動科論叢(13) : 75~87

METHODS FOR BODY COMPOSITION ANALYSIS IN SWINE

A. D. Mitchell

Growth Biology Laboratory
U. S. Department of Agriculture
Agricultural Research Service

Building 200, Room 205,
BARC-East Beltsville, MD 20705

Tel : 301-504-8868
Fax : 301-504-8623
e-mail : mitchell@ggpl.arsusda.gov

Introduction :

A major deterrent to reducing fat and increasing protein, is a lack of nondestructive techniques to assess dynamic changes in body composition of animals, which restricts our ability to design production systems that efficiently produce animal products of optimal composition. Thus, there is a need for validated and standardized direct and indirect nondestructive techniques for determining body composition.

An accurate and nondestructive means of determining body composition has been sought by nutritionists and physiologists for decades. Thus far, slaughter balance type trials have given the most accurate data on compositional change in animals. However this technique is very time consuming and necessitates large numbers of animals because of the terminal nature of the procedures. Other methods allow for repeated measurements to be made of the same animal, but all have lacked the sensitivity and reliability needed for the highly critical analysis required for research purposes.

Body composition of the pig varies as a result of breeding, age(stage of growth) and nutritional status. However, body compostion of the live animal is difficult to maesure accurately. In addition to meat(skeletal muscle) and fat (which can be external, internal or intramuscular), the body also contains variable amounts of : bone; blood; connective tissue and skin; hair and hooves; organs of the cardiovascular, respiratory, nervous, reproductive and urinary sys-

tems; and the gastrointestinal tract containing variable amounts of digesta. Also, it is usually difficult to control movement of the animal.

In order to achieve a suitable level of accuracy, the method will likely need to provide a direct measure of one or more of the major determinants of body composition, at either the tissue level(muscle, fat), chemical level(protein, lipid, water) or elemental level(carbon, hydrogen, nitrogen). Furthermore, the measurement should be based on total body rather than a localized sampling approach. In recent years, various instruments have been used to penetrate the animal in an attempt gather information on body composition. These include the use of ultrasound, x-rays, gamma rays, near-infrared rays, nuclear magnetic resonance, electrical impedance, electromagnetic conductivity and neutron activation. Many of these approaches are capable of providing accurate and useful information, but fail to meet the criteria for practical application; primarily the combination of accuracy and economy.

There are a number of criteria for an "ideal" method for measuring body composition : 1)rapid - depends on the application, $<$ one minute for evaluation of market animals to $<$ 20 minutes for selection of breeding stock, 2)non destructive - and preferably non invasive, 3)accurate - $>$ 95% accuracy, 4)easy to operate - minimal animal handling and restraint - no anesthesia - user friendly instrumentation and data processing, 5)economical - includes both initial and operating costs - the cost per measurement could depend on application and might range from a few cents for market animals to a few dollars for breeding animals, 6)real time measurement - minimal data manipulation and processing - ideally, results immediately available.

Research at the USDA-ARS Growth biology Laboratory in Beltsville, Maryland has focused on the development and refining of accurate non-invasive techniques for measuring the fat and lean content of animals over a wide range of body sizes and body composition. The primary emphasis is on the use of Dual-Energy X-ray Absorptiometry(DXA) and Magnetic Resonance Imaging (MRI). Both the MRI and DXA are diagnostic medical devices that have been found to be useful for measuring the body composition of both humans and animals. Clinically, MRI is used to visualize soft tissue abnormalities such as tumors or damage to the knee joint, etc., whereas, DXA is used most commonly to screen for osteoporosis by measuring bone mineral densities. Although a relatively new technique for measuring body composition of humans, DXA is rapidly being accepted as the "gold standard". This technique, based on the

relative attenuation of x-rays at two discrete energy levels, provides a measure of bone, fat and lean tissue masses. Without doubt, the single most powerful technique for studying composition of the live animal is MRI. MRI provides excellent contrast images that allows the visualization and measurement of fat, muscle and various other organs within the body. This is accomplished by placing the anesthetized animal within a high magnetic field and exciting the tissues with radio frequency waves.

The MRI instrument is very expensive to purchase and operate, thus, it's use is limited to research purposes. The DXA is inexpensive to operate, but the cost, although much less than the MRI, is enough that use would be limited primarily to large production units, cooperatives or research facilities. Thus, producers could ultimately benefit from more accurate assessment of superior breeding stock. This technique could also find application in the evaluation of valuable animals developed through transgenic research.

