Duration of feeding linseed diet influences expression of inflammation-related genes and growth performance of growing-finishing barrows

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ABSTRACT: The aim of the study was to investigate the effects of dietary linseed (rich in n-3 PUFA) on expression of inflammation-related genes and on growth performance of growing-finishing barrows. Two isonitrogenous and isocaloric diets were formulated, one as the basal diet and the other containing 10% linseed. Twenty-four Landrace × Yorkshire barrows weighing 35 ± 3.7 kg were randomly assigned to 1 of 4 treatment groups, with 6 pigs per group. During the entire experimental period of 90 d, these 4 groups of pigs were first fed the basal diet and then fed the linseed diet for 0, 30, 60, and 90 d before slaughter, respectively. Pig growth; messenger RNA (mRNA) expression of peroxisome proliferator-activated receptor-γ (PPARγ), IL-1β, IL-6, and tumor necrosis factor-α (TNF-α); and plasma concentrations of the 3 proinflammatory cytokines were measured and analyzed. Average daily feed intake did not differ among treatment groups (P > 0.05), but ADG (P < 0.05) and G:F (P < 0.01) responded quadratically to the duration of linseed diet feeding, and pigs in the 60-d treatment group had the greatest ADG and G:F. The mRNA expression of PPARγ in loin muscle and spleen increased linearly (P < 0.01) with the duration of linseed diet feeding, whereas its expression in adipose tissue was not affected (P = 0.095). Tumor necrosis factor-α and IL-6 mRNA expression in muscle, adipose, and spleen, as well as serum concentration of TNF-α, decreased linearly (P < 0.01) with the duration of linseed diet feeding. Peroxisome proliferator-activated receptor-γ mRNA abundance was negatively correlated with IL-1β, IL-6, and TNF-α mRNA abundance both in muscle (R² = 0.63, P < 0.001) and in spleen (R² = 0.69, P < 0.001), and PPARγ mRNA expression in spleen (R² = 0.59, P < 0.01) and muscle (R² = 0.52, P < 0.05) was negatively correlated with serum TNF-α concentration. There were also significant quadratic relations between ADG and expression of PPARγ (P < 0.05) and splenic TNF-α (P < 0.05). These data suggest that intake of n-3 PUFA from the linseed diet led to significant decreases in the expression of proinflammatory cytokine genes, which may stimulate growth in growing-finishing barrows, at least in part, through a PPARγ-dependent mechanism.

Key words: growth, linseed, n-3 polyunsaturated fatty acid, peroxisome proliferator-activated receptor-γ, pig, proinflammatory cytokine

INTRODUCTION

Cytokines are small polypeptide molecules that are important regulators of the immune and inflammatory responses. Three proinflammatory cytokines, IL-1β, IL-6, and tumor necrosis factor-α (TNF-α), have been reported to have profound behavioral, neuroendocrine, and metabolic effects (Johnson, 1997). It is well documented that proinflammatory cytokines increase muscle protein degradation, reduce muscle protein synthesis, and divert nutrients to the synthesis of components in the immune system, such as acute-phase proteins (Spurlock, 1997; Mitchell et al., 2001).

Interestingly, n-3 PUFA consumption could attenuate the growth-inhibitory effect by reducing the production of proinflammatory cytokines in several species (Korver and Klasing, 1997; Xi et al., 2001; Gaines et al., 2003). However, most of the n-3 PUFA effects mentioned above have been observed during inflammation or immune stress. Reports on the effects of dietary n-3 PUFA on the expression of inflammatory cytokines under physiological conditions are scarce. Although the antiinflammation effects of n-3 PUFA might come partly from their eicosanoid derivatives, which are able to regulate expression of inflammatory genes (Calder

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evidence is emerging that n-3 PUFA may exert some of their effects through activation of 1 or more transcription factors, such as peroxisome proliferator-activated receptor-γ (PPARγ; Olsen and Haldosén, 2006).

A potential commercial source of n-3 PUFA for pigs is whole linseed, which contains approximately one-third oil, of which more than 50% is α-linolenic acid (ALA, C18:3n-3) (Matthews et al., 2000). In this study, we used linseed as the source of n-3 PUFA and tested the hypothesis that dietary consumption of n-3 PUFA might activate the expression of nuclear transcription factor PPARγ; might downregulate the expression of the proinflammatory cytokines IL-1β, IL-6, and TNF-α; and consequently might stimulate the growth of growing-finishing barrows.

