**Dietary Linoleic and α-Linolenic Acids Affect Anxiety-Related Responses and Exploratory Activity in Growing Pigs**

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**Abstract**

**Background:** Growing evidence suggests that the dietary ratio of linoleic acid (LA) to α-linolenic acid (ALA), the precursors of arachidonic acid (AA) and docosahexaenoic acid (DHA), respectively, may affect behavior in mammals.

**Objective:** This study aimed at evaluating the impact of dietary LA and ALA intake on behaviors of growing pigs, a pertinent model for human nutrition.

**Methods:** At 7 wk of age, 32 pigs were allocated to 4 dietary treatments varying in daily intake of LA (1.3 and 2.6 g · kg body weight\(^{-0.75}\) · d\(^{-1}\)) for low- and high-LA groups, respectively, and ALA (0.15 and 1.5 g · kg body weight\(^{-0.75}\) · d\(^{-1}\)) for low- and high-ALA groups, respectively, for 4 wk. Between days 12 and 18, general behavior in the home pen was observed and pigs were subjected to an open field and novel object test. At 11 wk of age, brain fatty acid composition was analyzed.

**Results:** Compared with high LA intake, low LA intake increased the time spent on exploration, particularly nosing in the home pen (\(P < 0.05\)) and the open field (\(P < 0.05\)), and tended to reduce the time spent lying with eyes open in the home pen (\(P = 0.09\)). Time spent lying with eyes open also tended to be affected by the interaction between LA and ALA (\(P = 0.08\)). A high-LA/high-ALA intake (ratio of 2; \(P < 0.05\)) and a low-LA/high-ALA intake (ratio of 1; \(P = 0.06\)) decreased the latency to approach the novel object compared with a low-LA/low-ALA intake (ratio of 9). DHA in the frontal cortex was positively correlated with exploratory behaviors in the home pen (\(r_s = 0.56, P < 0.01\)), whereas AA was negatively correlated with time spent lying with eyes closed (\(r_s = -0.48, P < 0.01\)).

**Conclusions:** Low LA intake and a low dietary LA:ALA ratio increased exploration and decreased anxiety-related behaviors in pigs. It is suggested that changes in brain DHA and AA induced by dietary LA and ALA intake mediate these behavioral changes. *J Nutr* 2015;145:358–64.

**Keywords:** polyunsaturated fatty acids, omega-6, docosahexaenoic acid, novelty, pigs

**Introduction**

Omega-6 (n-6) and n-3 PUFAs are 2 distinct families of PUFAs that are derived from the precursors linoleic acid (LA; 18:2n-6) and α-linolenic acid (ALA; 18:3n-3), respectively. Arachidonic acid (AA; 20:4n-6) and DHA (22:6n-3), 2 long-chain PUFAs (LC-PUFAs) synthetized from LA and ALA, respectively, are major components of brain cell membrane phospholipids of vertebrates (1) and are known to have an important role in brain development and function and immune system and cardiovascular function (2–6). The conversion of LA and ALA into LC-PUFAs takes place by successive desaturation and chain elongation (7), with the n-3 and n-6 LC-PUFA biosynthetic pathways using the same enzymes and competing with each other for enzyme availability. Vertebrates are unable to synthesize the FA precursors, which must be obtained from the diet. Consequently, the relative proportion of LA and ALA in the diet determines the synthesis of LC-PUFAs in the brain, such as AA, DHA, or docosapentaenoic acid (DPA; 22:5n-3 and 22:5n-6), as we previously reported in pigs (8), and thus affects the composition and function of cell membranes (9).

Growing evidence suggests that dietary modulation of n-6 and n-3 PUFA balance may also influence behavioral functions and emotions. Several studies reported that dietary n-3 PUFA/LC-PUFA deficiency or supplementation affected anxiety-related responses in a novel environment in rodents, pigs, and nonhuman primates (10–15). A role of dietary n-3 PUFAs/LC-PUFAs in exploratory behaviors was also suggested in mice and...
nonhuman primates (13, 16, 17). Finally, consistent evidence showed that supplementation of or deficiency in n–3 PUFAs/LC-PUFAs and modulation of the dietary n–3:n–6 PUFA ratio induced changes in spontaneous locomotor activity in rhesus monkeys, rodents, and piglets (11, 12, 16, 18–21).

