n-6:n-3 PUFA ratio is involved in regulating lipid metabolism and inflammation in pigs

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Abstract

The objective of the present study was to investigate the optimal dietary n-6:n-3 PUFA ratios that regulate lipid metabolism and inflammation in pigs. A total of ninety-six cross-bred (Large White × Landrace) growing-finishing pigs (73.8 (SEM 1.6) kg) were chosen and fed one of the four isoenergetic diets with n-6:n-3 PUFA ratios of 1:1, 2:5:1, 5:1 and 10:1. The growth performance of pigs fed the diet with an n-6:n-3 PUFA ratio of 5:1 was the best, but the group fed the diet with an n-6:n-3 PUFA ratio of 1:1 had the highest muscle mass and the lowest adipose tissue mass (P<0.05). The concentrations of IL-6 and IL-1β of pigs fed the diet with an n-6:n-3 PUFA ratio of 1:1 were decreased compared with those of the other groups (P<0.05). The concentration of adiponectin of pigs fed the diet with an n-6:n-3 PUFA ratio of 1:1 was also markedly decreased, but the concentration of leptin was increased compared with that of the groups fed the diets with n-6:n-3 PUFA ratios of 5:1 and 10:1 (P<0.05). Additionally, the optimal dietary ratios of n-6:n-3 PUFA of 1:1 and 5:1 markedly suppressed the expression levels of lipid metabolism-related genes and proteins such as phosphoinositide-3-kinase-α, fatty acid transport protein-1 and PPARγ. They also significantly suppressed the expression levels of the inflammatory cytokines IL-1β, TNF-α and IL-6. The results indicated that the optimal n-6:n-3 PUFA ratios of 1:1 and 5:1 exerted beneficial effects on lipid metabolism and inflammatory system, leading to the availability of more energy and nutrients for high performance and homeostatic pathways.

Key words: n-6:n-3 ratios; PUFA; Lipid metabolism; Inflammation; Pigs

Essential fatty acids, including n-6 and n-3 PUFA, cannot convert into each other in the body. Hence, PUFA are crucial components of the food or diet1,2. It has been widely accepted that the present Western diet is low in n-3 fatty acids with a ratio of n-6:n-3 ranging from 15:1 to 20:1, instead of 1:1, while a value as much as possibly close to 1:1 is considered protective against degenerative pathologies3,4. Both n-6 and n-3 PUFA can regulate gene expression: n-3 PUFA exert suppressive effects on chronic diseases; conversely, n-6 PUFA increase the concentrations of inflammatory mediators5,6. On the one hand, it has been hypothesised that diets with high ratios of n-6:n-3 PUFA may increase the production of inflammatory mediators and lead to the pathology of the metabolic syndrome, such as cognitive impairment, Alzheimer’s disease and type 2 diabetes6–10. On the other hand, diets with higher ratios of n-6:n-3 fatty acids may lead to the pathology of the metabolic syndrome5. Therefore, lowering the n-6:n-3 PUFA ratio in diets is beneficial for the health of animals and humans.

A lower n-6:n-3 PUFA ratio is required for the prevention and management of chronic diseases2. Some previous studies have suggested that an n-6:n-3 PUFA ratio of 5:1 suppresses inflammation in patients with asthma11. It should be noted that the biological effects of n-6 and n-3 PUFA are not always in opposition. It is widely accepted that the n-6-derived lipoxins also exert anti-inflammatory effects12. Due to their opposing and coordinative effects, a proper balance between n-6 and n-3 fatty acids in the diet is very important to maintain the optimum growth and development of animals and also the health of humans11.

Abbreviations: FATP-1, fatty acid transport protein-1; PI3Kα, phosphoinositide-3-kinase-α.

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The morphology and physiology of the organs of humans and pigs are similar. Thus, the pig is an excellent animal model for studying human nutrition and metabolism. In the present study, we used a pig model to investigate the optimal dietary ratios of \( n-6:n-3 \) PUFA that regulate lipid metabolism and inflammation.

