Arch. Tierz., Dummerstorf **46** (2003) 2, 199-211

Ludwig-Maximilians-Universität München, Lehr- und Versuchsgut, Oberschleißheim[1], United States Department of Agriculture, Growth Biology Laboratory, Beltsville, MD, USA[2], Howard University Hospital, Department of Radiology, Washington, DC, USA[3]

ARMIN M. SCHOLZ[1], ALVA D. MITCHELL[2], HUAFU SONG[3] and PAUL C. WANG[3]

^{13}C nuclear magnetic resonance spectroscopy – a non-invasive *in vivo* method to measure muscle glycogen metabolism in pigs of different genotypes

Dedicated to Prof. Dr. agr. habil. Dr. h. c. mult. Georg Schönmuth on the occasion of his 75th birthday

Summary

The three *ryanodine receptor 1* gene variants (*NN*: homozygous normal, *Nn*: heterozygous, *nn*: homozygous defective) and the degree of Hampshire origin (0 %, 25 %, 50 %) serve as model for the investigation of the *in vivo* glycogen muscle metabolism in 27 pigs. The pigs originate from 4 different cross-breeding lines with an age varying between 41 and 58 days and a body weight between 7.3 and 19 kg. ^{13}C nuclear magnetic resonance spectroscopy was applied non-invasively *in vivo* and in a few pigs also *post mortem* to study the metabolic processes in the biceps femoris muscle after halothane exposure. In contrast to no visible effects of the halothane challenge test, the heterozygous defective allele carriers showed a drastic reduction in the level of glycogen (57 %) coupled with an increase in body temperature (1.36 °C). Overall, these changes were intermediate compared to the dramatic response in the homozygous *nn* genotype and to the very slow processes in *NN,* considering that the drastic glycogen depletion in the heterozygous genotype occurred after a rather long time of halothane exposure (>20 min). In addition, pigs with the highest degree of Hampshire origin (50% → *RN*$^{-}$ allele frequency: ~31.5 %) showed the slowest glycogen depletion compared to pigs with a lower degree of Hampshire origin (0 or 25 %).

Key Words: pigs, muscle metabolism, nuclear magnetic resonance spectroscopy, RyR1, Hampshire effect

Zusammenfassung

Titel der Arbeit: **^{13}C Magnetresonanz-Spektroskopie – Eine nicht-invasive *In-vivo*-Methode zur Messung des Muskel-Glykogen-Stoffwechsels in Schweinen verschiedener Genotypen**
Die drei *Ryanodin-Rezeptor-1*-Genvarianten (*NN*: homozygot normal, *Nn*: heterozygot und *nn*: homozygote Defektallelträger) sowie der Hampshire-Genanteil (0 %, 25 %, 50 %) dienen als Modell für die Untersuchung des Glykogen-Muskelstoffwechsels *in vivo* bei 27 Schweinen. Die Tiere stammen aus 4 verschiedenen Kreuzungslinien mit einem Alter zwischen 41 und 58 Tagen sowie einem Körpergewicht zwischen 7,3 und 19 kg. Ein ^{13}C-Magnetresonanz-Spektroskopie-Experiment wurde nicht-invasiv *in vivo* und bei einigen Tieren *post mortem* durchgeführt, um Stoffwechselvorgänge im M. biceps femoris nach Stressauslösung mittels Halothan untersuchen zu können. Im Gegensatz zu den in der Regel nicht sichtbaren Auswirkungen nach dem Halothanmasken-Test zeigten die heterozygoten *RyR1*-Defektallel-Träger einen drastischen Abfall des Glykogenniveaus (57 %) bei gleichzeitigem Anstieg der Körpertemperatur (1,36 °C). Generell lagen die heterozygoten Tiere mit dieser Reaktion in der Mitte zwischen den beiden homozygoten Genotypen, da der drastische Rückgang der Glykogenkonzentration erst nach einer relativ langen Halothangabe (>20 Minuten) erfolgte. Im Vergleich wies der homozygote *RyR1*-normale Genotyp einen sehr langsamen Glykogenabbau auf, während in den homozygoten *RyR1*-Defektallelträgern ein sehr schneller Glykogenabbau vonstatten ging. Schweine mit einem hohen Hampshire-Genanteil (50 % → *RN*$^{-}$ -Allelfrequenz: ~31.5 %) zeigen hingegen im Vergleich zu Tieren mit einem geringeren Hampshire-Genanteil (0 %, 25 %) den langsamsten Glykogenabbau.

Schlüsselwörter: Schwein, Muskelstoffwechsel, Magnetresonanz-Spektroskopie, RyR1, Hampshire-Effekt

Introduction

Glycogen, glucose, phosphocreatine and ATP belong to the major energetic components of the muscle metabolism. The glycogen levels at the time of slaughter and the speed of glycogenolysis *post mortem* are very important factors for the final pork quality -- especially for the processing quality of cured-cooked ham (FEDDERN et al., 1994; LAUBE, 2000; LE ROY et al., 2000 a, b). Only recently the originally called Rendement Napole (RN) gene or Hampshire effect gene -- associated with the above effects on meat quality -- has been localized at swine chromosome 15q2.5 (LOOFT et al., 2000, MILAN et al., 2000). Responsible mutations are a non-conservative substitution "R200Q" (MILAN et al., 2000 → high glycogen content → *RN⁻*) and the "I199V" substitution site (CIOBANU et al., 2001 → low glycogen content) in the PRKAG3 gene, which encodes a muscle-specific isoform of the protein kinase adenosine monophosphate-activated regulatory γ3–subunit. Results of biopsy studies *post mortem* show that low glycogen reserves at the time of slaughter result in poor meat quality (MÜLLER, 1994; STALDER et al., 1998). Pigs with Hampshire origin have a higher glycolytic potential and glycogen content *post mortem* than pigs without Hampshire "genes" (WASSMUTH and GLODEK, 1992; FEDDERN et al, 1994). *In vivo* no differences among different stress susceptible breeds and lines were found by LINDNER (1991), while LE ROY et al. (2000b) demonstrated significant differences in the glycolytic potential (GP=2([glycogen]+[glucose-6-phosphate]+[glucose])+[lactate]) among the three RN genotypes (RN^-/RN^-, RN^-/rn^+, rn^+/rn^+) with a dominant effect of RN^-. However, even *in vivo* biopsy studies do not allow a non-invasive and continuous investigation of muscle metabolic processes.

