Effects of Feeding Conjugated Linoleic Acid to Nursery Pigs of Low- and High-Health Status on Growth and Immune Competence

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ASL-R1555

Summary and Implications

Early weaned pigs allotted either into “clean” or into “dirty” environmental conditions and potentially subject to high or low levels of antigen exposure, respectively, were used to determine the impact of 0, .67, 1.33, and 2% conjugated linoleic acid (CLA-60) on the immune status and growth performance.

CLA levels modulate immune status in weanling pigs by decreasing the $CD4^+$:$CD8^+$ ratio due to an increase in $CD8^+$ and a decrease in $CD4^+$%. If the result of that ratio is favorable to the numerator, it means that the animal has a greater immune potential to fight against bacterial-type infections (serum antibodies produced by plasma cells) than against viral or other intracellular-type infections. The increase in $CD8^+$ indicates a potential increase in cytotoxic T lymphocytes. These cells play an important role in the development of the response of the animal against viral infections.

CLA caused an increase in alpha-1-acylglycoprotein (AGP), a serum acute-phase protein produced in the liver in response to stimulation from specific cytokines.

No statistical significance in feed efficiency was attributed to CLA. Pigs placed into the clean environment utilized feed more efficiently than those placed in the dirty environment.

Growth performance was shown to be independent of dietary treatments, but after a period of 42 days, pigs fed CLA become more viral immune competent than control animals.

Introduction

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds. Chemically it can be defined as a conjugated diene; two double bonds separated by a single bond in the carbon chain. CLA prevents carcinogenesis in rodents, is antiatherosclerotic in hamsters and rabbits, improves feed efficiency and reduces backfat thickness in pigs.

CLA inhibits immune-induced growth depression in mice (1). The muscular catabolic response to cytokines is responsible for enormous economical losses in the swine industry. Furthermore, cytotoxic T lymphocytes strongly contribute to kill neoplastic and virus infected cells.

Low immune stimulated pigs possessed lower T-lymphocyte $CD4^+$:$CD8^+$ ratio and serum acylglycoprotein concentrations than high immune stimulated pigs (3).

Therefore, nutrition together with type of microbial environment have been reported to influence the immune status, and this becomes a very important factor in the pathogeny of most infectious diseases.

Our experimental objectives were to demonstrate an immune-modulating effect of CLA and a growth performance response in early weaned pigs exposed to clean and dirty environments.

Materials and Methods

A factorial (2X4) arrangement within a completely randomized block design was used to pursue our objectives by using two management schemes to create a clean and a dirty environment, and using a total of 64 (5.3 ± 0.3 kg) individually-penned, ad libitum-fed, crossbred (Y X L X H X D) pigs. Eight pigs per treatment and 32 pigs per environment.

Experimental diets (Table 1) were formulated to contain increasing amounts of 0, .67, 1.33, and 2% CLA-60. Diets were fed for a period of 7 weeks that was divided into three Phases (phase I, 1 to 2; phase II, 3 to 5; and phase III, 6 to 7 weeks). CLA source was replaced by soy oil on an equal weight basis. Within periods, diets were assumed to be isocaloric and isonitrogenous. It was assumed that CLA and soy oil had the same value of metabolizable energy (ME). Between periods, diets contained decreasing amounts of lysine and milk products. NRC (1988) recommendations for swine were used to formulate the experimental diets.

Two management schemes were used to create a high (dirty) and a low (clean) microbial level environment. For the dirty environment, older pigs were introduced into the experimental room 1week before starting the experiment with the purpose of spreading microorganisms inside the experimental room and no biosecurity rules were applied

during the experimental period. For the clean environment, experimental animals were injected with a cephalosporin (Naxcel®) every other day from the farrowing date until day zero of the experiment, to avoid infection of the piglets during the lactation period, and strict biosecurity rules were established to avoid contamination during the experimental period.

Pigs and feeders were weighed in the metric system every week. Average daily gain (ADG), average daily feed intake (ADFI), G:F, and waste feed were measured for weekly periods and for cumulative periods during 7 weeks.

Pigs were bled by vena cava puncture on days 0, 14, 28, and 42. A fluorescence analysis conducted in a flow cytometer determined cell surface antigens ($CD4^+$ and $CD8^+$). A whole blood blastogenesis assay using concanavalin A as a mitogen was conducted as an indicator of lymphocyte proliferation. As an indirect indicator of cytokine release, AGP levels in serum were measured using a radial immunodiffusion test.

Results and Discussion

A lower (P=.02) $CD4^+$:$CD8^+$ ratio was found on day 42 in pigs fed CLA (Table 2). This response was due to a decrease (P=.03) in $CD4^+$ and an increase (P<.01) in $CD8^+$ in pigs fed CLA. Pigs in the clean environment had higher (P<.01) levels of $CD8^+$ on day 42, higher levels of $CD4^+$ on days 14 (P<.01) and 28 (P=.02), but lower (P=.02) levels on day 42 relative to pigs in the dirty environment. Younger pigs have more $CD4^+$ coreceptors on T lymphocyte membranes and when they become older there is an increase in $CD8^+$. This natural effect in the maturation process of T lymphocyte subpopulations might be affected by CLA, causing an accentuation of this natural trend.

Table 2. $CD4^+$:$CD8^+$ ratio, day 42.

	Environment	
CLA level, %	*Dirty*	*Clean*
0	1.4	1.2
.67	1.3	.8
1.33	1.3	.8
2	1.2	.5

SEM (n=8) = .10

Lymphocyte proliferation was shown to be higher (P<.01) in pigs the dirty environment, as indicated by the stimulation index (SI) on days 0, 14, and 42 (Table 3). No differences due to CLA were found on lymphocyte proliferation. In vitro experiments have shown that CLA lymphocytes do not respond to concanavalin A-induced proliferation, the mitogen used in our assay.

When measuring proliferation, the blastogenesis assay is also a crude indicator of the effects of interleukin-2, a cytokine that in mice has been reported to be related to a T helper-1-type immune response. In mice and pigs it is responsible for the inhibition of a T helper-2-type response. Therefore, our data might suggest that there is not the above mentioned inhibition in CLA pigs.

Table 3. Stimulation Index[1], day 42.

	Environment	
CLA level, %	*Dirty*	*Clean*
0	32.6	18.3
.67	18.8	16.0
1.33	32.6	10.0
2	29.3	8.8

[1]SI= [(counts per minute (CPM)in stimulated wells)/(CPM in nonstimulated wells)]
SEM (n=8) = 6.0

Serum AGP concentrations measured by using a radial immunodiffusion test were higher in pigs fed CLA than control pigs on days 28 (P<.01) and 42 (P=.01) (Table 4). Clean pigs had lower (P<.01) AGP concentrations than dirty pigs on days 28 and 42.

An (CLA X environment) interaction (P=.02) was described on day 42. CLA caused an increase in AGP levels regardless of the environment, but this increase was numerically higher in the dirty environment. The previous statistical interaction suggests that the higher level of antigen exposure in the dirty environment together with the improved immune competence induced by CLA have a synergistic effect.

A possible biological interpretation is that pigs fed CLA are more immune competent than control pigs regardless of the environment. Most of the pigs in the dirty environment ,however, have activated their immune potential, greater in CLA-treated than in control animals, to overcome the negative effects of a higher microbial load in it. Clean pigs in contrast, due to a lower microbial contamination in their environment, it did not need to activate the immune system.

Table 4. AGP concentrations (μg/ml), day 42.

	Environment	
CLA level, %	*Dirty*	*Clean*
0	612.8	448.7
.67	584.3	475.6
1.33	574.6	496.3
2	854.4	616.2

SEM (n=8) = 33.9

The fact that most of the described immune modulating properties of dietary CLA appear after a certain period of administration (minimum of 42 days under our experimental conditions) indicates that CLA, to be biologically active in vivo, requires a progressive and

accumulative incorporation into the organism (cell membranes) to develop its function.

No treatment differences in growth performance due to CLA were found in any phase of the feeding program (Table 5). Clean pigs had better feed efficiency than dirty pigs in phase I (P=.003) and in phase III (P=.05). No differences in ADG, ADFI and G:F due to CLA were found along the cumulative period. Pigs in clean environment, however, had higher (P=.0001) ADG, higher (P=.0028) ADFI, and a higher (P=.06) G:F than pigs in dirty environment (Table 6).

Table 1. Dietary composition (as-fed basis).

	Control Diets		
Ingredients, %	Phase, I[a]	Phase, II[a]	Phase, III[a]
Corn	30.26	48.72	60.68
Soybean meal (48%)	12.00	21.16	31.16
Dried whey	22.00	16.00	-
Spray-dried plasma	7.50	4.00	-
Dried blood cells	-	2.00	-
Dry skim milk	21.00	-	-
CLA-60[b]	-	-	-
Soy oil	3.33	3.33	3.33
Methionine	.18	.22	.09
Sodium chloride .	25	.25	.25
Dicalcium phosphate	1.09	1.72	1.95
Calcium carbonate	.78	.81	.74
Vitamin premix[c]	.20	.20	.20
Trace mineral[d]	.05	.05	.05
Selenium premix	1.36	1.36	1.36
Calculated analyses, %			
Crude protein	24.18	21.20	19.74
Lysine (total)	1.90	1.66	1.40
Met + cys	.96	.85	.71
Calcium	1.05	.90	.81
Phosphorus	.87	.79	.73
Ca:P ratio	1.21	1.14	1.11
ME (kcal/kg)	3,725.00	3,737.00	3,804.00

[a]Phase I, week 1–2; Phase II, week 3–5; Phase III, week 6– 7.
[b]Expressed as CLA-source, soy oil: 1.12, 2.21; 2.21, 1.12 and 3.33, 0 representing .67, 1.33 and 2% CLA-60 noncontrol experimental diets, respectively.
[c]Supplied 4,409 IU vitamin A; 1,102 IU vitamin D_3; 6.6 mg riboflavin; 17.6 mg pantothenic acid; 33 mg niacin; and 22 µg vitamin B_{12} per kilogram of diet.
[d]Supplied 165 ppm Zn; 193 ppm Fe; 66 ppm Mn; 19.29 ppm Cu; and .2 ppm Iodine per kilogram of diet.

Table 6. Environmental effects on growth performance, overall period.

Response	**CE**	**DE**	**SEM (n=8)**
ADG, kg	.587	.497	.020
ADFI, kg	.844	.754	.036
G:F	.731	.669	.024

Table 5. Effect of CLA on growth performance of weanling pigs.

		Dietary Treatments				
Item	**Period**	Control	.67	1.33	2% CLA-60	SEM
ADG, kg	Phase I	.262	.220	.271	.192	.023
	Phase II	.605	.616	.623	.543	.032
	Phase III	.777	.744	.781	.775	.027
	Cumulative	.556	.539	.563	.508	.020
ADFI, kg	Phase I	.319	.257	.325	.244	.028
	Phase II	.873	.843	.875	.760	.052
	Phase III	1.268	1.204	1.300	1.204	.050
	Cumulative	.829	.780	.834	.752	.036
G:F	Phase I	.830	.804	.900	.726	.038
	Phase II	.707	.740	.710	.732	.017
	Phase III	.632	.636	.652	.651	.031
	Cumulative	.686	.702	.719	.693	.024

[1]Phase I, week 1–2; Phase II, week 3–5; Phase III, week 6–7.

References

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