MAGNETIC RESONANCE IN CHEMISTRY, VOL. 35, S153–S158 (1997)

REVIEW

In Vivo NMR Studies of Animal Products: Body Composition. Qualitative Determination

Jean Pierre Renou*
STIM, INRA, Theix, 63122 Saint-Genès-Champanelle, France

One of the important objectives in animal product research has been the determination of body composition. NMR produces images of outstanding quality of transverse sections through the body. This method affords the best conditions for obtaining an exact measurement of lean content. Some studies have been performed *in vivo* to characterize muscle fibre types and fatty acid composition. NMR spectroscopy could be a useful technique for genetic selection and some NMR applications using muscle biopsies and biological fluids are reported. NMR has proved to be a powerful technique in research, but its application in industry has so far been relatively limited. © **1997 John Wiley & Sons, Ltd.**

Magn. Reson. Chem. **35**, S153–S158 (1997) No. of Figures: 8 No. of Tables: 1 No. of References: 30

Keywords: NMR; spectroscopy; imaging; *in vivo*; animals

Received 3 April 1997; accepted 6 June 1997

INTRODUCTION

It has been only about 40 years since preparative organic chemists began to exploit NMR spectroscopy. NMR emerged as a technique in meat research in 1980 when Gadian[1] discussed its potential for studying *post-mortem* metabolism. Considerable work has since been done in this area (for a review, see Ref. 2). In animal product research, an important objective has been the determination of body composition. However, some studies have been performed *in vivo* to characterize variables such as muscle fibre types, fatty acid composition and stallion semen quality.

Before reviewing applications of NMR to animal products, specifically to determine the body composition of live animals and qualitative characteristics, certain experimental problems will be discussed. *In vivo* NMR experiments require working with either whole animals or biopsies. This constraint raises many experimental problems besides the usual ones of pulse sequence and data processing.

EXPERIMENTAL CONSIDERATIONS

Biopsy or whole animal?

Some biopsies can be taken easily. Collecting biological fluids such as urine or plasma uses non-invasive methods, whereas muscle biopsies are more difficult to obtain. Surgery need not always be used, however, and shot biopsies can be carried out on live animals.[3] Nevertheless, the samples have to be representative and stable. It is well known that biochemical reactions occur after removal of tissue from animals. Large changes in energy metabolism are observed *post mortem*[4] and also some amino acids such as glutamine are not stable at temperatures over 4 °C. Representativeness is also a problem for muscle biopsies, as different fibre types exist in muscle. To compare animals, muscle biopsies have to be taken at the same specific location.

NMR experiments are time consuming and the animal must remain motionless throughout the experiment. Therefore, the animals must be sedated and anaesthetized. However, anaesthesia modifies metabolism, body temperature and breathing.[5] Monitoring is required and the risk of death is high. Another problem is localization. The spot where the study is to be performed has to be located with great accuracy. For comparison between animals of different size, anatomical references have to be taken.

Which magnet?

The choice of magnet depends on the sample and the measurement. Commercial magnets are available for animals up to pig size only. High fields are used for spectroscopy, whereas for imaging, low-field magnets are sufficient as the relaxation times are more favourable. The water signal is so strong that the signal-to-noise ratio is always high. The prices of magnets for different fields and different bore diameters are shown in Fig. 1. Prices do not increase linearly with these parameters. At 2.4 T, the price is three times more for a 100 cm than for a 40 cm bore.

* *Correspondence to*: J. P. Renou.

 CCC 0749–1581/97/SI0153–06 $17.50

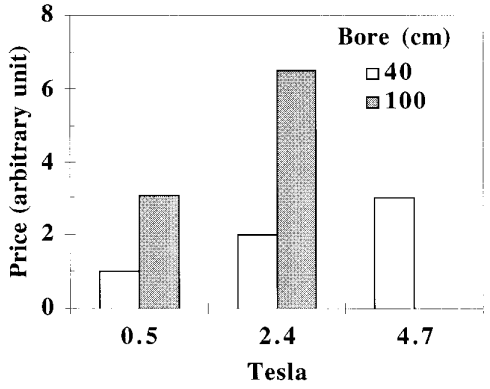

Figure 1. Prices of magnets for different fields and different bore diameters.