However, most of the studies on the role of dietary n–3 and n–6 PUFAs in behavioral functions used diets containing not only LA and ALA but also LC-PUFAs, including AA, DHA, or DPA (11, 16, 17, 19, 20), which makes it impossible to determine the specific role of LA and ALA intake. In the past decade, only a few studies used diets varying only in their contents in LA and ALA as unique sources of n–6 and n–3 PUFAs, respectively (14, 15, 21, 22). In addition, most of these studies focused their research on the effect of dietary n–3 PUFA (i.e., ALA) supplementation or deficiency, although excessive intake of LA is known to impair synthesis of LC-PUFAs from ALA (8, 23). However, the impact of a high LA intake on behavior is still unknown, which justifies the need for further work in this area.

Over the past century, a drastic change in food intake has occurred in modern industrialized societies. Today, the Western diet is characterized by a decrease in n–3 FA intake, including ALA, paired with an increased intake of saturated fat, trans FAs, and n–6 FAs, mostly due to the high content of LA in processed food and vegetable oils, including sunflower, soybean, corn, and safflower oil (24). This dramatic change in dietary LA and ALA intake results in an unbalanced dietary n–6:n–3 PUFA ratio of ~18:1, which is far from the recommended 1:1 ratio (24), which may eventually impair homeostasis and normal growth and development.

Considering the current change in dietary LA:ALA ratio in modern human societies, there is a need for studies in animal models to investigate the negative consequences of this drastic dietary change on behavior from a human nutrition and health perspective. Experimental studies in animal models, such as rodents and nonhuman primates, have provided valuable insight into the important role of dietary n–6 and n–3 PUFAs on behavioral functions. Nevertheless, the use of rats and nonhuman primates for biomedical research also suffers from some limitations. On the one hand, due to a large phylogenetic distance, rodents differ from humans in terms of nutrient needs, digestive physiology, and brain anatomy and development. On the other hand, studies in nonhuman primates (and humans) may be difficult to implement for ethical and practical reasons, and the limited data available in humans from intervention studies are inconsistent, mainly because of the difficulty to realize trials in controlled conditions (9). The pig, an omnivorous animal with high cognitive abilities, which is easy to maintain under controlled conditions, makes a good new alternative model to rodents to evaluate the effect of dietary manipulations on behavioral variables (25). In addition, as in humans (26), the conversion of ALA into DHA is very low in pig (27). In contrast, there is recent evidence that, in rodents, whole-body conversion of ALA into DHA is higher than originally predicted and more efficient than in humans (see reference 28), suggesting that the pig might be a better model than rodents for the investigation of dietary FA modulation.

Therefore, the aim of the current study was to evaluate the impact of dietary LA and ALA intake on general activity, locomotion, and exploratory behaviors, as well as anxiety-related responses in the home pen and in a novel environment in growing pigs, as a model for human nutrition. We postulated that a high-ALA diet and a low dietary LA:ALA ratio would have beneficial effects on anxiety-related responses and exploratory behaviors. We previously reported that LA and ALA intake tended to affect AA and DHA, and strongly affected DPA n–3 and DPA n–6 in the frontal cortex of pigs (8), and therefore, correlation analyses were also performed to explore the relations between brain FA profiles and behavior.

**Methods**

**Animals and housing.** Thirty-two gilts were 7 wk of age at the start of the study. They were selected from 8 litters and weighed 1.63 ± 1.8 kg (means ± SEMs). Pigs and housing conditions were identical to those reported previously (8). Briefly, during the 4-wk experimental period, the pigs were housed in individual pens (3.5 × 0.9 m) with half concrete and half slatted floors on a 12-h dark-light cycle (0700–1900 h). Long-stemmed straw (20 g/d) was provided daily to allow pigs to express natural foraging behaviors, and water was provided ad libitum. The experimental protocol was approved by the Animal Care and Use Committee of Wageningen University (The Netherlands).

**Diets.** After 2 d of habituation to the experimental pens and during which the pigs were fed a commercial diet, the gilts were randomly assigned to 4 dietary treatment groups (n = 8) in a 2 × 2 factorial arrangement, with daily intakes of LA (high vs. low) and ALA (high vs. low) as dependent variables. Allocation to the experimental groups was balanced for litter and initial weight (low-LA/low-ALA: 16.3 ± 0.6 kg; low-LA/high-ALA: 16.4 ± 0.7 kg; high-LA/low-ALA: 16.1 ± 0.8 kg; high-LA/high-ALA: 16.4 ± 0.5 kg).

