Peroxised dietary lipids impair intestinal function and morphology of the small intestine villi of nursery pigs in a dose-dependent manner

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Abstract
The objective of this study was to investigate the effect of increasing degrees of lipid peroxidation on structure and function of the small intestine of nursery pigs. A total of 216 pigs (mean body weight was 6.5 kg) were randomly allotted within weight blocks and sex and fed one of five experimental diets for 35 d (eleven pens per treatment with three to four pigs per pen). Treatments included a control diet without added lipid, and diets supplemented with 6% soyabean oil that was exposed to heat (80°C) and constant oxygen flow (1 litre/min) for 0, 6, 9 and 12 d. Increasing lipid peroxidation linearly reduced feed intake (P<0.001) and weight gain (P=0.024). Apparent faecal digestibility of gross energy (P=0.001) and fat (P<0.001) decreased linearly as the degree of peroxidation increased. Absorption of mannitol (linear, P=0.097) and D-xylose (linear, P=0.089), measured in serum 2 h post gavage with a solution containing 0.2 g/ml of D-xylose and 0.3 g/ml of mannitol, tended to decrease progressively as the peroxidation level increased. Increasing peroxidation also resulted in increased villi height (linear, P<0.001) and crypt depth (quadratic, P=0.005) in the jejunum. Increasing peroxidation increased malondialdehyde concentrations (quadratic, P=0.035) and reduced the total antioxidant capacity (linear, P=0.044) in the jejunal mucosa. In conclusion, lipid peroxidation progressively diminished animal performance and modified the function and morphology of the small intestine of nursery pigs. Detrimental effects were related with the disruption of redox environment of the intestinal mucosa.

Key words: Lipid peroxidation; Oxidative stress; Pigs; Small intestine

Dietary peroxidised lipids are of nutritional relevance because their intake has been linked to gastrointestinal disorders. Contribution of peroxidised lipids to human diets has markedly increased because of the elevated consumption of processed lipids and increased use of unsaturated oils in the cooking processes. Frying oils are heated to temperatures that range from 177 to 195°C for extended periods of time (up to 8 h for more than 1 week). Under such conditions, lipids become increasingly peroxidised, resulting in a large number of unstable and toxic compounds (hydroperoxides, aldehydes, alcohols, conjugated dienes, polymers and so on). In a recent study, Sebastian et al. determined that ‘in-use’ frying oils from 80% of the tested restaurants (n=20) in Canada were highly peroxidised (peroxide values up to 48 mEq O₂/kg and p-anisidine values (relative measurement of aldehydes content) up to 41). The degree of peroxidation of ‘discarded lipids’ was even greater (peroxide values up to 248 mEq O₂/kg and p-anisidine values up to 57). This is a great concern not only for human nutrition, as the content of peroxidised lipids in fried products (i.e. fried potatoes, tortilla chips and so on) may be up to 38%[5], but also for animal nutrition because discarded lipids are commonly recycled into livestock diets.

Understanding the effects of lipid peroxidation in the small intestine is particularly important because intestinal epithelial cells experience greater exposure to lipid peroxidation products. Early research provided evidence of the toxicity of peroxidised lipid products and cautioned their use in livestock feeds because this could result in a reduction of productivity. Recent studies investigated the deleterious effects of hydroperoxides, aldehydes and other lipid peroxidation subproducts on intestinal cell lines and animals. Dietary peroxidised lipids can induce oxidative stress and impair the antioxidant defence system. In the small intestine, oxidative stress and disruption of cellular redox status by peroxidised lipids increase cellular transition responses (proliferative, growth arrest and apoptosis), disrupting intestinal turnover and cell survival. Moreover, there is evidence that induction of oxidative stress and disruption of redox balance in intestinal cells contributes to a loss of intestinal integrity and activates proinflammatory transcription factors. Despite the recognition of lipid peroxidation as modulator of gastrointestinal disorders, little is known regarding the effects of lipid peroxidation on the structure and function of the small intestine.