APPLICATIONS

Body composition

Knowing the proportion of fat and lean on a carcass before the slaughter of an animal is very useful for both producer and carcass purchaser. Also, a knowledge of body composition and changes in composition that occur in growth have been research objectives for over 40 years. To understand the regulation of the daily balance of nutrients by the body, nutritionists and physiologists have used complex methods to measure the fluxes of substrates and their rate of uptake, storage and disposal.[6] Total body water is a measure of body composition since the water occupies a relatively fixed fraction, 73.2%, of the fat free mass.[7] To determine total body water, the isotopes of hydrogen, deuterium or tritium, and/or ^{18}O have been used.[8] For isotope dilution techniques, a small dose (D_i) of a tracer is introduced orally or intravenously into a volume V_c :

$$D_i = C_i V_i$$

where C_i is the concentration of isotope in the injection volume (V_i). After equilibration in total body water the final concentration of isotope is C_f, and V_c is given by

$$V_c = D_i/C_f$$

with $V_c \gg V_i$.

Isotopic ratio mass spectrometry (IRMS) is the usual method used to determine the initial and final concentrations. This method is very accurate but requires having the sample in gaseous form for analysis. Preparing the sample is cumbersome and time consuming. Also, IRMS equipment is not widely available. Can NMR operate with the same accuracy and comparable linearity over a wide range with untreated samples? ^2H NMR spectroscopy was investigated with water and urine samples. The NMR results correlated closely with those determined by IRMS, the correlation coefficient being 0.9997 and 0.9992 for water and urine, respectively (Fig. 2). However, the intercept is not zero for urine samples, which induces an overestimation of the dilution volume.

The *in vivo* measurement of body composition can be performed directly by magnetic resonance imaging (MRI).[9] The protons visible on an NMR image are associated with small, rapidly moving molecules. In muscle the main signal source is the water proton. Adipose tissue is rich in triglycerides, which have long chains of CH_2 groups. The proton density does not differ greatly between fat and lean tissues, and the contrast between these tissues in images is achieved from the differences in relaxation times in each tissue. The optimum image contrast between fat and lean depends on the pulse sequence used. In spin echo imaging, the signal intensity is given by

$$SI = \rho\left[1 - \exp\left(\frac{-T_R}{T_1}\right)\right]\exp\left(\frac{-T_E}{T_2}\right)$$

where ρ is the proton density, T_R the repetition time and T_E the echo time. An alternative image can be obtained by using the same sequence except that it is preceded by a spin inversion using adiabatic fast passage applied prior to the 90° pulse. In the interval (τ) between these two pulses, the spins relax back to the normal orientation by a spin–lattice process and pass through a null point at T-null. If the 90° pulse is delivered at this time, no signal will be detected for the population with T_1 equal to 1.442 times T-null (Fig. 3). The T_1 of muscle is much longer than that of fat. By using a T_1 weighted inversion–recovery sequence, a contrast ratio of six between fat and muscle was obtained (Fig. 4).[10] Data have to be processed for quantitative analysis. Using

