Research on the effects of a diet including rapeseed oil in the pig. Notes I and II

by L. Rampichini, A. Begliomini, E. Di Antonio, S. Ranucci, D. Rutili, and M. Severini

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RESEARCH ON THE EFFECTS OF A DIET INCLUDING RAPESEED OIL
IN THE PIG. NOTE I (*)

By

L. Rampichini; A. Begliomini; E. Di Antonio; S. Ranucci;
D. Rutili & M. Severini

SUMMARY

Pigs kept for 7 and 20 days on a diet containing 11.5% rapeseed oil (35% of erucic acid) showed slight erucic acid accumulation in the heart and skeletal muscle, but there were not found neither functional nor morphological specific changes in this and other organs examined.

KEY WORDS: Pigs, Rapeseed oil, erucic acid, heart, skeletal muscle.

(*)Some results of these investigations were communicated to the XXIX Congress of the Italian Society of Veterinary Sciences, Portoferaio (Begliomini et al., 1975)

The authors thank Messrs. O. Cortona and M. Spaccini for their technical collaboration.
INTRODUCTION

Diets containing rapeseed oil, more or less rich in erucic acid, cause, in different animal species, metabolic disturbances and myocardial alterations of varying importance.

The morphological investigations carried out on the rat and the pig have been numerous.

In the rat the alterations caused at a cardiac level have been described by various authors (Romn et al., 1960; Abdellatif et al., 1970; Beare-Rodgers et al., 1972; Vodovar et al., 1972; Rocquelin et al., 1973; Beare-Rodgers et al., 1974; Rauber et al., 1974 a, b; Cluzan et al., 1975; Dallocchio et al., 1975; Svaar et al., 1975; Vodovar et al., 1975) and it seems clear by now that this oil carries out a toxic action on the myocardium, perhaps because of its erucic acid content.

In the pig, to the contrary, the data available up to now (Roine et al., 1960; Beare-Rodgers et al., 1972; Rocquelin et al., 1973; Vodovar et al., 1973; Restuccia et al., 1974; Svaar et al., 1975; Vodovar et al., 1975) seem less harmonious in attributing to rapeseed oil a specific pathogenic effect on the heart.

The purpose of this work is to make a contribution to knowledge of the precocious alterations, especially at the cardiac level, induced in the pig by a diet including rapeseed oil, averagely rich in erucic acid, in a not excessively high percentage.

Olive oil was used as the control both because it is widely used for nutritional purposes in Italy, and also because its harmlessness has never been disputed.
PERSONAL OBSERVATIONS

Material and Techniques

Ten pigs of the Large White strain of both sexes, whole, approximately three months old, of an average weight of 25 kilograms at the beginning of the experiment.

All the pigs were fed for ten days with SM1 Petrini fodder, lacking methionine and antibiotics, in order to adapt them to the experimental diet.

Subsequently they were subdivided into three groups: the first group consisted of six subjects to which fodder mixed with rapeseed oil (containing 35% erucic acid) was administered at the rate of 11.5% by weight (24.2 cal. %); the second group comprised two subjects which were given fodder mixed with olive oil (lacking erucic acid) at the rate of 11.5% by weight (24.2 cal. %); the third group comprised two subjects which were only given fodder.

All the pigs received a constant dose of food equal to 40 kilos of live weight/pro die.

Fifty percent of the subjects of each group was sacrificed after seven days of experimental diet and the other fifty percent after twenty days.

All the pigs were subjected to electrocardiograph, enzyme, chemical, necroscopic, histological, histochemical and ultra-structural examinations.

The electrocardiograph examinations were carried out at the beginning of the adaptation diet and at the end of the experimental diet, immediately before butchering, with a "Galileo 2 Ca model" electrocardiograph calibrated to a band-width of $1mV = 1$ cm and a paper running velocity of 50 mm per second. The three bipolar derivations ($D_1, D_2, D_3$) and the three unipolar
derivations of the limbs (aVL, aVR, aVF) were registered. The ECG was carried out on the animals when they were at rest, in dorsal decubitus, with the limbs slightly flexed, the electrodes being attached above the foreleg and hock articulations. The tracings were carefully evaluated with regard to cardiac frequency, amplitude, duration, shape and direction of the individual waves and of the intervals between them.

