation. Piglet BW did not differ during the

Table 13. Effect of dietary CLA on pre-weaning sow and litter performance and piglet immunoglobulin status[1]

	Dietary Treatment			
Item	0% CLA	2% CLA	SEM	P-Value
Sow BW, kg				
Gestation				
d 85	197.5	223.4	14.1	0.22
d 112	220.1	242.3	13.4	0.27
Lactation				
d 1	205.1	224.4	16.0	0.43
d 17	198.8	216.8	14.6	0.40
Sow back fat, mm				
Gestation				
d 85	22.1	18.5	1.64	0.16
d 112	23.3	19.1	1.76	0.12
Lactation				
d 1	20.4	19.6	2.02	0.79
d 17	18.0	18.0	2.24	1.00
Sow ADFI, kg/d				
d 85 to d 112	3.04	2.96	0.11	0.63
d 112 to weaning	4.87	5.05	0.13	0.37
Overall	3.65	3.68	0.10	0.88
Piglets/litter				
Total born	7.33	12.6	1.07	0.005
Born alive	7.00	11.9	1.16	0.01
Still births	0.33	0.75	0.24	0.25
Weaned	6.83	9.75	0.99	0.06
Piglet BW, kg				
d 1	1.74	1.40	0.12	0.76
d 3	2.29	1.96	0.14	0.13
d 17	6.00	5.60	0.42	0.51
Piglet immunoglobulins, g/L				
IgG				
d 3	25.2	25.7	4.38	0.94
d 17	37.2	34.5	1.53	0.47
IgA				
d 3	2.10	2.01	0.14	0.68
d 17	0.18	0.17	0.02	0.66

[1] Values are LSmeans ; SEM are pooled

nursing period ($P > 0.10$).

At weaning there was a trend ($P = 0.09$) for piglets from CLA-supplemented sows and allocated to CLA diets to have reduced BW, but by d 28 BW were similar across treatment groups (Table 14). On d 36 piglets receiving 2% CLA-supplemented ND were lighter ($P < 0.04$) than piglets receiving the control diet. The main effects of sow lactation ration, ND and their interaction had no effect on ADG ($P > 0.10$). However, piglets weaned from CLA-supplemented sows had higher ($P = 0.05$) ADFI from d 17 to d 28 and tended ($P < 0.10$) to have reduced feed efficiency. Average daily feed intake and G:F was not affected by the main effects of lactation and ND nor their interaction (Table 14).

Visceral Organs, Intestinal Morphology, PUN and Immunoglobulins

From Table 15 it can be seen that spleen weight plus small intestine weight and length were not affected by dietary treatments ($P > 0.10$). Scours were detected at 8 h post-challenge in all treatment groups except the 2%-2% CLA group (Table 15). Scour severity reached a maximum level at 48 h post-challenge with the 0%-0% group (control piglets) having the most severe and the 2%-2% group the least severe scours. Piglets weaned from CLA-supplemented sows had reduced ($P < 0.05$) scours at each post-challenge period compared to piglets weaned from control sows. At 48 h, piglets receiving 2% CLA-supplemented ND tended to have reduced scour severity ($P < 0.10$) a benefit further enhanced in 2%-2% piglets at 56 h as evidenced by a significant interaction ($P < 0.01$) between lactation and ND.

Table 14. Growth performance for piglets born to control or 2% CLA-supplemented sows fed nursery diets with or without CLA and challenged with *E. coli* K88[+1]

	Dietary Treatment							
	0% CLA Lactation		2% CLA Lactation			P-Value[2]		
Item ND:	0% CLA	2% CLA	0% CLA	2% CLA	SEM	Lac	ND	LxN
BW, kg								
17 d	5.46	5.45	5.60	4.52	0.04	0.21	0.09	0.10
28 d	6.19	6.09	6.44	5.56	0.33	0.68	0.16	0.25
36 d	8.36	7.50	8.36	6.85	0.53	0.55	0.04	0.55
ADG, g/d								
17 to 28 d	185	214	189	173	18.9	0.35	0.76	0.25
28 to 36 d	226	198	234	184	26.3	0.92	0.16	0.67
Overall	563	692	630	552	98.4	0.72	0.80	0.31
ADFI, g/d								
17 to 28 d	372	307	453	437	51.3	0.05	0.44	0.64
28 to 36 d	781	743	928	766	92.8	0.37	0.30	0.51
Overall	570	520	684	594	66.9	0.18	0.31	0.77
G:F kg/kg								
28 d	0.54	0.64	0.47	0.39	0.09	0.10	0.91	0.36
36 d	0.31	0.30	0.26	0.26	0.04	0.34	0.97	0.94
Overall	0.28	0.24	0.20	0.22	0.04	0.24	0.75	0.45

[1] Values are LSmeans; SEM are pooled

[2] Main effects of Lactation (Lac) and ND or their interaction (L x N)

In terms of intestinal morphology, VH and CD were unaffected by dietary treatment ($P > 0.10$; Table 15). However, piglets weaned from CLA supplemented sows had thinner ($P < 0.04$) gut mucosa in comparison to control piglets.

Plasma urea N levels were greater for piglets from CLA-supplemented sows than from control sows ($P < 0.04$; Table 15). With a concentration of 7.44 mmol/L, 2%-2% piglets had the greatest PUN concentrations compared to the remaining treatment groups. Prior to weaning, there was no dietary effect ($P > 0.10$; Table 13) on piglet IgA or IgG concentrations on either d 3 or d 17 or nursing. However, after the *E. coli* $K88^+$ challenge, serum IgA and IgG levels were greater ($P < 0.05$; Table 16) in piglets weaned from CLA-supplemented sows compared to piglets weaned from control sows.