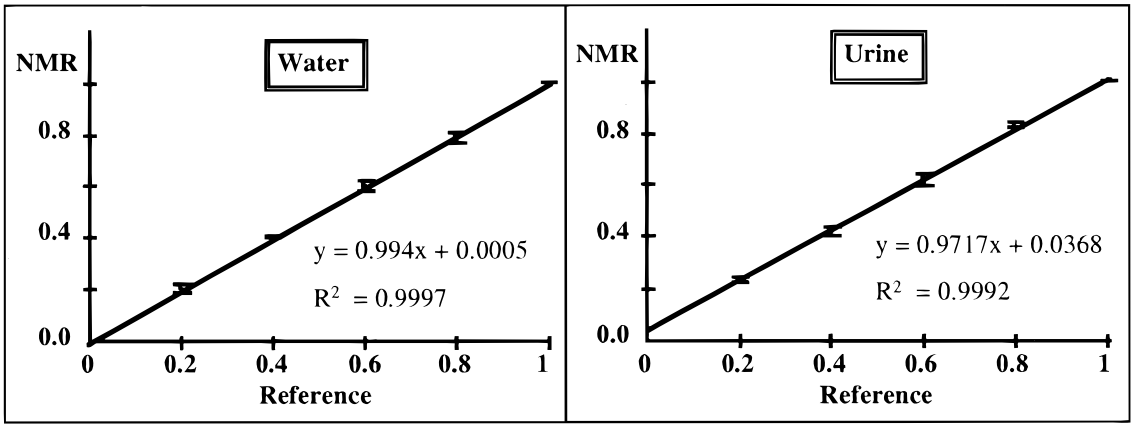

Figure 2. Relationship between ^2H NMR spectroscopy and IRMS results.

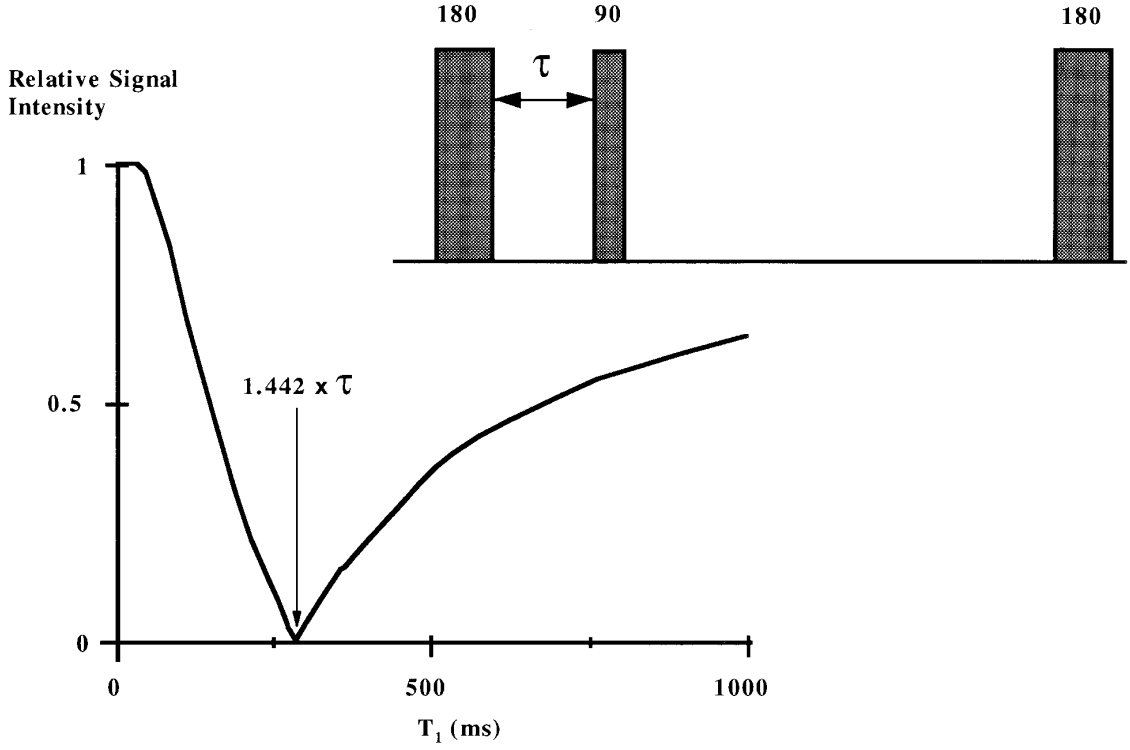

Figure 3. Signal size *vs.* T_1 value using a T_1 weighted inversion–recovery sequence.[10]

thresholding, muscle is segmented from fat. Intermuscular fat was separated from subcutaneous fat by scanning the segmented images. The fat/muscle ratio was determined by counting the number of voxels of each type. This thresholding method takes a few seconds.[11,12]