In addition to the above mentioned major gene effect on meat quality exists the even better studied Malignant Hyperthermia Syndrome (MHS) gene effect. The susceptibility to Malignant Hyperthermia is associated with an alteration of C1843 to T1843 in the DNA encoding the Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum (also called ryanodine receptor 1 -- *RYR1*) at swine chromosome 6p12-q22 (FUJII et al., 1991). Since dam lines (and to some extent sire lines) are being rigorously cleared from all *RYR1* defective allele (*n*) carriers in various pig breeding programs, it will be necessary to use supplementary phenotypic selection criteria based on the thorough knowledge of metabolic processes to achieve further genetic improvements.

Nuclear Magnetic Resonance (NMR) techniques like spectroscopy and imaging are very powerful tools to manifest such phenotypic selection criteria (RODEN and SHULMAN, 1999; BAULAIN and HENNING, 2001; MITCHELL and SCHOLZ, 2001). Carbon (^{13}C) and Phosphorous (^{31}P) NMR spectroscopy offer the opportunity to measure non-invasively, continuously *in vivo* and *post mortem* changes in glycogen, glucose, creatine (→ ^{13}C); phosphocreatine (PCr), inorganic phosphate (Pi), ATP and pH (→ ^{31}P) directly in relative and absolute quantitative terms over an "unlimited" period of time.

Previous *in vivo* ^{31}P NMR studies established that especially homozygous stress susceptible pigs (*RyR1 = nn*) respond to muscle stressors (like for example halothane) with faster phosphocreatine (PCr) decay, faster declining pH level (indicating a higher glycogen depletion), and a simultaneous increase of inorganic phosphate (Pi) combined with an increased adenosine triphosphate (ATP) depletion. Controversial inferences were made for the metabolism at rest and metabolic response of

heterozygous pigs (*Nn*) in comparison with the normal (*NN*) and homozygous stress susceptible genotype (*nn*), which were mainly caused by confounding *RYR1**line effects (GEERS et al. 1992a,b, 1996; JANZEN et al. 1994; SCHOLZ et al., 1995; KOHN, 1997; SCHOLZ, 2002).
The purpose of this ^{13}C NMR spectroscopy study was to test *in vivo* and to some extent *post mortem* whether the muscle metabolites (glycogen, creatine) monitored continuously and non-invasively during halothane exposure, would provide more basic knowledge for the effects of single or multiple gene polymorphisms like the ryanodine receptor 1 mutation or the Hampshire gene effect (*RN*$^-$ or *PRKAG3*).

Materials and Methods

The ^{13}C NMR study was performed with a Varian 4.7 T 33 cm horizontal bore magnet. The RyR1 genotypes (*NN*, *Nn*, *nn*) of 27 cross-bred pigs were identified by using a polymerase chain reaction technique (BRENIG and BREM, 1992; FÖRSTER et al., 1992) according to the procedure described in SCHOLZ et al. (1995). They originated from 4 different cross-breeding lines of the Iowa State University Experimental Station with an age between 41 and 58 days and a body weight between 7.3 and 19 kg at the time of the experiment:

I (line 730) → 5/8 Duroc, 1/4 Landrace, 1/8 Yorkshire origin; n = 9; (4 *Nn* - 5 *nn*) → 0 % Hampshire → 0 % Pietrain;
II (line 390) → 1/2 Hampshire, 1/4 Yorkshire, 1/8 Pietrain, 1/8 Spotted origin; n = 7; (4 *NN* - 2 *Nn* - 1 *nn*) → 50 % Hampshire → 12.5 % Pietrain;
III (line 700) → 1/4 Duroc, 1/4 Hampshire, 1/4 US-Landrace, 1/8 Spotted, 1/8 Yorkshire origin; n = 9; (3 *NN* - 4 *Nn* - 2 *nn*) → 25 % Hampshire → 0 % Pietrain;
IV (line 530) → 1/2 Spotted, 1/4 Yorkshire, 1/8 Pietrain, 1/8 Duroc origin; n = 2; (2 *nn*) → 0 % Hampshire → 12.5 % Pietrain.

Among the pigs were 17 male (4 *NN* - 9 *Nn* - 4 *nn*) and 10 female (3 *NN* - 1 *Nn* - 6 *nn*). The pigs were kept in groups of 6-8 animals at the USDA-ARS swine facilities in Beltsville, MD. A number of 21 pigs (6 *NN* - 8 *Nn* - 7 *nn*) underwent an *in vivo* study, while 6 animals (1- 2- 3) were studied *in vivo* and *post mortem* up to one hour after time of death.
Food was withheld for 18 hours prior to the experiment.
The intramuscular glycogen content *in vivo* was measured after a calibration with an external standard (phantom, Figure 1) -- correspondingly to the description by PRICE et al. (1996). The standard consists of pure glycogen[1)] (2 g), KCl (5 g) and H_2O (dist.) with a total volume of 268.5 ml. The molar mass for the "pure" glycogen was given as 179.7392 g/mol (contents: 99.5 % glucose at 180.15 g/mol and 0.5 % H_3PO_4 at 97.99 g/mol). The resulting glycogen content within the standard was 41.4422 µmol/g (0.00744879 g glycogen/ g solution):
■ $41.4422 = 2/268.5/179.7392 \bullet 1000000$.
Due to the juvenile age of the animals, a „fat correction" necessary for adult animals (BUCHLI and BOESIGER, 1993) was not used in the present study.

[1)] Sigma-Aldrich, Product-Nr.: G8876

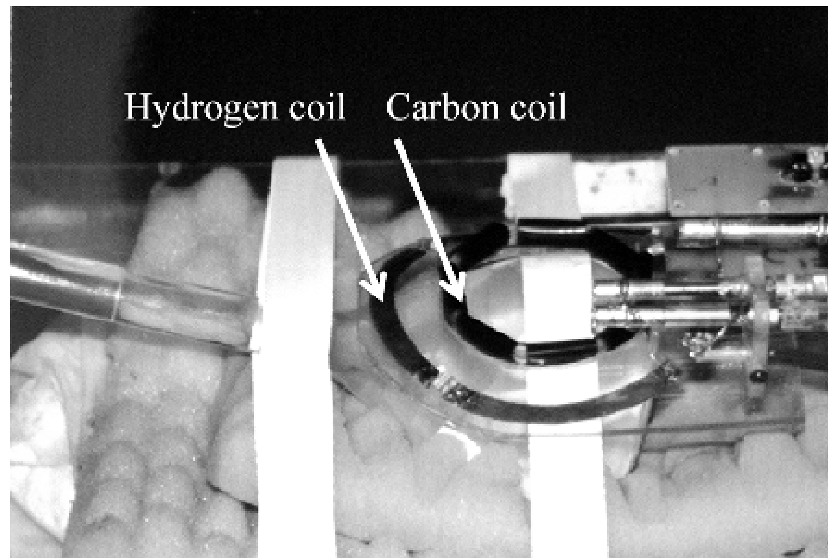

Fig. 1: Experimental setup for the ^{13}C-NMR equipment with proton decoupling [Experimentelle Anordnung für die ^{13}C-Magnetresonanz-Wasserstoff-Kohlenstoff-Entkopplungs-Spule]