Abbreviations: BW, body weight; MDA, malondialdehyde.
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Although the deleterious effects of lipid peroxidation are of biological relevance, experiments conducted using animal models have produced conflicting results. For instance, Varady et al.\(^\text{12}\) determined that lipid peroxidation activated inflammatory transcription factors in mice, whereas Ringsseis et al.\(^\text{13}\) did not observe inflammatory responses in pigs fed peroxidised lipids. Recently, Liu et al.\(^\text{14}\) concluded that supplementation of 10% peroxidised lipids to diets of young pigs did not affect intestinal integrity. Inconsistencies, when using animal models, may be because of differences in the methodology used, especially in regard to the extent of lipid peroxidation. Researchers have suggested that there is a minimum threshold for lipid peroxidation above which detrimental effects are noticeable\(^\text{8,16}\). However, there are very few studies that investigated the biological responses to increasing degrees of lipid peroxidation and it is unknown whether lipid peroxidation will exert gradual detrimental effects. Therefore, in the present study, we investigated the effects of increasing degrees of lipid peroxidation on growth response, intestinal morphology and function of the small intestine in young pigs.

Methods

All animal care and use procedures for the present experiment were approved by the Institutional Animal Care and Use Committee of North Carolina State University.

Experimental design and dietary treatments

A total of 216 crossbred barrows and gilts (6.5-kg body weight (BW)), weaned between 19 and 21 d of age, were randomly allotted within weight bands and sex to fifty-five standard nursery pens (three to four pigs per pen). Weaned pigs were fed a phase 1 nursery diet for 14 d, followed by a phase 2 diet for 21 d (Table 1). Dietary treatments in each of the phases included a negative control diet (no added soyabean oil), and diets supplemented with 6% soyabean oil previously exposed to peroxidation conditions (described below) for 0, 6, 9 and 12 d. Diets supplemented with peroxidised soyabean oil were manufactured from a common basal mix and formulated to a constant nutrient:metabolisable energy ratio. Titanium dioxide was included in all diets as an indigestible marker for determination of fat and gross energy digestibility. All nutrients exceeded the nutrient requirements suggested by the NRC\(^\text{17}\). Pigs were offered free access to water. Feed was available \textit{ad libitum}, with precaution taken to avoid feed wastage. All diets were offered in meal form. Body and feeder weights were recorded on d 0, 14 and 35 for computation of growth performance.

Peroxidation and chemical analysis of soyabean oil

Pure soyabean oil (60 kg) was placed into polyethylene drums equipped with immersion heaters. Soyabean oil was exposed to peroxidation by heating at 80°C while bubbling oxygen gas through the oil at a constant rate of 1 litre/min. After peroxidative exposure for 0, 6, 9 and 12 d, soyabean oil was stabilised with 0.5 g of an antioxidant (Rendox AT 20 Liquid; Kemin Industries) and stored under refrigeration (4°C) to prevent further peroxidation.

The chemical composition and peroxidation measures of the soyabean oil (Table 2) were determined in representative samples by a commercial laboratory (New Jersey Feed Laboratory Inc.) according to standard procedures of AOAC\(^\text{18}\) and American Oil Chemists’ Society\(^\text{19}\). Amounts of hydroperoxides in soyabean oil were estimated by measuring the initial peroxide value. The \(p\)-anisidine value is a relative measurement and was used to determine the aldehyde content of peroxidised oils. Concentrations of malondialdehyde (MDA, used as an index of lipid peroxidation) in samples were analysed using a commercial ELISA kit (Cell Biolabs). All analyses were conducted in duplicate, and intra- and inter-assay CV were <5%.

Nutrient digestibility

Faecal samples from eight pens per dietary treatment were collected from at least two pigs in each pen from d 11 to 14 and from d 32 to 35. Samples were then pooled within the pen and within the collection period and dried at 60°C in a forced-air

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### Table 1. Ingredient and chemical composition of the experimental diets on an as-fed basis

