Effect of introducing hemp oil into feed on the nutritional quality of pig meat

Jacques Mourot1,* and Mathieu Guillevic1,2

1 INRA UMR 1348 PEGASE, 35590 Saint-Gilles, France
2 VALOREX SA, 35210 Comboutillé, France

Received 6 May 2015 – Accepted 30 June 2015

Abstract – Research is being carried out to diversify the sources of n-3 fatty acid-rich lipids for animal feed. In this study, 3 batches of 12 pigs with between 50 and 105 kg of live weight, received isolipidic diets containing either palm oil (PO), or rapeseed oil (CO), or hemp oil (HO) (providing respectively 0.6; 1.9 and 3.4 g of C18:3 n-3 (ALA)/kg of feed). The quantity of ALA deposited in the meat is higher (p < 0.001) in the HO pigs. Hemp oil may be an interesting source of ALA to improve the nutritional quality of pork.

Keywords: n-3 fatty acids / hemp / pig / meat quality

1 Introduction

Breeding factors influence the quality of meat. There is a direct relationship between the nature of the fatty acids in feed and those which are deposited in the meat (Mourot and Hermier, 2001). This characteristic is used to modify the nutritional composition of the meat, and into the meat introduce fatty acids that are considered to be good for human health. The nutritional guidelines of the AFSSA recommend increasing the quantity of n-3 fatty acids in human consumption and decreasing the amount of n-6 (ANC, 2001). By providing n-3 fatty acids via the incorporation of linseed into pig feed, it is possible to increase the deposition of these fatty acids in the meat (Corino et al. 2014; Guillevic et al., 2009; Wood et al., 2008). The deposition in the meat will be in relation to the quantities ingested (Warnants et al., 1999) and/or the duration of distribution of the n-3 enriched diets (Haak et al., 2008). These products can also be processed and the nutritional advantage is preserved without modification to the sensory properties (Guillevic et al., 2009).

Although linseed is a good vector to provide more n-3 fatty acids, studies are being carried out to seek other plant sources of these fatty acids. Certain varieties of hemp seem to contain an interesting proportion of n-3 fatty acids and an interesting quantity of antioxidant factors (Oomah et al., 2002). What is more, hemp is a plant which has long been part of traditional crops in Europe to supply the fibres used for making ropes for ships and cloth for garments. Cultivation is easy and requires few inputs, which preserves the soil. The cultivation of this plant is developing again in the framework of sustainable agriculture programmes, and the fibres have a new outlet as insulation in building materials. The seeds and oil produced could therefore be used in animal nutrition if they have advantages of interest.

Hemp oil is rich in n-3 fatty acids, but it also contains a high proportion of n-6. Little work has been devoted to the use of this oil in animal feed. The aim of this study will be to...
monitor the animals’ growth performances and the fatty acid deposition in the meat.

2 Material and methods

2.1 Choice of the hemp oil

A comparison was made of the fatty acid composition of two varieties of hemp seeds (Tab. 1). The lipid contents of these seeds are 25.1 for Fedora 17 and 27.1 for Ferimon. The percentage of C18:3 n-3 is equivalent between the 2 seed varieties. We chose the Ferimon variety for our study, because of the slightly higher C18:3 n-3 content in this seed.

2.2 The animals and the diets:

36 pigs with a Large-White × Landrace dam and a Piétrain sire were divided into 3 batches of 12 pigs, each batch receiving a different diet. The diets were isoproteic and isocaloric, and the only difference was the source of fat content; either palm oil (PO), or rapeseed oil (CO), or hemp oil (HO). The palm diet contained 0.6 g of C18:3 n-3 (ALA)/kg of feed, the rapeseed diet contained 1.9 g and the hemp diet 3.4 g. The composition of the diets is shown in Table 2. All the diets provided 80 ppm of vitamin E and 0.25 ppm of selenium.

The animals received the diets at between 50 and 105 kg in weight. They were reared in individual housing and their consumption and growth performances were monitored. They were weighed each week.

At slaughter, the carcass muscle rate was determined. Liver, backfat adipose tissue, the longissimus dorsi (LD) muscle and blood were sampled to determine the lipid contents and the fatty acid composition.

The meat quality was measured (pH at 24 h, drip loss, losses during cooking).

2.3 The analyses:

The losses in draining and cooking at the level of the ham were analysed according to the Honikel technique (1987).