**MATERIALS AND METHODS**

The study was carried out according to Huazhong Agriculture University Animal Care and Use Committee guidelines.

**Animals and Diets**

Two isoenergetic and isonitrogenous diets were formulated, a basal diet and a linseed diet containing 10% linseed. Diet composition is presented in Table 1, and the fatty acid composition of the diets was analyzed and is listed in Table 2. Twenty-four Landrace × Yorkshire barrows weighing 35 ± 3.7 kg were randomly assigned to 1 of 4 treatment groups, with 6 pigs per group. Throughout the experimental period of 90 d, pigs in treatment groups 1, 2, 3, and 4 were first fed the control diet for 90, 60, 30, and 0 d and then fed the linseed diet for 0, 30, 60, and 90 d, respectively. Pigs were housed individually and fed ad libitum. Pigs were weighed individually on the beginning day and at the end of the trial. Feed consumption was recorded daily per pen. Average daily gain, ADFI, and G:F were calculated. At the end of the trial, the pigs were humanely slaughtered at 170 d of age by electrical stunning, coupled with exsanguination. Tissue samples were rapidly removed, wrapped in foil, and frozen in liquid nitrogen to be stored at −70°C. Skeletal muscle samples were collected from LM between the 10th and the last rib, adipose tissue samples containing the upper and middle layers were removed from the dorsal subcutaneous fat in the neck region, and a spleen sample was collected for subsequent RNA extraction. Another set of LM samples was also collected from each pig and stored at −20°C for fatty acid analysis.

Blood samples from each pig were collected at slaughter into 5-mL Vacutainer tubes containing 14.3 U/mL of lithium heparin (Becton Dickinson Canada, Ville Saint-Laurent, Quebec, Canada) and centrifuged (3,500 × g for 5 min at 4°C) to collect plasma, which was stored at −20°C until measurements for IL-1β, IL-6, and TNF-α concentration.

**Fatty Acid Analysis**

Fatty acid analysis was conducted on diet and backfat samples. Lipids were extracted by the chloroform:methanol procedure (Folch et al., 1957). The samples were analyzed by using a CP-3800 gas chro-

### Table 1. Composition and calculated analysis (as-fed basis) of diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Control diet</th>
<th>Linseed diet</th>
<th>Control diet</th>
<th>Linseed diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>48.70</td>
<td>60.50</td>
<td>52.90</td>
<td>65</td>
</tr>
<tr>
<td>Wheat middlings</td>
<td>18.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat bran</td>
<td></td>
<td>20.00</td>
<td>21.00</td>
<td>21.00</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>27.00</td>
<td>26.50</td>
<td>20.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Fat powder&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.30</td>
<td></td>
<td>3.10</td>
<td></td>
</tr>
<tr>
<td>Flaxseed</td>
<td></td>
<td>10.00</td>
<td></td>
<td>10.00</td>
</tr>
<tr>
<td>Premix&lt;sup&gt;4&lt;/sup&gt;</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Calculated analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP, %</td>
<td>18.07</td>
<td>18.01</td>
<td>16.05</td>
<td>16.08</td>
</tr>
<tr>
<td>Ether extract, %</td>
<td>5.59</td>
<td>5.87</td>
<td>5.56</td>
<td>6.02</td>
</tr>
<tr>
<td>DE, Mcal/kg</td>
<td>3.42</td>
<td>3.41</td>
<td>3.40</td>
<td>3.40</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>1.07</td>
<td>1.05</td>
<td>0.93</td>
<td>0.95</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.69</td>
<td>0.66</td>
<td>0.65</td>
<td>0.70</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>0.50</td>
<td>0.51</td>
<td>0.49</td>
<td>0.55</td>
</tr>
</tbody>
</table>

