Published June 25, 2015

Vitamin E and omega-3 fatty acids independently attenuate plasma concentrations of proinflammatory cytokines and prostaglandin E_2 in *Escherichia coli* lipopolysaccharide-challenged growing–finishing pigs[1]

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ABSTRACT: This study tested the hypothesis that vitamin E (Vit E) and omega-3 fatty acids will additively attenuate the production of proinflammatory cytokines and PGE_2 in immune system–stimulated growing–finishing pigs. A total of 80 mixed sex pigs weighing 50.7 ± 0.76 kg (mean ± SE) were blocked and stratified based on sex and BW to a 2 × 2 factorial design with the respective factors being 1) without and with 300 IU Vit E and 2) without and with 25% replacement of tallow to linseed oil as a source of *n*-3 fatty acids. Each treatment consisted of 4 replicate pens with 5 pigs (3 barrows and 2 gilts) per pen. All pigs were challenged with an intramuscular injection of *Escherichia coli* lipopolysaccharide (LPS; O111:B4) twice weekly over the 6-wk experiment. After LPS challenge, pigs fed a diet supplemented with *n*-3 fatty acids had fewer ($P < 0.05$) white blood cells and tended to show both a reduced ($P < 0.10$) proportion of lymphocytes and IgG concentration compared with pigs fed a diet without any supplements. Supplementation of *n*-3 fatty acids reduced ($P < 0.01$ and $P < 0.05$) serum concentrations of cortisol and tumor necrosis factor α (TNF-α), respectively. The serum concentration of PGE_2 was decreased ($P < 0.05$) with supplementation of both Vit E and *n*-3 fatty acids; however, the extent of the reduction was greater ($P < 0.001$) in pigs fed an *n*-3 fatty acid–supplemented diet. However, there were no additive effects of the combined supplementation of Vit E and *n*-3 fatty acids on serum concentrations of proinflammatory cytokines and PGE_2. The results suggest that *n*-3 fatty acids independently attenuate production of TNF-α and PGE_2 in immune system–stimulated growing–finishing pigs.

Key words: growing–finishing pigs, lipopolysaccharide challenge, omega-3 fatty acid, vitamin E

J. Anim. Sci. 2015.93:2926–2934
doi:10.2527/jas2014-8330

INTRODUCTION

Immune system stimulation is known to divert nutrient partitioning from body protein and lipid deposition to lymphoid tissues and, therefore, has significant effects on the growth of animals under commercial conditions (Klasing et al., 1991; Rakhshandeh et al., 2010; Kim et al., 2012). The acute phase following immune system stimulation is characterized by increased production of proinflammatory cytokines such as IL-1 and tumor necrosis factor α (**TNF-α**), mainly from activated monocytes and lymphocytes (Beisel, 1991). Released proinflammatory cytokines then alter nutrient partitioning by diverting nutrients needed for adequate immune function (Beisel, 1991; Klasing et al., 1991). Concurrently, the released proinflammatory cytokines stimulate production of the immunosuppressive molecule PGE_2, which is largely responsible for neurological infection responses such as anorexia and fever (Rivest, 2010).

Some nutrients are known to play important roles in the cellular mechanisms responsible for production of proinflammatory cytokines and PGE_2 (Kim et al., 2013). Vitamin E (**Vit E**) has been reported to block PGE_2 biosynthesis by antagonizing peroxidation of arachidonic acid, which is particularly facili-

[1]This work was supported by the Ministry of Education and National Research Foundation of Korea (NRF-2012K2A1A2032491).
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Received July 29, 2014.
Accepted March 24, 2015.

tated in states of immune system stimulation (Likoff et al., 1981). However, recent evidence suggests that dietary levels of Vit E found in commercial pig diets are suboptimal and may limit adequate immune function (Sivertsen et al., 2007; Lauridsen et al., 2011). Omega-3 fatty acids are known to competitively reduce PGE_2 biosynthesis under immune system stimulation by reducing availability of enzymes required for the conversion of omega-6 fatty acids to arachidonic acid, which is a precursor for PGE_2 production (Wall et al., 2010).