The total lipids of the lean or fatty tissues were extracted cold according to the method of Folch et al., (1957) in a chloroform-methanol mixture (2/1). For the pork rib, the whole of the meat (lean and fat) was removed from the bone, then mixed to make an homogeneous blend. Aliquot was taken to analyse the lipids. The fatty acid profile was determined by gas chromatography after derivation with Boron trifluoride (BF3) according to the Morrisson and Smith method (1964). The column was a 30 m long capillary tube made of fused silica on an internal diameter of 0.25 mm. The stationary phase was composed of 80% of biscyanopropyl and 20% of cyanopropylphenyl, and the mobile phase was hydrogen. The temperature of the oven was programmed for plates of 2 min, 7 min and twice 2 min at temperatures respectively of 45 °C, 195 °C, 220 °C and 240 °C with rises in temperature between the stages of 20 °C/min, 30 °C/min and 35 °C/min for a total analysis duration of 21.9 min. The temperatures of the injector and the detector were respectively 220 and 280 °C. The fatty acids (FA) were expressed as a percentage of the identified fatty acids and in total quantity calculated using an internal standard (C17:0).

The analysis results were compared by analysis of the variance with the diet as principal effect (SAS, 1999). The two-by-two comparison of the averages was carried out using the Bonferroni test.

2.4 Results

The zootechnical data and the body composition of the animals were identical between the different batches. The introduction of hemp oil into the diet did not modify the growth performances (Tab. 3). The cutting weights of the main pieces expressing in relation to the weight of the carcass were equivalent. There was therefore no modification to the body composition by the introduction of hemp oil into one of the diets.

The quality criteria of the meat were not modified by the diets. The 24 h pH concentrations were identical for the longissimus dorsi muscle and the semi-membranosus muscle (Tab. 4). The water losses by chilling and after cooking were identical for the longissimus dorsi muscle.

The lipid content of the analysed tissues was identical between the diets for the adipose tissue of the back and the liver (Tab. 4). On the other hand, it was higher in the longissimus dorsi muscle.

The circulating fatty acids were the reflection of those ingested (Tab. 5). The C18:2 n-6 and C18:3 n-3 contents were higher in the pigs receiving the diet with the hemp oil (p < 0.001). For the long-chain polyunsaturated fatty acids, the derivatives were not in relation to the quantity of precur- sors. There was a higher quantity of C20:4 n-6 arachidonic acid with the palm diet (p < 0.02), whereas for EPA and DHA the quantities were higher for the hemp diet with intermediate values for rapeseed (p < 0.001). The value of the DPA was identical between the diets.

### Table 1. Composition and fatty acid content of two varieties of hemp.

<table>
<thead>
<tr>
<th></th>
<th>Percentage of FA</th>
<th>mg FA/100 g of seed</th>
</tr>
</thead>
</table>
|                  | Fedora 17 | Ferimon           | Fedora 17 | Ferimon
| C14:0            | 0.12     | 0.07              | 28.4      | 17.5
| C16:0            | 7.27     | 7.37              | 1657      | 1810
| C16:1 (n-7)      | 0.24     | 0.32              | 55.7      | 78.3
| C18:0            | 3.01     | 2.67              | 685       | 656
| C18:1 (n-9)      | 13.14    | 13.57             | 2993      | 3408
| C18:2 (n-6)      | 55.34    | 55.15             | 12 609    | 13 551
| C20:0            | 3.93     | 4.40              | 894       | 1082
| C18:3 (n-3)      | 15.15    | 14.74             | 3401      | 3847
| C20:1(n-9)       | 0.85     | 0.93              | 193       | 228
| C20:2            | 0.08     | 0.08              | 18.2      | 20.3
| C20:3(n-3)       | 0.45     | 0.40              | 102.0     | 97.5
| Saturated FA     | 14.45    | 14.52             | 3292      | 3567
| Monounsaturated FA | 14.45  | 15.34             | 3293      | 3768
| Polyunsaturated FA | 71.10  | 70.15             | 16 200    | 17 236
| FA n-6           | 55.36    | 55.17             | 12 615    | 13 557
| FA n-3           | 15.65    | 14.89             | 3537      | 3958
| n-6/n-3          | 3.54     | 3.71              | 3.54      | 3.71

The losses in draining and cooking at the level of the ham were analysed according to the Honikel technique (1987).