<table>
<thead>
<tr>
<th>Ingredients (g/kg)</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Oil added</td>
</tr>
<tr>
<td>Yellow maize</td>
<td>435.2</td>
<td>331.8</td>
</tr>
<tr>
<td>Soyabean meal</td>
<td>194.2</td>
<td>237.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>150.0</td>
<td>150.0</td>
</tr>
<tr>
<td>Dried whey</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Fishmeal</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Plasma protein</td>
<td>40.0</td>
<td>40.0</td>
</tr>
<tr>
<td>L-Lys HCl</td>
<td>2·3</td>
<td>2·3</td>
</tr>
<tr>
<td>α-Met</td>
<td>1·4</td>
<td>1·9</td>
</tr>
<tr>
<td>L-Thr</td>
<td>0·8</td>
<td>1·1</td>
</tr>
<tr>
<td>Soyabean oil*</td>
<td>–</td>
<td>60·0</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>8·8</td>
<td>8·7</td>
</tr>
<tr>
<td>Ground limestone</td>
<td>6·0</td>
<td>5·8</td>
</tr>
<tr>
<td>Zinc oxide</td>
<td>2·5</td>
<td>2·5</td>
</tr>
<tr>
<td>Copper sulphate</td>
<td>0·8</td>
<td>0·8</td>
</tr>
<tr>
<td>Salt</td>
<td>1·0</td>
<td>1·0</td>
</tr>
<tr>
<td>Mineral premix†</td>
<td>1·5</td>
<td>1·5</td>
</tr>
<tr>
<td>Vitamin premix‡</td>
<td>0·5</td>
<td>0·5</td>
</tr>
<tr>
<td>Titanium dioxide§</td>
<td>5·0</td>
<td>5·0</td>
</tr>
</tbody>
</table>

**SID**, standard ileal digestibility; **ME**, metabolisable energy.

* Soyabean oil was exposed to peroxidation for 0, 6, 9 and 12 d.
† Supplied per kg of complete diet (mg): ferrous sulphate, 550; zinc oxide, 230; manganese sulphate, 133; copper sulphate, 65; ethylene diamine dihydroiodide, 0·38; sodium selenite, 0·66.
‡ Supplied per kg of complete diet (mg): retinol, 2·5; cholecalciferol, 0·029; α-tocopherol, 31·5; vitamin B₁₂, 0·03; riboflavin, 5·8; calcium pantothenate, 23·5; niacin, 35·2; menadione dimethylpyrimidinol bisulphate, 9·8; folic acid, 1·7; and biotin, 0·23.
§ Used as an indigestible marker for determination of dietary fat and gross energy digestibility.
Chemical analysis (%
jugular venepuncture using a vacuum tube with a 20 G
mannitol (Sigma-Aldrich). Pigs received a dose of 6
\( \cdot \)
calculated using the index method, as described by Adeola(22).
The apparent faecal digestibility of fat and gross energy was
selected. Pigs were deprived of feed for 9 h and then gavaged
and before pigs were switched to phase 2 diets, eight pigs per
measure the intestinal absorptive capacity. At d 14 of the study

Oil Chemists

acid hydrolysis (3 mol/l for 1 h at 90°C) according to American

Fatty acids (%

16 : 0 10.5 10.5 10.7 10.6
18 : 0 4.7 4.7 4.7 4.7
18 : 1n-9 21.9 22.0 22.1 22.3
18 : 1n-7 1.5 1.8 1.6 1.6
18 : 2n-6 53.1 53.0 52.5 52.5
18 : 3n-3 6.9 6.9 6.7 6.7
Other fatty acids‡ 1.4 1.2 1.8 1.5
Total fatty acids 98.8 98.6 98.2 97.1

Chemical analysis (%

Free fatty acids 0.1 0.1 0.1 0.1
Moisture 0.0 0.0 0.4 0.2
Insoluble impurities 0.0 0.0 0.0 0.0
Unsaponifiable matter 0.7 0.7 0.6 0.6
Peroxidation parameters

\( p \)-Anisidine value§ 4.0 19.0 25.0 39.0
MDA (mmol/l oil) 1.1 4.5 5.6 6.9
Peroxide value (mEq O₂/kg)

Initial AOM 1.0 1.0 1.0 1.0
4h AOM 11.0 90.0 94.0 84.0
24 h AOM 409.0 568.0 508.0 517.0

MDA, malondialdehyde; AOM, active oxygen method.
* Soybean oil was exposed to peroxidation for 0, 6, 9 and 12 d by exposing to heat (80°C) and constant oxygen flow (1 litre/min).
† Weight percentage of total fatty acids.
‡ Comprised <1 % of each of the following fatty acids: 14 : 0, 16 : 1, 17 : 0, 20 : 0, 20 : 1
and 22 : 0.
§ The \( p \)-anisidine value is a relative measurement used to determine the aldehydes content of peroxidised oils.