Therefore, we tested the hypothesis that dietary supplementation of Vit E and *n*-3 fatty acids will additively mitigate the production of proinflammatory cytokines and PGE_2 in immune system–stimulated pigs.

MATERIALS AND METHODS

Ethics Statement

The experiment was conducted at the swine experimental unit of Dankook University (Anseodong, Cheonan, Choongnam, Korea). The protocol for the current experiment was approved by the Animal Care and Use Committee of Dankook University.

Experimental Design, Animals, Housing, and Diets

A total of 80 mixed sex pigs ([Landrace × Yorkshire] × Duroc) with an initial BW of 50.7 ± 0.76 kg (mean ± SE) were used in a 6-wk trial. Pigs were blocked and stratified based on sex and BW to a 2 × 2 factorial arrangement with the respective factors being 1) without and with 300 IU Vit E and 2) without and with 25% replacement of tallow to linseed oil as a source of *n*-3 fatty acids. Each treatment consisted of 4 replicate pens with 5 pigs (3 barrows and 2 gilts) per pen. The diets were formulated to meet or exceed NRC (2012) recommendations for all nutrients (Table 1). To mimic the chronic immune system stimulation typically seen in commercially housed pigs (Pastorelli et al., 2012), all pigs were challenged with an intramuscular injection of *Escherichia coli* lipopolysaccharide (**LPS**; O111:B4; Sigma-Aldrich, St. Louis, MO) twice weekly over the 6-wk experiment (Rakhshandeh and de Lange, 2012). The LPS dose rate was 30 μg/kg BW at the first injection and was increased by 15% in each subsequent injection to counteract the development of any possible resistance to the LPS. Pigs were housed in environmentally controlled rooms at 24 ± 1°C and were allowed ad libitum access to feed and water throughout the experimental period, through a self-feeder and nipple drinker, respectively. The mean final BW was 76.0 ± 1.03 kg (mean ± SE).

Table 1. Composition of experimental diets and calculated and analyzed energy and nutrient contents (as-fed basis)

	n-3 Fatty acid[1]			
	No		Yes	
	Vitamin E[2]			
Item	No	Yes	No	Yes
Corn	57.61	57.55	57.61	57.55
Wheat, hard red spring	10.00	10.00	10.00	10.00
Wheat bran	5.00	5.00	5.00	5.00
Soybean meal, 44%	19.70	19.70	19.70	19.70
Tallow	3.00	3.00	2.25	2.25
Molasses	2.50	2.50	2.50	2.50
Limestone	0.90	0.90	0.90	0.90
Dicalcium phosphate	0.70	0.70	0.70	0.70
Salt	0.30	0.30	0.30	0.30
L-Lysine	0.09	0.09	0.09	0.09
Vitamin mix[3]	0.10	0.10	0.10	0.10
Mineral mix[4]	0.10	0.10	0.10	0.10
Vitamin E, 50%[5]	0.00	0.06	0.00	0.06
n-3 fatty acid (linseed oil)	0.00	0.00	0.75	0.75
Calculated value, %				
ME, MJ/kg	13.8	13.8	13.8	13.8
CP	16	16	16	16
Lysine	0.90	0.90	0.90	0.90
Methionine	0.29	0.29	0.29	0.29
Calcium	0.61	0.61	0.61	0.61
Total phosphorus	0.42	0.42	0.42	0.42
Analyzed value				
CP	16.2	16.2	16.3	16.2
Lysine	0.89	0.92	0.90	0.93
Methionine	0.28	0.29	0.29	0.30
Calcium	0.64	0.67	0.63	0.65
Total phosphorus	0.44	0.44	0.42	0.43

[1]25% replacement of tallow to linseed oil as a source of omega-3 fatty acid.

[2]300 IU.

[3]Provided per kilogram of complete diet: 8,000 IU vitamin A, 800 IU vitamin D_3, 40 IU vitamin E, 2 mg vitamin K, 4 mg vitamin B_2, 3 mg vitamin B_6, 20 μg vitamin B_{12}, 15 mg pantothenic acid, 20 mg niacin, and 0.02 mg biotin.