Dietary treatments were described previously (8). Differences between low and high intakes were designed to be identical for LA and ALA: low ALA and LA intakes were 0.16 and 1.32 and high ALA and LA intakes were 1.48 and 2.65 g · kg body weight$^{-0.75}$ · d$^{-1}$, respectively (Supplemental Table 1). LA and ALA represented 3.4% and 0.4% of energy in the low-LA/low-ALA diet, 3.3% and 3.7% of energy in the low-LA/high-ALA diet, 6.6% and 0.4% of energy in the high-LA/low-ALA diet, and 6.4 and 3.5% of energy in the high-LA/high-ALA diets, respectively. The low-LA/low-ALA, low-LA/high-ALA, high-LA/low-ALA, and high-LA/high-ALA diets had n–6:n–3 PUFA ratios of 9, 1, 17, and 2, respectively. LA and ALA were the only n–6 or n–3 PUFAs in the diets, and intakes of SFAs, MUFAs, and other nutrients were kept constant. To do so, the basal diet was supplemented with various doses of sunflower oil, linseed oil, high-oleic sunflower oil, and palm oil (Supplemental Table 2). Because LA and ALA were added on top of the basal diet, digestible energy intake varied among the treatments between 2.6 and 2.8 times the maintenance energy requirement. The pigs were fed the diets as mash twice daily for 4 wk.

**Home pen observations.** On days 12 and 18 after the onset of the dietary treatment, behaviors of the individual pigs were scored in their home pens by using live 2-min instantaneous scan sampling for 5 h/d during five 1-h sessions starting at 0800, 1000, 1230, 1430, and 1630 h. Data were collected by using a Psion hand-held computer with the Observer 5.0 software package (Noldus Information Technology). The behaviors are listed in Supplemental Table 3.

**Open field test.** Starting 15 d after the onset of the dietary treatment, the pigs were individually subjected to an open field test. The tests were carried out on 2 consecutive days with half of the pigs being tested on day 1 and the other half on day 2. The order of testing was balanced for treatment. The testing area was an unfamiliar arena of 5 × 5 m with 1.2-m-high wooden walls and a concrete floor. The arena was divided into 3 × 3 imaginary sections. The test started when the pig had entered the arena with all 4 paws and the door was closed. The tests were recorded on videotape. During the 300-s test, behavioral states (i.e., locomotion and exploration) and events (i.e., vocalizations, escape attempts, and eliminating) were live scored continuously with the Observer 5.0 software package. The behaviors are listed in Supplemental Table 4. In addition, the number of line crossings, the latency to first enter, and the percentage of time spent in the central section of the arena were scored
during video observations. The pig was considered to have entered a section when it had its head and both front legs in the section.

**Novel object test.** Starting 16 d after the onset of the dietary treatment, the pigs were individually subjected to a novel object test in the home pen. The tests were carried out on 2 consecutive days, with half of the pigs being tested on day 1 and the other half on day 2. The order of testing was balanced for treatment.

The test started when a bright red space hopper (diameter: 50 cm) was introduced in the home pen. During the 300-s test, latencies to approach and touch the object and frequencies of touching the object were live recorded. Approach was defined as ≥2 steps directly toward the space hopper. Touching was defined as contact between the space hopper and the head (nose and mouth), chest, or legs of the pig.

**Brain FA analysis.** At the end of the 4-wk dietary treatment, brain (hippocampus and frontal cortex) samples were collected for determining FA profiles. The procedure and the results of brain FA analyses are described fully in Smink et al. (8).

**Statistical analyses.** Statistical analyses were conducted with SAS version 9.1 (SAS Institute). A mixed model was used to determine the effect of LA, ALA, and LA × ALA interaction on the behavioral variables, followed by least-squares means differences when significant effects were found. The random effect of the litter was included in the model. For the home pen observation data, preliminary analyses were performed with the observation day (days 12 and 18) included as a dependent variable in the mixed model. Because no interaction was found between observation day LA and ALA intake (P > 0.1), except for a tendency for the percentage of time spent standing (ALA vs. LA intake), these behaviors were not analyzed. All of the behaviors were expressed as a percentage of time, except for the line crossings and low- and high-pitched vocalizations in the open field, which were expressed as total occurrences. The effect of dietary treatment on the vocalizations was found between observation day LA and ALA intake (P = 0.08). Lying did not occur during the open field test, and behaviors were investigated by Spearman’s rank correlation analysis, Model residuals were tested for normality, and if not normally distributed, the statistical analyses were performed on (arcsin) square root or logarithmically transformed data to obtain homogeneity of variances. P values are based on analyses of transformed data, and the threshold for statistical significance was set at P < 0.05, with 0.05 ≤ P < 0.1 considered as a trend. Data were expressed as untransformed means ± SEMs.