The enzyme examinations were carried out with the "Biochemia S.r.l." Boehringer, Mannheim methods and reagents, on the blood serum removed from the superficial jugular vein, immediately before slaughter.

The following were evaluated: aldolase (Ald), creatinphosphokinase (CPK), glutamic-oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and lacticodehydrogenase (LDH).

The chemical examinations were carried out on samples of the myocardium and quadriceps femoris muscle. The content in water and crude lipids (Official Methods of Analysis - 12th Ed. AOAC, Washington, 1975) and erucic acid (Official Analysis Methods for Oils and Fats - Italian Ministry of Agriculture and Forests - State Polygraphic Institute, 1964) was evaluated.

The quantity of water was determined by the difference between the wet weight and dry weight of the sample (100 - 105°C for 8 hours until a constant weight is obtained) referred to one hundred.

The content in crude lipids was determined by evaluating the dry weight, referred to one hundred, of the ethereal extract (eight hours in Soxhlet apparatus) of 15 grams of tissue (dried at 70°C for 20 hours).

The content in erucic acid was evaluated, on a sample of methylated ether extract, with Fractovap 2400 T (C. Erba) gaschromatograph with flame ionization detector, under the following working conditions; temperature of column 210°C, temperature of evaporator 310°C, temperature of detector
block 210°C, H₂ at a pressure of 0.5 kg/cm², air pressure 1.0 Kg/cm², carrier gas N₂ at a pressure of 1.1 Kg/cm² equal to a flow of 6 ml/minute. The metering was carried out through comparison with a standard solution of methylerucate.

The necroscopy was carried out immediately after slaughtering, with captive projectile pistol, and complete bleeding by cutting the jugular veins of the subjects which has been fasting for twelve hours.

The histological examinations were carried out on samples removed from the brain, thyroid, lung, heart, spleen, liver, pancreas, kidneys, suprarenal, stomach, from the small and large intestine, from the lymph nodes of the thoracic and abdominal organs and from the skeletal muscles of the posterior limbs fixed in formulin and enclosed in paraffin (wax). The sections prepared were coloured with hematoxylin and eosine for the general myocardium. Furthermore the sections of the myocardium were coloured with van Gieson's method for the connective (tissue).

The histochemical examinations were conducted on samples removed from the myocardium and from the medial Vast muscle of the left posterior limb, frozen in isopentane cooled with liquid nitrogen and sectioned in the cryostat. Sections of myocardium were coloured with Sudan III for the fats. Sections of myocardium and skeletal muscle were coloured with the PAS reagent in accordance with McManus or used for the survey of the phosphorylase activity according to the method described by Pierini et al. in 1970 (solvent 3 with and without alcohol), of the ATPase activity according to the method used by Padikula et al. (1955) and of the succinic-dehydrogenase activity according to the method of Nachlas et al. (1957).

The ultrastructural examinations were conducted on samples of
ventricular myocardium and medial Vast muscle placed in glutaraldehyde at 2% in phosphate buffer pH 7.4 for 2 hours, fixed afterwards in osmium tetroxide and enclosed in Epon 812. The ultra-thin sections, contrasted with lead hydroxide and uranyl acetate, were observed with an M.E. Philips 300.

RESULTS

For the entire duration of the experiment the subjects under examination showed no clinically perceivable pathological manifestations.

The examination of the basal electrocardiograph tracings and of those carried out immediately before slaughtering showed in the subjects of all the groups a substantial overlapping and the absence of pathological alterations, also as regards a certain variability in shape and amplitude of the waves in the individual pigs.