<sup>1</sup>BW range 30 to 60 kg.
<sup>2</sup>BW range 60 to 115 kg.
<sup>3</sup>Fat powder fatty acid profile: palmitic acid (C16:0), 70 to 80%; stearic acid (C18:0), 5 to 10%; oleic acid (C18:1), 8 to 15%.
<sup>4</sup>Provided per kilogram of premix: vitamin A, 11,250 IU; vitamin D<sub>3</sub>, 2,500 IU; vitamin E, 200 mg; niacin, 25 mg; d-pantothenic acid, 8 mg; vitamin B<sub>6</sub>, 3.0 mg; vitamin B<sub>12</sub>, 0.08 mg; d-biotin, 0.1 mg; folic acid, 12.5 mg; copper, 20 mg; iron, 50 mg; manganese, 30 mg; zine, 80 mg; iodine, 0.8 mg.
matograph (Varian Inc., Walnut Creek, CA) equipped with an 1177 injector, a flame-ionization detector, and a CPSil88 capillary chromatographic column for fatty acid methyl esters (50 m × 0.25 mm i.d. × 0.20 µm). The injector temperature was 250°C, the detector temperature was 270°C, column flux was 1.0 mL/min, and the split ratio was 1:100. The temperature program was as follows: increased from 100 to 200°C at 5°C/min and held 5 min, then increased to 225°C at 2°C/min and held 2 min. The total analysis time was 39.5 min. Peaks were identified by comparing their retention times with individual reference standard fatty acids.

RNA Isolation

Total RNA was extracted by using TRizol reagent (Invitrogen, Carlsbad, CA) according to the specifications of the manufacturer. The RNA samples were quantified spectrophotometrically at 260 and 280 nm. The ratio of light absorbance at 260 nm to that at 280 nm was between 1.8 and 2.0, indicating that they were pure and clean. The quality of RNA was also checked by 1.0% agarose gel electrophoresis and staining with 1 µg/mL of ethidium bromide.

Semiquantitative Reverse Transcription-PCR

A 2-step semiquantitative reverse transcription-PCR (RT-PCR) method (Spencer and Christensen, 1999) was used to measure gene expression at the time of slaughter. Oligo-(dT)$_{20}$n was used as a primer in the first step of complementary DNA (cDNA) synthesis. The reverse transcription reaction solution (20 µL) consisted of 4 µg of total RNA, 100 U of Moloney murine leukemia virus reverse transcriptase (Toyobo, Osaka, Japan), 20 U of an RNase inhibitor (Toyobo), 0.5 mmol/L of deoxyribonucleotide triphosphate, and 0.5 µL of oligo-(dT)$_{20}$n primers. The cDNA stock was stored at −20°C. The yield of cDNA was measured by the PCR signal generated for the internal standard housekeeping gene β-actin, which was amplified for 18 to 24 cycles starting with 0.1 µL of the cDNA solution. The volume of each cDNA pool was adjusted to give the same exponential-phase PCR signal intensity for β-actin after 20 cycles (Meadus et al., 2002).

Primer sequences and optimal PCR annealing temperatures for amplifying PPARγ, IL-1β, IL-6, and TNF-α messenger RNA (mRNA) are listed in Table 3. To ensure that no false-positive PCR fragments would be generated from pseudogenes in genomic DNA, primer sequences were designed to span intron regions when genomic sequence data were available. In addition, all PCR primer combinations were tested by using porcine genomic DNA as a negative control. The PCR were performed with a 2720 PCR instrument (Applied Biosystems, Foster City, CA) by using 100 ng of cDNA, 5 pmol of each oligonucleotide primer, 200 µM each dNTP, 1 U of Taq DNA Polymerase (Fermentas/Life Sciences, Vilnius, Lithuania), and 20 µL of 10× reaction buffer. The PCR program began with a 95°C denaturation for 5 min, continued with 28 to 32 cycles at 94°C for 1 min, optimal PCR annealing temperature for 1 min, 72°C for 1 min, and then a final extension at 72°C for 10 min. The linear amplification range for each gene was tested on the adjusted cDNA. The optimal cycle number was then considered to be 2 cycles less than the greatest cycle of linearity. The PCR samples were electrophoresed on a 2% agarose gel and stained with ethidium bromide. Pictures of the gels were digitally captured by using the BioImaging System GeneGenius (Syngene, Cambridge, UK), and densitometry values were measured by using Gene Tool software (Syngene). The RT-PCR values were presented as a ratio of the specified gene signal in the selected linear amplification cycle divided by the β-actin signal. Data for each gene represented the mean of 3 individual RT-PCR.