The first study on live animals was performed by Foster's group in Aberdeen[13,14] for the measurement of adipose tissue. Twelve female pigs were divided into two groups. Those in the obese group were fed a low-protein diet *ad libitum* and those in the lean group were given a high-protein diet in restricted quantities. There were no significant differences in the ages and weights of the two groups at the time of imaging. From anatomical marks (first thoracic vertebra and pelvic leg joint), 13 images were collected with a 0.04 T imager for each anaesthetized pig. Table 1 shows the percentage adipose tissue measured by MRI (13 slices) and by dissection for both pig groups.[13] These results demonstrate that adipose tissue quantities varied significantly between

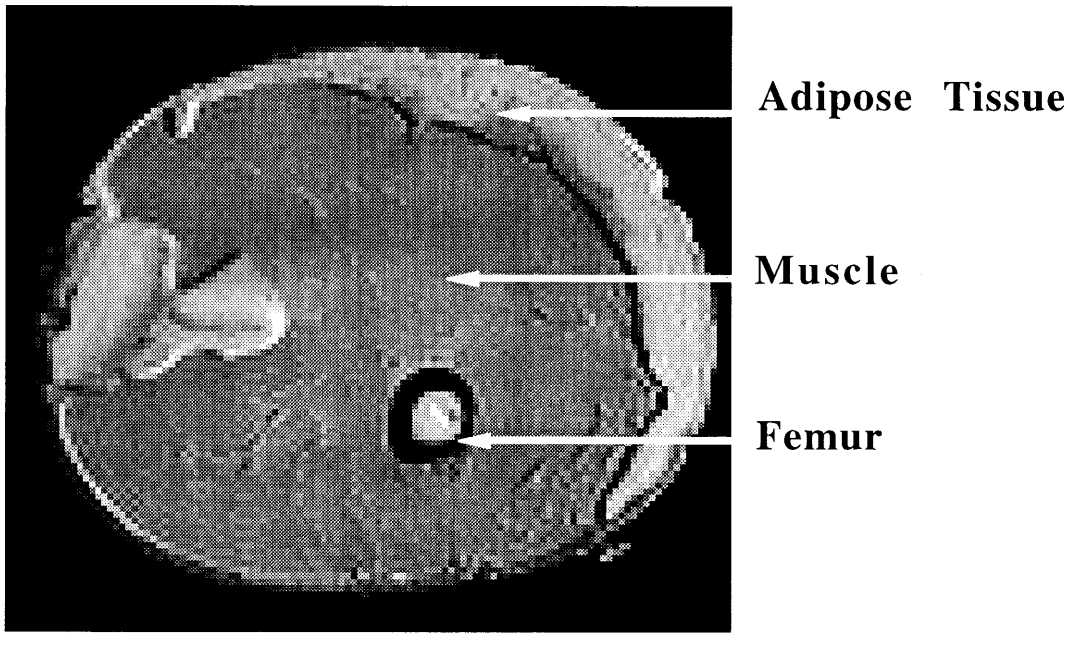

Figure 4. MR transverse section image of pig ham.

Table 1. Percentage adipose tissue by MRI and dissection in lean and obese pigs[13]

Pig type	MRI	Dissection
Obese	35.9 ± 2.0	39.6 ± 2.5
Lean	19.3 ± 3.8	22.7 ± 4.7
Lean/obese	0.54	0.57

obese and lean pigs even though lean and obese pigs were similar in external proportions. The agreement between percentage adipose tissue determined by MRI and dissection was very good ($r = 0.98$) with a mean square error of 2.1%. The distribution of adipose tissue along the pigs' bodies showed most discrepancy between the two methods (Fig. 5). MRI underestimated the percentage fat in the abdominal region and overestimated it in the neck and shoulders. Motion and alimentary canal contents can produce artefacts by producing a partial volume effect. Breathing and heart beat also induce movement artefacts.[15,16]