### Materials and methods

#### Animals and diets

All procedures followed in the present experiment were approved by the committee on animal care of the Institute of Subtropical Agriculture, the Chinese Academy of Sciences.

A total of ninety-six male cross-bred (Large White \( \times \) Landrace) pigs with a similar initial weight (75.8 (SEM 1.6) kg) were chosen and divided into four groups using a randomised complete block design based on body weight, with six replicates (pens) per group and four pigs per replicate. The pigs in the four groups were fed isoenergetic diets (3% fat) with different \( n-6:n-3 \) ratios, prepared using 3.00, 1.50, 0.75 and 0.30% of linseed oil to replace equivalent amounts of soyabean oil and nutrient levels of the four diets are listed in Table 1.

#### Sample collection

Body weights and feed intake of the pigs were recorded after an overnight fast to calculate weight gain and feed conversion.

### Table 1. Composition and nutrient levels of the diets (air-dry basis, %)

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>1:1</th>
<th>2:5:1</th>
<th>5:1</th>
<th>10:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>65-50</td>
<td>65-50</td>
<td>65-50</td>
<td>65-50</td>
</tr>
<tr>
<td>Soyabean meal</td>
<td>22-50</td>
<td>22-50</td>
<td>22-50</td>
<td>22-50</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>6-60</td>
<td>6-60</td>
<td>6-60</td>
<td>6-60</td>
</tr>
<tr>
<td>Soyabean oil*</td>
<td>0 1-50</td>
<td>2-25</td>
<td>2-70</td>
<td></td>
</tr>
<tr>
<td>Linseed oil</td>
<td>3-00</td>
<td>1-50</td>
<td>0-75</td>
<td>0-30</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0-50</td>
<td>0-50</td>
<td>0-50</td>
<td>0-50</td>
</tr>
<tr>
<td>Limestone</td>
<td>0-60</td>
<td>0-60</td>
<td>0-60</td>
<td>0-60</td>
</tr>
<tr>
<td>Salt</td>
<td>0-30</td>
<td>0-30</td>
<td>0-30</td>
<td>0-30</td>
</tr>
<tr>
<td>Premix†</td>
<td>1-00</td>
<td>1-00</td>
<td>1-00</td>
<td>1-00</td>
</tr>
<tr>
<td>Nutrient level (%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digestive energy (MJ/kg)</td>
<td>14-20</td>
<td>14-20</td>
<td>14-20</td>
<td>14-20</td>
</tr>
<tr>
<td>Crude protein</td>
<td>15-50</td>
<td>15-50</td>
<td>15-50</td>
<td>15-50</td>
</tr>
<tr>
<td>SID Lys</td>
<td>0.69</td>
<td>0.69</td>
<td>0.69</td>
<td>0.69</td>
</tr>
<tr>
<td>SID Met</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Ca</td>
<td>0.52</td>
<td>0.52</td>
<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>Available P</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
</tr>
</tbody>
</table>

SID, standardised ileal digestible.

*To replace equivalent amounts of soyabean oil, 3.00, 1.50, 0.75 and 0.30% of linseed oil were used, making the dietary \( n-6:n-3 \) ratios about 1:1, 2:5:1, 5:1 and 10:1, respectively (see Table 2).

From each replicate, one pig was chosen and killed at the end of the feeding test. Blood samples were collected via jugular vein puncture into 10 ml tubes, and serum was separated by centrifugation at 2000 \( \text{g} \) for 15 min at \( 4^\circ \text{C} \) and then stored at \( -20^\circ \text{C} \) until analysis. The pigs were electrically stunned, exsanguinated and eviscerated. Immediately, samples (about 5 g) of the longissimus lumborum muscle and subcutaneous adipose tissue dissected from the left side of the carcasses were placed in liquid \( \text{N}_2 \) and then stored at \( -80^\circ \text{C} \) until further analyses. Later, skeletal muscle and fat were dissected from the right side of the carcasses and weighed separately. The weights were used to calculate the total percentages of these components in the carcasses.