Fatty Acids

In Table 16 it can be seen that plasma SFA, MUFA and PUFA isomers remained relatively constant between treatment and control animals. However, piglets receiving 2% CLA-supplemented ND had greater ($P < 0.01$) circulating levels of 20:0 and 20:1 and lower concentrations of 20:3 isomers ($P = 0.01$) compared to piglets receiving control ND. In addition, piglets receiving 2% CLA-supplemented ND had greater ($P < 0.001$) concentrations of circulating *c*-9, *t*-11 and *t*-10, *c*-12 CLA isomers in comparison to piglets receiving control ND. Colostrum fatty acid profiles are shown in table 10. Due to limited sample numbers statistical analysis was restricted. Detectable levels of CLA isomers were only observed within the colostrum of animals receiving CLA supplemented diets (n = 5; Chapter 3, Table 10) with an average level of 5.95 g/100g of *cis*-9, *trans*-11 and 5.28 g/100g of and *trans*-10, *cis*-12 being measured respectively. In

Table 15. Effect of CLA supplementation on indices of enteric health and immune stimulation post *E. coli* K88$^+$ challenge[1]

	Dietary Treatment							
	0% CLA Lactation		2% CLA Lactation			P-Value[2]		
Item Nursery diet:	0% CLA	2% CLA	0% CLA	2% CLA	SEM	Lac	ND	LxN
Visceral organs								
Spleen wt, g	23.0	20.7	25.7	22.5	2.30	0.34	0.26	0.84
Small Intestine Wt, g	533	513	540	460	40.5	0.58	0.23	0.47
Small Intestine length, cm	928	994	981	933	40.6	0.92	0.82	0.18
Scour scores, h post-challenge[3]								
8	0.17	0.33	0.06	0.00	0.09	0.03	0.59	0.24
24	0.58	0.42	0.25	0.07	0.14	0.03	0.24	0.97
48	1.58	1.42	1.19	0.71	0.18	0.01	0.10	0.42
56	0.92	1.25	0.78	0.07	0.0	<0.001	0.33	0.01
Intestinal morphology								
Villus height (VH), µm	276	309	283	310	30.5	0.89	0.34	0.92
Crypt depth (CD), µm	311	295	276	282	20.1	0.25	0.81	0.61
Mucosal thickness[4]	7.10	6.97	5.62	6.62	0.43	0.04	0.33	0.21
PUN, mmol/L[5]	4.97	5.05	5.29	7.44	0.62	0.04	0.09	0.11
Immunoglobulins, g/L								
IgG	34.1	35.7	41.2	42.2	2.80	0.03	0.65	0.93
IgA	0.24	0.33	0.42	0.46	0.05	<0.001	0.21	0.56

[1] Values are LSmeans; SEM are pooled

[2] Main effects of Lactation (Lac) and ND or their interaction (L x N)

[3] Scour scores: 0 = no scours, 1 = soft feces, 2 = mild diarrhea, 3 = severe diarrhea

[4] Mucosal thickness: calculated as ratio of crypt depth to crypt width

[5] Plasma urea nitrogen was measured 7 d post *E. coli* K88$^+$ challenge

Table 16. Plasma fatty acid profiles post *E. coli* K88$^+$ challenge for piglets born to control or 2% CLA-supplemented sows[1]

	Dietary Treatment							
	0% CLA Lactation		2% CLA Lactation			P-Value[2]		
ND:	0% CLA	2% CLA	0% CLA	2% CLA	SEM	Lac	ND	LxN
Fatty Acid[3]	g/100g Total Fatty Acids							
SFA								
16:0	3.13	2.07	2.22	3.24	0.67	0.86	0.98	0.14
18:0	3.18	2.11	2.14	3.00	0.64	0.91	0.87	0.15
20:0	0.03	0.80	0.05	1.22	0.15	0.15	<0.001	0.20
MUFA								
16:1	3.25	2.56	2.56	3.33	0.54	0.94	0.94	0.19
18:1	0.44	0.34	0.28	0.30	0.08	0.23	0.66	0.49
20:1	0.44	0.64	0.32	0.94	0.15	0.54	0.01	0.17
PUFA								
18:2	7.64	3.86	5.83	5.79	1.50	0.97	0.22	0.23
20:2	0.07	0.04	0.06	0.06	0.01	0.80	0.37	0.24
18:3	0.09	0.06	0.05	0.08	0.02	0.65	0.87	0.11
20:3	1.23	0.38	0.95	0.53	0.22	0.76	0.01	0.35
20:4	0.01	0.01	0.02	0.02	0.01	0.55	0.78	0.90
CLA								
c-9, *t*-11	nd	0.05	nd	0.07	0.01	0.08	<0.001	0.19
t-10, *c*-12	nd	0.08	0.01	0.15	0.03	0.22	<0.001	0.36

[1] Values are are LSmeans; SEM are pooled, nd = not detected

[2] Main effects of Lactation (Lac) and ND or their interaction (L x N)

[3] SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = poly-unsaturated fatty acids

addition, whereas saturated isomers remained relatively constant between treatment groups, mono and poly-unsaturated isomers appeared to be reduced in CLA supplemented groups.

Microbial Populations and Fermentation Products

Total coliforms and lactic acid bacterial populations were unaffected by dietary treatments ($P > 0.05$; Table 17). Ammonia N levels were greatest in 0%-0% piglets and were approximately 75.6% greater than in the 0%-2%, 2%-0% and 2%-2% treatment groups, even though concentrations were not significantly different between dietary groups ($P \geq 0.25$). In general, VFA concentrations were unaffected by dietary treatment. Although not significant, acetic and proprionic concentrations were greatest in 2%-2% piglets in comparison to the remaining treatment groups.

DISCUSSION

Animal Performance

Dietary supplementation of CLA in post-weaning diets did not affect piglet growth performance, results in agreement with Weber et al. (2001) and Bontempo et al. (2004). Furthermore, Bee (2000b) reported that piglets weaned from CLA-supplemented sows showed significantly increased weight gain, feed intake and final weight at d 70 of age, results not observed in the current study or by Bontempo et al. (2004). Piglets in the study of Bee (2000b) were weaned at d 35 versus d 17 in this study. This extended time presumably allowed piglets to ingest more CLA isomers prior to weaning which may have accounted for their superior post-weaning growth performance.

Table 17. Microbial enumeration and intestinal fermentation measurements in piglets born to control or CLA-supplemented sows post *E. coli* K88$^+$ challenge[1]

	Dietary Treatment							
	0% CLA Lactation		2% CLA Lactation			P-Value[2]		
Item ND:	0% CLA	2% CLA	0% CLA	2% CLA	SEM	Lac	ND	LxN
Microbial Counts, log_{10} cfu/g								
Total coliforms	4.42	4.53	4.46	4.18	0.41	0.72	0.85	0.64
Total lactic acid bacteria	5.00	4.92	4.81	4.97	0.34	0.84	0.90	0.73
Ammonia N, mg/L	78.4	46.6	45.0	42.5	15.6	0.25	0.29	0.37
Volatile Fatty Acids, mmol/L								
Acetic	36.0	39.7	42.1	50.8	5.96	0.17	0.32	0.69
Propionic	20.5	18.3	20.8	27.6	3.83	0.23	0.57	0.26
Iso-butyric	0.12	0.10	0.05	0.08	0.04	0.30	0.89	0.53
Butyric	10.3	7.32	7.08	8.71	2.00	0.67	0.75	0.28
Iso-valeric	0.26	0.46	0.34	0.31	0.13	0.77	0.51	0.37
Valeric	5.54	3.42	2.92	3.06	1.40	0.31	0.49	0.44
Lactic	23.9	17.4	18.5	23.9	12.7	0.71	0.77	0.91
Total	87.7	86.7	91.8	114.4	16.4	0.35	0.53	0.49

[1] Values are LSmeans; SEM are pooled

[2] Main effects of Lactation (Lac) and ND or their interaction (L x N)

Immune stimulation & Indicators of gut health

Based on scour severity, piglets weaned from CLA-supplemented sows appeared to be healthier than piglets weaned from control sows during the first 56 hr after oral ETEC challenge. However, it is important to note the inherent subjectivity of scour scoring. Here, although score rarely exceeded a level of mild diarrhea which suggests all piglets remained relatively healthy, it is likely that piglets were sicker than the scour score indicated.