oven for 48 h. After drying, the samples were finely ground in a
Wiley mill (Thomas Scientific) through a 0.8-mm mesh screen
and mixed. Contents of titanium dioxide in feed and faecal
samples were determined according to the protocol described by
Myers et al.(20) Total fat in samples was determined after
acid hydrolysis (3 mol/l for 1 h at 90°C) according to American
Oil Chemists’ Society(21) for rapid determination of fat using
paraffin, sectioned (5 µm) and stained with haematoxylin–eosin.
Slides were read using an Olympus Vanox-S microscope and
analysed using SPOT Basic Imaging software (Diagnostic Instru-
ments). Measurements of villus height and width and crypt depth
were performed as previously described by Moeser et al.(25). The
surface area of the villus was calculated using the formula for the
surface area of a cylinder and subtracting the area of the base of
the villus.

**Mucosal sample analyses**

Oxidative stress in jejunum mucosa was assessed by measuring
concentrations of MDA. Collected samples were homogenised in
PBS containing butylated hydroxytoluene to prevent further
oxidation. Supernatants were collected and analysed for MDA
concentrations using a commercial ELISA kit according to the
manufacturer’s protocol. Concentrations of MDA in samples
were expressed as \( \mu \)mol per g of mucosal protein. Total
antioxidant capacity (TAC) was used as an estimation of the
collective capacity of biomolecules in mucosal samples to exert
antioxidant activity. To assess TAC, mucosal samples were
homogenised in cold PBS. Supernatants were collected and
analysed by using a commercial ELISA kit. Results were
expressed as \( \mu \)mol Cu-reducing equivalents per g of mucosal
protein. Mucosal protein was measured using a commercial
ELISA kit (Thermo Scientific). To determine the concentrations
of TNF in jejunum mucosa, samples were homogenised in cold
PBS and the supernatant was collected and analysed using a
commercial ELISA kit (R&D Systems). Results were expressed
as pg per mg of mucosal protein.

**Intestinal absorptive capacity**

A procedure adapted from Doerfler et al.(23) was used to
measure the intestinal absorptive capacity. At d 14 of the study
and before pigs were switched to phase 2 diets, eight pigs per
dietary treatment (one pig with average BW in each pen) were
selected. Pigs were deprived of feed for 9 h and then gavaged
with a solution containing 0.2 g/ml of p-xylene and 0.5 g/ml of
mannitol (Sigma-Aldrich). Pigs received a dose of 6-5 ml of
solution per kg of BW. Pigs were bled 2 h after gavage via
jugular venepuncture using a vacuum tube with a 20 G \( \times \)
3.81 cm needle. This procedure was repeated on d 35 using the
same selected pigs.

Serum samples were frozen at −20°C until analysis was
conducted. For analysis, samples were spiked with fucose
solution (Sigma-Aldrich), which served as an internal standard
for calculation. Samples were then diluted 100-fold with
deonised water. Diluted samples were filtered through
OnGuard-H cartridges (Dionex) to remove free amino acids.
HPLC analysis was performed according to the method of
Jahnau et al.(24). The HPLC system (Dionex) consisted of a
250×4 mm Dionex PA-1 column, a gradient pump, an auto-
sampler and a pulsed amperometric detector. The quantifica-
tion was performed by calculating the ratio of the unknown
peak height/fucose peak height.

**Tissue and mucosal sample collection**

At d 35 of the study, seven pigs per treatment (different from pigs
used in the absorptive capacity test) were randomly
selected and euthanised by captive bolt gun followed by
immediate exsanguination. Tissue samples were collected from
the proximal jejunum, the main site of digestion and absorption
of lipids, and used for analysis of intestinal histology. Mucosal
scrapings from the proximal jejunum were collected and frozen
immediately in liquid N₂. Samples were stored under −80°C
until biochemical analyses were undertaken.