TRADITIONAL METHOD OF BODY COMPOSITION ANALYSIS

The following are the traditional methods for assessing body composition of the live pig :

1) VISUAL APPRAISAL
2) WEIGHT AND LENGTH MEASUREMENT
3) BACKFAT PROBE
4) SLAUGHTER ANALYSIS

and more recently -

5) ULTRASOUND

EXPERIMENTAL METHODS OF BODY COMPOSITION ANALYSIS :

The following is a list of some of the experimental techniques that have been used to measure body composition of the live pig :

DILUTION TECHNIQUES-(total body water)

- ^3H_2O
- D_2O
- UREA

VOLUMETRICS(SPECIFIC GRAVITY)

- Acoustics
- Displacement

ELECTRICAL CONDUCTIVITY OR IMPEDANCE

TOBEC(Total Body Electrical Conductivity)

EI(Electrical Impedance)

SPECTROSCOPY

NIR(Near-Infra Red)

NMR(Nuclear Magnetic Resonance)

IMAGING

CT(Computerized Tomography)

MRI(Magnetic Resonance Imaging)

Ultrasound

DXA(Dual-Energy X-Ray Absorptiometry)

OTHERS

^{40}K(Whole body counting)

NA(Neutron Activation-nitrogen, carbon, oxygen)

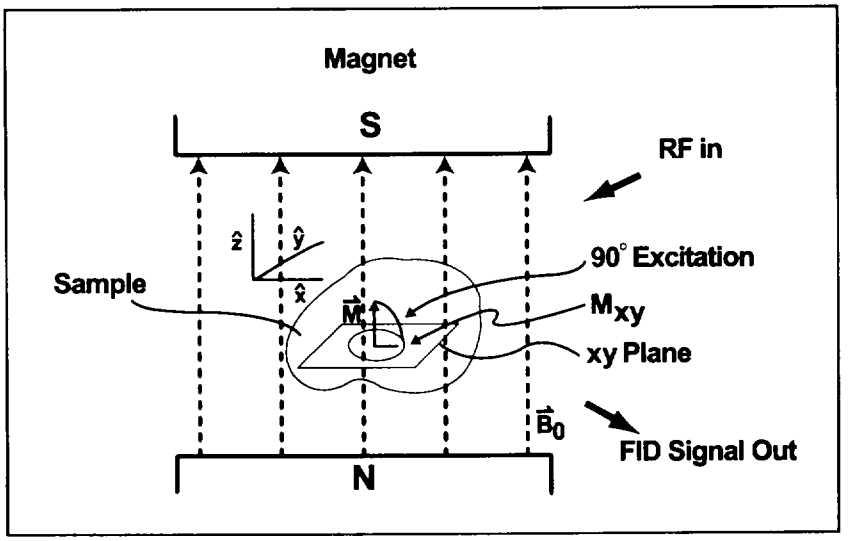

Figure 1. The protons in sample of tissue align with the magnetic field B_0 to produce a net magnetization M in the z-direction. A 90° pulse at a resonant frequency rotates M into the x-y plane. Mxy precesses about the B_0 direction at the resonant frequency($f = y/2\pi \cdot B_0$) due to the torque on M caused by B_0. A current is induced in a coil of wire around the sample (not shown) due to the changing magnetic flux. This signal is called the free induction decay(FID).

Body Composition Analysis of the Pig by Magnetic Resonance Imaging

Background :

The visualization of soft tissue characteristics or anatomy by NMR imaging as routinely used clinically is based on differences in the behavior of hydrogen protons associated with water and other macromolecules(primarily fat) when tissues are placed in a magnetic field and irradiated with radiofrequency(RF) waves at the resonant frequency determined by the magnetic field strength and the nucleus to be studied(Larmor frequency). After excitation, the sample emits a RF signal that can be detected by a receiver coil placed close to the sample.

The intensity of the emitted signal is related to the number of protons present in a given volume and the relaxation times of the excited sample. The NMR image is generated by imposing a magnetic field gradient upon the sample during the excitation and relaxation time periods to establish spatial encoding(the unique relationship between frequency and location for all volume elements of the object). Differences in the hydrogen content, mobility of the water molecules and the NMR relaxation times of tissues permit excellent contrast between fat, muscle and other soft tissues.