<table>
<thead>
<tr>
<th>No. of Pigs</th>
<th>Days</th>
<th>Diet Admin'd.</th>
<th>ADH</th>
<th>CK</th>
<th>GOT</th>
<th>GPT</th>
<th>LDH</th>
<th>HbO₂</th>
<th>Heart fat (l)</th>
<th>H₂O</th>
<th>Muscle Fat (l)</th>
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<tr>
<td>1</td>
<td>7</td>
<td>rapeseed</td>
<td>5.18</td>
<td>47.60</td>
<td>38.66</td>
<td>12.96</td>
<td>133.80</td>
<td>77.20</td>
<td>1.01</td>
<td>75.19</td>
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<tr>
<td>2</td>
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<td>&quot;</td>
<td>6.80</td>
<td>79.00</td>
<td>20.60</td>
<td>19.06</td>
<td>238.75</td>
<td>77.29</td>
<td>1.02</td>
<td>76.95</td>
<td>3.78</td>
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<td>&quot;</td>
<td>5.48</td>
<td>51.58</td>
<td>27.52</td>
<td>16.20</td>
<td>229.20</td>
<td>73.83</td>
<td>5.06</td>
<td>73.13</td>
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<td>7.58</td>
<td>62.70</td>
<td>16.13</td>
<td>11.61</td>
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<td>62.21</td>
<td>35.35</td>
<td>12.55</td>
<td>162.50</td>
<td>72.39</td>
<td>6.71</td>
<td>73.31</td>
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<tr>
<td>4</td>
<td>7</td>
<td>olive</td>
<td>6.39</td>
<td>71.39</td>
<td>22.20</td>
<td>11.16</td>
<td>181.59</td>
<td>79.56</td>
<td>3.85</td>
<td>75.83</td>
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<td>55.00</td>
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<td>13.68</td>
<td>114.10</td>
<td>75.33</td>
<td>5.07</td>
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<td>6.50</td>
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<td>12.16</td>
<td>155.30</td>
<td>76.10</td>
<td>4.82</td>
<td>72.35</td>
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<td>4.61</td>
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<td>77.40</td>
<td>5.15</td>
<td>76.13</td>
<td>3.12</td>
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</tbody>
</table>

(1) grams/100 g of fresh tissue.
The data relating to the seric enzymes, those relating to the content of water and crude lipids in the myocardium and in the skeletal muscle overall show a substantial similarity in values between the pigs of the various groups (Table no. 1).

Erucic acid was found to be present in small quantities in the myocardium and in the skeletal muscle of the pigs fed for twenty days with fodder and rapeseed oil (Table no. 2).

Table No. 2.

| Pigs: Content of erucic acid in the heart and skeletal muscles in mg/kg of fresh tissue. |
|---|---|---|---|
| FJ | L | Hart | Skeletal muscle 1st group |
| Heart and skeletal muscle 2nd and 3rd groups |

The necroscopic examinations showed in all subjects the presence of lobular alterations in the lung (enzootic pneumonia) and, in the pigs of the first two groups, a moderately pallid (pale) aspect of the kidney and sometimes of the muscular groups of the posterior limbs. In a pig fed for 7 days with fodder and olive oil, hemorrhagic gastritis was present.

The histological examination revealed the presence of constant alterations only with respect to the heart, liver, kidneys, intestine and lung. In the myocardium of the pigs fed with rapeseed oil the alterations were extremely modest, slightly more accentuated in the subjects sacrificed
after 20 days of diet and consisting of the presence of scant and isolated fibers with homogeneous cytoplasm, intensely eosinophile, without striation and with a nucleus which seemed sometimes to be pyknotic, of infrequent fibers with clear (pale) areas containing amorphous material, fine-grained, localized at the apexes of the nuclei and of infrequent interstitial infiltrations of histiocyte cells, for the most part in the form of small foci.

In one single subject, sacrificed after 20 days of diet some isolated fibers were present with homogeneous and intensely basophile cytoplasm. In the heart of pigs fed with olive oil the same alterations, substantially, were found. Pale perinuclear halos and infrequent cytoplasmatic vacuoles optically empty, were present in the myocardium of the subjects belonging to the three groups. Found in the liver of all the pigs examined were microvacuolization phenomena in the centrolobular areas, often associated with the presence of small interstitial foci formed of mononucleate cells, and in the kidneys degenerative alterations in the epithelium of the contorted tubules. The extent of these alterations was always extremely modest and difficult to quantify, however, it appeared to be greater in the pigs belonging to the first and second groups as compared with those belonging to the first group.

Found in the intestine was a modest catarrhal enteritis, for the most part affecting the small intestine, in numerous subjects fed with rape-seed oil or with olive oil.

The presence of lesions characteristic of enzootic pneumonia was found in the lungs of all the pigs of the various groups.

From time to time lesions affecting other organs were found.

Present in the pancreas of a pig fed for 7 days with rape-seed oil were some small foci of necrosis of the exocrine glands; in another subject
of the same group sacrificed after 7 days there were found in the skeletal muscle widespread infiltration foci composed of mononucleate cells, while in a pig given a diet including olive oil for 20 days a hemorrhagic gastritis was present.