### Table 2. Fatty acid composition (g/100 g of total fatty acids) of diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Control diet</th>
<th>Linseed diet</th>
<th>Control diet</th>
<th>Linseed diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>1.09</td>
<td>0.11</td>
<td>1.10</td>
<td>0.11</td>
</tr>
<tr>
<td>C16:0</td>
<td>69.50</td>
<td>11.40</td>
<td>69.60</td>
<td>10.54</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.10</td>
<td>0.38</td>
<td>0.11</td>
<td>0.39</td>
</tr>
<tr>
<td>C18:0</td>
<td>4.00</td>
<td>4.76</td>
<td>4.09</td>
<td>4.25</td>
</tr>
<tr>
<td>C18:1n-9</td>
<td>11.73</td>
<td>24.81</td>
<td>10.74</td>
<td>24.18</td>
</tr>
<tr>
<td>C18:2n-6</td>
<td>12.49</td>
<td>24.17</td>
<td>11.96</td>
<td>25.90</td>
</tr>
<tr>
<td>C18:3n-3</td>
<td>0.97</td>
<td>30.34</td>
<td>1.08</td>
<td>32.59</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.19</td>
<td>0.21</td>
<td>0.18</td>
<td>0.21</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.06</td>
<td>0.06</td>
<td>0.04</td>
<td>0.23</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.04</td>
<td>0.12</td>
<td>0.05</td>
<td>0.11</td>
</tr>
<tr>
<td>C22:6n-3</td>
<td>0.03</td>
<td>0.39</td>
<td>0.09</td>
<td>0.20</td>
</tr>
<tr>
<td>SFA$^3$</td>
<td>74.82</td>
<td>16.6</td>
<td>75.02</td>
<td>15.22</td>
</tr>
<tr>
<td>PUFA$^4$</td>
<td>13.49</td>
<td>54.9</td>
<td>13.13</td>
<td>58.69</td>
</tr>
</tbody>
</table>

$^1$BW range 30 to 60 kg.  
$^2$BW range 60 to 115 kg.  
$^3$Percentage of SFA is the sum of 14:0, 16:0, 18:0, 20:0, and 22:0.  
$^4$Percentage of PUFA is the sum of 18:2n-6, 22:6n-3, and 18:3n-3 (α-linolenic acid).
Measurement of Blood Cytokine Concentrations

Serum IL-1β, IL-6, and TNF-α concentrations were measured by using commercially available porcine ELISA kits (Quantikine PLB00, P6000, and PTA00, respectively, R&D Systems, Minneapolis, MN) according to the instructions of the manufacturer (http://www.rndsystems.com/pdf/plb00.pdf, http://www.rndsystems.com/pdf/p6000.pdf, and http://www.rndsystems.com/pdf/pta00.pdf, respectively). The intraassay and interassay precision, sensitivity, recovery, and linearity of the assays were described in the instructions of the manufacturer. Each sample was assayed in triplicate and the absorbance values at 450 nm were recorded. The minimum detectable concentration was 15 pg/mL for IL-1β, 10 pg/mL for IL-6, and 20 pg/mL for TNF-α.

Statistical Analyses

Experimental animals were assigned to different treatments in a completely randomized design. The basic variable was linseed addition time (d). Linear and quadratic relationships with day were determined for parameters collected from treatment groups. The data variances were analyzed by using PROC GLM (SAS Inst. Inc., Cary, NC). The equations that describe the relations between gene expression and ADG were generated by iterative nonlinear least squares regression.

RESULTS

Production Performance

Average daily feed intake was not affected (P = 0.87) by treatment. However, ADG (P < 0.05) and G:F (P < 0.05) exhibited a quadratic response; treatment group 3 had the greatest ADG value among the 4 groups (Table 4).

Gene Expression

Expression data of PPARγ and the 3 proinflammatory cytokine genes (IL-1β, IL-6, and TNF-α) are presented in Figure 1 and Table 5. The expression of PPARγ in muscle and in spleen increased (P < 0.01; Figure 1A and 1C, respectively) linearly with increasing duration of linseed diet feeding, but its expression in adipose tissue was not affected (P = 0.095; Figure 1B). In contrast, TNF-α and IL-6 expression in muscle, adipose, and spleen decreased (P < 0.01) linearly with duration on the linseed diet, and the expression of IL-1β decreased quadratically in adipose tissue (P < 0.01; Figure 1E) and spleen (P < 0.01; Figure 1F).