The total lipids of the lean or fatty tissues were extracted cold according to the method of Folch et al., (1957) in a chloroform-methanol mixture (2/1). For the pork rib, the whole of the meat (lean and fat) was removed from the bone, then mixed to make an homogeneous blend. Aliquot was taken to analyse the lipids. The fatty acid profile was determined by gas chromatography after derivation with Boron trifluoride (BF3) according to the Morrisson and Smith method (1964). The column was a 30 m long capillary tube made of fused silica on an internal diameter of 0.25 mm. The stationary phase was composed of 80% of biscyanopropyl and 20% of cyanopropylphenyl, and the mobile phase was hydrogen. The temperature of the oven was programmed for plates of 2 min, 7 min and twice 2 min at temperatures respectively of 45 °C, 195 °C, 220 °C and 240 °C with rises in temperature between the stages of 20 °C/min, 30 °C/min and 35 °C/min for a total analysis duration of 21.9 min. The temperatures of the injector and the detector were respectively 220 and 280 °C. The fatty acids (FA) were expressed as a percentage of the identified fatty acids and in total quantity calculated using an internal standard (C17:0).

The analysis results were compared by analysis of the variance with the diet as principal effect (SAS, 1999). The two-by-two comparison of the averages was carried out using the Bonferroni test.
Table 2. Composition and fatty acid content of diets (% of identified FA and g FA per kg of feed).

<table>
<thead>
<tr>
<th>Diets</th>
<th>Percentage of FA</th>
<th>g FA per kg of feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm</td>
<td>Palm</td>
<td>Hemp</td>
</tr>
<tr>
<td>Fat matter (g/kg)</td>
<td>43.6</td>
<td>45.5</td>
</tr>
<tr>
<td>C12:0</td>
<td>0.11</td>
<td>0.00</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.66</td>
<td>0.09</td>
</tr>
<tr>
<td>C16:0</td>
<td>29.22</td>
<td>10.48</td>
</tr>
<tr>
<td>C16:1 (n-7)</td>
<td>0.29</td>
<td>0.28</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.24</td>
<td>1.96</td>
</tr>
<tr>
<td>C18:1 (n-9)</td>
<td>29.52</td>
<td>40.31</td>
</tr>
<tr>
<td>C18:2 (n-6) LA</td>
<td>34.59</td>
<td>38.77</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.18</td>
<td>0.41</td>
</tr>
<tr>
<td>C20:1 (n-9)</td>
<td>2.08</td>
<td>6.19</td>
</tr>
<tr>
<td>Σ SFA</td>
<td>33.57</td>
<td>13.14</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>29.86</td>
<td>41.90</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>36.67</td>
<td>44.96</td>
</tr>
<tr>
<td>LA/ALA</td>
<td>16.60</td>
<td>6.26</td>
</tr>
</tbody>
</table>

Table 3. Effect of diets on growth performance and body composition of pigs.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Palm</th>
<th>Rapeseed</th>
<th>Hemp</th>
<th>Rsd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight, kg</td>
<td>46.8</td>
<td>46.4</td>
<td>46.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Final weight, kg</td>
<td>102</td>
<td>104</td>
<td>106</td>
<td>3.7</td>
</tr>
<tr>
<td>Carcass weight, kg</td>
<td>80.2</td>
<td>80.8</td>
<td>80.9</td>
<td>2.7</td>
</tr>
<tr>
<td>Carcass yield, %</td>
<td>78.7</td>
<td>77.8</td>
<td>76.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Duration in days</td>
<td>63</td>
<td>62</td>
<td>61</td>
<td>5.1</td>
</tr>
<tr>
<td>Average daily gain, g</td>
<td>904</td>
<td>943</td>
<td>946</td>
<td>90</td>
</tr>
<tr>
<td>Food conversion ratio</td>
<td>2.60</td>
<td>2.67</td>
<td>2.61</td>
<td>0.25</td>
</tr>
<tr>
<td>Lean meat content, %</td>
<td>57.3</td>
<td>55.3</td>
<td>56.3</td>
<td>2.9</td>
</tr>
<tr>
<td>Loin weight, %</td>
<td>26.9</td>
<td>26.5</td>
<td>26.3</td>
<td>1.33</td>
</tr>
<tr>
<td>Back fat weight, %</td>
<td>6.55</td>
<td>7.39</td>
<td>6.40</td>
<td>1.24</td>
</tr>
<tr>
<td>Ham weight, %</td>
<td>23.9</td>
<td>23.2</td>
<td>23.4</td>
<td>0.99</td>
</tr>
</tbody>
</table>

N = 12/treatments. No significant effect was observed.