### Measurement of the concentrations of secreted adipokines by ELISA

The serum concentrations of IL-6 (R&D), TNF-\( \alpha \) (Endogen), IL-1\( \beta \), leptin, total adiponectin (Usnsc) and insulin (Mercodia) were quantified using ELISA kits for porcine assay according to the manufacturers’ instructions. All the samples were measured in six replicates.

### Real-time PCR

Total RNA was extracted from the harvested tissue using the TRIzol reagent (Invitrogen). Primers for the selected genes (Table 2) were designed using the Oligo 6.0 software. RT was performed using the AMV Reverse Transcriptase Kit (Promega). The relative expression levels of the target genes were determined using quantitative real-time PCR, performed with an ABI 7900 PCR system (ABI Biotechnology). The final volume of the reaction mixtures (20 \( \mu \)l) contained diluted complementary DNA and SYBR Green I (Molecular Probes) as a PCR core reagent. \( \beta\)-Actin was used as a housekeeping gene or an internal control to normalise the expression of target genes.

The relative quantification of gene amplification by RT-PCR was performed using the value of the threshold cycle (\( \text{Ct} \)). The comparative \( \text{Ct} \) value method using the formula \( 2^{-\Delta \Delta \text{Ct}} \) was employed to quantify the expression levels of phosphoinositide-3-kinase-\( \alpha \) (\( \text{PI3K}\alpha \)), fatty acid transport protein-1 (\( \text{FATP-1}\)), \( \text{PPAR}_{\gamma} \), IL-1\( \beta \), TNF-\( \alpha \) and IL-6 relative to those of \( \beta\)-actin using the following formula:

\[
2^{-\Delta \Delta \text{Ct}} = \frac{C_{\text{gene of interest}} - C_{\beta\text{-actin}}}{C_{\text{untreat}} - C_{\text{untreat}}}.
\]

### Western blotting

Tissue samples (about 500–800 mg) were powdered in liquid \( \text{N}_2 \) to extract total protein. Approximately 30 \( \mu \)g of the protein sample were size-fractionated on SDS–PAGE gel and transferred onto polyvinylidene difluoride membranes (Millipore) under the conditions of 30 mA and \( 4^\circ \text{C} \) overnight. Later, the membranes were blocked with 5% bovine serum albumin (BSA) for 1 h and then probed overnight at \( 4^\circ \text{C} \) with the antibodies against FATP-1 (ab69458; Abcam) at 1:800 dilution and...
Table 2. Primers used for real-time PCR

<table>
<thead>
<tr>
<th>Genes</th>
<th>Forward</th>
<th>Reverse</th>
<th>Size (bp)</th>
<th>T&lt;sub&gt;a&lt;/sub&gt; (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P&lt;sub&gt;3&lt;/sub&gt;Kα</td>
<td>CCAGTGTAAGGAGAAACCGG</td>
<td>TTGCTTGATGGTGGGTAAT</td>
<td>334</td>
<td>60</td>
</tr>
<tr>
<td>FATP-1</td>
<td>GGAGTGAAACCGG</td>
<td>TTGCTTGATGGTGGGTAAT</td>
<td>208</td>
<td>64</td>
</tr>
<tr>
<td>PPARγ</td>
<td>AGGCTGCGGCGGCTGCTGAA</td>
<td>TTGCTTGATGGTGGGTAAT</td>
<td>381</td>
<td>58</td>
</tr>
<tr>
<td>IL-1β</td>
<td>GCTAACTAGGTGAGCTAAGG</td>
<td>TTGCTTGATGGTGGGTAAT</td>
<td>196</td>
<td>64</td>
</tr>
<tr>
<td>TNF-α</td>
<td>TTCCTACGGGCTGTCTCCTC</td>
<td>TTGCTTGATGGTGGGTAAT</td>
<td>395</td>
<td>64</td>
</tr>
<tr>
<td>IL-6</td>
<td>CAGCAAAGTCAATGATGTAAG</td>
<td>TTGCTTGATGGTGGGTAAT</td>
<td>494</td>
<td>64</td>
</tr>
<tr>
<td>β-Actin</td>
<td>TGGCGGACATCAAGAAGAAG</td>
<td>TTGCTTGATGGTGGGTAAT</td>
<td>216</td>
<td>64</td>
</tr>
</tbody>
</table>

T<sub>a</sub>, annealing temperature; P<sub>3</sub>Kα, phosphoinositide-3-kinase-α; FATP-1, fatty acid transport protein-1.