Table 3. Oligonucleotide PCR primers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession no.</th>
<th>Primer source</th>
<th>Primer sequence (5′→3′)</th>
<th>Orientation</th>
<th>Product size, bp</th>
<th>Ta, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARγ</td>
<td>L40904</td>
<td>Human</td>
<td>TCTCATAACGCCATCAGG</td>
<td>Forward</td>
<td>442</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GACGCGCATATTTAGGA</td>
<td>Reverse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>M86725</td>
<td>Pig</td>
<td>CATACCCAGAGTCCACAT</td>
<td>Forward</td>
<td>409</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TCACAGACACTGGCTGCTTC</td>
<td>Reverse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>M80258</td>
<td>Pig</td>
<td>ATGACTCCCTCTCCACAAAGC</td>
<td>Forward</td>
<td>493</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TGCTTTGTCGGATGGATTTCGCC</td>
<td>Reverse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>NM214022</td>
<td>Pig</td>
<td>TGAGCCCAATGTCGAAGCC</td>
<td>Forward</td>
<td>399</td>
<td>59</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>TGGCCAGATTCGCAAAAGTG</td>
<td>Reverse</td>
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<td>β-Actin</td>
<td>SSU07786</td>
<td>Pig</td>
<td>GACATTCCGAGCAGGAGATGG</td>
<td>Forward</td>
<td>233</td>
<td>—</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>GCACCGTGTTGGCGTAGAGG</td>
<td>Reverse</td>
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<td></td>
</tr>
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</table>

1PPARγ = peroxisome proliferator-activated receptor-γ; and TNF-α = tumor necrosis factor-α.

2Ta = optimal PCR annealing temperature.

Table 4. Effects of linseed on growing-finishing pig performance

<table>
<thead>
<tr>
<th>Item</th>
<th>0 (T1)</th>
<th>30 (T2)</th>
<th>60 (T3)</th>
<th>90 (T4)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADG, g</td>
<td>662</td>
<td>725</td>
<td>733</td>
<td>657</td>
<td>8.2</td>
<td>Q*</td>
</tr>
<tr>
<td>ADFI, g</td>
<td>2,040</td>
<td>2,028</td>
<td>2,065</td>
<td>2,160</td>
<td>26.0</td>
<td>NS</td>
</tr>
<tr>
<td>G:F, g/kg</td>
<td>326</td>
<td>358</td>
<td>366</td>
<td>323</td>
<td>3.5</td>
<td>Q*</td>
</tr>
</tbody>
</table>

1The 4 treatment groups, each with 6 pigs, included a control diet group (T1) and three 10% linseed supplementation diet groups (T2, T3, and T4) that differed in diet duration.

2The n used to calculate the SEM was 6.

3NS = not significant; Q = quadratic effect for days on the linseed diet.

*P < 0.05.
Plasma Cytokine Concentrations

Serum concentration of TNF-α decreased ($P < 0.01$) linearly with increasing duration of feeding the linseed diet, but the concentrations of IL-1β and IL-6 in serum were not significantly different ($P > 0.05$) between different diet treatments (Table 6).

Correlations Between Expression of PPARγ and Proinflammatory Cytokine Genes

Significant negative linear correlations were found between expression of PPARγ and expression of the 3 proinflammatory cytokines (IL-1β, IL-6, and TNF-α) both in muscle ($R^2 = 0.75$, $P < 0.001$; $R^2 = 0.63$, $P = 0.001$; and $R^2 = 0.70$, $P < 0.001$, respectively) and in spleen ($R^2 = 0.73$, $P < 0.001$; $R^2 = 0.70$, $P < 0.001$; and $R^2 = 0.77$, $P < 0.001$, respectively), as well as in adipose tissue for IL-1β ($R^2 = 0.54$, $P = 0.007$), but not in adipose tissue for IL-6 ($R^2 = 0.30$, $P = 0.166$) and TNF-α ($R^2 = 0.32$, $P < 0.138$).

Correlation Between PPARγ Expression and Plasma Cytokine Concentrations

Significant negative linear correlations were found between splenic PPARγ expression and serum TNF-α concentration ($R^2 = 0.59$, $P < 0.01$), but not between splenic PPARγ expression and serum IL-13 ($R^2 = 0.15$, $P = 0.52$) or IL-6 ($R^2 = 0.25$, $P = 0.28$) concentration, respectively. Similarly, significant negative linear correlations were detected between PPARγ expression and
serum TNF-α concentration ($R^2 = 0.52, P = 0.02$) in muscle, but not between PPARγ and IL-1β ($R^2 = 0.12, P = 0.62$) or IL-6 ($R^2 = 0.33, P = 0.16$). In adipose tissue, there were no significant linear negative correlations between PPARγ expression and serum proinflammatory cytokine concentrations (IL-1β: $R^2 = 0.15, P = 0.52$; IL-6: $R^2 = 0.13, P = 0.44$; and TNF-α: $R^2 = 0.33, P = 0.16$).