Procedure :

The anesthetized pig is placed inside the magnet. Conditions are optimized for the spin-echo imaging sequence. This sequence provides good contrast between fat and lean tissue. A scout image is obtained to verify positioning and to establish location for cross-sectional imaging. Up to 40 cross-sectional images (1cm slice thickness) may be obtained in one imaging sequence. The animal is then repositioned(i.e. moved forward 40cm) for the next series of cross-sectional images. After the scanning is completed the animal is removed from the magnet to recover from the anesthesia.

Images may be processed from either transparency film or as image files. From each cross-sectional image, areas of interest are traced and a series of images are then processed to yield volumetric measurements of specific areas such as subcutaneous fat, muscles or groups of muscles and internal organs.

Results:

MRI was used th visualize, define, and quantify major fat and muscle areas of the pig(Mitchell et al., 1993). This is analogous to being able to dissect the pig without killing it. A typical MRI cross-sectional image of a pig is shown in Figure 2.

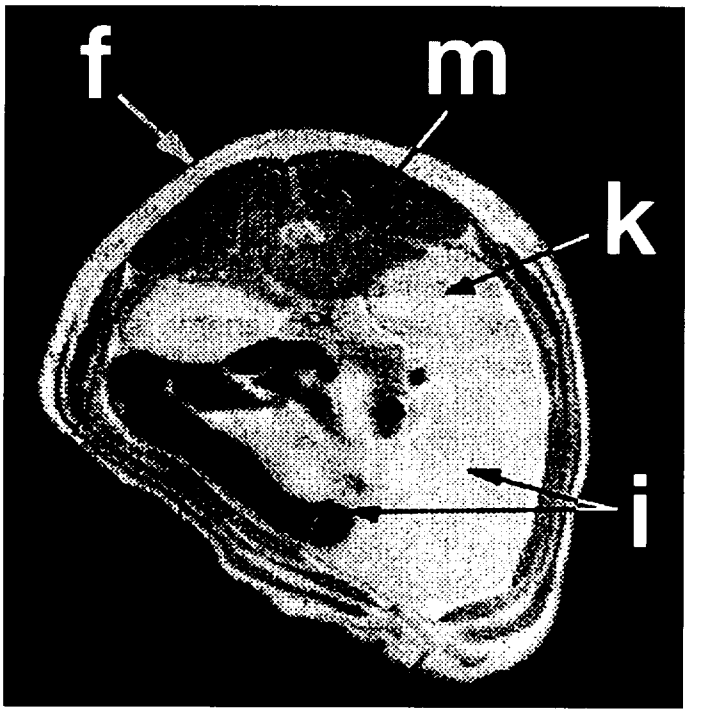

Figure 2. Cross sectional image through the abdominal region of a pig(f = fat, m = muscle, k = kidney, i = intestines).

Areas of fat or lean can be reconstructed from a series of such images taken throughout the body(Figure 3) and then used to calculate amounts of fat or lean in a particular region.

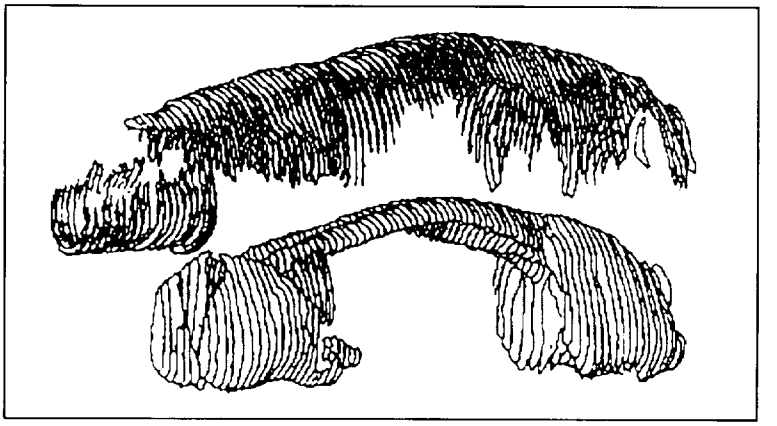

Figure 3. Three-dimensional reconstruction(from a series of MRI cross-sectional images) showing the major areas of subcutaneous fat and muscle in the pig.

The amount of tissue measured by MRI analysis agrees very closely with the amount weighed following dissection, and in some cases may be more accurate.