[4]Provided per kilogram of complete diet: 150 mg Cu, 150 mg Fe, 50 mg Zn, 40 mg Mn, 0.5 mg I, 0.5 mg Co, and 0.3 mg Se.

[5]Promix 50, providing 500 g dl-a-tocopheryl acetate per kg product (DSM Nutritional Products Korea Pty Ltd, Seocho-ku, Seoul).

Sampling and Measurements

Body weight was measured individually at 0, 2, 4, and 6 wk of age. Feed consumption was recorded on a pen basis during the experiment to calculate the ADG, ADFI, and G:F. Five pigs per treatment (3 barrows and 2 gilts), whose initial BW were close to the median weight, were selected at the beginning of the experiment and bled via jugular venipuncture (0 d). The same pigs were then bled at 14, 28, and 42 d of the experiment. Blood samples (5 mL) were collected approximately 24 h after *E. coli* LPS injection into vacuum tubes containing no additive and tubes containing K_3EDTA (Becton Dickinson Vacutainer Systems;

Becton, Dickinson and Company, Franklin Lakes, NJ) to obtain serum and whole blood, respectively. The red blood cells, white blood cells (**WBC**), and lymphocyte counts of whole blood samples were determined using an automatic blood analyzer (ADVIA 120; Bayer Corp., Tarrytown, NY). The lymphocytes were then expressed as a percentage of the total WBC. The serum was separated by centrifugation for 15 min at 3,000 × *g* at 4°C and stored at –4°C until determination of serum IgG, cortisol (Rodent Cortisol ELISA Kit; Endocrine Technologies, Minneapolis, MN), TNF-α (R and D Porcine ELISA kit; R&D Systems, Minneapolis, MN), IL-1β (R and D Porcine IL-1β ELISA kit; R&D Systems), IL-6 (R and D Porcine IL-6 ELISA kit; R&D Systems), and PGE_2 (Cayman Prostaglandin E_2 EIA kit; Cayman, Ann Arbor, Michigan). Crude protein, AA, calcium, and phosphorus contents of experimental diets were analyzed as described by Wang et al. (2013).

Before the commencement and at the end of the experiment, the back fat thickness and lean percentage of all pigs (n = 20 per treatment) were measured 5 cm from the right-hand side of the midline from 3 different sites (shoulder, mid back, and loin at a position directly above the point of elbow, last rib, and last lumbar vertebra, respectively) using a real-time ultrasound instrument (Piglog 105; SFK Technology, Herlev, Denmark; Kim et al., 2004). The mean value was taken and used for subsequent statistical analysis.

Statistical Analysis

Data were subjected to 2-way ANOVA according to the factorial arrangement of the treatments (Genstat, 16th edition; VSN International Ltd., Hemel Hempstead, UK). The pig (n = 5) was used as the experimental unit for blood measures and lean percentage content, and the pen (n = 4) was used as the experimental unit for performance indices. The time-dependent data were analyzed as a 3-way ANOVA with time being the repeated measure. For statistical analysis of blood parameters (n = 5) measured every 14 d over 6 wk, repeated measure ANOVA was used to test the dietary effects using baseline values measured on Day 0 as covariates. For back fat thickness and lean percentage data (n = 20), measurements at the start and end of the experiment were analyzed using repeated measure ANOVA with the initial values at the start of the experiment used as covariates. Pearson's correlation analysis was conducted using all blood data to examine relationships between blood cell counts, serum concentrations of proinflammatory cytokines, and PGE_2. Linear regression analysis was conducted to examine the relationships between carcass traits and blood measures. Variability in the data was expressed as the SEM. A probability level of $P < 0.05$ was considered to be statistically significant and $P < 0.10$ was considered a trend.