PPARγ (C2435; Cell Signaling Technology) and P<sub>3</sub>Kα (C4255; Cell Signaling Technology) at 1:1000 dilution. The membranes were then rinsed with Tris-buffered saline plus 0·1% Tween 20 three times and incubated with peroxidase-conjugated goat anti-rabbit or anti-mouse IgG for 1 h at 1:5000 dilution at room temperature; β-actin monoclonal antibody (sc47778) at 1:2000 dilution was used to normalise the amount of proteins (Santa Cruz Biotechnology). The protein bands were visualised using a chemiluminescent reagent. The density of the protein bands was determined using the Alpha Imager 2200 software (Alpha Innotech Corporation).

Statistical analyses

All the results are expressed as means with their standard errors. Statistical analyses were carried out using one-way ANOVA, SAS 8.2 (SAS Institute, Inc.). followed by a Tukey test of multiple comparisons. In case of a P value <0·05, differences were considered to be statistically significant.

Results

Effects of dietary n-6:n-3 PUFA ratios on the growth performance and body composition of pigs

The fatty acid contents of the experimental diets are listed in Table 3. The measured values coincided better with the calculated values. The growth performance and body composition of pigs fed diets with different n-6:n-3 PUFA ratios are summarised in Table 4. Compared with those of the other groups, the body weight and daily weight gain of pigs fed the diet with an n-6:n-3 PUFA ratio of 5:1 were increased significantly (P<0·05), while the daily intake and feed conversion rate of this group were decreased (P<0·05). However, the group fed the diet with an n-6:n-3 PUFA ratio of 1:1 had high muscle mass and low adipose tissue mass. We speculated that an optimal n-6:n-3 PUFA ratio could regulate the crosstalk between the muscle and adipose tissue of pigs.

Effects of different n-6:n-3 PUFA ratios on serum glucose and cytokine concentrations

As shown in Table 5, the concentrations of glucose, TNF-α and insulin were not different among the treatment groups. The concentrations of IL-6 and IL-1β of pigs fed the diet with an n-6:n-3 PUFA ratio of 1:1 were decreased by 12·3 and 37·9% (P<0·05), respectively, compared with those fed diets with an n-6:n-3 PUFA ratio of 10:1. Furthermore, the serum concentrations of adiponectin of pigs fed the diet with an n-6:n-3 PUFA ratio of 1:1 were also decreased by 13·5% compared with those of pigs fed the diet with an n-6:n-3 PUFA ratio of 10:1 (P<0·05); on the contrary, the concentration of leptin of this group was increased by 16·4% (P<0·05).

Effects of dietary n-6:n-3 PUFA ratios on the gene expression levels of pigs

The expression levels of genes in the muscle and adipose tissue of pigs are shown in Fig. 1(A) and (B). The expression levels of P<sub>3</sub>Kα mRNA were lower (P<0·05) in the groups fed diets with n-6:n-3 PUFA ratios of 1:1 and 2:5:1, and there was no difference between these two groups (P>0·05). The expression levels of the FATP-1 gene in the muscle and adipose tissue of pigs fed diets with an n-6:n-3 PUFA ratio of 1:1 were the lowest (P<0·05), and those fed diets with n-6:n-3 PUFA ratios of 1:1 and 2:5:1 exhibited down-regulated expression levels of the PPARγ gene in the muscle and adipose tissue (P<0·05); also, there was no difference (P>0·05) between these two groups. Interestingly, the diet with an n-6:n-3 PUFA ratio of 1:1 markedly down-regulated the expression levels of IL-1β, TNF-α and IL-6 genes in the skeletal muscle and adipose tissue of pigs (P<0·05).