**Results**

**Behaviors in the home pen.** LA and ALA intake affected general behavior in the home pens. Compared with high LA intake, low LA intake significantly increased the time spent on exploratory activities [Table 1; 10.6 ± 1.0% (high-LA) vs. 13.7 ± 1.0% (low-LA); P = 0.038], and more specifically, the time spent nosing [7.8 ± 0.8% (high-LA) vs. 10.4 ± 0.7% (low-LA); P = 0.031]. Time spent lying with eyes open tended to be affected by LA intake (P = 0.09) and LA × ALA interaction (Table 1; P = 0.08). The interaction trend was related to less time spent lying with eyes open in pigs fed the low-LA/high-ALA diet compared with pigs fed the high-LA/high-ALA, the low-LA/low-ALA, and the high-LA/low-ALA diets. No effects of LA and ALA intake were found on the time spent in activity/inactivity or on locomotor activity in the home pen (P > 0.1).

**Open field test.** Compared with a high LA intake, low LA intake induced a significant increase in the time spent nosing during the open field test [Table 2; 59.7 ± 4.0% (high-LA) vs. 69.7 ± 2.5% (low-LA); P = 0.046]. No effects of LA and ALA intakes were found on the percentage of time spent in the center of the arena (Figure 1A). The latency to enter this central section was affected by LA intake (Figure 1B; P = 0.008) and tended to be affected by the LA × ALA interaction (P = 0.06). The interaction trend was related to a higher latency to first enter the center of the arena in pigs fed the low-LA/high-ALA diet compared with pigs fed the high-LA/high-ALA, the high-LA/low-ALA, and the low-LA/low-ALA diets. A tendency was found for the effect of LA × ALA interaction on the percentage of time spent standing alert [Figure 1C; P = 0.08]. The numerical

| TABLE 1 | General activity of pigs fed diets varying in LA and ALA contents in the home pen
<table>
<thead>
<tr>
<th><strong>Behaviors</strong></th>
<th><strong>Time spent, % of total time</strong></th>
<th><strong>P</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low-LA/low-ALA</td>
<td>Low-LA/high-ALA</td>
</tr>
<tr>
<td>Standing</td>
<td>9.2 ± 1.4</td>
<td>9.0 ± 1.5</td>
</tr>
<tr>
<td>Sitting, kneeling</td>
<td>2.6 ± 1.0</td>
<td>2.2 ± 0.8</td>
</tr>
<tr>
<td>Lying</td>
<td>53.6 ± 3.1</td>
<td>50.6 ± 3.9</td>
</tr>
<tr>
<td>With open eyes</td>
<td>22.5 ± 2.3</td>
<td>16.4 ± 1.9</td>
</tr>
<tr>
<td>With closed eyes</td>
<td>31.1 ± 3.2</td>
<td>34.1 ± 4.8</td>
</tr>
<tr>
<td>Activity²</td>
<td>34.6 ± 2.8</td>
<td>38.1 ± 3.7</td>
</tr>
<tr>
<td>Exploration</td>
<td>12.8 ± 2.5</td>
<td>14.7 ± 1.5</td>
</tr>
<tr>
<td>Eating, drinking, eliminating</td>
<td>17.8 ± 1.7</td>
<td>20.7 ± 3.1</td>
</tr>
</tbody>
</table>

¹ Values are means ± SEMs, n = 8. Data were analyzed by a mixed model, followed by least-squares means differences when significant interaction effects were found. Labeled means in a row without a common letter differ, P < 0.05 (a, b), or tend to differ, 0.05 ≤ P < 0.1 (y, z); ALA, α-linolenic acid; LA, linoleic acid; Σ, total.
² Activity includes all of the behaviors, with the exception of standing, sitting, kneeling, and lying with eyes open or closed.
data suggested that the pigs fed the low-LA/high-ALA diet spent less time standing alert than the pigs fed the high-LA/high-ALA and low-LA/low-ALA diets. No effect of LA and ALA intake was found on the total occurrences of line crossings (Table 2) or low- (low-LA/low-ALA, 8.1 ± 5.1; low-LA/high-ALA, 1.3 ± 0.9, high-LA/low-ALA, 2.0 ± 1.7; and high-LA/high-ALA, 5.0 ± 2.8 occurrences) and high-pitched vocalizations (Figure 1D), although the numerical data suggested that the pigs fed the low-LA/high-ALA diet had less high-pitched vocalizations than pigs fed the low-LA/low-ALA diet. No other effect of LA and ALA intake on behaviors during the open field test was found.