**Histological measurements**

Collected tissue samples were fixed in 10 % neutral-buffered
formalin, transferred to 70 % ethanol after 24 h, embedded in
paraffin, sectioned (5 µm) and stained with haematoxylin–eosin.
Slides were read using an Olympus Vanox-S microscope and
analysed using SPOT Basic Imaging software (Diagnostic Instru-
ments). Measurements of villus height and width and crypt depth
were performed as previously described by Moeser et al.(25). The
surface area of the villus was calculated using the formula for the
surface area of a cylinder and subtracting the area of the base of
the villus.
Statistical analysis

Animal performance data were analysed as a randomised complete block design using the MIXED procedure of SAS statistical package (version 9.3; SAS Institute Inc.). The fitted model included dietary treatment and sex and their interaction as the fixed effects and weight blocks as random effect. Intestinal absorptive capacity and nutrient digestibility data were assessed at d 14 and 35 of the study and analysed using the MIXED procedure of SAS with repeated measures. Data for intestinal histology, metabolic oxidative stress, antioxidant activity and cytokine analysis corresponded to a complete randomised design and were analysed using the general linear model procedure of SAS. Pen was considered the experimental unit for the analysis of animal performance and nutrient digestibility data, whereas individual pig was considered as the experimental unit for other measurements. The main effect of lipid peroxidation was tested. Df were partitioned into orthogonal contrasts to evaluate linear and quadratic effects of lipid peroxidation. All data were expressed as least square means with their standard errors. P values ≤0.05 were considered to be statistically significant, and values >0.05 but <0.10 were considered as tendencies towards differences.

Results

Peroxidation analysis of soyabean oil

As shown in Table 2, analysis of the soyabean oil indicated that peroxidative exposure for increasing lengths of time resulted in increasing p-anisidine values and MDA concentrations. Initial peroxide value of soyabean oil increased until d 9 and remained relatively similar on d 12. Contrary to these observations, exposure to peroxidation did not affect moisture, free fatty acids, insoluble impurities or unsaponifiable matter of soyabean oil.

Growth performance

For the entire period of the study (d 0–35), nursery pigs fed diets supplemented with soyabean oil (0 d of peroxidation) grew faster (P ≤ 0.027) and displayed improved feed efficiency (P ≤ 0.006) when compared with those fed the negative control diet without soyabean oil (Table 3). In addition, feed intake (linear, P < 0.001), energy intake (linear, P < 0.001) and weight gain (linear, P = 0.024), but not feed efficiency (P = 0.923), decreased as the added soyabean oil was increasingly peroxidised. During the first 14 d, increased peroxidation of soyabean oil reduced the feed intake (quadratic, P < 0.001), but not weight gain (P = 0.915) or feed efficiency (P = 0.990), of pigs. For d 15–35 of the study, detrimental effects of lipid peroxidation observed on animal performance were more evident. Increasing soyabean oil peroxidation linearly reduced feed intake (P = 0.002), energy intake (P = 0.002) and weight gain (P = 0.016), but not the efficiency of feed utilisation (P = 0.374) of pigs.

Nutrient digestibility

During the first 14 d (Fig. 1(A)) and 35 d (Fig. 2(B)) of the study, the apparent faecal digestibility of dietary fat and gross energy for nursery pigs fed diets supplemented with soyabean oil (0 d of peroxidation) was much greater than for those fed the...
negative control diet without soyabean oil ($P < 0.001$). During the first 14 d of the study, apparent faecal digestibility of fat ($P = 0.023$) and gross energy ($P = 0.040$) in nursery pigs decreased in a quadratic manner as the added soyabean oil was increasingly peroxidised. As experimental diets were fed to pigs for longer periods (d 15–35), increasing peroxidation linearly and quadratic effects of lipid peroxidation.

**Intestinal absorptive capacity**

At d 14 of the study, dietary inclusion of peroxidised soyabean oil did not affect the concentrations of mannitol ($P = 0.800$) or $\alpha$-xylose ($P = 0.944$) measured in blood samples collected from pigs 2 h after oral administration (Fig. 2(A)). In contrast, concentrations of mannitol (linear, $P = 0.097$) and $\alpha$-xylose (linear, $P = 0.089$) in blood samples collected on d 35 tended to decrease as the added soyabean oil was increasingly peroxidised (Fig. 2(B)).

**Histological measurements**

Increasing soyabean oil peroxidation resulted in longer (linear, $P < 0.001$) and thinner (linear, $P = 0.002$) villi and deeper crypts (quadratic, $P = 0.005$) in the jejunum (Table 4). This resulted in greater villus height: crypt depth ratios, as the added soyabean oil was increasingly peroxidised (quadratic, $P = 0.022$). Moreover, effects of peroxidised soyabean oil on villus area were not detected ($P = 0.851$).