RESULTS

Serum Indices Associated with Immune System Stimulation

Blood indices associated with immune system stimulation measured 24 h after LPS challenge over the 6-wk experiment are presented in Table 2. Blood cell number count showed that *n*-3 fatty acid supplementation independently reduced ($P < 0.01$) numbers of WBC after LPS challenge and tended to reduce ($P < 0.10$) the proportion of lymphocytes within WBC. Likewise, pigs fed a diet supplemented with *n*-3 fatty acids independently had decreased ($P < 0.01$) serum concentrations of cortisol and tended to have decreased ($P < 0.10$) serum IgG compared with pigs fed a diet without any supplements. Supplementation of *n*-3 fatty acids also reduced ($P < 0.05$) the TNF-α concentration in serum whereas serum concentrations of IL-1β and IL-6 were not statistically different. The serum concentration of PGE_2 was reduced ($P < 0.05$) with supplementation of both Vit E and *n*-3 fatty acids ($P < 0.001$); however, the extent of the reduction was greater in pigs fed an *n*-3 fatty acid–supplemented diet (Fig. 1). However, no additive effects of the combined supplementation of Vit E and *n*-3 fatty acids were observed for any of the serum parameters.

The effects of time, Vit E, and *n*-3 fatty acids were statistically analyzed using repeated measure ANOVA to examine, first, whether chronic immune system stimulation was achieved and then maintained over the 6-wk period of the study and, second, whether there was an interaction between time and dietary treatments. There were significant time effects for numbers of WBC and serum concentrations of TNF-α and PGE_2 such that the measured mean values after LPS injection (Day 14, 28, and 42) were greater ($P < 0.001$) than baseline values measured before the LPS injection (Day 0). However, there were no interactions between time and treatments for all measured blood parameters (Table 2).

Correlations between Serum Indices Associated with Immune System Stimulation

Correlations between serum indices and whole blood cell counts observed over 6 wk are presented in Table 3. Numbers of WBC were positively correlated ($P < 0.05$) to the serum PGE_2 concentration. The proportion of lymphocytes was positively correlated

Table 2. Effect of vitamin E (Vit E) and *n*-3 fatty acid–supplementation on serum concentrations of cytokines and immunity-related indices measured on d 0, 14, 28, and 42 in *Escherichia coli* lipopolysaccharide-challenged growing–finishing pigs[1]

	n-3 Fatty acid[2]					Significance[4]					
	No		Yes								
	Vit E[3]					Vit E	*n*-3 Fatty acids	Vit E × *n*-3 fatty acids	Time	Time × Vit E	Time × *n*-3 fatty acids
Item	No	Yes	No	Yes	SEM						
WBC,[5] 10^3/μL	20.9	19.3	17.4	16.2	1.55	NS	**	NS	***	NS	†
Lymphocyte,[6] %	44	43	39	38	3.3	NS	†	NS	***	NS	NS
IgG, mg/dL	496	482	471	451	18.1	NS	†	NS	NS	NS	NS
Cortisol, μg/dL	3.8	3.6	3.2	3.0	0.25	NS	**	NS	***	NS	NS
IL-1β, pg/mL	12.9	12.2	11.9	11.6	1.63	NS	NS	NS	***	NS	NS
IL-6, pg/mL	43	41	42	41	3.0	NS	NS	NS	NS	NS	NS
TNF-α,[7] pg/mL	50	45	44	42	3.0	NS	*	NS	***	NS	NS
PGE_2, pg/mL	87	78	71	71	3.8	*	***	*	***	NS	NS

[1]Values are mean of 5 samples per treatment collected from the same pigs selected at the commencement of experiment.

[2]25% replacement of tallow to linseed oil as a source of omega-3 fatty acid.

[3]300 IU.

[4]Data were analyzed using a repeated measure ANOVA with initial value at the start of the experiment as a covariate and there were no significant 3-way interaction between time, Vit E, and *n*-3 fatty acids for measured variables. NS = not significant.

[5]WBC = white blood cells.

[6]Lympocytes are expressed as a percentage of the total WBC count.

[7]TNF-α = tumor necrosis factor α.

†$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

to serum concentrations of IL-1β ($P < 0.01$), TNF-α ($P < 0.05$), and cortisol ($P < 0.001$). The serum TNF-α concentration was positively correlated to serum concentrations of IL-6 ($P < 0.05$) and cortisol ($P < 0.001$) but negatively correlated ($P < 0.05$) to serum IL-1β concentration. The serum concentration of PGE_2 was negatively correlated ($P < 0.05$) to the proportion of lymphocytes within the total WBC count.