This technique enables the researcher study the growth and development of specific tissues and to compare the effects of various treatments or genetic selection. In addition to providing detailed images, some magnetic resonance instruments can provide chemical information(NMR spectroscopy) that can be sued to evaluate localized composition(Mitchell et al., 1991) and metabolic processes that are occurring in the live animal(Scholz et al., 1995, 1998).

The following comparison have been made between MRI volumes and measurements by dissection/chemical analysis:

- Subcutaneous fat volume - backfat and jowl fat
- Muscle volumes - shoulder muscles, longissmus dorsi muscle, psoas muscle and ham muscles
- Internal organs - brain, heart, liver, kidneys
- Total body volume - total body weight

Applications - MRI has been used to measure body composition of pigs in the following studies:

- Evaluation of GH treated pigs(Mitchell et al., 1991d)
- Evaluation of energy deposition(Mitchell et al., 1991c)
- Evaluation of pigs carrying the defective ryanodyne receptor gene(Scholz et al., 1995, 1998)

Summary:

- Using MRI scans of the entire body, information can be obtained on the size, shape and distribution of a number of organs and tissues.
- Accuracy of volume measurements depend on the ability to define the boundaries of tissues, which is influenced by the image quality(resolution and contrast) and the number or choice of slices analyzed.
- MRI volume measurements of the major fat and muscle areas of the body can be used to predict the total lipid and protein content of the body.

NMR spectroscopy can be used to make the following measurements in the live animal :

- ^{1}H : water and lipid proton spectra
- ^{13}C : lipid and glycogen carbon spectra
- ^{31}P : high energy phosphate metabolites, ATP, creatine phosphate, Pi

Body Composition Analysis of the Pig by Dual Energy X-ray Absorptiometry

Background :

The measurement of body composition by the DXA system used in these studies is based on the differential attenuation of low-(38keV) and high-energy (70keV) x-rays by fat and other soft tissues. The fat and lean content is determined for each pixel(0.46㎠) of a total body scan that does not overlie bone and is reported to be virtually independent of tissue thickness. The soft tissue attenuation ratio(R_{St}) is the ratio of the mass attenuation coefficients(μ) at 38 and 70 keV. Calibration studies at DPX energies of 38 and 70keV report that R_{St} values range from 1.2 for fat to 1.4 for 100% lean. In addition to whole body composition values for fat and lean content, DXA measurement also estimates bone mineral content(BMC), total mass of soft tissues(TMST), and by analyzing only those pixels within a defined area, composition can be determined on a regional basis. Because of the low radiation dose and the ability to detect the differential attenuation of the radiation by bone, fat and lean tissue, both DPA and DXA have received considerable attention for the measurement of human body composition.

Initial flux I_o of photons undergo attenuation with resultant exponential reduction in flux by absorption in tissues :

$$I^{38} = I_o^{38} e^{-(\mu_{ST}^{38} M_{ST} + \mu_B^{38} M_B)}$$

$$I^{70} = I_o^{70} e^{-(\mu_{ST}^{70} M^{ST} + \mu_B^{70} M_B)}$$

Where :

I_o	=	Unattenuated photon fluence rate(photons/sec)
I	=	Attenuated photon fluence rate(photons/sec)
M	=	Mass of given substance(g/㎠)
μ	=	Mass attenuation coefficient(㎠/g)
38, 70	=	Subscripts for 30 and 70 keV photon energies
S, B	=	Subscripts for soft-tissue and bone

The two masses are calculated as follows :

$$M_{ST} = \frac{R_B \cdot \ln(I^{70}/I_o^{70}) - \ln(I^{38}/I_o^{38})}{\mu_{ST}^{38} - \mu_{ST}^{70} \cdot R_B}$$

$$M_B = \frac{R_{ST} \cdot \ln(I^{70}/I_o^{70}) - \ln(I^{38}/I_o^{38})}{\mu_B^{38} - \mu_B^{70} \cdot R_{ST}}$$

The soft tissue attenuation coefficient(R_{ST}) is the ratio of the mass attenuation coefficients at 38 and 70 keV :

$$R_{ST} = \mu_{ST}^{38}/\mu_{ST}^{70} = \ln(I_o^{38}/I^{38})/\ln(I_o^{70}/I^{70})$$

Calibration studies at DXA energies of 38 and 70 keV report that R_{ST} values range from 1.2 for fat to 1.4 for 100% lean.