The total percentage lipid was closer overall to percentage adipose tissue by dissection and by MRI than slice percentage lipid. The total percentage lipid is a more accurate reflection of lipid content in the pig than the data subset represented by slice percentage lipid. These results clearly demonstrate that MRI can produce accurate estimates of percentage adipose tissue and reduce the residual variance significantly below that achieved by the use of age and weight.[14] Recently, Geers *et al.*[17] compared three methods (gravimetry, Fourier transform IR spectrometry after total fat extraction from a biopsy and *in vivo* NMR spectroscopy) to determine *in vivo* intramuscular fat content. The NMR results were lower than the others. The difference was explained by the fact that localized NMR spectroscopy measured only triglycerides. Moreover, NMR spectroscopy using the ISIS localization technique[18] investigates a small volume (4 cm^3), which is not very representative when there is great heterogeneity in intramuscular fat content.

Besides whole-body composition, MRI was applied to study the development of individual tissues such as the mammary glands. The *in vivo* determination of mammary gland size and gross composition in goats was performed using MRI.[12,19] In lactating goats, the mammary parenchyma volume was significantly greater than the weight of parenchyma determined *post mortem*. The 54% overestimation was due to the difficulty in differentiating mammary parenchyma from stroma and the rest of the body. However, the volume determined by MRI was closely correlated with the parenchyma weight. Hence it was possible to follow parenchyma volume for goats during the first and second gestation. For primiparous goats, parenchyma volume followed an exponential pattern whereas for the second gestation goats, no increase was observed until after week 15. The study demonstrated the feasibility of using MRI to monitor mammogenesis *in vivo* and generate quantitative data.

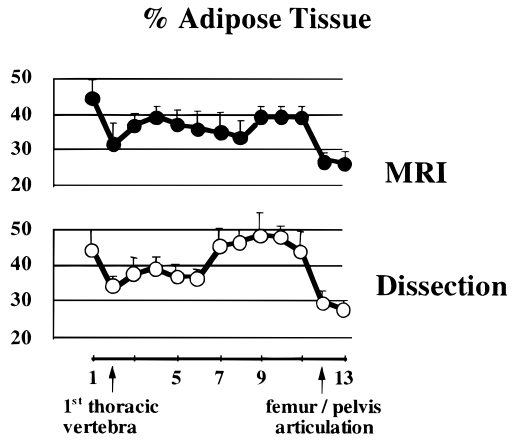

Figure 5. Distribution at 13 locations along the body of adipose tissue percentage by MRI and dissection in lean and obese pigs.[13]

Qualitative and quantitative determination

Knowledge of body composition can be improved by qualitative measurements of muscle type, fatty acid composition and technological and organoleptic qualities of meat. Magnetic resonance spectroscopy is very useful for qualitative determination, but MRI gives a spatial resolution that allows the characterization of muscle fibre types. Muscle fibres are generally not homogeneous for phenotypes and display a wide variety of types depending on muscle metabolism and innervation. The most widely used classification system is based on physiological behaviour: slow-twitch oxidative or type I, fast-which oxidative glycolytic or type IIa, and fast-twitch glycolytic or type IIb.[20] The proportions of the different types are related to muscle function and the type of innervation. The usefulness of a non-invasive method to determine changes in muscle fibres during growth is obvious. T_1 and T_2 values were determined *in vivo* in pure slow-twitch and fast-twitch rabbit muscles and in rabbit muscles with different fibre types. Figure 6 displays the T_2 values measured from the parametric images *vs.* the percentage of type I fibre. Muscle with high proportions of oxidative slow-twitch fibres

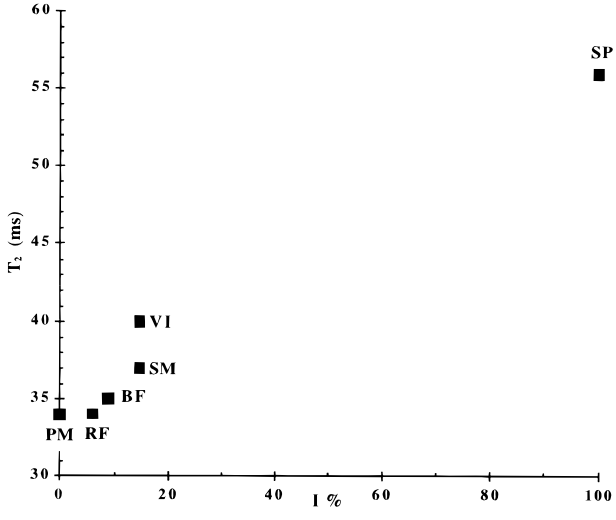

Figure 6. T_2 *vs.* type I fibre.[21] BF = biceps femoris; PM = psoas major; RF = rectus femoris; SM = semimembranosus; SP = semimembranosus proprius; VI = vastus internalis.