Microbial populations within the upper gut of pigs are affected by many variables including age, diet and health status and a dynamic relationship exists between commensal and pathogenic micro-flora that are affected by these variables (Fairbrother et al., 2005). In young pigs, proliferation of opportunistic pathogenic microbes may lead to antigenic stimulation of intestinal tissue resulting in inflammation and reduced availability of nutrients required for growth (Nyachoti et al., 2006). In the current study, it was speculated that dietary CLA may allow beneficial microbes such as *Lactobacillus* species to populate the intestinal wall leading to improved gut health in the face of an ETEC challenge. The control and CLA-supplemented diets were formulated to contain the same protein and carbohydrate concentrations, which may have accounted for the lack of changes observed in LAB or total coliform populations. The fact that these feed constituents are known substrates for enteric microorganisms and were kept consistent across rations may explain why microbes populating the intestinal wall were unchanged. It should also be noted that long chain fatty acids such as CLA are not favorable fermentation substrates for most microbes, and in some cases may even be bactericidal

(Knapp and Melly, 1986). Nonetheless, a thorough microbial census was not performed and other micro-flora which were not detected may have been affected.

Volatile fatty acid results coincide with microbial results in that there were no significant differences observed between treatments. However, acetic acid and total VFA levels were greatest in piglets weaned from CLA supplemented sows. As well, propionic acid was greatest in 2%-2% piglets in comparison to the remaining treatment groups. These differences may have been beneficial, in that if these piglets had reduced intestinal pH it would create an inhospitable environment for ETEC. Because both acetic and propionic acids have relatively high pK_a values (4.76 and 4.88, respectively) and their production could lower overall digesta pH (Partanen and Mroz, 1999). However, this can not be confirmed since digesta pH was not directly measured in this experiment.

Production of ammonia from AA and other nitrogenous compounds is a result of enteric microbial fermentation, and coliform bacteria such as *E. coli* have been shown to contribute to ammonia production within the upper gut of pigs (Dierick et al., 1986). Elevated intestinal ammonia is thus a biomarker for the proliferation of potentially pathogenic bacteria. As mentioned previously, microbial enumeration performed in this study was limited and biased in favor of culturable bacteria. That ammonia was elevated in 0%-0% piglets suggests proliferation of pathogenic *E. coli*, which may explain advanced incidence of scours and impaired enteric health in these treatment animals.

In addition to being used as a marker of protein absorption and AA utilization, PUN has been used to indicate the extent of lean tissue catabolism, immune induction and acute phase protein synthesis in diseased piglets (Owusu-Asiedu et al., 2003). Although little work had been done establishing normal PUN values in the suckling piglet, elevated

PUN levels could result from the metabolism of dietary AA which are not incorporated into muscle tissue. Because 2%-2% piglets had levels approximating normal (7.4 to 21.4 mmol/L; CCAC, 1993), an alternative explanation could be that PUN concentrations of piglets nursing control sows could be depressed, potentially as a result of reduced protein absorption.

Maternally derived colostrum and milk immunoglobulins contribute to intestinal development and serve as a primary means of disease resistance for the hypo-immune piglet (Le Dividich et al., 2005). However, these large macro-molecules can only be absorbed for a brief period postpartum after which absorption is no longer possible due to a shift in intestinal cell populations known as gut closure (Rooke and Bland, 2002). Thus, insufficient intake of colostrum or consumption of poor quality colostrum and milk by the neonatal piglet is associated with reduced survivability and subsequently, economic losses to producers (Le Dividich et al., 2005).

Dietary CLA had no effect on circulating immunoglobulin concentrations prior to weaning, results which are in disagreement with previous research showing that dietary CLA can significantly increase piglet IgG at d 2, 10 and 20 of age (Bontempo et al., 2004). However, the study of Bontempo et al. (2004) showed no effect of lactational CLA-supplementation on post-weaning piglet IgG titers, whereas nursery diet supplementation was only significant at 46 d of age versus elevated IgA and IgG at d 36 of age in the current study. The fact that immunoglobulins were not affected by dietary CLA in our study, may be due in part to the use of primiparous sows which are less efficient at mobilizing plasma fatty acids and immunoglobulins into colostrum and milk (Slevin and Wiseman, 2003) than the multi-parity sows used by Bontempo et al. (2004).

Nevertheless, the sows used by Bontempo et al. (2004) only received CLA 8 d prior to farrowing versus 30 d in the current study. The extended gestational supplementation in the present study may have contributed to a greater post-weaning carry over immune stimulation resulting in piglets weaned from CLA-supplemented sows having greater serum IgA and IgG concentrations at d 36 of age.

During enteric infections, secretion of IgA by intestinal lymphocytes serves as an important defense mechanism by reducing the ability of invading pathogens to attach to epithelial receptors and deactivating bacterial toxins (Mestecky et al., 1999; Salmon, 1999). The fact that circulating IgA in pigs weaned from CLA-supplemented sows were significantly greater than controls could indicate a correlation with mucosal IgA concentrations (O'Shea et al., 2004) and thus an indicator of stimulated secretory immunity in these piglets.