$$\text{Lean fraction} = (R_{ST}-R_{FAT})/(R_{LEAN}-R_{FAT})$$

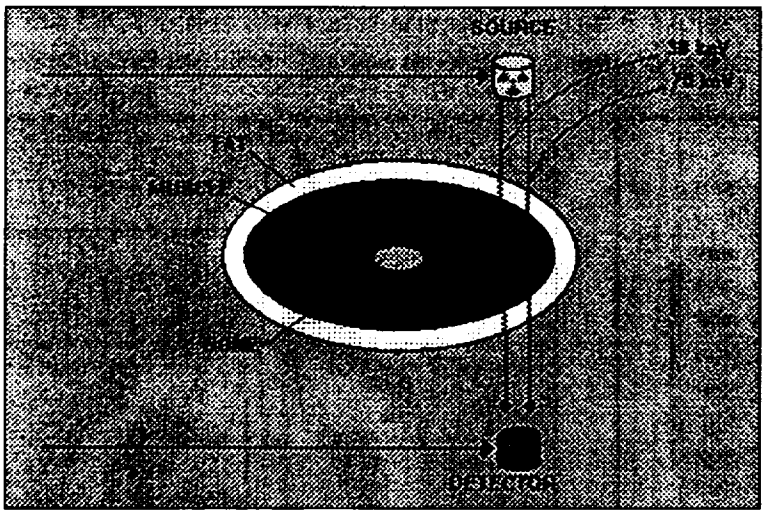

Figure 4. Schematic representation of the attenuation of 38 and 70 keV x-rays by fat and lean tissue during a DXA scan.

Procedure :

The anesthetized pig is placed on the scanning table. The scanning mode is selected, based on the size of the animal. During the scanning procedure the x-ray source and detector pass simultaneously across the animal, advancing approximately 0.9cm with each pass until the entire body has been scanned. After the scanning procedure is complete the pig is removed to recover from the anesthesia.

The software program automatically calculates the amount of fat, lean and bone mineral content of the whole body. By using pre-programmed or manual

region of interest analysis, these components will also be analyzed for various regions of the body(i. e., arms, legs or body trunk). Based on calibration studies, the DXA lean mass can be used to predict total body water and protein content.

Results :

Studies have been conducted to validate the use of DXA for measuring the fat and lean content of the whole body and the front and back leg regions (Mitchell et al., 1996b) and to evaluate the use of DXA for monitoring changes

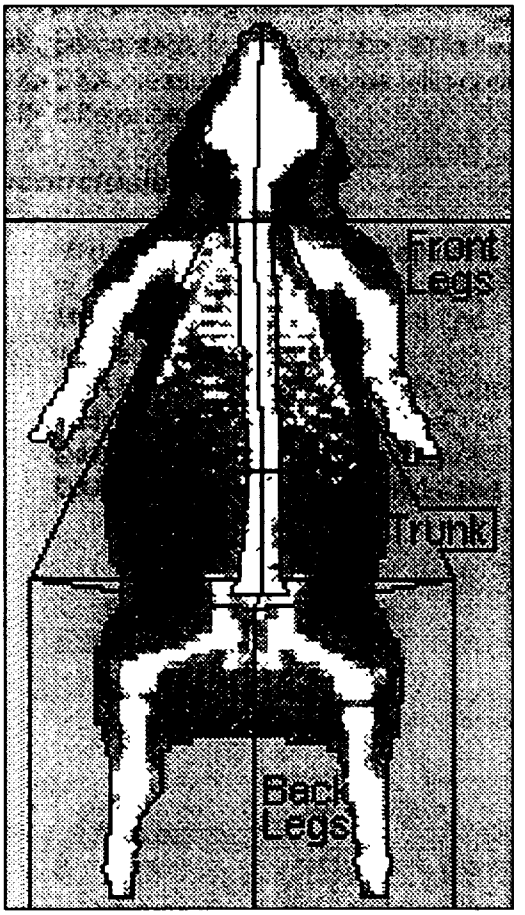

Figure 5. DXA scan image of a pig showing the typical regions of interest that are analyzed separately.