The colouring with Sudan III showed, in one single subject sacrificed after 20 days of a diet including rapeseed oil, the presence of minute fat droplets spread irregularly over the cytoplasm of extremely infrequent and isolated myocardial fibers.

The histochemical investigations undertaken to reveal the positive PAS material and enzyme activities showed, in all subjects of the three groups, substantially overlapping pictures.

The positive PAS material, in the myocardium, was present in relatively few fibers, with a colour intensity which was modest in general, and only in a few perinuclear areas of those sectioned longitudinally or at the level of the cells of the conduction bundles, did it take on the look of intensely coloured minute granules. At the skeletal muscle it was present in variable quantity in numerous fibers with a reticulated filamentous look only sometimes having the look of minute granules. The chromatic differences between fiber and fiber were vague and contained within extremely reduced limits while they were clearly obvious in only four pigs belonging to each of the three groups (two of the 1st group, one of the 2nd and one of the 3rd) and more specifically in those animals which had borne the slaughtering manipulations with limited reactions.

The phosphorylase activity in the myocardium showed itself with a colour tending towards brown (solvent without alcohol) or blue (solvent with alcohol) and was irregularly distributed both within the sphere of the section (areas with positive fibers next to areas with almost negative fibers)
and within the sphere of a same fiber sectioned longitudinally (positive zones alternated with other negative ones). In the skeletal muscle it was absent in some fibers and present in numerous other ones of the same section with a colour tending towards brown or blue-violet.

The ATPase activity was present and was very intense in the cytoplasm of all the myocardial fibers, over their entire length. Only rarely were areas found in which it was possible to find a clear transversal striation. In the skeletal muscle it was, to the contrary, absent in some fibers and present, in considerable quantity, in all the others.

Also the activity of the Succinic-dehydrogenase was very intense in the myocardium, present in all the fibers, localized in the cytoplasm in the form of granules aligned in parallel fashion to their length. It was absent in some fibers in the sections of the skeletal muscle and present in all the others in the form of cytoplasmatic granules which varied in number and chromatic intensity.

Ultrastructural examinations revealed a substantial integrity of the fibers in the hearts of all the pigs belonging to the three groups. Only rarely in some sections of the myocardium belonging to subjects fed with rapeseed oil or olive oil, were pictures found in which fibers with clear cytoplasmatic areas were present, without myofibrillae (Fig. 1) often surrounded by a thin membrane which gave them the look of vesicles, for the most part localized close to the nuclear apexes (Figs. 2 and 3).

There sometimes appeared in these fibers altered mytochondria and pyknotic nuclei (Fig. 1). The frequency of these alterations appeared to be slightly more accentuated in the subjects given the two diets for a longer period of time. Small, scanty and isolated lipid droplets slightly (electron) charged infrequently surrounded by a clear thin membrane, were observed in a low percentage of fibers.
Pig fed for 7 days with rape seed oil.
Contrast: lead hydioxide and uranyl acetate.
X 2400
Pig fed for 20 days with rape-seed oil.
Contrast: lead hydroxide and uranyl acetate.
X 10600

Figure 2.

Pig fed for 20 days with rape-seed oil.
Contrast: lead hydroxide and uranyl acetate.
X 5000

Figure 3.
The calculation of the average percentage of fibers concerned and
the extent of the phenomenon enabled us to reveal a modest increase in the
lipid change in the pigs fed with rapeseed oil or olive oil while it did
not show any difference between the subjects subjected to these two diets.
In the pigs belonging to the first two groups there were also found histiocytic
cells in the interstitial spaces between fiber and fiber, but we never had
the opportunity to observe these cells in the form of agglomerates. No
significant alterations were found in the skeletal muscle in any of the
subjects examined.

CONCLUSIONS AND CONSIDERATIONS

The results of our first research permit us to draw very limited
conclusions. The data obtained show that the diet including rapeseed oil
determined, in pigs, a modest accumulation of erucic acid in the myocardium
and skeletal muscle without the simultaneous occurrence of characteristic
functional and morphological alterations in these and in the other organs
examined, even if we did note, both in the subjects fed with rapeseed oil and
in those fed with olive oil, the precocious beginning of extremely modest
morphological alterations in the liver, kidney, intestine and perhaps in the
heart, which seem to confirm a certain sensitivity in the pig to a hyperlipid
feeding regime (Roine, et al., 1960; Svaar et al., 1975).