As *E.coli* $K88^+$ colonizes the small intestine during infection, local inflammation is facilitated through the synthesis and secretion of heat-liable (LT) toxins (Fairbrother et al., 2005). A consequence of this colonization and resulting inflammation is that local tissue damage manifest as erosions, villi atrophy and mucosal thickening. Conjugated linoleic acid has the ability to reduce inflammation through multiple means, such as the production of a less active series of eicosaniods (Belury, 2002). One outcome of *E. coli* $K88^+$ infection is activation of phospholipase-A by LT leading to liberation of membrane bound arachidonic acid and downstream synthesis of pro-inflammatory prostaglandin-E_2 (PGE_2; de Haan and Hirst, 2004). Once CLA becomes incorporated into cellular membranes it is metabolized in a similar fashion as linoleic acid, resulting in the formation of a conjugated isomer of arachidonic acid (Banni, 2002). Enzymatic

oxidation of this compound by cyclooxidase (COX) produces PGE_4, a less potent inflammatory agent (O'Shea et al., 2004). Additionally, CLA has been shown to competitively inhibit COX activity resulting in reduced PGE_2 production and less inflammatory response initiation (Bulgarella et al., 2001). Although CLA was not detected in plasma of 2%-0% piglets this does not preclude residual CLA remaining within gut tissue which accumulated during nursing. Lack of mucosal thickening and reduced scours observed in 2%-0% and 2%-2% piglets suggests synthesis of less active prostaglandins within intestinal tissue, which may have contributed to reduce local inflammation and superior epithelial integrity.

The duration for which dietary CLA must be supplied in order to stimulate immunity in nursery pigs is currently unknown. For example, 42 d of post-weaning CLA supplementation was required for lymphocyte proliferation to be affected in immune challenged piglets (56 d of age; Bassaganya-Riera et al., 2001). Whereas in immune competent piglets suckling CLA-supplemented sows, 25 d (46 d of age) was required to increase serum IgG (Bontempo et al., 2004). The ability of young pigs to digest complex lipid nutrients such as CLA is limited during the first week post weaning, which may explain why immune stimulation is not observed in piglets less than 8 wk of age. However, our results appear to be in line with previous data which indicates that immune stimulation can be accelerated when piglets are weaned from sows fed CLA-supplemented diets.

Conjugated linoleic acid is a known ligand for peroxisome proliferator-activated receptor gamma (PPAR-γ; O'Shea et al., 2004) whose activation has been associated with reduced intestinal inflammation in pigs (Hontecillas et al., 2002; Bassaganya-Riera and

Hontecillas, 2006). Although not measured in this study PPAR-γ activation may have been responsible for reduced mucosa thickening observed in 2%-0% and 2%-2% piglets may have been the result of. It is also possible that PPAR-γ activation along with reduced production of proinflammatory eicosanoids contributed to superior enteric health observed in 2%-0% and 2%-2% piglets. However, future studies will need to be conducted to confirm this mode of action.

Supplementation of sow gestation and lactation diets with CLA appears to be a practical strategy to improve the enteric health and immune status of nursery piglets. The results of the present study indicate that piglets weaned from CLA-supplemented sows had superior intestinal health and immune status markers. The fact that nursery diet supplementation with CLA did not improve intestinal health markers may have been related to piglet age or feeding duration. In light of these beneficial findings, further studies are required to determine the specific routes by which dietary CLA supplementation is facilitating these favorable effects.

CHAPTER 5

GENERAL DISCUSSION

As modern swine management systems continue to increase the pressure to wean more piglets per year, combined with the growing trend to reduce in-feed antibiotic administration, there is a growing need to improve the immunity of young piglets. Research focused on passive immune transfer indicates that the amount and composition of colostrum and milk consumed by new-born pigs is a major contributor to the immune status of piglets post-weaning (Le Dividich et al., 2005). That is, piglets that are able to consume sufficient amounts of colostrum and milk rich in immunoglobulins and other immune compounds stand to have more vigorous immune systems later in life (Drew and Owen, 1988). Because of the multiple health benefits that have been attributed to dietary CLA supplementation, including studies focused on breeding animals (Bee, 2000a; Bontempo et al., 2004), provision of CLA to gestating and lactating sows, as well as to piglets post-weaning may be a practical strategy of improving sow and litter performance as well as piglet immunity pre- and post-weaning.

The objectives of the research conducted in manuscript one were to assess the ability of supplemental CLA to improve sow and litter performance through monitoring changes in body condition, as well as circulating levels of plasma fatty acids and serum immunoglobulins. It was hypothesized that supplemental CLA would improve sow and litter performance and improve passive immunity transfer to piglets. In terms of sow performance, the most noteworthy improvements were observed when immature sows were provided dietary CLA. During lactation, immature sows retained greater back fat

reserves compared to control sows. Although piglets nursing CLA-supplemented sows were lighter than piglets nursing control sows, total litter weight weaned was not affected by dietary CLA. Dietary CLA also had no effect on sow or piglet circulating immunoglobulin levels.

It is unknown how dietary CLA produced the observed biological effects in this study. For example, the fact that immunoglobulin concentrations within sows' serum and colostrum was not affected by dietary CLA, yet fatty acid profiles did appear to have changed within colostrum, suggests it is the consumption of these isomers that may facilitate heightened immunity. Although immunoglobulin titers were not affected by dietary CLA within piglet serum by d 3, this does not preclude elevated concentrations at d 1 or 2 when uptake of large immune compounds is maximal. Indeed, Bontempo et al. (2004) observed elevated IgG in piglets suckling CLA supplemented sows at d 2 as well as elevated serum and colostrum IgG within sows consuming CLA supplemented diets throughout lactation. The inherent variability of the parameters under measurement could explain why such a discrepancy exists between the current and previous study.

In manuscript two, the extent to which CLA improves passive immunity post-weaning was evaluated through the use of an oral *E. coli* $K88^+$ challenge model. It was hypothesized that piglets receiving dietary CLA from birth, through lactation and post-weaning would have a superior response to an immune challenge. From this study it was concluded that piglets weaned from CLA supplemented sows showed the greatest health improvements with no visible additive benefits to those piglets receiving CLA in nursery diets. While gross performance parameters were not improved in these piglets, markers of enteric health and immune stimulation were apparent. Most obvious were the

reductions in scour onset and severity observed following ETEC challenge. Although there were no changes in the intestinal micro-flora which was cultured, enteric biomarkers such as acetic and propionic acid as well as ammonia production suggested a shift towards non-pathogenic bacterial colonization within the small intestine. Furthermore, intestinal inflammation was reduced in piglets weaned from CLA-supplemented sows with marked increases in circulating concentrations of both IgG and IgA as well.