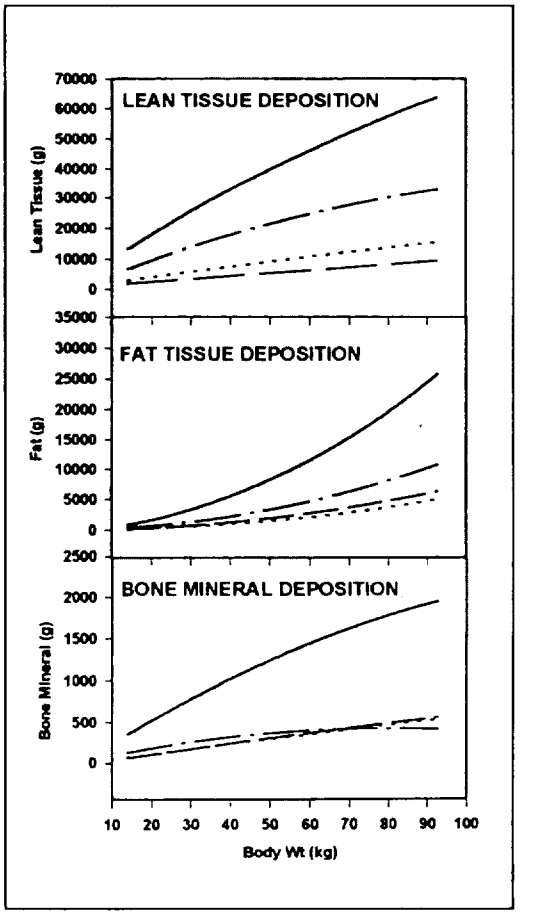

Figure 6. DXA measurements of lean, fat and bone mineral in total body(——), front legs(— —), back legs(— — — —) and trunk(— — —) during growth from 15 to 90kg.

in body composition occurring during growth and for measuring the rates of fat, lean and bone mineral deposition in the total body and in specific regions of the body(Mitchell et al., 1996a). Total body DXA scans were performed on anesthetized pigs using a Lunar DPX-L(Madison, WI) instrument(see Figure 5). For the growth study, each pig was scanned by DXA at the beginning of the study and at 14 day intervals until reaching a final weight of approximately 90kg. The results of the DXA scans are summarized in Figure 6. After the final scan each pig was slaughtered and the carcass analyzed chemically for lipid, Water, protein and ash content.

Applications - DXA has been used to measure body composition of pigs in the following studies :

- Evaluation of ryanodyne receptor genotypes(Mitchell and Scholz, 1997, 1998)
- Measurement of energy deposition(Mitchell et al., 1994, Mitchell and Scholz, 1998)
- Evaluation of transgenic pigs(Mitchell et al., 1998d)
- Analysis of half-carcass composition(Mitchell et al., 1998c)

Summary :

- DXA underestimates the fat content of both the live pig and carcass - most notably smaller and leaner pigs - but, appears to be accurate for larger pigs.
- However the correlation between DXA and chemical measurements of percentage fat is high($r > .9$) in both the live pig and carcass.
- DXA measurements agree with chemical results in evaluating genotype differences in pigs.

Conclusions :

- MRI is a highly accurate method for detailed body composition measurement of selected body tissues(sections) and the whole body.
- MRI requires high investment and maintenance costs and labor intensive image processing.
- DXA is an accurate method for whole body composition measurements and body parts. It requires relatively low investment and maintenance costs. Data can be processed quickly and easily.
- DXA requires some calibration and provides no volume information

Selected Bibliography :

Mitchell, A.D., P.C. Wang and T.H. Elsasser. 1991a. Determination of fat and water content in vitro and in vivo by proton magnetic resonance spectroscopy. J. Sci. Food Agric. 56:265~276.

Mitchell, A.D., P.C. Wang, R.W. Rosebrough, T.H. Elsasser and W.F. Schmidt. 1991b. Assessment of body composition of poultry by nuclear magnetic resonance imaging and spectroscopy. Poultry Sci. 70:2494~2500.

Mitchell, A.D., P.C. Wang, T.H. Elsasser and W.F. Schmidt.1991c. Application of NMR spectroscopy and imaging for body composition analysis as related to sequential measurement of energy deposition. Energy Metab. Farm Anim., Proceedings of the 12th symposium. EAAP No. 12:222~225.

Mitchell, A.D., P.C. Wang and C.M. Evock. 1991d. Body composition analysis of control and pGH treated pigs by NMR imaging. J. Anim. Sci. 69(Suppl. 1)308.