**Novel object test.** A significant LA × ALA interaction was found on the latency to approach the novel object (Figure 2), with the latency to approach the novel object being lower in the pigs fed the high-LA/high-ALA diet (P = 0.02) and, to a lesser extent, the pigs fed the low-LA/high-ALA diet (P = 0.06) than in the pigs fed the low-LA/low-ALA diet. No significant effect of LA and/or ALA intake was found in the frequency of contact with the novel object (P > 0.1).

**Correlation between brain FAs and behaviors.** Significant correlations were found between DHA and AA concentrations in the frontal cortex and the general activity in the home pens. The pigs that spent less time lying with eyes closed had a higher AA content in the frontal cortex (Figure 3A; r_s = −0.48, P = 0.006). The pigs that spent more time exploring and nosing the home pen had higher DHA concentrations in the frontal cortex (Figure 3B; r_s = 0.56, P = 0.001, and r_s = 0.49, P = 0.004, respectively). The pigs that spent more time rooting also had higher DHA content in the frontal cortex (r_s = 0.35, P = 0.047). Furthermore, the pigs that had less frequent contact with the novel object also had higher DHA concentrations in the frontal cortex (r_s = −0.35, P = 0.049). No other correlations were found between frontal cortex AA or DHA concentrations and behavior. No correlations were found between DPA n-6 and n-3 contents in the brain and behaviors in the home pen or during the open field and novel object test (P > 0.05).

**Discussion**

This study investigated the effects of 4-wk exposure to diets varying in their LA and ALA contents on anxiety and behavior in individually housed growing pigs. Compared with a high LA intake, low LA intake increased exploratory behaviors, and particularly nosing, both in the home pen and in a novel environment. Exploratory behaviors such as nosing, rooting, and chewing are known to be “natural behavioral needs” strongly related to welfare in pigs (29–31). The increase in exploration in the home pen found in pigs fed the low-LA diets may reflect an increased motivation to investigate the environment, i.e., inquisitive exploration, sometimes referred as

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**TABLE 2** Behaviors of pigs fed diets varying in LA and ALA contents during the open field test

<table>
<thead>
<tr>
<th>Behaviors</th>
<th>Low-LA/low-ALA</th>
<th>Low-LA/high-ALA</th>
<th>High-LA/low-ALA</th>
<th>High-LA/high-ALA</th>
<th>LA</th>
<th>ALA</th>
<th>LA × ALA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standing</td>
<td>40.1 ± 5.1</td>
<td>40.5 ± 4.8</td>
<td>35.0 ± 2.2</td>
<td>44.5 ± 5.5</td>
<td>0.95</td>
<td>0.29</td>
<td>0.35</td>
</tr>
<tr>
<td>Walking, running</td>
<td>59.7 ± 5.1</td>
<td>59.5 ± 4.8</td>
<td>65.0 ± 2.2</td>
<td>55.5 ± 5.5</td>
<td>0.94</td>
<td>0.30</td>
<td>0.34</td>
</tr>
<tr>
<td>Standing alert</td>
<td>8.4 ± 6.3</td>
<td>0.9 ± 0.7</td>
<td>3.2 ± 2.6</td>
<td>10.9 ± 7.8</td>
<td>0.65</td>
<td>0.81</td>
<td>0.08</td>
</tr>
<tr>
<td>Σ Exploration</td>
<td>73.3 ± 4.6</td>
<td>83.3 ± 2.5</td>
<td>76.0 ± 5.6</td>
<td>72.6 ± 5.9</td>
<td>0.49</td>
<td>0.57</td>
<td>0.14</td>
</tr>
<tr>
<td>Nosing</td>
<td>68.1 ± 4.5</td>
<td>71.2 ± 2.4</td>
<td>60.8 ± 7.2</td>
<td>58.5 ± 4.0</td>
<td>&lt;0.05</td>
<td>0.97</td>
<td>0.51</td>
</tr>
<tr>
<td>Chewing</td>
<td>5.3 ± 1.5</td>
<td>12.2 ± 3.2</td>
<td>15.2 ± 5.9</td>
<td>14.1 ± 3.7</td>
<td>0.17</td>
<td>0.36</td>
<td>0.24</td>
</tr>
<tr>
<td>Line crossing</td>
<td>44.9 ± 7.7</td>
<td>40.4 ± 6.2</td>
<td>47.1 ± 6.4</td>
<td>41.1 ± 5.8</td>
<td>0.80</td>
<td>0.39</td>
<td>0.90</td>
</tr>
</tbody>
</table>