As outlined above, CLA has been shown to increase piglet IgG concentrations during lactation (Bontempo et al., 2004). The current study has shown what Bontempo and colleagues speculated, namely that post-weaning immunity can be improved when sow diets are supplemented with CLA. The antigenic challenge used to induce an immune response was specific to newly weaned pigs, as intestinal receptors for *E. coli* $K88^+$ decline with age (Fairbrother et al., 2005). As a result, piglets were challenged at approximately 28 d of age. Because the ability of young pigs to digest complex lipid nutrients such as CLA is limited during the first week post weaning (Gu and Li, 2003), there may not have been sufficient time for post-weaning CLA supplementation to garner an effect. This could explain why piglets receiving CLA supplementation during lactation and post-weaning had a comparable immune response to piglets receiving CLA only during lactation.

From these studies it is evident that dietary CLA can stimulate the immune system and promote gut health, however, it is unclear how these responses are achieved. Because of its chemical structure and metabolism pattern, CLA or its metabolites have been proposed as ligands for peroxisome proliferator-activated receptor gamma (PPAR-γ;

Yu et al., 2002b). Expression products associated with PPAR-γ activation include a wide variety of cytokines involved in immune regulation, tissue accretion and repair as well as general cell systems management (Dubuquoy et al., 2006). Cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1α (IL-1α), IL-6 and IL-8 are typically associated with local tissue damage and micro-inflammation, while TNF-α, interferon- γ (INF-γ) and IL-4 and IL-10 have been associated with local and peripheral T-cell sub-population modification (Yu et al., 2002a; O'shea et al., 2004). Previous studies have shown that feeding CLA to enteric antigen challenged pigs activated PPAR-γ and reduced the expression of TNF-α, INF-γ and IL-10 resulting in reduced intestinal inflammation (Hontecillas et al., 2002; Bassaganya-Riera et al., 2004; Bassaganya-Riera and Hontecillas, 2006). It has also been shown that CLA can affect the microstructure of the intestine, modulating the synthesis of proteins such as occulins that contribute to tight junction formation, which in turn may affect intestinal permeability, nutrient uptake and antigen entry (Lindmark et al., 1998; Donnet-Hughes et al., 2001; Roche et al., 2001). Additionally, CLA has been shown to affect peripheral T-cell sub-populations (Bassaganya-Riera et al., 2001) which can have effects on the type of immunoglobulin that is produced and secreted by B-cell populations (O'Shea et al., 2004). As well, cytokines associated with T-cell distribution and differentiation such as IL-4, IL-10 and INF-γ have also been shown to be affected by supplemental CLA (O'Shea et al., 2004). Therefore, the activation of PPAR-γ by CLA may represent the pivotal mechanism whereby immune modulation and anti-inflammation is achieved.

Alternative to the proposed PPAR-γ mediated effects of CLA is the notion that CLA can modulate the formation of prostaglandins. Prostaglandins are derived from

arachidonic acid, and it has been suggested that the metabolic pathway of CLA results in conjugated isoforms of arachidonic acid (Banni et al., 2004) resulting in a series of prostaglandins possessing different activities (Belury, 2002). As was postulated in manuscript two, the reduced intestinal inflammation could have been due to the production of PGE_4 versus the typical pro-inflammatory PGE_2. However, it should be noted that PGJ_2 is a known PPAR activator. With this in mind, future studies should be guided towards examining potential synergies between formation of prostaglandin series with modulated activity and PPAR activation and associated changes in expression products. It is likely that given the differing responses CLA has been observed to elicit that multiple mechanisms are playing contributing roles.

CHAPTER 6

CONCLUSIONS

The following conclusions can be drawn from the research contained within this thesis:

1. When CLA is supplemented to sows from late-gestation until weaning, immature sows receiving CLA-supplemented diets retained more back fat during lactation in comparison to immature sows receiving control diets.
2. As a result of dietary supplementation, CLA isomers are detectable in sow plasma by d 105 of gestation, in colostrum and can be observed by d 3 of lactation in piglet plasma.
3. Dietary CLA had no effect on circulating concentrations of sow and piglet immunoglobulins during gestation and lactation.
4. Piglets nursing CLA-supplemented sows were lighter than piglets nursing control sows, however, total litter weight weaned was not affected by dietary CLA.
5. Piglet growth performance measured as ADG, ADFI and G:F is not affected by post-weaning supplementation of CLA.
6. Piglets weaned from CLA-supplemented sows had greater circulating concentrations of IgG and IgA after being challenged with *E. coli* $K88^+$, which may indicate a greater propensity to combat disease.
7. The onset and severity of scours associated with enteric infection of *E. coli* $K88^+$ were significantly reduced in piglets weaned from CLA supplemented sows.
8. Additional indicators of enteric health such as reduced intestinal inflammation and reduced production of toxic microbial metabolites with simultaneous production of

potentially beneficial metabolites were observed in piglets weaned from CLA supplemented sows following *E. coli* $K88^+$ challenge.

9. A mixed-isomer CLA supplement at 2% into gestating and lactating sow diets can effectively enhance passive immune transfer to piglets resulting in greater post-weaning immune stimulation and gut health when challenged with *E. coli* $K88^+$.

Recommendations for future study:

1. Establish how dietary CLA affects compositional changes in milk and colostrum, including activity levels of active cells and compounds as well shifts in total fat and lipid constituents. Estimating intake levels of these active compounds through the use of digestibility studies would also be beneficial.
2. Establish effects of supplemental CLA on piglet gut environment during the lactational period. Measurements of intestinal histology, culturable and non-culturable microbes as well as utilizing molecular techniques to estimate key mediators of disease onset, inflammation and development could all be implemented.
3. Whole body nutrient partitioning in the sow should be examined directly as opposed to indirectly, as in the current study.
4. Long term studies focused on continuous dietary supplementation to examine sow longevity as well as life-time performance measurements.
5. Evaluate how weaning piglets from CLA supplemented sows affects nutrient digestibility post-weaning.
6. The current study indicated no affect on intestinal micro-flora communities under antigenic challenge using traditional culture techniques. The use of molecular

techniques would provide a better estimate of these populations before and during enteric challenge.

7. Increased serum IgA concentrations detected in piglets weaned from CLA supplemented sows during *E. coli* $K88^+$ challenge suggests intestinal IgA levels should also be elevated. Estimating the extent of this potential elevation should be examined through the use of molecular and/or immuno-histological techniques.
8. The activation of molecular mediators of inflammation and immune modulation, such as PPAR and associated compounds by dietary CLA has been investigated in pig colon in multiple studies, but has not been rigorously examined in the small intestine. This should be examined since the principal site of ETEC infection occurs in the upper-gut.