Mitchell, A.D., P.C. Wang, H.F. Song and W.F. Schmidt. 1993. Body composition analysis of the pig by magnetic resonance imaging. Human Body Composition:In Vivo Methods, Models, and Assessment.(K.J. Ellis and J.D. Eastman, Eds.) 105~108.

Mitchell, A.D. and J.M. Conway. 1994. Measurement of energy deposition and body composition of pigs by dual-energy x-ray absorptiometry(DEXA). Energy Metab. Farm anim., Proceedings of the 13th symposium. EAAP No. 76:31~34.

Scholz, A.M., A.D. Mitchell, P.C. Wang, H. Song and Z. Yan. 1995a Muscle metabolism and body composition of pigs with different ryanodine receptor genotypes studied by means of 31P nuclear magnetic spectroscopy and 1H magnetic resonance imaging. Archiv. Anim. Breeding. 38:539~552.

Scholz, A.M., A.D. Mitchell, P.C. Wang, H. Song and Z. Yan and W. Scholz. 1995b. NMR study on muscle metabolism and body composition of pigs with different ryanodine receptor genotypes. SMR/ESMRMB P1858.

Mitchell, A.D., J.M. Conway and A.M. Scholz. 1996a. Incremental changes in total and regional body composition of growing pigs measured by dual-energy x-ray absorptiometry. Growth, Dev. Aging. 60:95~105.

Mitchell, A.D., J.M. Conway and W.J.E. Potts. 1996b. Body composition analysis of pigs by dual-energy x-ray absorptiometry. J. Anim. Sci. 74:2663~2671.

Mitchell, A.D. and A.M. Scholz. 1997. Dual-energy x-ray absorptiometry(DXA) analysis of growth and body composition of pigs of different ryanodine receptor genotypes. Archiv. Anim. Breeding 40:11~21.

Mitchell, A.D., M.B. Solomon and T.S. Rumsey. 1997a. Compositional analysis of beef 9, 10, 11-rib sections by dual-energy x-ray absorptiometry. Meat Sci. 47:115~124.

Mitchell, A.D., R.W. Rosebrough and J.M. Conway. 1997b. Body composition analysis of chickens by dual-energy x-ray absorptiometry. Poultry Sci. 76: 1746~1752.

Mitchell, A.D., A.M. Scholz and J.M. Conway. 1998a. Body Composition Analysis of Pigs From 5 to 95kg by Dual Energy X-ray Absorptiometry. In : (M. Alpsten and S. Mattsson, Eds.) International Symposium on In Vivo Body Composition Studies. Appl. Radiat. Isot. 49:521~523.

Mitchell, A.D., A.M., Scholz. 1998. Energy Deposition and Body Composition Measurement of Pigs of Different Ryanodine Receptor Genotypes by Dual Energy X-ray Absorptiometry. In : (McCracken, Unsworth and Wylie, Ed.) Energy Metabolism Farm Animals(Proceedings of the 14th Symposium on Energy Metabolism, September 14-20, 1997, Newcastle, Northern Ireland). CAB International p249~252.

Scholz, A.M., A.D. Mitchell, P.C. wang, H. Song and Z. Yan. 1998. In Vivo Muscle Glycogen and Phosphate Metabolism in Relation to Body Composition in Young Pigs of Different Genotypes Studied by Nuclear Magnetic Resonance Spectroscopy. 6th World Congress on Genetics Applied to Livestock Production(Armidale, Australia, 11th-16th January 1998).

Mitchell, A.D., A.M. Scholz and J.M. Conway. 1998b. Body Composition Analysis of Small Pigs by Dual-Energy X-ray Absorptiometry. J. Anim. Sci.(in press)

Mitchell, A.D., A.M. Scholz, V.G. Pursel and C.M. Evock-clover. 1998c. Composition Analysis of Pork Carcasses by Dual-Energy X-ray Absorptiometry. J. Anim. Sci.(in press)

Mitchell, A.D., V.G. Pursel and G. Bee. 1998d. Evaluation of body composition of transgenic pigs using dual-energy x-ray absorptiometry(DXA). J. Anim. Sci.(Suppl. 1, in press)

Mitchell, A.D., P.C. Wang, H.F. song, J.M. Conway and A.M. Scholz. 1998e. Application of magnetic resonance imaging and dual-energy x-ray absorptiometry for the measurement of body composition of pigs. WCAP, Seoul Korea, June 28~July 4, 1998.