The signal of pure glycogen *in vitro* is divided into three peaks in a NMR spectrum (Figure 2). The signals of C1 and C6 are separately visible in the spectra due to their positions in the glucose molecules. The signals of the nuclei C2 - C5 are combined within one peak and could not be split up in more detail under the given experimental conditions. Since the *in vivo* signals of the C1 nuclei of glycogen are not visibly influenced by other signals (C containing metabolites like glucose or fatty acids), the measurement procedure was set up in such a way to detect the signal of the C1 glycogen at the position 100.5 ppm within the spectrum (Figure 2).

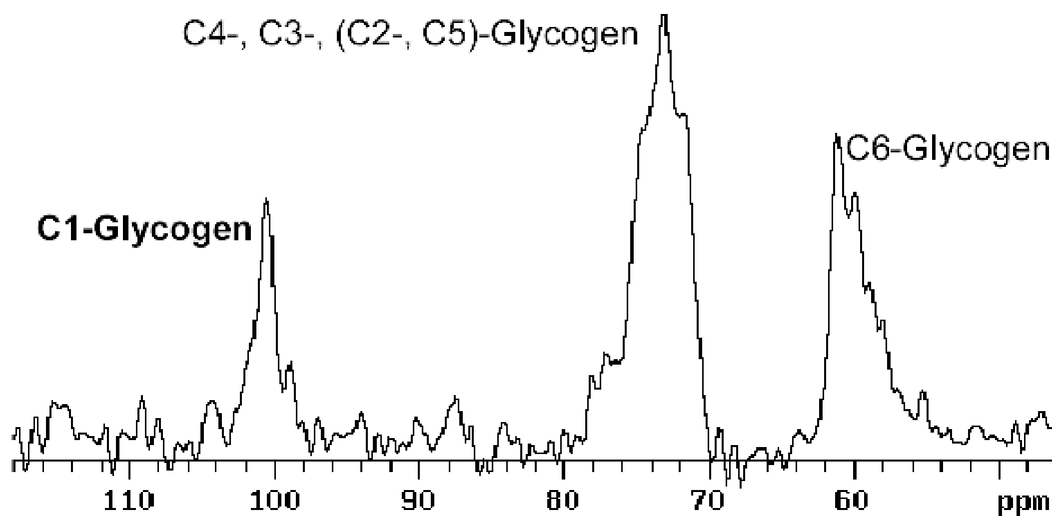

Fig. 2: *In vitro* ^{13}C NMR spectrum of pure glycogen with C1 at 100.5 ppm [*In-vitro*-^{13}C Magnetresonanz-Spektrum von reinem Glykogen mit C1 bei 100,5 ppm]

A series of ^{13}C spectra were collected every 4.5 minutes with an acquisition time of 0.026 s for each free induction decay, a recovering time of 0.150 s, spectral width 20000 Hz, and total of 1480 acquisitions for each spectrum at 50.295 MHz with proton decoupling (Figure 3). The inner coil (55 mm diameter) is the carbon coil and the outer coil (96 mm diameter) is the proton (hydrogen) coil (Figures 1 and 4). Broad band

proton noise decoupling was activated only during the acquisition time to avoid the development of the nuclear Overhauser effect.

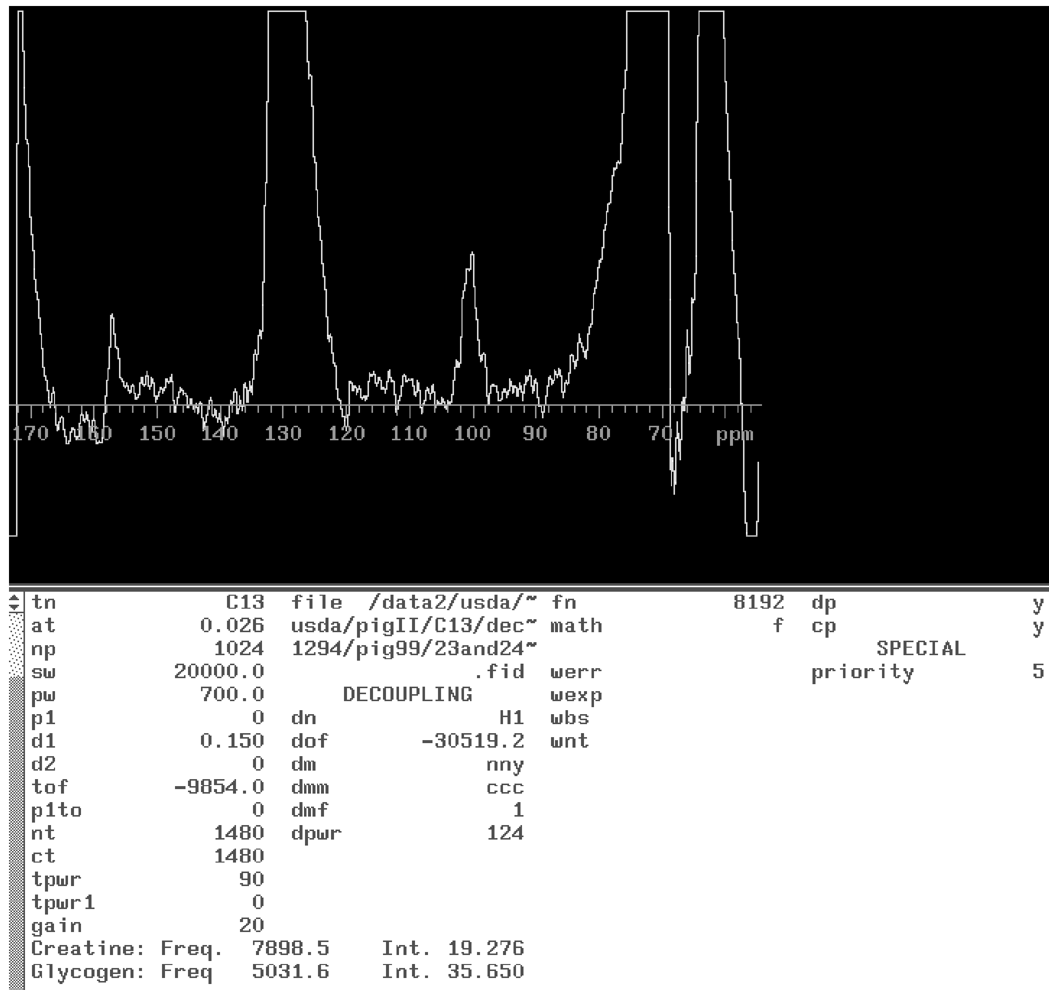

Fig. 3: Screen dump for acquisition settings and example for a Glycogen spectrum *in vivo* (C1-Glycogen at 100.5 ppm and Creatine at 157 ppm) [Bildschirm-Kopie der Aufnahmeparameter und Beispiel für ein Glykogen-Spektrum *in vivo*]