CHAPTER 7

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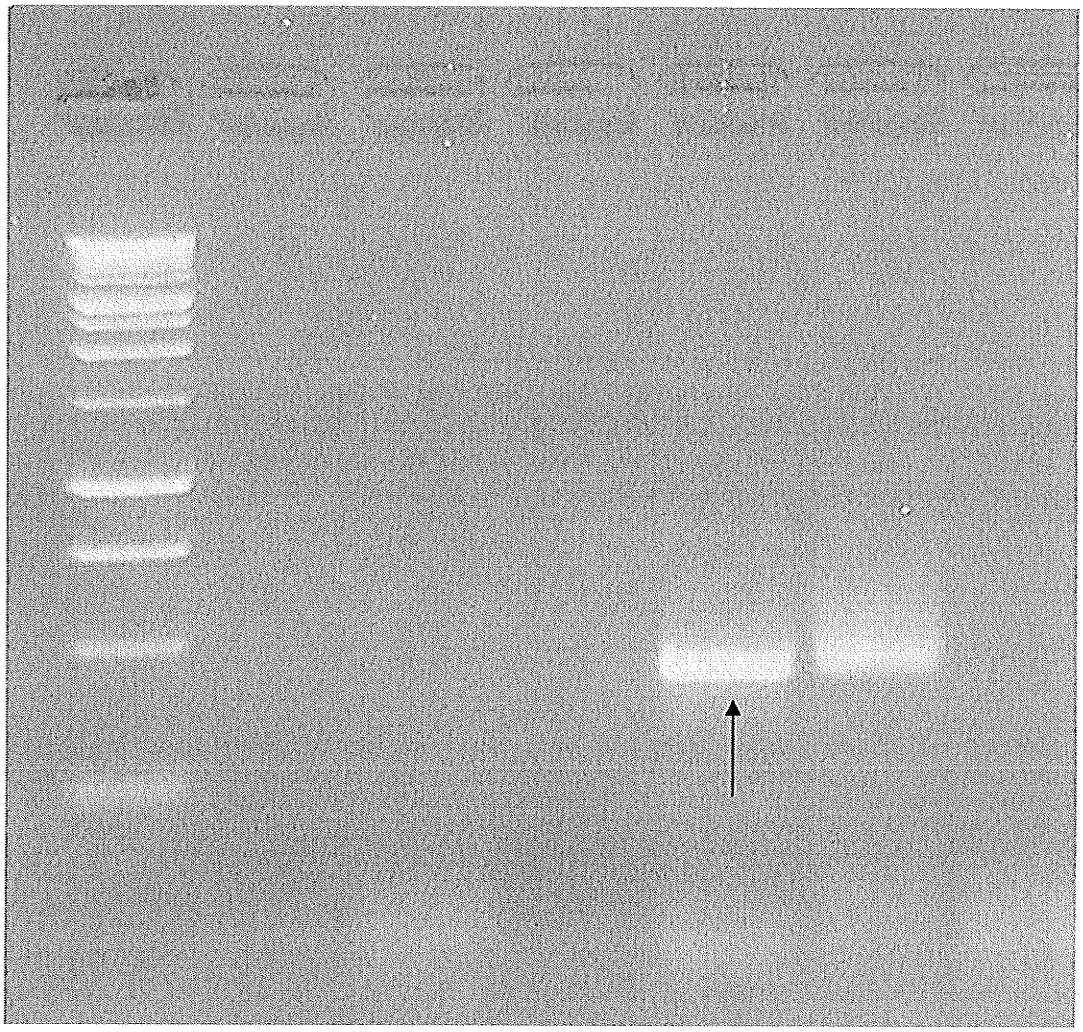
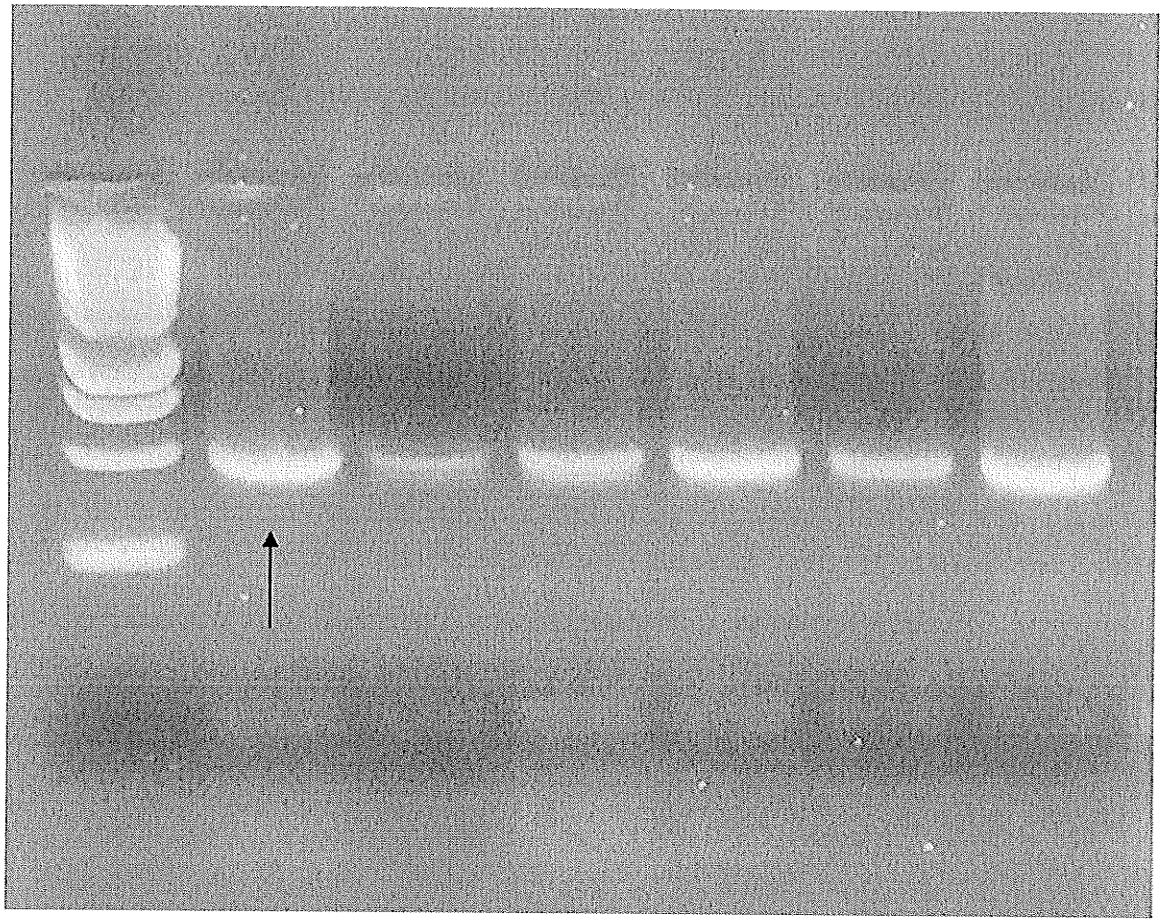
APPENDIX 1