Relation Between Gene Expression and Growth Performance

The mRNA expressions for PPARγ and proinflammatory cytokines in treatment groups 1, 2, 3, and 4 ranged from 0.72 to 2.79. These mRNA expression data were pooled and compared with ADG to evaluate a possible correlation between expression of inflammation-related genes and growth performance.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Source</th>
<th>0 (T1)</th>
<th>30 (T2)</th>
<th>60 (T3)</th>
<th>90 (T4)</th>
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</thead>
<tbody>
<tr>
<td>PPARγ</td>
<td>Muscle</td>
<td>0.87</td>
<td>1.31</td>
<td>1.54</td>
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<td></td>
<td>Adipose</td>
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<td>1.77</td>
<td>2.07</td>
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<td></td>
<td>Spleen</td>
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<td>1.75</td>
<td>1.99</td>
<td>2.10</td>
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<tr>
<td>IL-1β</td>
<td>Muscle</td>
<td>1.89</td>
<td>1.74</td>
<td>0.99</td>
<td>1.12</td>
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<tr>
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<td>2.79</td>
<td>1.65</td>
<td>0.72</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>2.74</td>
<td>1.05</td>
<td>0.83</td>
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</tr>
<tr>
<td>IL-6</td>
<td>Muscle</td>
<td>1.83</td>
<td>1.61</td>
<td>1.48</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>Adipose</td>
<td>1.85</td>
<td>1.70</td>
<td>1.30</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>2.05</td>
<td>1.80</td>
<td>1.30</td>
<td>1.21</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Muscle</td>
<td>1.27</td>
<td>1.17</td>
<td>0.91</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Adipose</td>
<td>1.92</td>
<td>1.73</td>
<td>1.35</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>1.95</td>
<td>1.63</td>
<td>1.46</td>
<td>1.31</td>
</tr>
</tbody>
</table>

1PPARγ = peroxisome proliferator-activated receptor-γ; and TNF-α = tumor necrosis factor-α.
2The 4 treatment groups, each with 6 pigs, included a control diet group (T1) and three 10% linseed supplementation diet groups (T2, T3, and T4) that differed in diet duration.
3The n used to calculate the SEM was 6.
4NS = not significant; L and Q indicate a significant linear and quadratic effect, respectively, for days on the linseed diet.
5Values for semiquantitative reverse transcription-PCR are presented as a ratio of the specified gene signal in the selected linear amplification cycle divided by the β-actin signal.
**P < 0.01.

There were statistically significant ($P < 0.05$) quadratic relations between expression of muscular PPARγ or splenic TNF-α mRNA and ADG (Figure 2 and 3, respectively). Average daily gain increased as the expression of PPARγ mRNA in muscle increased (Figure 2), whereas the optimal expression level of TNF-α (relative to β-actin) in spleen ranged from 1.4 to 1.8, beyond or below which the growth performance of pigs declined (Figure 3).

DISCUSSION

The results of the present study indicating that feeding a 10% linseed diet for 30 to 90 d at the finishing stage positively affected growth performance of pigs was partially in agreement with several previous reports. Romans et al. (1995a,b) reported that feeding pigs with up to 15% linseed in the diet for 25 d did not sig-

Table 5. Effects of linseed diet on expression of inflammation-related genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Source</th>
<th>0 (T1)</th>
<th>30 (T2)</th>
<th>60 (T3)</th>
<th>90 (T4)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARγ</td>
<td>Muscle</td>
<td>0.87</td>
<td>1.31</td>
<td>1.54</td>
<td>1.49</td>
<td>0.023</td>
<td>L**</td>
</tr>
<tr>
<td></td>
<td>Adipose</td>
<td>1.57</td>
<td>1.77</td>
<td>2.07</td>
<td>1.97</td>
<td>0.054</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>1.45</td>
<td>1.75</td>
<td>1.99</td>
<td>2.10</td>
<td>0.021</td>
<td>L**</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Muscle</td>
<td>1.89</td>
<td>1.74</td>
<td>0.99</td>
<td>1.12</td>
<td>0.013</td>
<td>L**</td>
</tr>
<tr>
<td></td>
<td>Adipose</td>
<td>2.79</td>
<td>1.65</td>
<td>0.72</td>
<td>0.79</td>
<td>0.029</td>
<td>Q**</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>2.74</td>
<td>1.05</td>
<td>0.83</td>
<td>0.62</td>
<td>0.026</td>
<td>Q**</td>
</tr>
<tr>
<td>IL-6</td>
<td>Muscle</td>
<td>1.83</td>
<td>1.61</td>
<td>1.48</td>
<td>1.33</td>
<td>0.018</td>
<td>L**</td>
</tr>
<tr>
<td></td>
<td>Adipose</td>
<td>1.85</td>
<td>1.70</td>
<td>1.30</td>
<td>1.18</td>
<td>0.017</td>
<td>L**</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>2.05</td>
<td>1.80</td>
<td>1.30</td>
<td>1.21</td>
<td>0.022</td>
<td>L**</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Muscle</td>
<td>1.27</td>
<td>1.17</td>
<td>0.91</td>
<td>0.79</td>
<td>0.013</td>
<td>L**</td>
</tr>
<tr>
<td></td>
<td>Adipose</td>
<td>1.92</td>
<td>1.73</td>
<td>1.35</td>
<td>1.33</td>
<td>0.044</td>
<td>L**</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>1.95</td>
<td>1.63</td>
<td>1.46</td>
<td>1.31</td>
<td>0.032</td>
<td>L**</td>
</tr>
</tbody>
</table>