In substance we did not find significant and constant pathological
aspects which could certainly be related to the presence of rapeseed oil in
feed. This datum contrasts with what was referred by other authors according
to whom diets including rapeseed oil determine the precocious appearance of
accumulations of fat in the myocardium, which can be evidenced both histochemically
(Beare-Rodgers et al., 1972) and by observation with the E.M. (Vodovar et al.,
1973; Vodovar et al., 1975). This discordance is difficult for us to explain
because it does not seem to be linked to the low percentage of oil administered
by us because researchers who have worked under experimental conditions substantially similar to ours, describe in the heart of the pig serious alterations considered specific and connected to the action of erucic acid (Beare-Rodgers et al., 1972).

We therefore think that we should undertake further research work on pigs subjected to diets including a greater percentage of rapeseed oil, richer in erucic acid, and protracted for a longer time period for the purpose of offering an organic contribution to the definition of the real toxicity of the erucic acid in this animal species.

(UREVISED)
BIBLIOGRAPHY


RESEARCH ON THE EFFECTS OF A DIET INCLUDING RAPESEED OIL IN THE PIG—NOTE II

By

L. Rampichini; M. Severini; A. Begliomini; S. Ranucci; D. Rutili and E. Di Antonio

SUMMARY

Researches about the effects of a diet containing rapeseed oil in pig. II communication

The authors report about results of research work on pigs kept for 7-15-20-10 and 100 days on a diet containing 13% of rapeseed-oil (10% of erucic acid) or 13% of olive-oil.

The animals fed with rapeseed-oil showed an early accumulation of the erucic acid in the heart and skeletal muscle, but there was no early and notable lipidosis in the heart.

Slight morphological changes in the myocardium were found in all the animals of both groups, but they were more accentuated — even if retarded — in the animals fed with rapeseed-oil.

Key words: Pig, rapeseed oil, erucic acid, heart, skeletal muscle.

INTRODUCTION

In a previous note (Rampichini et al., 1976 a) we showed that pigs subjected to a diet including 11.5% of rapeseed oil (with 35% of erucic acid) did not present, during the first 20 days, characteristic functional
and morphological alterations.

This datum has created an interpretative problem over the real toxicity of erucic acid in the pig.

In this work we shall illustrate the results of further research conducted on pigs subjected for a longer period of time to a feeding regime including a high percentage of rapeseed oil, richer in erucic acid.

For this experiment we used the same rapeseed oil, which in the rat, in a lesser dose, was capable of inducing significant morphological alterations, represented by a serious and precocious lipidosis in the myocardium and kidneys (Rampichini et al., 1976 b).

PERSONAL OBSERVATIONS

Materials and techniques

Sixteen Large White race pigs, entire females, approximately 3 months old, of an average weight of 25 kilograms at the beginning of the experiment.

The subjects were fed for 10 days with SM 1 PETRINI fodder, without methionine and antibiotics, for the purpose of obtaining their adaptation to the experimental diet.

Subsequently they were subdivided into three groups: the first group comprised nine pigs to which fodder integrated with rapeseed oil (containing 40% of erucic acid) at the rate of 13% by weight (27 cals.%); the second group comprised five subjects to which fodder integrated with olive oil (without erucic acid) at the rate of 13% by weight (27 cal. %) was given; the third group comprised two pigs to which only fodder was administrated.

All the subjects received a constant measure of feed equal to 40/kg of living weight/pro die.
The pigs were sacrificed after the following time intervals:

<table>
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<th>15</th>
<th>20</th>
<th>40</th>
<th>100</th>
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</thead>
<tbody>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>number of subjects in second group</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>number of subjects in third group</td>
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<td>0</td>
<td>0</td>
<td>1</td>
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All the pigs were subjected to electrocardiograph, enzyme, chemical, necroscopic, histological, histochemical and ultrastructural examinations.

The electrocardiograph examinations were carried out at the beginning and at the end of the adaptation diet and at the end of the experimental diet, immediately before slaughtering, according to the methods already described (Rampichini et al., 1976).

(*) Some results of this investigation were communicated to the XXIX Congress of the Italian Society of Veterinary Sciences (Begliomini et al., 1975), Portoferraio, Italy.

The authors thank Messrs. O. Cortona and M. Spaccini for their technical assistance.