The backfat adipose tissues of the pigs from the hemp batch (Tab. 6) had less saturated (p < 0.08) and mono-unsaturated (p < 0.001) fatty acids than the others, but they had more polyunsaturated AG (p < 0.001). The percentage of C18:3 n-3 deposited was in relation to the quantity ingested, these values differing among the 3 diets. The deposition of C18:2 n-6 was higher with the hemp diet (p < 0.001) but there was no difference between the palm and rapeseed diets. There was no difference for the long-chain derivatives whose values were identical between all the batches of animals. The LA/ALA ratio was close to 13 for the palm diet and 7 for the n-3 fatty acid-rich diets.

For the muscle, the effects observed were not as marked as at the level of the fat cover tissue (Tab. 7). Only the ALA precursor was increased in the lean part of the meat with the hemp and rapeseed diets compared to the palm diet (p < 0.001). But the values were not significantly different between these 2 diets whereas the quantities ingested were higher for hemp. The long chain n-6 and n-3 derivatives were identical between the animals. The LA/ALA ratio was higher than the values observed in the fat tissue. It was lower in animals receiving rapeseed and hemp compared to the palm diet.

The fatty acids present in the whole rib of pork were the reflection of the compositions expressed as a percentage in the fat tissue and the muscle.

The quantity of ALA for 100 g of pork was higher with the animals receiving the hemp diet. It was intermediate for rapeseed and lowest for the hemp diet (Fig. 1). It reached more than 200 mg for the hemp diet. But the long chain fatty acid contents were very low. They varied from 5 to 10 mg for EPA depending on the diets and from 13 to 20 mg for the DPA and they were equivalent for the DHA (5 mg/100 g of meat). The LA content was close to 1.1 g in the pigs of the palm and rapeseed batches and reached 1.6 g in the pigs in the hemp batch. The value of the C18:2/C18:3 ratio was 14 for the animals in the palm batch; 8 for the pigs in the rapeseed batch and 7 for the hemp diet.

The fatty acid composition of the liver was also modified for certain fatty acids depending on the diets (Tab. 8). The percentage of saturated fatty acids was lower in the pigs from the hemp batch (p < 0.06) and the percentage of polyunsaturated...
fatty acids was higher ($p < 0.002$). Overall, the percentage in arachidonic acid was much higher than what was observed in the other tissues, but this value was not affected by the treatment. On the other hand, as for the other tissues, the ALA percentage was in relation to the diets ($p < 0.001$). The long-chain EPA and DPA derivatives were also increased with the diets providing the most n-3 precursor, but the DHA was not modified.
3 Discussion

The growth performances were not modified by the introduction of n-3 fatty acids into the animal feed, which confirmed similar studies carried out with the addition of other fats than hemp such as linseed (Corino et al., 2008; Kouba et al., 2003; Matthews et al., 2000).

The pH values and the colour of the meat were not modified, which was in agreement with other studies, as the impact of the lipids seemed to have little effect on these parameters (Haak et al., 2008; Romans, Wulf et al., 1995; Romans, Johnson et al., 1995).

The circulating fatty acids were the reflection of those provided by the feed. Among the n-3 fatty acids, the C18:3 n-3 precursor was in the majority. But the content of long-chain derivatives was also significantly increased as has already been shown in man for EPA and DHA (Weiß et al., 2002). This may be explained by the importance of collecting these fatty acids for incorporation into the membranes of the erythrocytes. The increase in the concentration was in relation to the quantity provided by the feed; the pigs with the palm diet were different from those with the rapeseed diet, and they in their turn were different from pigs with the hemp diet for ALA and EPA.

The effect of the nature of the fat content in the diet on the nutritional quality of the animal products was once again demonstrated (Mourot and Hermier, 2001). The n-3 content in the meat was multiplied by 2.6 with the addition of hemp oil and by 1.8 with rapeseed oil compared with animals receiving a diet low in n-3. These results confirmed other studies showing a relationship to the quantity of n-3 ingested (Corino et al., 2014; Warnants et al., 1999). However, with an equivalent quantity of fat content in the diets, the deposits of n-3 fatty acids obtained with hemp oil were lower than those obtained with the introduction of extruded linseed into the diet (Corino et al., 2008), the increase varying from 4 to 7 according to the tissues or products analysed.

The ALA precursor was essentially found in this form in the lean and fat tissues. The long chain n-3 fatty acids were found in a small quantity, which confirms the low conversion of the precursor (Alessandri et al., 2009). In the fat tissue, an increase in C22:5 n-3 was shown (p < 0.08) with the hemp diet, which indicated a limiting stage in the transformation into DHA. This accumulation of DPA is not observed in cattle (Geay et al., 2001; Raes et al., 2004). This may be related to the location of lipogenesis synthesis which is preponderant...
in the liver in these animals, compared to the pig where it is essentially located in fat tissue.