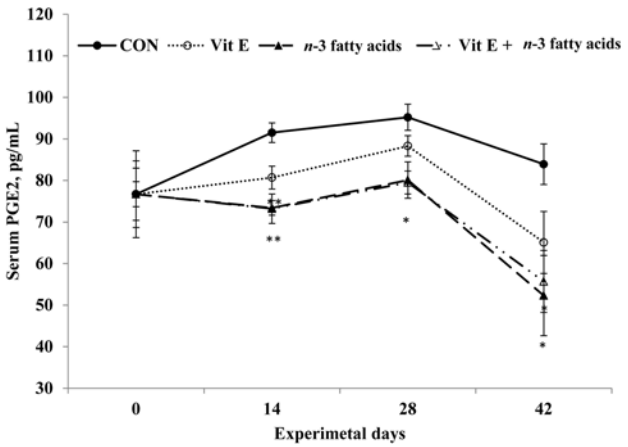

Figure 1. Effect of individual or combined supplementation of vitamin E (Vit E; 300 IU) and *n-3* fatty acids on the plasma PGE_2 concentration in *Escherichia coli lipopolysaccharide* (*LPS*)-challenged growing–finishing pigs over 6 wk of the experimental period (n = 5). Pigs received intramuscular injection of *e. coli* LPS twice weekly and blood samples were collected 24 h after the injection. Plasma PGE_2 content on Day 0 was used as a covariate for statistical analysis. *P < 0.05; **P < 0.01. CON = Control, basal diet without n -3 fatty acids and vitamin E.

Growth Performance and Carcass Traits

Pigs fed a diet supplemented with *n*-3 fatty acids improved ($P < 0.05$) their ADG in the final 14 d and tended to improve ($P < 0.10$) overall ADG compared with pigs fed a diet without any supplements (Table 4). There were no differences in ADFI or G:F ratio over the 6-wk experiment.

At the end of the experiment, the back fat thickness was increased ($P < 0.001$) in all dietary treatments; however, supplementation of *n*-3 fatty acids tended to increase back fat thickness more compared with pigs that did not receive additional *n*-3 fatty acids (time × *n*-3 fatty acid interaction, $P < 0.10$). The lean percentage was increased ($P < 0.01$) by supplementation of both Vit E and *n*-3 fatty acids ($P < 0.001$) compared with pigs fed a diet without any supplements (Table 5).

Relationship between Carcass Traits and Blood Measures

Linear regression analysis was conducted to examine the relationships between carcass lean percentage and blood measures on Day 42 (Table 6). The lean percentage was negatively related ($P < 0.10$) to the number of WBC and the proportion of lymphocytes within WBC ($P < 0.05$). Although statistically not significant, a weak negative relationship between the carcass lean percentage and PGE_2 concentration was observed ($P = 0.111$).

Table 3. Pearson's correlation coefficient between measures of blood parameters determined every 14 d over the 6-wk experimental period[1]

Item	WBC,[2] 10^3/μL	Lymphocyte, %	IL-1β	IL-6	TNF-α[3]	Cortisol
Lymphocyte,[4] %	0.077					
IL-1β	0.136	0.316**				
IL-6	0.027	–0.013	–0.141			
TNF-α	–0.076	0.239*	–0.468***	0.266*		
Cortisol	0.108	0.548***	–0.011	0.173	0.549***	
PGE_2	0.291*	–0.226*	–0.001	–0.001	–0.158	–0.148

[1]Correlation coefficient was derived from 80 observations (5 pigs per treatment × 4 treatments × 4 collection times).

[2]WBC = white blood cells.

[3]TNF-α = tumor necrosis factor α.