(semimembranosus proprius muscle) had higher T_2 values than the others whereas T_1 did not vary significantly.[21]

Knowledge of the fatty acid chain composition in relation to diet is limited because of the invasive sampling that is required. Until recently it was necessary to obtain body tissue samples through surgery followed by solvent extraction of the lipids. The fatty acid composition of body fat can change drastically, but the turnover rate can be very slow.[22,23] Magnetic resonance spectroscopy is a non-invasive method that can detect gradual changes over the long term. Investigations by ^{1}H NMR spectroscopy are impeded by the intense water resonance and the overlapping of many resonances in ^{1}H NMR spectra. These limitations were overcome by Desmoulin and Seelig.[24] They used a pulse sequence combining homonuclear shift correlated and spatially localized spectroscopy to study adipose tissue in rats. *In vivo* ^{13}C NMR has been applied to monitor the fatty acid chain composition in the adipose fat of rats fed saturated fatty acids, vegetable oils and fish oils.[25] The results indicated that a large portion of the diet fatty acyl chains were incorporated directly into adipose tissues, although some were also metabolized.[26] ^{13}C NMR resonances at 130 and 128 ppm are characteristic of the unsaturated carbons of polyunsaturated fatty acids whereas the unsaturated carbons of monounsaturated fatty acids are found at 130 ppm. The results demonstrate that a large proportion of the diet fatty acyl chains was incorporated directly into adipose tissues. The 130/128 ppm signal intensity ratio is an accurate indicator of the consumption and synthesis of monounsaturated fatty acids *vs.* consumption of polyunsaturated fatty acids.[25] As the unsaturation of the dietary fat and hence the adipose tissue increased, the 130/128 ppm ratio decreased (Fig. 7). Changes in the T_1 of fatty acid carbons were also measured. Thus the segmental mobility of acyl chains was sensitive to changes in diet-derived fatty acyl chain composition and especially the central region of the acyl chain.[26]

The second example concerns pork quality. The rate of *post-mortem* catabolism in pig muscle determines pork quality. This trait is under partial genetic control. Methods usable in live animals for the prediction of metabolism intensity in muscle after slaughter are of great importance. The muscle metabolic defect connected with porcine halothane sensitivity (also known as malignant hyperthermia syndrome) leads to a pale, soft, exudative (PSE) meat of low technological and organoleptic quality. A 2 g biopsy sample was taken by shot biopsy on pigs and analysed by ^{31}P NMR spectroscopy.[27] Figure 8 shows ^{31}P NMR spectra from biopsies obtained from two pigs with slow (normal) and fast (PSE) rates of *post-mortem* metabolism. By 30 min after biopsy, the spectrum of the 'normal' pig contained seven resonances corresponding to sugar phosphates (SP), inorganic phosphates (Pi), glycerophosphoryl choline (GPC) phosphocreatine (PC) and the three phosphate groups of ATP. For the PSE biopsy most of the resonances disappeared and only the SP and Pi resonances were observed.

^{31}P NMR measurements on skeletal muscle biopsies provide an effective tool for the assessment of halothane sensitivity and prediction of meat quality in pigs. The level of several metabolites, reflecting the rate of muscle glycogenolysis and glycolysis, was measured *post mortem* in the biceps femoris muscle of normal and PSE pigs.[28] The study demonstrated a more than threefold accelerated PC decay in heterozygote malignant hyperthermia compared with normal pigs. Combining ^{31}P NMR with a rapid and efficient technique for taking biopsies allows the prognosis of these defects in live animals, which is particularly useful for genetic selection.