The elongase allowing the synthesis of C24:5n-3 was certainly underactive. There is competition between the n-6 and n-3 fatty acids for the synthesis of long-chain derivatives. The transformation of the n-6 fatty acids seems favoured compared to the n-3, which may explain the low transformation observed. Studies adding a larger quantity of n-3 to the diet than this one using linseed (55 g ALA per kg feed vs. 34 g at present) have shown a significant increase in DHA (Corino et al., 2008). But the total quantity, even if it was sometimes doubled, still remained very limited, the content passing from 5 to 6 mg of DHA per 100 g of meat to between 10 and 15 mg. This confirmed the low conversion into long-chain polyunsaturated fatty acids from the ALA precursor observed in humans and animals, including fish.

In the muscle, the percentages of long-chain n-3 derivatives were higher than those observed in the fat tissues. This is explained by the fact that lipids in the muscle are half composed of polar lipids coming from membrane structures that are rich in polyunsaturated fatty acids unsaturated long chain to maintain membrane fluidity (Warmants et al. 1999). Moreover, Hertzman et al., (1988) suggested that the phospholipidic membranes of the muscle tissue incorporate polyunsaturated fatty acids particularly well, unlike fat tissues which comprise vacuoles of lipids, much less selective as to the incorporation of fatty acids.

As for blood plasma, the nature of fatty acids in food modifies the composition of lipids in the membranes. A larger input of n-3 fatty acids in the diet can therefore have an impact on membrane fluidity.

However, although the fatty acids are expressed in quantity, the low total lipid content in the muscle, approximately 2%, compared to the fat tissue (65 to 70%), means that the muscle will have a limited impact on the input of these long chain fatty acids in the consumer’s plate. With the introduction of hemp oil into the diet, the global contents of n-3 fatty acids in the pork rib will be near to 250 to 300 mg for 100 g of meat, whereas with the introduction of linseed selected for its rich position of muscle, adipose tissue. liver and sausages. membrane content, whatever the nature of the fat content of the feed. The liver will therefore be an important source of arachidonic acid in the human diet in particular through pâtés and prepared meat products rich in lipids (Guillevic et al., 2009).

The effect of the diets was also found in the liver, but a concentration of n-6 fatty acids was highlighted compared to other tissues (Enser et al., 2002). This is probably the particular place for transforming the n-6 family into derivates, since a high increase can be seen in the C20:4 n-6 fatty acid concentration, whatever the nature of the fat content of the feed. The liver will therefore be an important source of arachidonic acid in the human diet in particular through pâtés and prepared meat products rich in lipids (Guillevic et al., 2009).

In the meat, the value of the LA/ALA ratio was close for animals receiving the rapeseed and hemp diets whereas the ALA content was higher in pigs with the hemp diet. This can be explained by the fact that these animals also had a high deposition of LA provided by the hemp oil. The value of this ratio is higher than that of other studies using linseed (7 vs. 4 to 5) (Corino et al., 2008; Guillevic et al., 2009) and also higher than the ANSES guidelines which recommend a ratio of 5 (ANC). But these animals receiving rapeseed or hemp oils had a ratio decreased by 2 in comparison with animals receiving standard feed; so this leads to better food balance for human consumption.

4 Conclusion

The use in animal feed of fat content providing a notable quantity of n-3 fatty acids increases the deposition of these fatty acids in the meat and to a lesser degree the deposition of the long-chain derivates, without modifying the growth performances of the animals. The C18:2/C18:3 ratio is better balanced and provides what specialists in human nutrition are looking for. If the ratio is equivalent in the meat of pigs receiving rapeseed or hemp oil, the animals receiving hemp oil will have higher n-3 content than that obtained with the rapeseed diet. Hemp oil can therefore be used favorably in pig feed. Current production of this plant is not as intense as at the beginning of the 20th century. But the new development of cultivation of this plant to supply agro-materials may make hemp oil an interesting co-product for animal nutrition. Research on extruded hemp products would appear to be a worthwhile project.

Acknowledgements. The authors declare having no conflict of interest.

Important point. This study concentrates on the effect of hemp oil as a source of n-3 fatty acids in pig feed. This oil comes from sustainable production, and has interesting effects for the increase of n-3 fatty acid content in the meat. Relatively speaking, these effects are higher than those obtained with the incorporation of rapeseed oil into animal feed.

References