1PPARγ = peroxisome proliferator-activated receptor-γ; and TNF-α = tumor necrosis factor-α.
2The 4 treatment groups, each with 6 pigs, included a control diet group (T1) and three 10% linseed supplementation diet groups (T2, T3, and T4) that differed in diet duration.
3The n used to calculate the SEM was 6.
4NS = not significant; L and Q indicate a significant linear and quadratic effect, respectively, for days on the linseed diet.
5Values for semiquantitative reverse transcription-PCR are presented as a ratio of the specified gene signal in the selected linear amplification cycle divided by the β-actin signal.
**P < 0.01.

Table 6. Effects of linseed diet on serum concentrations (pg/mL) of proinflammatory cytokines

<table>
<thead>
<tr>
<th>Item</th>
<th>0 (T1)</th>
<th>30 (T2)</th>
<th>60 (T3)</th>
<th>90 (T4)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>4.12</td>
<td>4.12</td>
<td>4.15</td>
<td>4.08</td>
<td>0.131</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.61</td>
<td>0.04</td>
<td>0.30</td>
<td>0.01</td>
<td>0.232</td>
<td>NS</td>
</tr>
<tr>
<td>TNF-α</td>
<td>73.25</td>
<td>67.97</td>
<td>54.31</td>
<td>51.89</td>
<td>4.143</td>
<td>L**</td>
</tr>
</tbody>
</table>

1TNF-α = tumor necrosis factor-α.
2The 4 treatment groups, each with 6 pigs, included a control diet group (T1) and three 10% linseed supplementation diet groups (T2, T3, and T4) that differed in diet duration.
3The n used to calculate the SEM was 6.
4NS = not significant; L = significant linear effect for days of linseed feeding.
5Log-transformation was used to normalize data.
**P < 0.01.
nificantly influence feed intake. Other researchers also found that the differences in feed intake among pigs fed linseed oil diets were not significant (Fontanillas et al., 1998; Kouba et al., 2003). However, the results for ADG and G:F in the present study were in contrast to the reports of Fontanillas et al. (1998) and Kouba et al. (2003), who showed no statistically significant differences of ADG and G:F by feeding linseed. Nguyen et al. (2004) reported that feeding linseed appeared to affect the ADG of pigs, but this failed to reach statistical significance ($P = 0.077$). Indeed, the stimulatory effect of linseed on growth may not be significant when the amount added is less than 5% or the feeding duration is shorter than 60 d (Romans et al., 1995a,b; Fontanillas et al., 1998; Kouba et al., 2003; Nguyen et al., 2003, 2004). However, additional intake of ALA may stimulate growth in growing-finishing pigs (Nguyen et al., 2004). In the current study, linseed supplementation in the diet increased the growth of growing-finishing barrows, which was consistent with a previous report by Liu et al. (2003), who showed that pigs fed fish oil (rich in n-3 PUFA) had greater BW gain independent of a lipopolysaccharide challenge.

It seems unlikely that the stimulatory effect of linseed oil on growth is due to an ALA deficiency in the control diet because LM, backfat lipids, and liver, in all cases, contained a moderate concentration (at least more than 0.5, 0.9, and 2.1%, respectively; data for backfat, muscle, and liver ALA content not presented) of ALA at the end of the experiment. However, the duration of feeding the linseed diet did affect the expression of inflammation-related genes in the current study. Previous studies in humans and pigs have demonstrated that consumption of n-3 PUFA from linseed oil or fish oil inhibits IL-1β and TNF-α production (Caughey et al., 1996; Gaines et al., 2003). Therefore, feeding the 10% linseed diet to pigs might improve growth performance partially by modulating the production of proinflammatory cytokines.