For selecting leaner broiler chickens, a study using ^{31}P magnetic resonance spectroscopy was performed with 18-day-old embryos from two chicken lines, differing in body fat content.[29] The ATP phosphodiester ratio was obtained *in vivo*. The same birds were slaughtered at 8 weeks of age and the body fat content was measured. A linear negative relationship was found between the body fat content and embryonic ATP : phosphodiester ratio ($r^2 = 0.62$–0.78). NMR spectroscopy could be a useful technique for selecting broiler chickens.

NMR spectroscopy has also been used in genetics for the evaluation of equine seminal plasma.[30] Quantitative

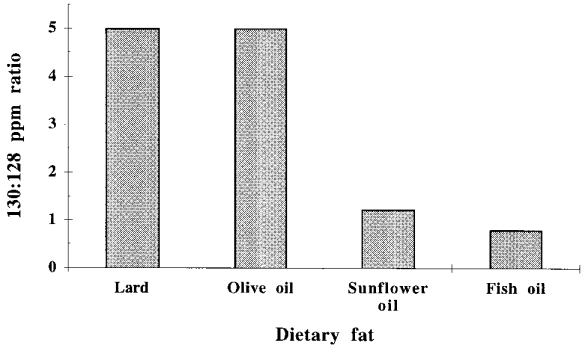

Figure 7. Influence of dietary fat on relative heights of peaks from unsaturated carbons of body fat.[25]

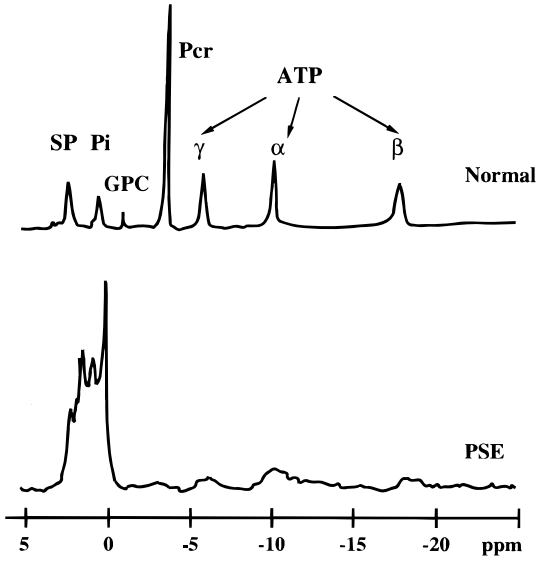

Figure 8. ^{31}P spectra obtained (30 min after biopsy) from a muscle biopsy from 'normal' and 'PSE' pigs.[27] SP = sugar phosphates; Pi = inorganic phosphate; GPC = glycerophosphorylcholine; Pcr = creatine phosphate; ATP = adenosine triphosphate.

and qualitative variations in equine seminal plasma were observed according to season, stallion and ejaculates per stallion. The evaluation of these variations in semen composition have important consequences in the selection of a stallion and in the prediction of his fertility. Interactions between some components and macromolecules or metal ions resulted in the disappearance of resonances from ^{1}H NMR spectra. By acidification of plasma, these metabolites were detected and their concentrations determined. The mean values measured by NMR were comparable to those published from biochemical measurements.

NMR has proved to be a powerful technique in research, but its application in industry has so far been relatively limited. Some quality parameters and body composition can be assessed by NMR. However, this method of investigation is still too sophisticated and the high cost of the equipment restricts its application.

Acknowledgements

I thank G. Bielicki, P. Ritz and C. Troquier for their contribution to the ^{2}H MRS study.