Photograph of $K88^+$ after PCR amplification and 2% agarose gel electrophoresis



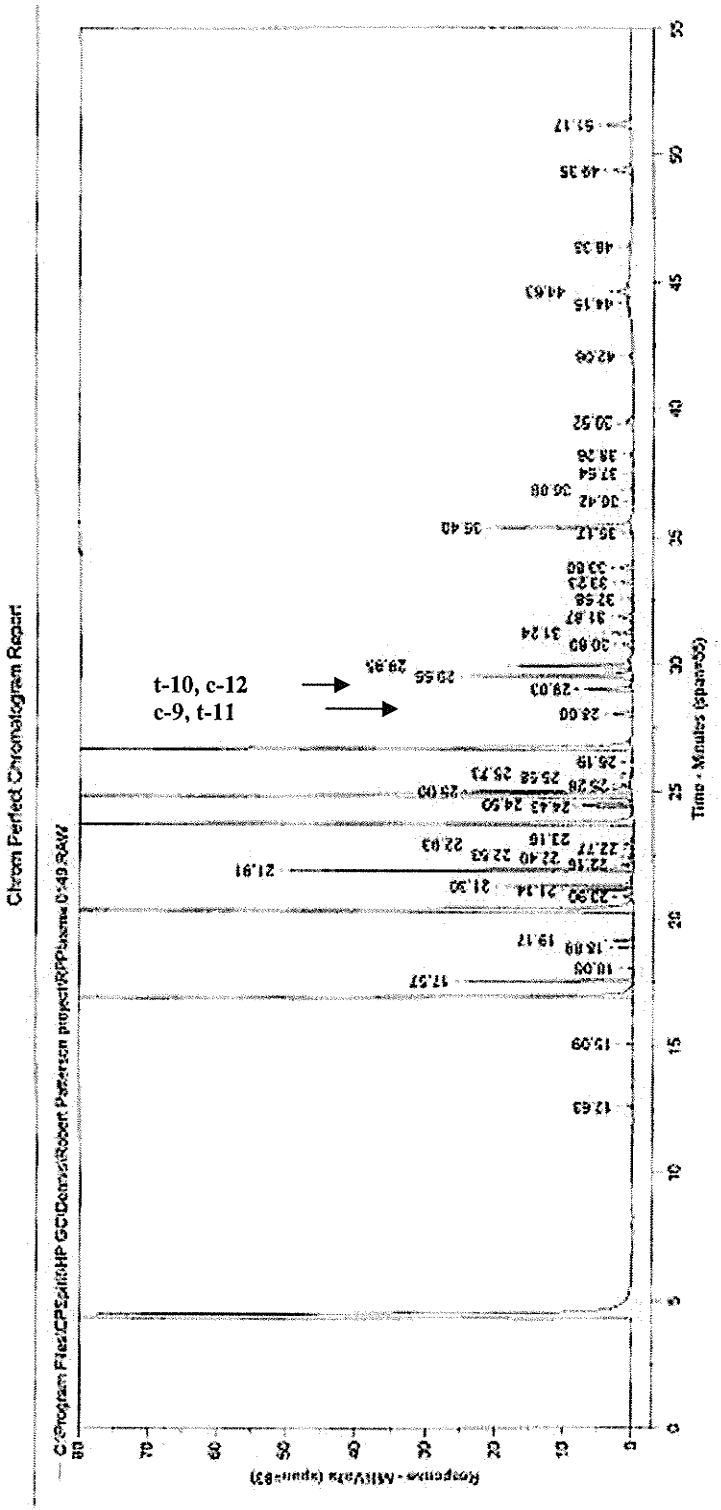
APPENDIX 2

Photograph of heat liable toxin (LT) after PCR amplification and 2% agarose gel electrophoresis



APPENDIX 3

Sample gas chromatogram with demarcated CLA isomers



APPENDIX 4

Manuscript 1 additional data - Condition scores for mature and immature sows fed control or CLA-supplemented diets during gestation and lactation[1]

	Dietary Treatment							
	0% CLA		2% CLA			P-Value[2, 3]		
Item	Immature	Mature	Immature	Mature	SEM	D	M	D x M
Gestation								
d 85	3.30	3.50	3.30	3.30	0.14	0.24	0.59	0.37
d 105	3.30	3.40	3.25	3.10	0.09			
d 112	3.25	3.50	3.25	3.25	0.15			
Lactation								
d 1	3.25	3.40	3.10	3.30	0.13	0.48	0.06	0.48
d 3	3.25	3.50	3.10	3.30	0.12			
d 17	3.00	3.25	3.20	3.20	0.13			
4 d pw	3.00	3.40	3.40	3.25	0.10			

[1] Values are LSmeans ± average SEM for each period; pw = post-weaning
[2] Main effects of diet (D), maturity (M), sampling period or their interaction (D x M)
[3] Period (P), plus two-way (D x P, M x P) and three-way (D x M x P) interactions were included in statistical model but were not significant ($P > 0.10$)

APPENDIX 5

Manuscript 1 additional data – Plasma fatty acids (g/100g) for mature (M) and immature (I) sows fed control or CLA-supplemented diets during gestation[1]

		Dietary Treatment					P-Value[2]					
		0% CLA		2% CLA								
Item	Parity:	I	M	I	M	SEM	D	M	D x M	P	D x P	M x P
18:0												
d 85		3.80	4.01	4.68	3.85	6.27	NS	NS	NS	NS	NS	NS
d 105		5.64	4.57	3.43	2.31	6.27						
d 112		8.16	5.66	15.6	20.9	6.27						
18:1												
d 85		0.51	0.51	0.65	0.61	0.07	NS	NS	NS	NS	**	NS
d 105		0.75	0.59	0.37	0.22	0.11						
d 112		0.82	0.68	2.06	2.71	1.57						
18:2												
d 85		6.54	6.86	7.87	6.85	0.67	NS	NS	NS	†	†	NS
d 105		7.49	7.11	5.90	5.08	1.27						
d 112		12.4	7.09	10.1	11.0	3.97						
Σ SFA												
d 85		7.47	7.25	8.84	7.71	0.53	NS	NS	NS	†	NS	NS
d 105		9.04	7.41	7.03	5.64	1.47						
d 112		16.4	8.89	13.1	13.1	5.48						
Σ MUFA												
d 85		4.41	4.82	5.19	5.44	0.35	NS	NS	NS	**	†	NS
d 105		4.37	4.38	3.78	3.18	0.70						
d 112		8.31	4.78	6.45	7.11	2.38						
Σ PUFA												
d 85		8.59	8.78	9.78	9.07	0.69	NS	NS	NS	*	†	NS
d 105		8.98	8.66	7.20	6.22	1.51						
d 112		14.8	8.50	11.3	12.6	4.71						
18:1/18:0												
d 85		0.14	0.13	0.14	0.16	0.01	NS	NS	NS	*	**	NS
d 105		0.13	0.13	0.11	0.09	0.001						
d 112		0.10	0.12	0.12	0.10	0.01						