1Values are means ± SEMs, n = 8. Data were analyzed by a mixed model, followed by least-squares means differences when significant interaction effects were found. Labeled means in a row without a common letter differ, P < 0.05. ALA, α-linolenic acid; LA, linoleic acid; Σ, total.
“curiosity” (32). Exploratory behaviors in an unfamiliar environment are known to reflect fear and anxiety, with lower levels of exploration being indicative of higher anxiety (33). Thus, low dietary LA intake may decrease anxiety and increase exploration, thereby potentially increasing well-being both in familiar and unfamiliar/stressful environments in pigs.

The tendency of pigs fed the low-LA diets to spend less time lying with eyes open than pigs fed the high-LA diets supports this assumption. In mink, pigs, and mice, the time spent lying awake increases with changes in housing environment (i.e., stressful situation) and decreases with housing enrichment, suggesting that lying inactive but awake may reflect stress/anxiety (34–37). Schouten (38) also postulated that lying with eyes open is an alert posture allowing pigs to be more quickly aware of potential threats. The decreased time spent lying with eyes open in the pigs fed the low-LA diets may therefore reflect a lower vigilance toward the observers during (live) observation, which further supports the possibility that low dietary LA intake decreases anxiety and vigilance in pigs.

Not only dietary LA but also modulation of dietary LA and ALA balance induced alterations in behaviors in the home pen and during the novelty tests, as indicated by LA × ALA interactions. The pigs fed the high-LA/high-ALA (n–6:n–3 PUFA ratio of 2) and, to a lesser extent, the low-LA/high-ALA diet (n–6:n–3 PUFA ratio of 1) took less time to first approach the novel object in the home pen than did the pigs fed the low-LA/low-ALA diet (n–6:n–3 PUFA ratio of 9). Because latency to approach and touch a novel object is indicative of fear in pigs (39–41), this result suggests that a low dietary LA:ALA ratio decreases anxiety in pigs.

Consistent with this suggestion, a trend for LA × ALA interaction suggested that the pigs fed the low-LA/high-ALA diet (n–6:n–3 PUFA ratio of 1) spent less time lying with eyes open than the pigs fed the 3 other diets in the home pen (n–6:n–3 PUFA ratios of 2, 9, and 17). An LA × ALA interaction trend also suggested that the pigs fed the low-LA/high-ALA diet (n–6:n–3 FA ratio of 1) spent less time standing alert and had numerically less high-pitched vocalizations than the pigs fed the low-LA/low-ALA diet (n–6:n–3 FA ratio of 9) during the open field test. Standing alert [“freezing behavior” (42, 43)] and high-pitched vocalizations (44) are thought to demonstrate anxiety levels in a novel environment in pigs. Taken together, these trends further support the postulate that not only low LA intake but also a low dietary n–6:n–3 PUFA ratio decrease anxiety both in familiar and unfamiliar environments in pigs.

Our results are consistent with previous studies in adult mice and rats showing that dietary n–3 PUFA/LC-PUFA deficiency, or a high n–6:n–3 PUFA ratio, reduced exploratory activity and increased anxiety in a novel environment (10, 11, 13–15, 22). In contrast, other studies reported that dietary n–3 PUFA supplementation reduced exploratory activity in aged nonhuman primates (17), young mice (16) and rats (19), or had no effect on exploratory behaviors in mature/old mice (16) and anxiety in aged nonhuman primates (17). As suggested previously (13, 17), it is possible that modulation of the dietary n–6/n–3 PUFA balance affects exploratory behavior and anxiety differently across aging.
Although substantial evidence suggests that a high dietary n–6:n–3 PUFA ratio may induce higher levels of anxiety and reduce exploration in mammals, the large majority of the studies focused on dietary n–3 PUFA modulation and used mixed sources of dietary n–3 and n–6 PUFAs, including not only LA and ALA but also LC-PUFAs such as DHA and AA, at different inclusion rates, which makes it impossible to determine the specific role of LA and ALA intake. Some recent studies on the effects of dietary LA:ALA ratio on exploration and anxiety in rodents reported results similar to our findings. In these studies, however, the effects were mostly due to dietary ALA modulation (14, 15, 22), whereas in our study differences in exploratory behaviors and stress were found between low and high LA intakes.