REFERENCES

1. D. G. Gadian, *Developments in Meat Science*. Elsevier, London (1980).
2. J. P. Renou, *Annu. Rep. NMR Spectrosc.* **31**, 313 (1995).
3. R. Lahucky, *Pig News Inform.* **83**, 103 (1987).
4. J. P. Renou, P. Canioni, P. Gatellier, C. Valin and P. J. Cozzone, *Biochimie* **68**, 543 (1986).
5. J. D. de Certaines, V. A. Larsen, F. Podo, G. Carpinelli, O. Briot and O. Henriksen, *NMR Biomed.* **6**, 345 (1993).
6. J. F. Sutcliffe, *Phys. Med. Biol.* **41**, 791 (1996).
7. N. Pace and E. N. Rathburn, *J. Biol. Chem.* **158**, 685 (1945).
8. J. Robelin, *In vivo Measurement of Body Composition in Meat Animals*. Elsevier Applied Science, London (1984).
9. R. Ross, *Can. J. Physiol.* **74**, 778 (1996).
10. M. A. Foster, J. M. S. Hutchinson, J. R. Mallard and M. F. Fuller, *Magn. Reson. Imaging* **2**, 187 (1984).
11. A. Colin, E. Erbland, C. Datin, J. Y. Boire, A. Veyre and M. Zanca, *IEEE*/EMBS **15**, 421 (1995).
12. P. A. Fowler, C. H. Knight, G. C. Cameron and M. A. Foster, *J. Reprod. Fertil.* **89**, 359 (1990).
13. P. A. Fowler, M. F. Fuller, C. A. Glasbey, G. C. Cameron and M. A. Foster, *Am. J. Clin. Nutr.* **56**, 7 (1992).
14. M. F. Fuller, P. A. Fowler, G. McNeill and M. A. Foster, *J. Nutr.* **124**, 1546S (1994).
15. J. C. Seidell, C. J. G. Bakker and K. Van der Kooy, *Am. J. Clin. Nutr.* **51**, 953 (1990).
16. P. A. Fowler, C. E. Casey, G. C. Cameron, M. A. Foster and C. H. Knight, *Br. J. Obstet. Gynaecol.* **97**, 595 (1990).
17. R. Geers, C. Decanniere, H. Villé, P. Van Hecke and L. Bosschaerts, *Meat Sci.* **40**, 373 (1995).
18. R. J. Ordidge, A. Connely and J. A. B. Lohman, *J. Magn. Reson.* **66**, 283 (1986).
19. P. A. Fowler, C. H. Knight, G. C. Cameron and M. A. Foster, *J. Reprod. Fertil.* **89**, 367 (1990).
20. A. M. Pearson and R. B. Young, *Muscle and Meat Biochemistry*. Academic Press, San Diego (1989).
21. J. M. Bonny, M. Zanca, O. Boespflug-Tanguy, V. Dedieu, S. Joandel and J. P. Renou, *Magn. Reson. Imaging* in press.
22. J. Hirsch, J. W. Farquhar, E. H. Ahrens, M. L. Peterson and W. Stoffel, *Am. J. Clin. Nutr.* **8**, 499 (1960).
23. S. Dayton, S. Hashimoto, W. Dixon and M. L. Pearce, *J. Lipid Res.* **7**, 103 (1966).
24. F. Desmoulin and J. Seelig, *Magn. Reson. Med.* **14**, 160 (1990).
25. S. C. Cunnane, T. Allman, J. Bell, M. Barnard, G. Coutts, S. C. R. Williams and R. A. Iles, *Human Body Composition*. Plenum Press, New York (1993).
26. T. W. M. Fan, A. J. Clifford and E. M. Higashi, *J. Lipid Res.* **35**, 678 (1994).
27. R. Lahucky, J. Mojti, J. Poltarsky, J. P. Renou, A. Miri, A. Talmant and G. Monin, *Meat Sci.* **33**, 373 (1993).
28. B. Moesgaard, B. Quistorff, V. G. Christensen. I. Therkelsen and P. F. Jørgensen, *Meat Sci.* **39**, 43 (1995).
29. Z. Liu, A. Lirette, R. Fairfull and B. W. McBride, *Poult. Sci.* **733**, 1633 (1994).
30. F. Seguin, M. Magistrini, P. Beau, S. Akoka, E. Palmer and A. Le Pape, *J. Magn. Reson. Anal.* **1**, 53 (1995).