[4]Percentage of the total WBC count.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

DISCUSSION

Disease challenge, whether it is clinical or subclinical, under commercial conditions of pig production can lead to the production of numerous immune system–related compounds such as acute phase proteins (Wright et al., 2000) and cytokines (Curfs et al., 1997; Paradis et al., 2012; Rakhshandeh and de Lange, 2012; Rakhshandeh et al., 2014). The increased release of proinflammatory cytokines in immune system–challenged pigs causes reduced feed intake and growth performance and deterioration in feed efficiency, mainly due to altered nutrient partitioning and neurological infection responses such as anorexia and fever (Webel et al., 1997; Kim et al., 2013). In the current study, *E. coli* LPS was chosen to mimic immune system stimulation in pigs kept under commercial conditions (Rakhshandeh and de Lange, 2012) to evaluate the dietary effects of Vit E and *n*-3 fatty acids in mitigating the pigs' responses to immune system stimulation and to examine effects on production and carcass composition. *Escherichia coli* LPS is a component of the outer membrane of *E. coli* and has been reported to cause fever (Johnson and von Borell, 1994; Leininger et al., 2000) and anorexia

Table 4. Effect of vitamin E (Vit E) and *n*-3 fatty acids supplementation on growth performance in *Escherichia coli* lipopolysaccharide-challenged growing–finishing pigs[1]

	n-3 Fatty acid[2]							
	No		Yes			Significance		
	Vit E[3]							
Item	No	Yes	No	Yes	SEM	Vit E	*n*-3 Fatty acids	Vit E × *n*-3 fatty acids
0 to 2 wk								
ADG, g	494	537	556	545	27.3	NS[4]	NS	NS
ADFI, g	1,635	1,667	1,646	1,642	16.0	NS	NS	NS
G:F	0.30	0.32	0.34	0.33	0.015	NS	NS	NS
2 to 4 wk								
ADG, g	572	589	613	604	25.4	NS	NS	NS
ADFI, g	1,805	1,991	1,868	1,772	68.9	NS	NS	†
G:F	0.32	0.30	0.33	0.34	0.023	NS	NS	NS
4 to 6 wk								
ADG, g	618	661	701	758	31.4	NS	*	NS
ADFI, g	2,256	2,278	2,325	2,340	153.5	NS	NS	NS
G:F	0.27	0.29	0.31	0.33	0.024	NS	NS	NS
0 to 6 wk								
ADG, g	561	595	623	635	18.6	NS	†	NS
ADFI, g	1,899	1,979	1,946	1,918	55.6	NS	NS	NS
G:F	0.30	0.30	0.32	0.33	0.017	NS	NS	NS

[1]Values are mean of 4 pens per treatment with 5 pigs per pen.

[2]25% replacement of tallow to linseed oil as a source of omega-3 fatty acid.

[3]300 IU.

[4]NS = not significant.

†$P < 0.10$; *$P < 0.05$.

Table 5. Effect of vitamin E (Vit E) and *n*-3 fatty acid supplementation on back fat thickness and lean percentage in *Escherichia coli* lipopolysaccharide-challenged growing–finishing pigs[1]

	n-3 Fatty acids[2]					Significance[4]					
	No		Yes								
	Vit E[3]										
Item	No	Yes	No	Yes	SEM	Vit E	*n*-3 Fatty acids	Vit E × *n*-3 fatty acids	Time	Time × Vit E	Time × *n*-3 fatty acids
Back fat thickness, mm											
Initial	10.6	10.4	10.4	10.3							
Final	12.0	11.9	12.4	12.5	0.24	NS	NS	NS	***	NS	†
Lean percentage											
Initial	59.2	59.3	59.3	59.2							
Final	59.5	60.5	60.8	61.3	0.17	**	***	NS	***	**	***

[1]Values are mean of 20 pigs per treatment.

[2]25% replacement of tallow to linseed oil as a source of omega-3 fatty acid.

[3]300 IU.

[4]Data were analyzed using a repeated measure ANOVA with initial value at the start of the experiment as a covariate and there were no significant 3-way interactions between time, Vit E, and *n*-3 fatty acids for measured variables. Initial value was used as a covariate for statistical analysis. NS = not significant.

†$P < 0.10$; **$P < 0.01$; ***$P < 0.001$.