**Mucosal sample analyses**

Increasing soyabean oil peroxidation increased the concentrations of MDA (quadratic, $P = 0.035$; Table 4) and reduced the antioxidant capacity in the mucosal samples from the jejunum (linear, $P = 0.044$). To explore the impact of dietary inclusion of peroxidised soyabean oil on intestinal inflammation, we measured mucosal concentrations of TNF in the jejunum of pigs. Mucosal TNF did not differ among treatments (peroxidation, main effect $P = 0.739$).

**Discussion**

This study involved incremental increases in peroxidation (rather than a static dose) of soyabean oil on the growth performance of pigs and on selected variables related to structure
and function of the small intestine. Peroxidation of soyabean oil was induced by heating at 80°C with constant oxygen flow, similar to DeRouchey et al. (8). Using this approach, we successfully obtained increasing degrees (or dose) of peroxidation. Therefore, we speculate that the responses observed in this study were caused directly by increasing the degree of peroxidation.

During lipid peroxidation, many reactions occur that result in a large number of unstable compounds (hydroperoxides, aldehydes, alcohols, conjugated dienes, polymers and so on) (6). The degree of lipid peroxidation is very difficult to characterise because these unstable compounds decompose as peroxidation progresses, and their concentrations in lipids depend on the conditions of peroxidation (i.e. heating at low or high temperature) (26). A reliable characterisation must include the conditions of peroxidation (i.e. heating at low or high temperature) (26). A reliable characterisation must include the conditions of peroxidation (i.e. heating at low or high temperature) (26). A reliable characterisation must include the conditions of peroxidation (i.e. heating at low or high temperature) (26). A reliable characterisation must include the conditions of peroxidation (i.e. heating at low or high temperature) (26). A reliable characterisation must include the conditions of peroxidation (i.e. heating at low or high temperature) (26).

Table 4. Effects of peroxidised dietary soyabean oil on oxidative stress, antioxidant activity, TNFα and villus morphology in the jejunum of nursery pigs (Least squares means with their standard errors)

<table>
<thead>
<tr>
<th>Items</th>
<th>Days of peroxidative exposure*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control†</td>
<td>SEM</td>
</tr>
<tr>
<td>MDA (µmol/g protein)</td>
<td>35b</td>
<td>0.273</td>
</tr>
<tr>
<td>TAC (µmol CRE/g protein)</td>
<td>52b</td>
<td>0.155</td>
</tr>
<tr>
<td>TNF (pg/mg protein)</td>
<td>8</td>
<td>0.546</td>
</tr>
</tbody>
</table>

Villus morphology (µm)

Villus height 529c 504d 531b 572a 563a 10.0 0.077 <0.001 <0.001 0.479
Villus width 139b 147a 143b 141b 135b 3.0 0.025 0.019 0.002 0.560
Crypt depth 167b 168a 182b 187a 177b,c 182a 188a 4.0 0.761 0.004 0.028 0.005
Villus area 0.25 0.25 0.25 0.26 0.25 0.01 0.659 0.851 0.735 0.796
Villus height:crypt depth 2.0 2.0 0.11 0.11 0.11 0.11 0.11 0.01 0.004 0.004 0.004

MDA, malondialdehyde; TAC, total antioxidant capacity; CRE, Cu-reducing equivalents.

α,b,c Least squares means within a row with unlike superscript letters were significantly different (P ≤ 0.05).

* Soyabean oil added to diets was exposed to peroxidation for 0, 6, 9 and 12 d by exposing to heat (80°C) and constant oxygen flow (1 litre/min) and fed to pigs for 35 d.

† Control diet was not supplemented with soyabean oil.

‡ P Value corresponding to the comparison of control diet with 0 d peroxidised lipid-added diet.

§ The main effect of lipid peroxidation was tested. Orthogonal contrasts were performed to evaluate linear and quadratic effects of lipid peroxidation.