Effect of dietary n-6:n-3 PUFA ratios on the protein expression levels of pigs

The relative expression levels of P<sub>3</sub>Kα, FATP-1 and PPARγ proteins are shown in Fig. 2(A) and (B). The expression level of the P<sub>3</sub>Kα protein was higher in the muscle of pigs fed the diet with an n-6:n-3 PUFA ratio of 10:1 (P<0·05). The trend of the expression levels of the FATP-1 protein was the same as those of the gene in the muscle. However, the trend of the expression levels of the FATP-1 protein in the adipose tissue was the reverse. The diets with n-6:n-3 PUFA...
ratios of 1:1 and 2.5:1 significantly down-regulated the expression levels of the PPARγ protein in the muscle and adipose tissue (*P < 0.05*), also partially corresponding to the expression levels of the gene.

**Discussion**

In the present study, with an accompanying decline in the average daily feed intake and feed conversion rate, the final body weight and daily gain of pigs fed the diet with an n-6:n-3 PUFA ratio of 5:1 improved significantly. This result is in agreement with earlier reports showing that a diet with a lower n-6:n-3 PUFA ratio, rich in n-3 PUFA, is beneficial for the growth performance and health of animals\(^{(12-15)}\).

The main components of adipose tissue are fatty acids, which may influence the expression of adipokines, such as adiponectin and leptin\(^{(15)}\). Interestingly, the results of the present experiment showed that the serum concentrations of adiponectin of pigs decreased gradually as the dietary n-6:n-3 PUFA ratio decreased. Adiponectin is an adipokine exclusively derived from the adipose tissue\(^{(16,17)}\). It has been reported that fish oil rich in n-3 PUFA increases the serum concentrations of adiponectin in mice 2–3-fold in a dose-dependent manner and also in a PPARγ-dependent manner\(^{(17)}\). However, the present results showed that a low n-6:n-3 PUFA ratio could reduce the serum concentrations of adiponectin. The results led us to hypothesise that the serum concentrations of adiponectin are affected by different ratios of dietary n-6:n-3 PUFA. Leptin circulates in the body at a concentration highly correlated with white adipose tissue mass and may be of great importance in the regulatory action on body fat\(^{(15,16)}\). It has been shown that the serum concentrations of leptin are significantly reduced in mice fed diets with an n-6:n-3 PUFA ratio of 1:1, but these are not significantly reduced in mice fed diets with n-6:n-3 PUFA ratios of 5:1, 10:1 and 20:1\(^{(17)}\). In the present study, the concentrations of leptin of the group fed the diets with n-6:n-3 PUFA ratios of 1:1 and 2.5:1 were higher, indicating that the optimal ratio may vary in different animal models. However, no difference in the serum concentrations of insulin was observed in the present study. We speculated that n-6:n-3 PUFA ratios could stimulate the negative feedback regulatory mechanism of adiponectin and leptin.

Immune stimulation in the rearing environment results in the production of potent pro-inflammatory cytokines, which antagonise anabolic growth factors and thus suppress growth. IL-6, IL-1β and TNF-α, which are all inflammatory cytokines, initiate the production of an array of inflammatory mediators, thus leading to an inflammatory response. The concentrations of these cytokines are increased on increasing n-6 fatty acid intake and decreased on increasing n-3 fatty acid intake in bovine chondrocytes and in mouse kidney, spleen and peritoneal macrophages, as well as in human monocytes\(^{(20)}\). The circulating levels of IL-6 might reflect, at least in part, the production of IL-6 in the adipose tissue, although it is also secreted by the exercising muscle\(^{(21)}\). The concentrations of IL-6 decrease by 10.5% on altering the n-6:n-3 ratio to 1:3\(^{(22)}\). Moreover, the concentrations of TNF-α decline significantly by 30% in response to a flaxseed oil diet rich in n-3 PUFA and decrease by 74% after fish oil supplementation\(^{(23)}\).