(Johnson and von Borell, 1994; Webel et al., 1997) and to increase plasma TNF-α and cortisol levels (Webel et al., 1997; Sauber et al., 1999). In response to the proinflammatory cytokines, PGE_2 production is also stimulated (Akaogi et al., 2006). To test the hypothesis that Vit E and *n*-3 fatty acids will additively attenuate the production of proinflammatory cytokines and PGE_2 in immune system–stimulated growing–finishing pigs, we attempted to mimic chronic immune system stimulation over the 6-wk experimental period by repeatedly injecting increasing amounts of *E. coli* LPS. Mild chronic immune system stimulation was successfully achieved as numbers of WBC and serum TNF-α and PGE_2 concentrations measured on Days 14, 28, and 42 were greater compared with the baseline concentrations measured before the LPS injection on Day 0 in pigs fed a diet without any supplements.

The acute phase response of immune system stimulation is characterized by the production of proinflammatory cytokines and PGE_2, and under the conditions of this experiment, additive reductions in proinflammatory cytokines and PGE_2 were anticipated in pigs fed a diet supplemented with both Vit E and *n*-3 fatty acids, as Vit E and *n*-3 fatty acids are known to mitigate production of these compounds through independent mechanisms (Likoff et al., 1981; Wall et al., 2010; Kim et al., 2013). Conversion of the 18-carbon *n*-6 fatty acid arachidonic acid into PGE_2 is facilitated by transcription of cyclooxygenase-2 (**COX-2**) on signaling mechanisms through proinflammatory cytokines such as TNF-α or IL-1β (Rivest, 2010; Kalinski, 2012). In a study using LPS-stimulated rats, 100 mg of Vit E supplementation depressed TNF-α production in peritoneal macrophages and the degree of TNF-α suppression was correlated to serum Vit E levels (Bulger et al., 1997). A mouse study also demonstrated that 500 mg Vit E supplementation reduced age-related *ex vivo* production of PGE_2 in a spleen homogenate (Meydani et al., 1986; Wu et al., 1998). In human colon Caco2 cells, α-tocopherol (Vit E) did not affect COX-2 mRNA expression but inhibited COX-2 activity, indicating that Vit E acts posttranscriptionally on COX-2 activity (O'Leary et al., 2004). On the other hand, *n*-3 fatty acids are known to attenuate production of proinflammatory cytokines and PGE_2 through reducing the availability of enzymes needed for the conversion of omega-6 fatty acids to arachidonic acid, the precursor for PGE_2 (Wall et al., 2010). For example, Carroll et al. (2003) and Gaines et al. (2003) demonstrated that menhaden fish oil (containing *n*-3 fatty acids) reduced levels of serum cortisol and TNF-α

Table 6. Linear regression results for carcass lean percentage (*y*) and measures of blood parameters (*x*) determined on Day 42

Item	Intercept	Slope	R^2	RSD[1]	Number of observations	Significance
WBC,[2] $10^3/\mu L$	–0.255	65.01	0.115	5.69	20	0.079
Lymphocyte,[3] %	–0.1118	66.86	0.152	5.45	20	0.050
PGE_2, pg/mL	–0.0459	63.51	0.087	5.87	20	0.111

[1]RSD = residual SD.

[2]WBC = white blood cells.

[3]Percentage of the total WBC count.

in immune system–stimulated pig given LPS. Fritsche et al. (1993) also reported decreased PGE_2 biosynthesis in piglets suckled from sows fed a diet supplemented with fish oil. Based on the literature, we therefore hypothesized that a combined supplementation of Vit E and *n*-3 fatty acids would additively attenuate LPS-induced biosynthesis of proinflammatory cytokines and PGE_2. However, our results suggested that Vit E and *n*-3 fatty acids act independently to reduce the biosynthesis of proinflammatory cytokines and PGE_2. The lack of additive effect between Vit E and *n*-3 fatty acids on inflammatory responses was mainly caused by the weak impact of Vit E on PGE_2 production, whereas *n*-3 fatty acids effectively attenuated PGE_2 production.