[1] Values are LSmeans ± average SEM for each period

[2] Main effects of diet (D), maturity (M), sampling period (P) or their two-way interactions (D x M, D x P, M x P); three-way (D x M x P) interactions were not significant ($P > 0.10$)

APPENDIX 6

Manuscript 1 additional data – Plasma fatty acids (g/100g) for mature (M) and immature (I) sows fed control or CLA-supplemented diets during lactation[1]

		Dietary Treatment					P-Value[2]					
		0% CLA		2% CLA								
Item	Parity:	I	M	I	M	SEM	D	M	D x M	P	D x P	M x P
18:0												
d 1		5.97	6.66	3.74	2.57	1.78	NS	†	NS	**	NS	NS
d 3		5.31	6.22	4.32	7.41	1.71						
d 17		1.53	0.85	0.54	2.03	1.00						
4 d pw		3.32	4.59	6.14	10.4	2.49						
18:1												
d 1		1.06	0.76	1.43	0.18	0.69	NS	NS	NS	†	**	NS
d 3		0.65	0.74	0.56	0.80	0.25						
d 17		0.21	0.69	0.11	0.18	0.20						
4 d pw		0.43	0.27	0.60	1.65	0.45						
18:2												
d 1		7.96	8.03	4.97	3.55	2.26	*	NS	NS	NS	*	NS
d 3		5.45	7.24	7.32	9.83	2.74						
d 17		2.20	6.56	2.09	3.78	2.87						
4 d pw		4.15	6.81	13.4	15.3	3.40						
Σ SFA												
d 1		10.1	12.2	7.20	5.13	3.17	NS	NS	NS	*	NS	NS
d 3		7.84	10.1	9.13	14.1	3.43						
d 17		2.63	4.10	1.92	4.32	2.33						
4 d pw		5.46	7.53	14.7	21.2	5.40						
Σ MUFA												
d 1		6.60	6.63	7.54	5.56	1.62	*	NS	NS	*	NS	NS
d 3		6.83	6.83	8.91	10.5	2.83						
d 17		1.97	4.97	1.71	3.02	2.03						
4 d pw		3.40	5.13	9.30	11.8	2.72						
Σ PUFA												
d 1		9.86	9.67	6.25	4.70	2.81	*	NS	NS	NS	†	NS
d 3		7.85	9.04	10.3	13.0	3.62						
d 17		2.87	8.37	2.54	4.64	3.61						
4 d pw		5.95	9.40	17.8	20.6	4.60						
18:1/18:0												
d 1		0.15	0.04	0.56	0.13	0.25	NS	NS	NS	†	NS	NS
d 3		0.14	0.12	0.13	0.13	0.01						
d 17		0.14	0.56	0.13	0.10	0.13						
4 d pw		0.13	0.13	0.10	0.14	0.01						

[1] Values are LSmeans ± average SEM for each period; $P < 0.10$ = †, $P < 0.05$ = *, $P < 0.01$ = **

[2] Main effects of diet (D), maturity (M), sampling period (P) or their two-way interactions (D x M, D x P, M x P); three-way (D x M x P) interactions were not significant ($P > 0.10$)

APPENDIX 7

Manuscript 1 additional data – Piglet plasma fatty acids (g/100g) for mature (M) and immature (I) sows fed control or CLA-supplemented diets while nursing[1]

		Dietary Treatment					P-Value[2]					
		0% CLA		2% CLA								
Item	Parity:	I	M	I	M	SEM	D	M	D x M	P	D x P	M x P
16:0												
	d 3	16.5	14.8	28.1	29.3	5.04	*	NS	NS	NS	*	NS
	d 17	18.9	19.2	15.7	17.0	2.41						
16:1												
	d 3	5.89	5.60	7.13	9.15	1.12	*	NS	NS	NS	NS	NS
	d 17	5.37	6.57	6.68	6.26	0.89						
18:0												
	d 3	20.0	15.8	23.4	24.0	4.11	NS	NS	NS	**	*	NS
	d 17	14.0	12.0	7.42	9.31	1.46						
18:1												
	d 3	2.81	2.24	3.25	3.95	0.73	NS	NS	NS	**	**	NS
	d 17	2.79	2.34	0.92	1.42	0.32						
18:2												
	d 3	17.8	16.2	25.9	26.4	4.54	NS	NS	NS	NS	*	NS
	d 17	21.2	21.3	17.5	20.6	2.76						
Σ SFA												
	d 3	37.0	31.0	55.1	55.6	9.40	NS	NS	NS	**	*	NS
	d 17	33.1	31.3	24.9	27.9	3.91						
Σ MUFA												
	d 3	10.1	9.14	13.9	15.4	2.25	†	NS	NS	**	*	NS
	d 17	9.28	10.0	9.04	8.96	1.27						
Σ PUFA												
	d 3	23.9	21.1	33.2	34.2	5.85	NS	NS	NS	NS	*	NS
	d 17	26.6	26.1	21.1	25.5	3.47						
16:1/16:0												
	d 3	0.39	0.40	0.29	0.34	0.03	NS	NS	NS	NS	**	NS
	d 17	0.30	0.34	0.45	0.38	0.02						
18:1/18:0												
	d 3	0.14	0.15	0.21	0.15	0.05	NS	NS	NS	NS	†	NS
	d 17	0.20	0.19	0.12	0.14	0.01						

[1] Values are LSmeans ± average SEM for each period; $P < 0.10$ = †, $P < 0.05$ = *, $P < 0.01$ = **

[2] Main effects of diet (D), maturity (M), sampling period (P) or their two-way interactions (D x M, D x P, M x P); three-way (D x M x P) interactions were not significant ($P > 0.10$)