**Table 4. Effect of dietary n-6:n-3 PUFA ratios on the growth performance of pigs**

<table>
<thead>
<tr>
<th>Items</th>
<th>1:1</th>
<th>2.5:1</th>
<th>5:1</th>
<th>10:1</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (kg)</td>
<td>74.58</td>
<td>72.75</td>
<td>73.92</td>
<td>73.83</td>
<td>0.64</td>
<td>0.27</td>
</tr>
<tr>
<td>Final body weight (kg)</td>
<td>125.58</td>
<td>125.77</td>
<td>129.75</td>
<td>126.03</td>
<td>0.98</td>
<td>0.04</td>
</tr>
<tr>
<td>Daily weight gain (kg/d)</td>
<td>0.85</td>
<td>0.88</td>
<td>0.93</td>
<td>0.87</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Feed intake (kg/d)</td>
<td>2.70</td>
<td>2.78</td>
<td>2.56</td>
<td>2.77</td>
<td>0.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Feed conversion rate (gain:feed)</td>
<td>3.17</td>
<td>3.15</td>
<td>2.75</td>
<td>3.18</td>
<td>0.06</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Muscle mass (%)</td>
<td>34.30</td>
<td>32.00</td>
<td>31.38</td>
<td>31.25</td>
<td>0.58</td>
<td>0.02</td>
</tr>
<tr>
<td>Adipose tissue mass (%)</td>
<td>8.21</td>
<td>9.02</td>
<td>10.51</td>
<td>12.99</td>
<td>1.03</td>
<td>0.04</td>
</tr>
</tbody>
</table>

\(^{ab}\) Values with unlike letters within a row were significantly different (*P* < 0.05).

\(^{a}\) The ratio represents the muscle or adipose tissue mass: carcass weight.

**Table 5. Effect of dietary n-6:n-3 ratios on serum glucose and cytokine concentrations of pigs**

<table>
<thead>
<tr>
<th>Items</th>
<th>1:1</th>
<th>2.5:1</th>
<th>5:1</th>
<th>10:1</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>3.56</td>
<td>3.13</td>
<td>3.62</td>
<td>3.63</td>
<td>0.24</td>
<td>0.44</td>
</tr>
<tr>
<td>IL-6 (ng/ml)</td>
<td>27.39</td>
<td>29.49</td>
<td>30.15</td>
<td>31.23</td>
<td>0.54</td>
<td>0.01</td>
</tr>
<tr>
<td>TNF-α (ng/ml)</td>
<td>0.23</td>
<td>0.22</td>
<td>0.24</td>
<td>0.26</td>
<td>0.01</td>
<td>0.30</td>
</tr>
<tr>
<td>IL-1β (ng/ml)</td>
<td>0.18</td>
<td>0.26</td>
<td>0.28</td>
<td>0.29</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Adiponectin (μg/ml)</td>
<td>22.07</td>
<td>23.84</td>
<td>25.08</td>
<td>25.52</td>
<td>0.69</td>
<td>0.03</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>2.34</td>
<td>2.12</td>
<td>1.96</td>
<td>2.01</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>23.48</td>
<td>23.80</td>
<td>25.01</td>
<td>25.13</td>
<td>0.96</td>
<td>0.55</td>
</tr>
</tbody>
</table>

\(^{ab}\) Values with unlike letters within a row were significantly different (*P* < 0.05).
The present results also indicated that higher n-3 PUFA could reduce the serum concentrations of IL-6 as well as IL-1β, but not of TNF-α. Additionally, we also found that the expression levels of IL-6, IL-1β and TNF-α mRNA in the skeletal muscle and adipose tissue of pigs fed the diet with an n-6:n-3 PUFA ratio 1:1 were markedly down-regulated. Numerous studies have reported that n-3 PUFA can decrease the production of these inflammatory cytokines. It has been shown that an optimal n-6:n-3 PUFA ratio could regulate several cytokines to reduce inflammatory events in the body.