Correlations between proinflammatory cytokines and immunity-related indices such as numbers of WBC, the proportion of lymphocytes, and concentrations of cortisol and PGE_2 were investigated. Interestingly, there was a strong negative correlation between TNF-α and IL-1β concentrations in serum, indicating that LPS-induced immune system stimulation may primarily activate TNF-α rather than IL-1β. However, previous studies showed increased production of both TNF-α and IL-1β after LPS challenge in pigs (Wright et al., 2000; Rakhshandeh and de Lange, 2012). The serotype of *E. coli* LPS, sampling timing, and dose rate of LPS might have influenced serum concentrations of cytokines and immunity-related molecules, as the response of pigs to LPS was reported to be dose dependent (Webel et al., 1997). For example, Wright et al. (2000) and Rakhshandeh and de Lange (2012) used LPS extracted from *E. coli* B55:B5 and used either 100 μg/kg via intraperitoneal injection or 60 μg/kg via intramuscular injection, whereas our study used LPS extracted from *E. coli* O111:B5 and 30 μg/kg via intramuscular injection. In addition, and from a continuous blood sampling study, Wright et al. (2000) showed that a single dose of LPS induced an immune response for only a short period of time (i.e., less than 24 h).

Under the conditions of LPS-induced chronic immune system stimulation used in this study, we anticipated that nutritional attenuation of the immune response on feeding either Vit E or *n*-3 fatty acids individually or in combination would improve muscle growth of pigs and, therefore, ADG and G:F. This would most likely occur through nutrient partitioning toward muscle growth from lymphoid tissues (Carroll et al., 2003; Klasing, 2004). Our results showed that Vit E alone did not improve ADG or lean percentage content; however, *n*-3 fatty acids alone or in combination with Vit E improved these indices without affecting back fat thickness. This improvement is thought to be associated with a reduced infection response due to mitigation of PGE_2 production, as demonstrated in Fig. 1. Results of the regression analysis also partly support this notion as carcass lean percentage was negatively related to WBC, the proportion of lymphocytes, and PGE_2, albeit the relationships were weak. In agreement with our findings, Liu et al. (2003) demonstrated, in a weaner pig study, that fish oil–derived *n*-3 fatty acids decreased PGE_2 production and improved ADG and ADFI following LPS-induced immune system stimulation. Moreover, Zhan et al. (2009) showed that increased duration of feeding linseed-derived *n*-3 fatty acids linearly decreased gene expression of inflammatory cytokines such as TNF-α and improved ADG and feed efficiency. Therefore, performance and leanness data suggest that under our experimental condition, *n*-3 fatty acids mitigated LPS-induced muscle growth in growing–finishing pigs.

The physiological response of growing pigs to the LPS challenge is reported to last from only a few hours to 72 h. For example, Wright et al. (2000) challenged growing pigs with an intraperitoneal injection of 100 μg *E. coli* O55:B5 LPS/kg BW and examined changes in plasma concentrations of immunity-related proteins. A single dose of LPS increased plasma levels of TNF-α, PGE_2, cortisol, growth hormone, and haptoglobin for 4, 4, 16, 48, and 72 h, respectively. The LPS challenge also reduced ADFI for 24 h and plasma concentration of IGF-1 for 64 h (Wright et al., 2000). These responses can most likely vary with repeated injection of LPS, although we are not aware of any study that measured the duration of the innate immune response after this intervention. Nevertheless, the time after LPS injection is a critical factor influencing measurements associated with innate immunity, and in the present study, blood samples were collected approximately 24 h after injection but an individual pig's blood sampling time could have varied from 24 to 25 h. Therefore, the dietary effects of *n*-3 fatty acids and Vit E on serum indices of inflammation reported in the present article warrants cautious interpretation.

Conclusions

The findings from the present study indicate that supplementation of 300 IU Vit E and replacing 25% of tallow with linseed oil as *n*-3 fatty acids significantly reduced the production of proinflammatory cytokine and PGE_2 and improved muscle growth and ADG of growing–finishing pigs under a model of LPS-induced immune system stimulation. However, no additive effects were observed in inflammatory responses and growth of pigs on supplementation of Vit E and *n*-3 fatty acids in combination, indicating that under the conditions of the present study, the mechanisms behind mitigation of the immune system markers and production indices observed were independent and not additive.

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