Cytokines play important roles in immunoregulation. The 3 cytokines IL-1β, IL-6, and TNF-α could induce great metabolic changes (Melchior et al., 2004). Although they are mostly derived from lymphocytes and macrophages, it is now clear that cytokines are also produced by cells not traditionally considered part of the immune system, such as adipocytes and myofibers, which are effective sources and targets of cytokines (Pié et al., 2004; Jacobi et al., 2006). Production of appropriate amounts of proinflammatory cytokines is beneficial in response to infection, but insufficient production or overproduction of these cytokines can be dangerous (Simopoulos, 2002). Proinflammatory cytokines mediate “reprogramming” of metabolism and shift the partitioning of dietary nutrients away from skeletal muscle accretion toward metabolic responses that support the immune system (Spurlock, 1997; Melchior et al., 2004). However, the majority of previous results were observed under conditions of inflammation or immune stress. Interestingly, the present study showed that during normal physiological processes, feeding the linseed diet suppressed the expression of proinflammatory cytokines and decreased the circulatory TNF-α concentration of growing-finishing barrows. However, the total circulatory TNF-α did not show a significant correlation with ADG. The reason for this requires further study.

Both human and marine studies have demonstrated that dietary n-3 PUFA are able to regulate expression of inflammatory genes. The n-3 PUFA might exert their effects through direct actions on intracellular signaling pathways, which lead to activation of 1 or more transcription factors, including PPAR (Xi et al., 2001; Calder, 2002). Peroxisome proliferator-activated receptor-γ is a member of the nuclear receptor superfamily of PPAR, and its main function was originally thought to be regulation of adipocyte differentiation (Hammad et al., 2004; Pegorier et al., 2004). Recently,

- **Figure 2.** Relation between muscular peroxisome proliferator-activated receptor-γ (PPARγ) expression and ADG. The duration of linseed diet feeding was 0 (□), 30 (■), 60 (▲), or 90 (▲) d, respectively. Numbers on the x-axis are ratios of PPARγ gene signals divided by the corresponding β-actin signals in semiquantitative reverse transcription-PCR. The equation, which describes the relation, was generated by iterative nonlinear least squares regression.

- **Figure 3.** Relation between splenic tumor necrosis factor-α (TNF-α) expression and ADG. The duration of linseed diet feeding was 0 (□), 30 (■), 60 (▲), or 90 (▲) d, respectively. The numbers on the x-axis are ratios of TNF-α gene signals divided by the corresponding β-actin signals in semiquantitative reverse transcription-PCR. The equation, which describes the relation, was generated by iterative nonlinear least squares regression.
growing evidence points to their involvement in regulating the immune response, particularly in inflammation control (Sokolowska et al., 2005). In the present study, significant negative linear correlations were found between expression of PPARγ and expression of proinflammatory cytokines in muscle and spleen, and a significant negative linear correlation was also observed between PPARγ and IL-1β expression in adipose tissue. These suggest that feeding linseed might decrease the expression of proinflammatory cytokines by activating PPARγ.

The increased expression of PPARγ in skeletal muscle indicated that feeding linseed induced the development of preadipocytes from stromal-vascular stem cells to promote intramuscular fat content (Meadus et al., 2002). Surprisingly, the stimulatory effect of the linseed diet on PPARγ expression was weaker in subcutaneous fat than in skeletal muscle. This conflict might be due to prolonged PPARγ stimulation by dietary n-3 PUFAs increasing the apparent intramuscular fat content, because more stem cells are available for intramuscular fat locations than for subcutaneous fat locations (May et al., 1994; Meadus et al., 2002).

Recently, PPARγ has also attracted attention as a key factor in insulin sensitivity (Sokolowska et al., 2005). Furthermore, IL-1 has been shown to inhibit the anabolic effects of insulin on skeletal muscle (Klasing and Johnstone, 1991). Meadus et al. (2002) have demonstrated that the glucose supply of muscle cells could be increased with CLA. This finding suggests that the growth-enhancing effect of ALA may also be related to an increase in muscle glucose uptake by a mechanism similar to that of CLA.

**LITERATURE CITED**