The PI3K pathway controls essential cellular functions such as signal transduction, cytoskeletal dynamics and membrane trafficking. The expression levels of the PI3Kα gene in mononuclear cells of healthy human subjects have been reported to decrease after supplementation with fish oil. In the present study, the expression levels of PI3Kα gene and protein in the muscle and adipose tissue of pigs fed the diet with a lower n-6:n-3 PUFA ratio were the lowest and the PI3K pathway was activated. In mammals, FATP-1 transports long-chain fatty acids actively across adipocyte cell membranes. In the present study, it was found that the expression levels of FATP-1 mRNA and protein in the muscle and adipose tissue were down-regulated significantly in pigs fed the diet with a lower n-6:n-3 PUFA ratio. We speculated that n-3 PUFA could suppress adipogenic processes by down-regulating the expression levels of FATP-1 and the optimal dietary ratios of n-6:n-3 PUFA might be 1:1 and 5:1.

PPARγ regulates genes involved in adipocyte differentiation and lipogenesis, while n-3 PUFA and their metabolites have been shown to suppress the transcription of lipogenic genes.

In the present study, the expression levels of P13Kα gene and protein in the muscle and adipose tissue of pigs fed the diet with an n-6:n-3 PUFA ratio of 1:1 were the lowest and the P13K pathway was activated. In mammals, FATP-1 transports long-chain fatty acids actively across adipocyte cell membranes. In the present study, it was found that the expression levels of FATP-1 mRNA and protein in the muscle and adipose tissue were down-regulated significantly in pigs fed the diet with a lower n-6:n-3 PUFA ratio. We speculated that n-3 PUFA could suppress adipogenic processes by down-regulating the expression levels of FATP-1 and the optimal dietary ratios of n-6:n-3 PUFA might be 1:1 and 5:1. PPARγ regulates genes involved in adipocyte differentiation and lipogenesis, while n-3 PUFA and their metabolites have been shown to suppress the transcription of lipogenic genes.

The present results also indicated that higher n-3 PUFA could reduce the serum concentrations of IL-6 as well as IL-1β, but not of TNF-α. Additionally, we also found that the expression levels of IL-6, IL-1β and TNF-α mRNA in the skeletal muscle and adipose tissue of pigs fed the diet with an n-6:n-3 PUFA ratio 1:1 were markedly down-regulated. Numerous studies have reported that n-3 PUFA can decrease the production of these inflammatory cytokines. It has been shown that an optimal n-6:n-3 PUFA ratio could regulate several cytokines to reduce inflammatory events in the body.

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by functioning as natural ligands for PPAR\(^\text{127–31}\). Interestingly, \(n\)-3 PUFA and their metabolites can activate the extracellular signal-regulated kinase pathway\(^{29,31,52}\), which primarily regulates cellular growth and differentiation\(^{33,54}\). Some previous studies have already demonstrated that PPAR\(\gamma\) ligands inhibit the production of IL-6, TNF-\(\alpha\) and IL-1\(\beta\)\(^{28,35,56}\) and that TNF-\(\alpha\) can inhibit adipocyte differentiation and adipogenesis by suppressing the expression of the PPAR\(\gamma\) gene\(^{120,36}\).

In the present study, the expression levels of PPAR\(\gamma\) gene and protein in both the muscle and adipose tissue of pigs fed the diets with \(n\)-6:3 PUFA ratios of 1:1 and 2:5:1 were markedly reduced. It was observed that a diet with a lower \(n\)-6:3 PUFA ratio could reduce the expression levels of PPAR\(\gamma\), which further suppress the transcription of lipogenic genes and lipogenesis.

**Conclusion**

On the whole, \(n\)-6:3 PUFA ratios regulate lipid metabolism and inflammation differently and the optimal ratios are 1:1 to 5:1, which vary based on the roles under considerations. Optimal \(n\)-6:3 PUFA ratios could inhibit immune stimulation to ensure the availability of more energy and nutrients for high performance and homeostatic pathways. We speculated that there was a common pathway shared by energy metabolism and inflammation modulation. However, further research is necessary to confirm the results and to illustrate the underlying metabolic pathways.

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The authors’ contributions are as follows: Y. Y., L. L. and L. L. were in charge of the whole trial; Y. D. and F. L. wrote the manuscript; J. F. and X. S. assisted with the animal trial and biochemical analyses. The authors have no conflicts of interest to declare.

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