The surface area (µm²) of the villus was calculated using the formula for the surface area of a cylinder and subtracting the area of the base of the villus.
across the brush-border membrane by a carrier-mediated process or by paracellular diffusion\(^{30-35}\). These sugars have been used extensively as indicators of malabsorption in birds\(^{25,34}\) and to test intestinal barrier function in multiple species\(^{35-37}\). In the present study, we observed that the capacity to absorb mannitol and \(\alpha\)-xylose tended to progressively reduce as peroxide dose increased. These results are consistent with the gradual reduction in the digestion of fat and gross energy and with the modified structure of the small intestine. Apparent fat digestibility is defined as the net disappearance of ingested dietary fat from the digestive tract and reflects the enzymatic hydrolysis, micelle formation and absorption of fat from the gastrointestinal lumen. In the present study, increasing dose of lipid peroxidation progressively reduced the apparent fat digestibility. Our observations are in agreement with those presented by DeRouchey \textit{et al.}\(^{12}\), who reported a reduction (in a quadratic manner) in the digestibility of nutrients when pigs were fed lipids exposed to peroxidation. Our findings on the intestinal absorptive capacity and apparent digestibility of fat and gross energy suggest that dietary soyabean oil exposed to peroxidation impaired intestinal function and that these detrimental effects are accentuated by increasing degree and duration of peroxidative challenge.

The intestinal cell transition to proliferative, growth arrest or apoptotic states is thought to be governed by the biological redox balance of cellular signalling events\(^{39-44}\). Any disturbance in the redox balance results in cellular oxidative stress and cell transition status\(^{45}\). Tian \textit{et al.}\(^{46}\) provided evidence that oxidised colonic mucosal redox status promoted cell growth and proliferation. The researchers induced alterations in the redox balance of colonic mucosal in rats by inhibiting the enzyme GSH/GSSG. In our study, the changes in villus structure and villus height: crypt depth ratios suggest that lipid peroxidation accentuated enterocyte proliferation as a result of oxidative stress in the small intestine of pigs. This contention was supported by the increased concentrations of MDA and progressive reduction of antioxidant capacity in the intestinal mucosa of pigs. Other studies have demonstrated that dietary lipid peroxidation products can induce cellular oxidative stress\(^{5,11,13}\). However, to our knowledge, this is the first study to demonstrate that increasing dose of lipid peroxidation products progressively disrupted the redox environment in the intestine and proposed this induction of oxidative stress as the mechanism for the impairment of the structure and function of the small intestine.

Strong oxidative stress, as a result of dietary peroxidative challenge, could disrupt cell integrity and activate proinflammatory transcription factors\(^{2}\). We explored the potential effects of lipid peroxidation on intestinal inflammation by measuring mucosal concentrations of TNF and did not observe any significant effects. Our results are in agreement with those presented by Ringseis\(^{11}\), who demonstrated that dietary peroxidised lipids induced oxidative stress in pigs, without causing inflammation. In contrast, Varady \textit{et al.}\(^{112}\) administered orally by gavage peroxidised lipids to female mice and concluded that ingestion of peroxidised lipids activated proinflammatory transcription factors. It is plausible that the lack of inflammatory response to lipid peroxidation was because of the limited analysis performed in our study and that other inflammatory markers, including lipid-mediated markers (i.e. PG and leukotrienes), could be elevated\(^{47,48}\). Future studies that investigate TNF and other inflammatory mediators are needed to clarify the extent and duration of peroxidative challenge that may cause intestinal inflammatory responses \textit{in vivo}.

In conclusion, lipid peroxidation reduced animal growth, reduced digestion and absorption of nutrients and modified villus morphology of the small intestine. We infer that disruption of the redox environment in the intestine is the mechanism by which the structure and function of the small intestine were impaired. Detrimental effects of lipid peroxidation were progressively accentuated by a dose-related increase in lipid peroxidation products. In this context, we suggest that the biological response to lipid peroxidation, to the degree tested in the present study, is progressive so that the extent to which measures are affected must be stated in relation to the dose of peroxidation products. This principle should be considered in future studies pertaining to the effects of significant lipid peroxidation, which could include viability and medical treatment.

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D. S. R. and E. v. H. designed the research study. D. S. R. conducted the study, analysed data and wrote the paper. J. O., A. J. M., R. D. B. and E. v. H. were involved in portrayal of the data, interpretation of the analysis and they conducted a critical review of the manuscript. All authors read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

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