The hardware and software settings for calibration were used during the *in vivo* experiment in order to measure the glycogen concentration(s) during the experiments. Ten minutes prior to every spectroscopy experiment, the pigs were sedated by using a mixture of Ketamine (100 mg/10 kg body weight), Telazol and Rompun (50 mg/10 kg body weight) and positioned in a cradle. The ^{13}C spectra were collected *in vivo* by placing the proton-carbon coils above the skin of the biceps femoris muscle (Figure 4). The measurement region (M. biceps femoris) is always positioned at the center of the magnet for better magnetic field homogeneity. During the experiment, halothane (3 Volume% per 3 Liters O_2/minute) was used to trigger a metabolic stress situation according to the former halothane mask test procedure (EIKELENBOOM and MINKEMA, 1974), though the time of halothane administration with ~ 30 minutes differed largely from the original duration of the test (≤5 minutes). The acquisition of spectra *in vivo* was always started 18 minutes before halothane administration in order to provide data characterizing the metabolism under resting conditions. Therefore, the mean of the first four spectra was normalized to 100% as a background reference. A typical spectrum is shown in Figure 5. Furthermore, at least 5 spectra were collected

after stopping the halothane administration to monitor either the recovering of the metabolism *in vivo* or changes *post mortem*. Halothane administration was stopped immediately -- before reaching the designated time limit of 30 minutes halothane administration -- if a significant drop in glycogen was observed. Thus, the total number of spectra depended on the effect of the anesthesia. The areas (integrals) of the C1 glycogen signal -- which are proportional to the actual present glycogen in the muscle -- were calculated after a “baseline” correction.

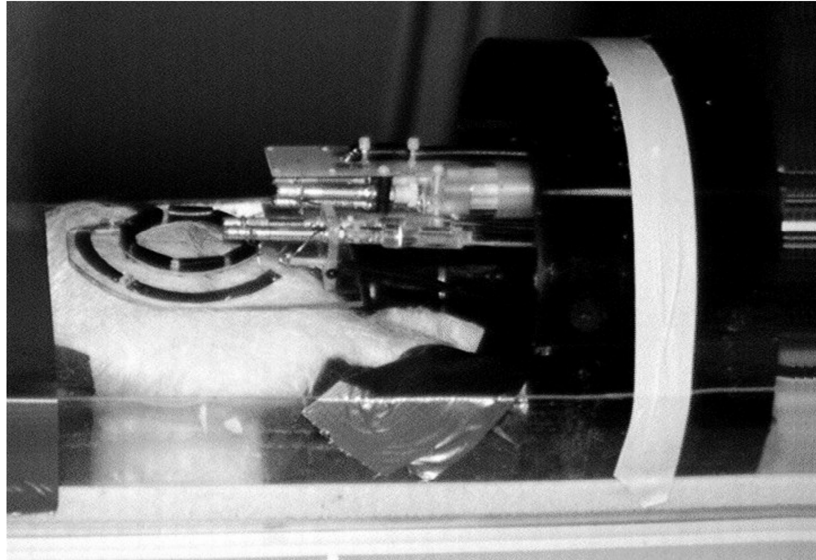

Fig. 4: ^{1}H-^{13}C-coil placement above the biceps femoris muscle *in vivo* [Platzierung einer ^{1}H-^{13}C-Spule über dem M. biceps femoris *in vivo*]

In order to study the effects of *RyR1* genotype and Hampshire origin on the metabolic response, the following General Linear Model (SAS 8.1) was applied:

$Y_{ijkl} = R_i + L_j + S_k + E_l + R_i \bullet E_l + \beta P\,(P_{ijkl} - \bar{P}) + e_{ijkl}$

- Y_{ijkl} = observation
- R_i = *RyR1*-genotype (fix), i = 1 - 3
- H_j = degree of Hampshire origin (fix), j = 1 - 3 (0 %, 25 %, 50 %)
- S_k = gender (fix), k = 1 - 2 (male, female)
- E_l = finals status (fix), l = 1 - 2 (*in vivo, post mortem*)
- $R_i \bullet E_l$ = interaction effect between R_i und E_l (fix)
- P_{ijkl} = degree of Pietrain origin (covariate)
- e_{ijkl} = random error.

Final status was not used in the statistical model for the trait “metabolic level at rest”. Age, body weight, and time of halothane exposure did not affect the response intensity. The theoretical gene portions of the other breeds present in the 4 cross-breeding lines like Duroc, US-Landrace, Spotted, and Yorkshire were not considered in this model due to an unfavorable class structure.

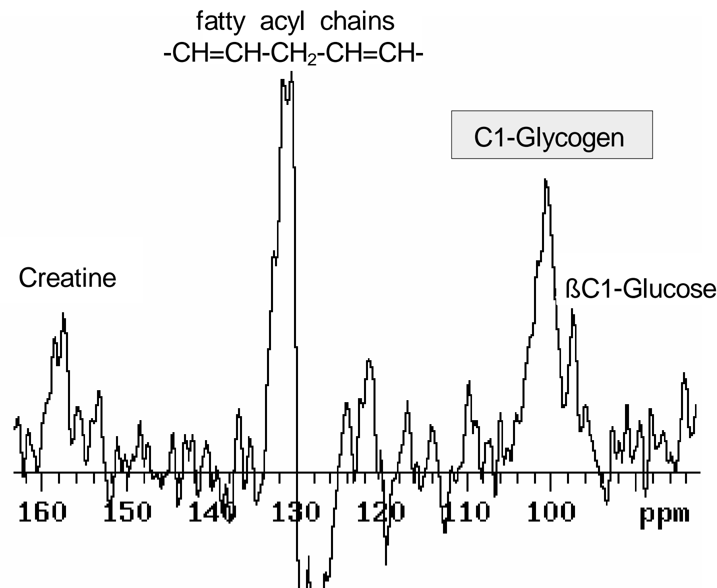

Fig. 5: Example for a typical ^{13}C spectrum at the C1 Glycogen position *in vivo* [Beispiel für ein typisches ^{13}C-Spektrum an der C1-Glykogen-Position *in vivo*]

Results

Besides the significant effect of the *RyR1* genotype (p = 0.007, Table 1), the gender affected the glycogen level at rest (start) to a large extent (p = 0.0057). Both effects together explain 41.5 % of the variation in the glycogen level at rest, while unexpectedly the degree of Hampshire origin had no significant effect on the glycogen level at rest -- with only a slightly higher level in pigs with the highest degree of Hampshire origin (Ha = 50%, Table 2).

Table 1
Comparison of the results from ^{13}C NMR spectroscopy for the *RyR1* genotypes (LSM ± SEE#) [Vergleich der Ergebnisse aus der ^{13}C Magnetresonanz-Spektroskopie für die RyR1-Genotypen]

	NN	***Nn***	***nn***
Glycogen at start (µmol/g)#	72.21 ± 4.41[a]	73.36 ± 4.22[a]	55.31 ± 3.71[b]
n (total)	7	10	10
Glycogen change (%) *in vivo*	-19.10 ± 4.36[a]	35.03 ± 3.37[b]	-33.05 ± 3.80[b]
Creatine change (%) *in vivo*	-7.86 ± 12.74	-1.47 ± 9.8	+10.99 ± 11.09
Temperature change (°C) *in vivo*	+0.28 ± 0.54	1.36 ± 0.41	+1.59 ± 0.47
Halothane (min) *in vivo*	34.85 ± 5.28[a]	21.19 ± 4.07[a]	6.53 ± 4.60[b]
n (*in vivo* study)	6	8	7
Glycogen change (%) *post mortem*	-17.53 ± 11.56[a]	-73.42 ± 6.59[b]	-49.71 ±10.29[ab]
Creatine change (%) *post mortem*	+24.94 ± 33.72	-41.49 ± 19.21	+18.89 ± 30.01
Temperature change (°C) *post mortem*	-1.47 ± 1.42[a]	+5.00 ± 0.81[b]	+5.85 ±1,26[b]
Halothane (min) *post mortem*	54.67 ± 13.98	25.98 ± 7.96	11.13 ± 12.44
n (*post mortem* study)	1	2	3

#Different superscripts characterize significance with p≤ 0.05.

Female pigs had a significantly higher level of glycogen (74.87 ± 3.95 µmol/g) than male pigs (59.04 ± 2.99 µmol/g) at the start of the experiment. After halothane administration, the glycogen level in female pigs appeared to decrease proportionally less than in the male pigs (-35.73 ± 4.71 % vs. -44.87 ± 3.37 %). Despite these proportional discrepancies, significant differences in the glycogen level were still present at the end of the experiment with 48.12 µmol/g for female and 32.54 µmol/g

for male pigs, due to an almost similar glycogen depletion with 26.75 and 26.49 µmol glycogen per gram muscle tissue over a period of approximately 130 minutes. The average glycogen depletion rate after approximately 25 minutes halothane administration amounts to 0.20 µmol/g • min^{-1}.
All *nn* pigs for the *RyR1* genotype showed a significant decline in the muscle glycogen level combined with a slight increase in the creatine level after a very short time of halothane exposure (≈8 min, *in vivo* and *post mortem*). The *Nn* genotype also showed a drastic decline in the glycogen level, but after a significantly longer time of halothane exposure (≈ 22 min, Table 1). The time course of the glycogen depletion shown in Figure 6 reflects the average pattern for each *RyR1* genotype independent of the other effects like the degree of Hampshire or Pietrain origin, and sex. The least squares means (and standard errors of estimation) of the glycogen levels at the start of the experiment and after the stress response shown in Table 1 confirm the average pattern comparing the three ryanodine receptor genotypes. In contrast to a previous ^{31}P experiment (SCHOLZ et al., 1995), where a total recovering of PCr, ATP and pH could be observed in the majority of the pigs after a significant stress response, the glycogen level did not return to the starting level as shown in Figure 6. Only in a very few pigs did the glycogen level recover almost completely after a decline was observed due to halothane exposure.
The results of the *post mortem* study (Table 1) do not allow strait-forward conclusions due to the small number of animals investigated. They do, however, confirm in tendency the *in vivo* results and are in agreement with the results of a previous ^{31}P NMR study for the change of the body temperature (SCHOLZ et al., 1995).

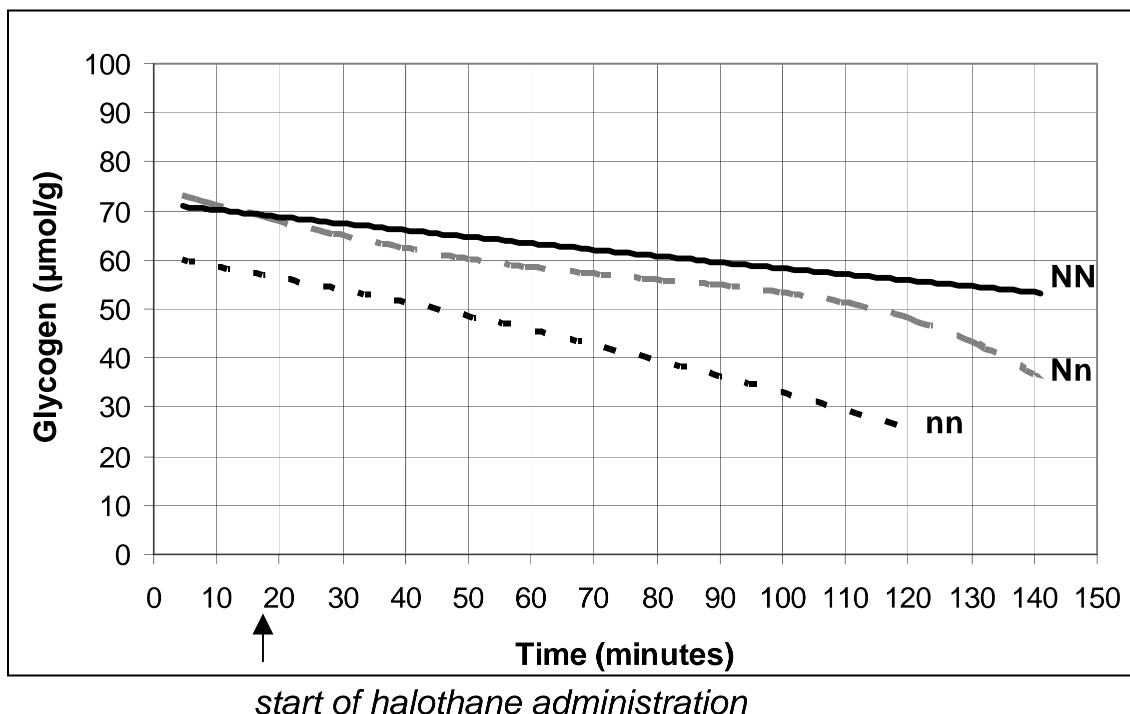

Fig. 6: Average time course of the glycogen level in pigs of different ryanodine receptor 1 genotypes (NN: n=7, Nn: n=10, nn: n=10) [Durchschnittlicher Verlauf des Glykogen-Niveaus in Schweinen unterschiedlicher *RyR1*-Genotypen]

For the comparison of the metabolic response among the different Hampshire origins, an interaction effect between the degree of Hampshire origin and final status was not considered in the model (Table 2).

Table 2
Comparison of the results from ^{13}C NMR spectroscopy depending on the degree of Hampshire origin (LSM ± SEE#) [Vergleich der Ergebnisse aus der ^{13}C MR-Spektroskopie in Abhängigkeit vom Hampshire-Genanteil]

	Ha 0 % (*4 Nn/ 7 nn*)	*Ha 25 %* (*3NN/ 4 Nn/ 2 nn*)	*Ha 50 %* (*4 NN/ 2 Nn/ 1nn*)
Start Glycogen (µmol/g)	66.70 ± 4.36	65.44 ± 4.25	68.90 ± 4.85
Glycogen change (%)	-44.74 ± 4.45 $^{ab°}$	-48.50 ± 4.79 ^{b}	-20.68 ± 9.47 ^{a*}
Creatine change (%)	+15.93 ± 13.01	-6.16 ± 28.55	-16.36 ± 14.03
Temperature change (°C)	+1.29 ± 0.55	+1.79 ± 0.59	+2.90 ± 1.21
Halothane exposure (min)	23.02 ± 4.29	26.63 ± 4.19	22.53 ± 4.79
Final Glycogen (µmol/g) +)	36.86	33.70	**54.65**
Total n (*n post mortem*)	11 (*4*)	9	7 (*2*)

#) Different superscripts characterize significance with p≤ 0.05.
Least Squares Means (LSM) with ° and * exceed the significance level minimally with p ≤ 0.07.
+) calculated from the Glycogen level at „start" and Glycogen depletion %

Discussion

The homozygous defective genotype (*nn*) and the heterozygous genotype (*Nn*) for the *RyR1* gene showed dramatic changes in their muscle energy metabolites (glycogen) after halothane exposure, while the concentration of muscle glycogen altered only to a small extent in the *NN* genotype (Table 1). However, as described by SCHOLZ et al. (1995) and KOHN (1997) for the results of ^{31}P NMR studies, the *in vivo* metabolic processes in the *Nn* genotype seem to be more similar to the *NN* genotype due to the very long time of halothane exposure until there was a visible -- but drastic -- response in the ^{13}C spectra. The time of response onset seems to depend on the duration of halothane exposure (dose) and on the number of defective alleles. While the metabolic situation (glycogen content) changes drastically in both defective allele carriers, there is only a very slow and less drastic response in *NN*.

The *nn* genotype showed already at rest (start) a lower glycogen level than *NN* and *Nn*. In contrast to these results, LINDNER (1991) found no significant differences in the glycogen level (glycolytic potential) at rest between stress stable and stress susceptible pigs using *in vivo* biopsy samples. Behavioral differences observed in a few pigs prior to anesthesia may have resulted in different 'starting values' among the genotypes. Differences among all three genotypes became more evident *post mortem*, though the *Nn* genotype showed an even larger response (glycogen depletion %) than *nn* (Table 1).

The following glycogen depletion rates result for the three RyR1 genotypes by considering *in vivo* and *post mortem* results together:

15.47 µmol/g during in the average 135 minutes for ***NN*** → 0.11 µmol/g • min^{-1};
43.16 µmol/g during in the average 130 minutes for ***Nn*** → 0.33 µmol/g • min^{-1} and
26.71 µmol/g during in the average 100 minutes for ***nn*** → 0.27 µmol/g • min^{-1}.

However, when comparing the glycogen depletion rates, it must be kept in mind that the time of halothane exposure differed significantly between *nn* with 6.53 (11.13) minutes vs. 21.19 (25.98) minutes for *Nn*.
Mainly in the *NN* genotype and partly in *Nn*, the glycogen level decreased independent of a change (increase) in creatine (Table 1). Obviously, the "energy" originates in those cases first from glucose and glycogen and with a time delay from phosphocreatine. These observations agree with the results from the previous ^{31}P NMR study (SCHOLZ et al., 1995), where a decrease in the pH value -- as an indirect indicator for the time pattern of glycolytic processes -- occurred independent of changes in the concentration of phosphocreatine. Generally, changes in the level of creatine were not significantly different from zero with a very wide range of variation (Table 1, Table 2). The level of creatine increased very rapidly, specifically in a few *nn* pigs immediately after starting the halothane administration. This increase led to an immediate stop of the halothane administration in order to maintain the metabolic homoeostasis in the stress susceptible pigs. An increase in the creatine level was assumed to signal a consumption of phosphocreatine, which indicates energy depletion. However, the glycogen level decreased continuously, independent of a timely restricted increase in the creatine level.
Since MILLER et al. (2000) describe a high frequency of the dominant *RN*⁻ allele in American Hampshire (p=0.63), it can be assumed that the likelihood of the presence for this allele is highest in the group with theoretically 50 % Hampshire origin (Ha=50% → *RN*⁻ frequency = 31.5 %). Therefore it is not surprising that this group showed the lowest glycogen depletion (%), though the body temperature increased in tendency more than in the two other groups (Table 2). These two groups with 0 % or 25 % Hampshire origin do not differ in the glycogen depletion suggesting the absence or a very low frequency of the *RN*⁻ allele (theoretically 0 % or 15.75 %). In contrary to the results of the studies by LEBRET et al. (1999 -- biopsy sampling 5 min *post mortem)* and LE ROY et al. (2000b -- biopsy sampling *post mortem* and *in vivo*) that pigs with the dominant *RN*⁻ allele have an increased glycogen level or glycolytic potential, the pigs with the highest degree of Hampshire origin in the present ^{13}C NMR study did not show a significant higher glycogen concentration at the start of the experiment compared to the two other groups. This observation would support the results by LE ROY et al. (2000), that the glycogen level -- or more exactly the glycolytic potential -- *in vivo* is not generally increased in all muscles -- especially not significantly in "red" muscles like the *semispinalis capitis*. However as found in this ^{13}C NMR study within the *biceps femoris* muscle, the glycogen depletion is significantly slower in the *RN*⁻ carriers under a stress situation (like for example the slaughtering process; other manipulation *in vivo*) or *post mortem*. MILAN et al. (2000) indicate, in agreement with the results of this ^{13}C NMR study, that the R200Q substitution could be a dominant negative mutation inhibiting Adenosine Monophosphate (AMP) activation and glycogen degradation by interfering with multiple isoforms of PRKAG.
In addition to the single effects of *RyR1* genotype and degree of Hampshire origin (*RN* gene effect), an interaction effect between *RyR1* and *RN* can not be rejected. LE ROY et al. (2000a) support this thesis with an interaction of *RyR1* and *RN* for meat quality traits. Carriers of the *RyR1 d*efective allele (*n*) and of the dominant *RN*⁻ allele (characterized by a higher muscle glycogen content/ slower glycogen depletion) show

a lower pH value 35 minutes *post mortem* and a lower drip loss than carriers of the *RyR1* defective allele (*n*) combined with the „normal" rn^+ allele (rn^+/rn^+, characterized by a „normal" muscle glycogen content). In contrary, carriers of the RN^- allele showed a lower cooking loss in combination with the homozygous „defective" *RyR1 g*enotype (*nn*) compared to the combination with the homozygous „normal" *RyR1* genotype (*NN*). That means also for this ^{13}C NMR study that the different (unknown) combination of RN^- and rn^+ alleles with *N* or *n* alleles of the *RyR1* gene will affect the glycogen metabolism differently within and among the different Hampshire origin groups.
^{13}C (and ^{31}P) NMR spectroscopy offer an appropriate tool to measure muscle metabolic processes more profoundly than biopsy studies would allow to do. The metabolic processes can be monitored *in vivo,* non-invasively over a long period of time. Thus -- in combination with DNA and/or RNA typing techniques -- NMR spectroscopy can provide more knowledge about the "phenotypic" function of single or multiple gene polymorphisms *in vivo* and finally allows a selection for *in vivo* "meat" quality.

Acknowledgement

This research work of A.M. Scholz was funded by the Alexander von Humboldt-Foundation (Bonn, Germany) and by the OECD Co-operative Research Program: Biological Resource Management for Sustainable Agricultural Systems.

References

BAULAIN, U.; HENNING, M.:
Untersuchungen zur Schlachtkörper- und Fleischqualität mit Hilfe von MR-Tomographie und MR-Spektroskopie. Arch. Tierz., Dummerstorf **44** (2001), 181-192
BRENIG, B.; BREM, G.:
Molecular cloning and analysis of the porcine "halothane" gene. Arch. Tierz., Dummerstorf **35** (1992), 129-135
BUCHLI, R.; BOESIGER, P.:
Comparison of Methods for the Determination of Absolute Metabolite Concentrations in Human Muscles by ^{31}P MRS. Magnetic Resonance in Medicine **30** (1993), 552-558
CIOBANU, D.; BASTIAANSEN, J.; MALEK, M.; HELM, J.; WOOLLARD, J.; PLASTOW, G.; ROTHSCHILD, M.:
Evidence for New Alleles in the Protein Kinase Adenosine Monophosphate-Activated γ3-Subunit Gene Associated With Low Glycogen Content in Pig Skeletal Muscle and Improved Meat Quality. Genetics **159** (2001), 1151–1162
EIKELENBOOM, G.; MINKEMA, D.:
Prediction of pale, soft, exudative muscle with a non-lethal test for the halothane-induced porcine malignant hyperthermia syndrome. Neth. J. Vet. Sci. **99** (1974), 421-426
FEDDERN, E.; KRIETER, J.; KALM, E.:
Verlauf der postmortalen Glykogenolyse und Merkmale der Fleischbeschaffenheit bei Hampshire-Reinzuchttieren und verschiedenen Kreuzungskombinationen. Arch. Tierz., Dummerstorf **37** (1994), 229-243
FÖRSTER, M.; BRENIG, B.; BREM, G.; WITTMANN, W.; LITTMANN, B.; PESCHKE, W.:
Der MHS-Gentest für die sichere Streßresistenzzüchtung beim Schwein. Schweinezucht und Schweinemast **40** (1992), 4-6
FUJII, J.; OTSU, K.; ZORZATO, F.; DE LEON, S.; KHANNA, V.K.; WEILER, J.; O'BRIEN, P.J.; MACLENNAN, D.H.:
Identification of a mutation in the porcine ryanodine receptor that is associated with malignant hyperthermia. Science **253** (1991), 448-451
GEERS, R.; DECANNIERE, C.; VILLÉ, H.; VAN HECKE, P.; GOEDSEELS, V.; VANSTAPEL, F.; BOSSCHAERTS, L.; DE LEY, J.; ZHANG, W.; JANSSENS, ST.:

In vivo muscle ^{31}P nuclear magnetic resonance spectroscopy during treatment of halothane-sensitive and halothane-nonsensitive pigs. Am. J. Vet. Res. **53** (1992a), 613-616

GEERS, R.; DECANNIERE, C.; VILLÉ, H.; VAN HECKE, P.; GOEDSEELS, V.; BOSSCHAERTS, L.; DE LEY, J.; JANSSENS, ST.; NIERYNCK, W.:
Identification of halothane gene carriers by use of in vivo ^{31}P nuclear magnetic resonance spectroscopy in pigs. Am. J. Vet. Res. **53** (1992b), 1711-1714

GEERS, R.; DECANNIERE, C.; ROSIER, A.; VILLÉ, H.; VAN HECKE, P.; VANDESANDE, F.; JOURQUIN, J.:
Variability of energy metabolism and nuclear T3-receptors within the skeletal muscle tissue of pigs with respect to the halothane gene. J. Anim. Sci. **74** (1996), 717-722

JANZEN, E.G.; GAREAU, P.J.; STEWART, W.A.; TOWNER, R.A.:
The use of in vivo magnetic resonance imaging and spectroscopy to study porcine stress syndrome in young, halothane-susceptible pigs: Preliminary results. Can. J. Anim. Sci. **74** (1994), 37-43

KOHN, G.:
In-vivo-Untersuchungen zur Muskelphysiologie von Schweinen mit Hilfe der Magnet-Resonanz-Spektroskopie. Landbauforschung Völkenrode, Sonderheft **171** (1997), 133 S.

LAUBE, SIGURD:
Die Eignung spezieller Schweinekreuzungen zur Qualitätsverbesserung von Markenschweinefleisch unter besonderer Berücksichtigung von MHS-Status, Hampshirefaktor und intramuskulärem Fettgehalt. Dissertation, TiHo Hannover (2000), 78 S.

LEBRET, B.; LEROY, P.; MONIN, G.; LEFAUCHEUR, L.; CARITEZ, J.C.; TALMANT, A.; ELSEN, J.M.; SELLIER, P.:
Influence of the Three RN Genotypes on Chemical Composition, Enzyme Activities, and Myofiber Characteristics of Porcine Skeletal Muscle. J. Anim. Sci. **77** (1999), 1482–1489

LE ROY, P.; MORENO, C.; ELSEN, J.M.; CARITEZ, J.C.; BILLON, Y.; LAGANT, H.; TALMANT, A.; VERNIN, P.; AMIGUES, Y.; SELLIER, P.; MONIN, G.:
Interactive effects of the HAL and RN major genes on carcass quality traits in pigs: preliminary results. In: Quality of meat and fat in pigs as affected by genetics and nutrition (Eds.: C. Wenk, J.A. Fernández, and M. Dupuis). EAAP publication No. **100** (2000a), 139-142

LE ROY, P.; ELSEN, J.M. ; CARITEZ, J.C.; TALMANT, A.; HERVE, J.; SELLIER, P.; MONIN, G.:
Comparison between the three porcine RN genotypes for growth, carcass composition and meat quality traits. Genet. Sel. Evol. **32** (2000b), 165-186

LINDNER, R.:
Untersuchungen über die Beziehungen zwischen Glykolyse und Fleischbeschaffenheit beim Schwein - zugleich Beschreibung der Basis eines Selektionsexperimentes. Dissertation Universität Hohenheim (1991), 179 S.

LOOFT, CH.; MILAN, D.; JEON, J.T.; PAUL, S.; REINSCH, N.; ROGEL-GAILLARD, C.; REY, V.; AMARGER, V.; ROBIC, A.; KALM, E.; CHARDON, P.; ANDERSSON, L.:
A high-density linkage map of the RN region in pigs. Genet. Sel. Evol. **32** (2000), 321-329

MILAN, D.; JEON, J.T.; LOOFT, CH.; AMARGER, V.; ROBIC, A.; THELANDER, M.; ROGEL-GAILLARD, C.; PAUL, S.; IANNUCCELLI, N.; RASK, L.; RONNE, H.; LUNDSTRÖM, K.; REINSCH, N.; GELLIN, J.; KALM, E.; LE ROY, P.; CHARDON, P.; ANDERSSON., L.:
A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. Science **288** (2000), 1248-1251

MILLER, K.D.; ELLIS, M.; McKEITH, F.K.; BIDNER, B.S.; MEISINGER, D.J.:
Frequency of the Rendement Napole RN^{-} allele in a population of American Hampshire pigs. J. Anim. Sci. **78** (2000), 1811–1815

MITCHELL, A.D.; SCHOLZ, A.M.:
Techniques for Measuring Body Composition of Swine (Chapter 41). In: Swine Nutrition, 2nd Edition (Eds.: A.J. Lewis and L. Lee Southern), CRC Press, Boca Raton, London, New York, Washington, D.C. (2001), 917-960

MÜLLER, E.:
Untersuchungen über die Beziehungen von Fleischanteil, Fleischbeschaffenheit und Streßresistenz bei verschiedenen Schweineherkünften 2. Mitteilung: Vergleich von Enzymaktivitäten und Metabolitkonzentrationen des Glykogenabbaus im M. longissimus dorsi. Züchtungskunde **66** (1994), 349-358

PRICE, TH.B.; PERSEGHIN, G.; DULEBA, A.; CHEN, W.; CHASE, J.; ROTHMAN, D.L.; SHULMAN, R.G.; SHULMAN, G.I.:
NMR studies of muscle glycogen synthesis in insulin-resistant offspring of parents with non-insulin-dependent diabetes mellitus immediately after glycogen-depleting exercise. Proc. Natl. Acad. Sci. (USA) **93** (1996), 5329-5334

RODEN, M.; SHULMAN, G.I.:

Applications of NMR Spectroscopy to study muscle glycogen metabolism in man. Annu. Rev. Med. **50** (1999), 277–290

SCHOLZ, A.M.; MITCHELL, A.D.; WANG, P.C.; SONG, H.; YAN, Z.:
Muscle metabolism and body composition of pigs with different ryanodine receptor genotypes studied by means of ^{31}P nuclear magnetic resonance spectroscopy and ^{1}H magnetic resonance imaging. Arch. Tierz., Dummerstorf **38** (1995), 539-552

SCHOLZ, A.M.:
In-vivo-Methoden zur Analyse von Muskelstoffwechsel und Körperzusammensetzung beim Schwein unter besonderer Berücksichtigung genetischer Einflüsse. Habilitationsschrift, Tierärztliche Fakultät der Ludwig-Maximilians-Universität München (2002), 402 S.

STALDER, K.J.; MAYA, J.; CHRISTIAN, L.L.; MOELLER, S.J.; PRUSA, K.J.:
Effects of Preslaughter Management on the Quality of Carcasses from Porcine Stress Syndrome Heterozygous Market Hogs. J. Anim. Sci. **76** (1998), 2435-2433

WASSMUTH, R.; GLODEK, P.:
Untersuchungen zum “Hampshirefaktor” in der Fleischbeschaffenheit von Schweinen 3. Mitteilung: Einfluß der Standzeit vor der Schlachtung auf das glykolytische Potential des Muskels und die Fleischbeschaffenheit bei Schweinen mit und ohne Hampshire-Genanteil. Züchtungskunde **64** (1992), 129-135

Received: 2003-01-13

Accepted: 2003-02-26

Author's addresses
PD Dr. ARMIN M. SCHOLZ
Lehr- und Versuchsgut der Tierärztlichen Fakultät der
Ludwig-Maximilians-Universität München
Hubertusstr. 12
D-85764 Oberschleißheim, Germany

E-Mail: A.S@lmu.de

Dr. ALVA D. MITCHELL
United States Department of Agriculture
Growth Biology Laboratory
Beltsville, MD 20705, USA

Prof. Dr. PAUL C. WANG, HUAFU SONG
Howard University
Department of Radiology
Washington, DC 20007, USA