

PORCINE STRESS SYNDROMES

Paul Hermann Heinze

A thesis submitted to the Faculty of Science,
University of the Witwatersrand, Johannesburg,
in fulfillment of the requirements for the
degree of Doctor of Philosophy
Johannesburg 1989

DECLARATION

I declare that this thesis is my own work. It is being submitted for the degree of Doctor in Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University. All experiments were approved by the Animal Ethics Committee of the University of the Witwatersrand (AEC nr. 85/94).

Heinze

PAUL HERMANN HEINZE

18th day of April 1989

ABSTRACT

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The aim of this study was to determine the possible use of blood variables and muscle metabolites in young pigs for the identification of stress susceptibility in pigs compared to the use of the halothane test: the influence of stress resistant (SR) and stress susceptible (SS) pigs on growth, carcass and meat characteristics, and on muscle fibre characteristics; and to determine the influence of halothane exposure and treadmill exercise on blood variables, muscle metabolites and rectal temperature.

Sixty-six pigs were used in this study after being screened using the halothane screening test (4% halothane in oxygen for 3 minutes). At the age of 11 weeks, blood was obtained under manual restraint, and used for the determination of various blood variables. At the age of 13 weeks, muscle was taken under barbiturate anaesthesia from the *M. semitendinosus* for the determination of various muscle metabolites. At the age of 21 weeks, 47 pigs were challenged with halothane. Blood and muscle (*M. semitendinosus*) were obtained for the determination of the various blood variables and muscle metabolites. Nineteen pigs were subjected to treadmill exercise at the age of 21 weeks. Pigs surviving the treadmill exercise were exposed to halothane. For statistical purposes, all pigs that died as a result of the second halothane exposure or as a result of the treadmill exercise, were designated SS, and the survivors SR.

It is concluded that the use of the halothane test is superior to tests using blood variables and muscle metabolites. SS pigs have certain advantageous carcass and meat characteristics, but the production of PSE meat negates these advantages. It is advisable to identify SS pigs using the halothane test, and to keep the SS pigs from breeding herds. The blood variables, muscle metabolites and rectal temperatures indicate that both halothane exposure and treadmill exercise are perceived as stressful situations. The results suggest that the mechanisms of stimulation of malignant hyperthermia, glycolysis and glycogenolysis are different on exposure to halothane and treadmill exercise.

The SS pigs had a similar average daily gain than the SR pigs, but a lower feed conversion rate than the SR pigs. The SS pigs had higher slaughter-out percentages than the SR pigs, and a lower chilling loss. The SS pigs had thinner backfats and were shorter, and produced pale, soft,

oxidative (PSE) meat, which was more tender. The SS pigs were found to have higher percentages of white muscle fibres, but lower red and intermediate muscle fibre percentages than the SR pigs.

Major differences were found in the blood variables between halothane exposed and treadmill exercised pigs. The treadmill exercise resulted in more severe muscle damage, and was perceived as being more stressful. Halothane exposure resulted in a shift of fluid from the vascular compartment into the extravascular spaces. These changes were exacerbated in SS pigs.

The measurements of muscle metabolites indicated a higher degree of stimulation of glycolysis and glycolysis during treadmill exercise. This was also found in the SR pigs, but the changes in the SS pigs were more severe, with an indication of a higher degree of anaerobic metabolism during halothane anaesthesia in SS pigs. No difference in rectal temperature was found between SR and SS pigs on exposure to stress. Treadmill exercise generally resulted in higher rectal temperatures than halothane exposure.

This thesis is dedicated to my father, Horst Norbert Paul Heinze (1925-1988), for the opportunities given to me, his loyal support, motivation and enthusiasm.

ACKNOWLEDGMENTS

To the Holy Father, for the ability and time given to complete this study, I express my deepest gratitude.

I would also like to express my appreciation towards the following institutions and persons:

The Department of Agriculture and Water Supply, and the Animal and Dairy Science Research Institute for the use of the data from facet VS 3121/50/1/11 for this study.

The University of the Witwatersrand for allowing me to register as a student for post graduate study.

Prof. G. Mitchell, my supervisor, for his interest in the study, his support and constructive criticism.

Dr. R.T. Naudé for his advice and interest shown in the study.

Mrs. J.D. Snyman and F. Visser, Mr. O. Bergé, C.D. Knottenbelt and A.E. Makubela, the personnel from the Pig Performance Testing Station, the abattoir and Histology section at Irene, for their kind assistance.

My mother, for her loyal support.

My wife Ria, for the support and encouragement throughout this study.

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PREFACE

Various authors have researched the characteristics of stress susceptibility in pigs. These characteristics have ranged from the effect of stress susceptibility on growth, carcass, and meat characteristics, to the effect of stress susceptibility on blood and muscle variables and properties and its economic consequences. The lesion responsible for stress susceptibility has also received attention.

SS pigs have been subclassified into the Porcine Stress Syndrome (PSS) group, and the Malignant Hyperthermia Syndrome (MHS) group. This grouping of the SS pigs is made according to the response of the pig on exposure to an agent or stressor. PSS pigs succumb to physical exercise. MHS pigs succumb after exposure to drugs. Both PSS and MHS have a common denominator in death and *post mortem* development of pale, soft, exudative (PSE) musculature, associated with the stimulation of glycolysis and glycogenolysis. It has been assumed by various researchers that PSS and MHS are identical, with the difference depending on whether the trigger was a physical stressor, or a pharmacological agent. However, some researchers have observed that the rate of lactate accumulation in muscles of the PSS and MHS pigs on premedication with various drugs differ. It is therefore possible that PSS and MHS are not identical, or at least may be stimulated via different mechanisms.

The primary aim of my study was to determine whether SS pigs would react differently to exposure of the anesthetic halothane and to forced treadmill exercise, as measured by changes in blood variables, muscle metabolites and rectal temperatures. Identification of pigs prior to exposure was important. The possible use of blood variables and muscle metabolites in predictive tests for stress susceptibility was therefore, also investigated. