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Effects of supplementation of nursery diets with an essential fatty acid on immunity in artificially reared pigs

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K EFFECTS OF SUPPLEMENTATION OF NURSERY DIETS WITH AN **S** ESSENTIAL FATTY ACID ON IMMUNITY IN **U** ARTIFICIALLY REARED PIGS

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Summary

Twenty four pigs were weaned immediately at farrowing, reared artificially for 21 d, and then used in a 35-d nursery experiment to determine the effects of essential fatty acid deficiency on immune function. Treatments were: 1) a semi-purified diet deficient in essential fatty acids and 2) diet 1 with 2% added linoleic acid. Conversion of linoleic acid to linolenic and then arachidonic acid is a normal step in fatty acid metabolism. Metabolites of arachidonic acid are thought to have a role in mediating immune function. On d 28 of the experiment, pigs were orally dosed with *Salmonella choleraesuis* to challenge their immune systems. At d 35, pigs fed linoleic acid had greater concentrations of several fatty acids in both small intestine and liver tissues. Also, several measures of arachidonic acid metabolites in the plasma, which activate inflammatory reactions and stimulate white blood cell activity, were greater for pigs fed diets with added linoleic acid. However, no gross lesions were noted at necropsy that would result from infection with *S. choleraesuis*. Thus, for the short period of this experiment (35 d), deficiency of essential fatty acids apparently had minimal effect on ability of nursery pigs to resist disease.

(Key Words: Starter, Essential Fatty Acid, Immunity, *Salmonella choleraesuis*.)

Introduction

Swine producers are well aware of the stressful period that pigs experience at weaning, with the abrupt changes in environment, social structure, and diet. These stressors are especially problematic because the pigs no longer have access to the passive immunity supplied by antibodies from sow's milk, combined with a relatively undeveloped immune system in the pigs themselves. Researchers focused primarily on the use of antibiotics and vaccines to assist weanling pigs during this time of stress, until recent advances in the use of specialty diets (e.g., with 30 to 60% milk products, emulsified and blended fats, dried plasma protein, specialty soybean products, etc.) stimulated interest in manipulation of dietary ingredients to facilitate early weaning with minimum morbidity and mortality.

One area of interest common to veterinarians and nutritionists results from the understanding that the essential fatty acids (linoleic, linolenic, and arachidonic acid) are precursors for prostaglandins that mediate activity of the immune system. The experiment reported herein was designed to determine the potential for improving immune function in compromised pigs by addition of an essential fatty acid to their diets.

Procedures

Twenty-four pigs were removed from sows immediately at birth and orally dosed with

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porcine blood plasma to ensure consumption of antibodies without exposure to possible disease organisms from the sows. The pigs were housed in isolation for 21 d and given a diet of canned milk. At d 21, the pigs were moved to individual cages in a disease control facility and given the experimental diets (Table 1) for 35 d. The two diets were: 1) a semi-purified control diet, formulated to be nearly devoid of fat (.45%) and essential fatty acids and 2) diet 1 with 2% linoleic acid (20 × the published NRC requirement) added at the expense of cornstarch.

Blood samples were collected 2 d before and 27 d after initiation of feeding the experimental diets (i.e., start of the experiment). On d 28 of the experiment, the pigs were challenged by oral infusion with viable *S. choleraesuis* organisms. Blood samples were collected on d 32 and 35. Pigs were euthanized on d 35 for collection of tissue from the small intestine and liver. The intestines, spleen, liver, lungs, and mesenteric lymph nodes were examined for lesions associated with *S. choleraesuis* infection and scored on a scale from 1 (no symptoms) to 3 (moderate to severe lesions). Response criteria were plasma concentrations of protein, albumin, leukotriene B₄ (LTB₄), prostaglandin E₂ (PGE₂) and thromboxane B₂ (TXB₂); white blood cell counts; neutrophil chemotaxis; neutrophil chemiluminescence; neutrophil synthesis of eicosanoids; fatty acid concentrations in the small intestine and liver; and severity of lesions in the intestines, spleen, liver, lungs, and mesenteric lymph nodes.

Results and Discussion

Intestine tissue had greater concentration of myristic acid and numerically greater amounts of palmitoleic and linoleic acid than liver tissue (Table 2). Liver tissue had greater concentrations of palmitic, stearic, oleic, linolenic, and arachidonic acid than intestine tissue.

There were no tissue by treatment interactions, indicating that the slight increases in fatty acid concentrations for pigs fed the diet

Table 1. Diet Composition^a

<u>Ingredient, %</u>	<u>Deficient diet</u>
Cornstarch	38.98
Soy protein isolate	14.70
Dried skim milk	20.00
Dried whey (edible grade)	20.00
Cellulose (solka-floc)	3.00
Monocalcium phosphate	1.88
Limestone	.44
Salt	.10
Vit/Min mix	.52
Lysine-HCl	.13
Chromic oxide	.25

^aFor the diet with adequate essential fatty acid concentration, 2% linoleic acid was used in place of cornstarch.

with added linoleic acid were consistent in the intestine and liver. When pooled across tissues (intestine and liver), feeding diets with adequate linoleic acid resulted in numerical increases in concentrations of all fatty acids, with statistically significant increases for palmitoleic and oleic acid. These data indicate that diets deficient in essential fatty acids and nearly devoid of any fat tend to reduce fatty acid concentrations in tissues of nursery pigs. However, large changes in fatty acid concentrations were not observed, indicating that pigs had substantial reserves of fatty acids after artificial rearing to 21 d of age with canned milk.

Plasma concentrations of total protein, albumin, and TXB₂ were greater at d 27 for pigs fed the adequate diet compared to pigs fed the diet that was deficient in essential fatty acids (Table 3). These changes suggest that deficiency of essential fatty acids impairs protein synthesis in the liver. Plasma concentrations of

the arachidonic acid metabolites LTB₄, PGE₂, and TXB₂ were greater at d 32 (after challenge) for pigs fed the adequate diet than pigs fed the deficient diet. These compounds activate inflammatory reactions and stimulate white blood cells to recognize and destroy infectious microorganisms. However, these advantages in plasma concentrations of arachidonic acid metabolites could not be correlated with increased synthesis in isolated white blood cells (Table 4). Total and differential white blood cell counts were not different for pigs fed the experimental diets. No differences were noted for the ability of white blood cells to migrate toward infectious organisms (chemotaxis). Luminol-dependent chemiluminescence (a measure of white blood cells' ability to kill) decreased significantly in all pigs after *S. choleraesuis* challenge, but pigs fed the adequate diet had four times the activity at d 35 compared to pigs fed the deficient diet (Figure 1).

Apparently, the attempt at moderate infection by oral challenge with *S. choleraesuis* was met with sufficient immune function by pigs in both treatment groups to prevent all but mini-

mal pathological tissue damage. At necropsy, the only tissue reactions observed were indicative of mild inflammation that cleared the infecting organisms. There were no differences in frequency or severity of lesions for pigs fed the adequate or deficient diets. This observation is consistent with effective white blood cell function in both groups.

These data indicate that diets deficient in essential fatty acids and nearly devoid of fat tend to reduce fatty acid concentrations in tissues of nursery pigs. However, wholesale changes in fatty acid concentrations were not observed, indicating that pigs had substantial reserves of fatty acids after artificial rearing to 21 d with canned milk. Furthermore, although the pigs fed adequate diets had small advantages in some measures of immune function (i.e., plasma concentrations of total protein, albumin, LTB₄, PGE₂, and TXB₂), pigs in both treatment groups were able to thwart attempts at inducing a mild infection with *S. choleraesuis*. Thus, short-term deficiency of essential fatty acids (i.e., for a 35-d nursery experiment) appears to have minimal effect on immune function of artificially reared pigs.

Table 2. Effect of Feeding a Diet Deficient in Essential Fatty Acids on Fatty Acid Profiles of the Small Intestine and Liver

Fatty acid, % of tissue wt	Small intestine		Liver		CV
	Deficient	Adequate	Deficient	Adequate	
Myristic (14:0) ^d	.07	.09	.04	.05	56.7
Palmitic (16:0) ^a	2.29	2.67	2.76	2.93	25.2
Palmitoleic (16:1) ^f	.70	.76	.51	.73	32.1
Stearic (18:0) ^d	1.79	2.19	3.67	3.57	27.4
Oleic (18:1) ^{ac}	4.06	4.91	4.96	5.31	24.3
Linoleic (18:2) ^g	1.55	1.68	1.48	1.52	32.3
Linolenic (18:3) ^b	.44	.47	.11	.28	103.3
Arachidonic (20:4) ^c	2.42	2.49	3.57	3.51	37.3

^{abcd}Intestine vs liver (P < .10, P < .05, P < .01, P < .001, respectively).

^{ef}Deficient vs adequate (P < .10, P < .05, respectively).

^gNo treatment effect (P > .44).

Table 3. Clinical Chemistry

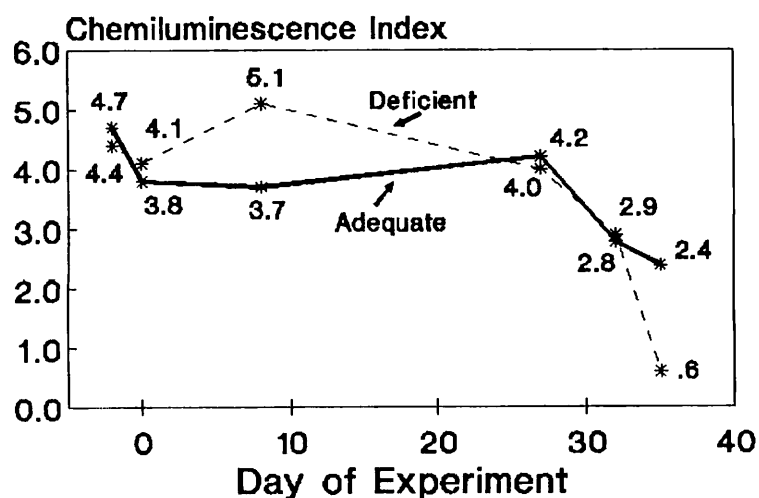
Item	Deficient diet	Adequate diet
Total protein, g/dl (d 27) ^a	3.9 ± .51	4.5 ± .36
Albumin, g/dl (d 27) ^a	2.3 ± .42	2.8 ± .16
Leukotriene B ₄ , pg/100 μL (d 32) ^a	6.3 ± .8	10.8 ± 1.1
Prostaglandin E ₂ , pg/100 μL (d 32) ^a	80 ± 21	112 ± 15
Thromboxane B ₂ , ng/100 μL		
d 2	.8 ± .2	1.2 ± .3
d 27 ^a	.9 ± .1	2.7 ± .4
d 32 ^a	.9 ± .4	2.4 ± .1

^aEffect of diet treatment (P < .05).

Table 4. White Blood Cell (Neutrophil) Synthesis of Eicosanoids (In Vitro), Day 27^a

Metabolite	Treatment diet	Stimulant	
		Phorbolmyristic acid	A23187, calcium ionophore
LTB ₄	Deficient	1.14	1.64
LTB ₄	Adequate	1.27	1.87
PGE ₂	Deficient	1.34	1.14
PGE ₂	Adequate	1.74	1.69
TXB ₂	Deficient	1.21	1.06
TXB ₂	Adequate	1.10	.94

^aAll concentrations are total pg of metabolite secreted into 1.0 ml of medium. There were no differences (P > .05).

**Figure 1. Chemiluminescence of White Blood Cells (Neutrophils).**