Contrary to what would have been predicted from other behavioral indicators, low LA intake increased the latency to first enter the central section of the open field, although no difference was found in the time spent in the center. In addition, a trend for LA × ALA interaction suggested that the pigs fed a diet with an n–6:n–3 PUFA ratio of 1 had a higher latency to enter the center section of the open field than did pigs fed diets with higher n–6:n–3 PUFA ratios. A decreased latency to enter and a reduced time spent in the central area of the open field are strong indicators of anxiety in rodents (45), suggesting that the intake of a diet low in LA or with a low LA:ALA ratio increased anxiety levels in our study, contrary to what was previously stated. However, diazepam, a strong anxiolytic drug, failed to affect the number of entries into the center of an open field in pigs (46), whereas pigs treated with azapropazone, a drug used to prevent stress in husbandry systems, spent more time in the periphery of an open field than did control pigs (47). These findings raise the possibility that the time spent in the center of the open field may not be a pertinent measure of anxiety in pigs, contrary to rodents which tend to remain close to the walls as an antipredator strategy [i.e., thigmotaxis (45)]. Although a relatively small number of animals were used in the present study, the majority of the variables investigated seemed to indicate consistent and congruent effects on anxiety. It is thus reasonable to assume that a 4-wk intake of a diet low in LA or with a low LA:ALA ratio decreased anxiety and increased exploratory behaviors in the home pen and a novel environment in pigs.

Analyses of brain FAs showed that low LA intake tended to increase DHA and decrease AA concentrations in the frontal cortex [see previously published results (8)]. Interestingly, DHA and AA concentrations in this brain region correlated with anxiety-related behaviors. Pigs that spent more time exploring (nosing and rooting) in the home pen had higher DHA concentrations in the frontal cortex and pigs that spent more time lying with eyes closed (i.e., possibly with low anxiety levels) had lower AA contents in this brain region. The correlation between exploratory behaviors and DHA concentrations in the frontal cortex is consistent with findings in rats showing correlations between brain DHA content and exploration in the open field (48). Moreover, Ng and Innis (11) reported a relation between reduced activity and decreased DHA or increased AA concentrations in the frontal cortex in piglets fed a low-PUFA diet. Taken together, those results suggest that increased exploratory activity (and lower anxiety levels) induced by low LA intake may be related to high DHA and low AA concentrations in the brain and provide further evidence suggesting that brain phospholipid FA composition may be a critical factor underlying behavioral changes induced by modulation of dietary n–6 and n–3 PUFA content (21, 49).

Although several studies reported inhibiting effects of dietary n–3 PUFAs on locomotor activity in adult nonhuman primates and rodents (12, 16, 20), we failed to report any effect of dietary LA and ALA intake on locomotor activity in the present study. It is possible, however, that the effects of dietary n–3 and n–6 PUFAs vary with age because contrasting effects have been reported in immature (50) and adult (50, 51) rats, as well as in young, mature, and old mice (16).

In conclusion, we demonstrated that the intake of a diet low in LA and the intake of a diet with a low LA:ALA ratio decreased anxiety-related responses and increased exploratory behaviors but had no effect on locomotor activity in the home pen and in potential stressful situations in growing piglets. These findings suggest that dietary n–3 PUFAs might not be the only determinant of behavioral functions and emphasize the need for further research on the specific effects of dietary n–6 FAs and the balance between n–3 and n–6 PUFAs on behavioral development. Our study also indicates that changes in DHA or AA contents in the frontal cortex associated with dietary LA intake may be a critical factor underlying these behavioral alterations. Further studies should consider using the pig as a suitable animal model to determine to what extent DHA and AA concentrations in the brain mediate the behavioral alterations induced by changes in dietary n–6 FA intake. The present findings in pigs could help to reinforce current recommendations and support the development of dietary measures to decrease intake of LA, and thus optimize the beneficial effects of a high ALA intake, particularly in young humans suffering from anxiety-related disorders (9).

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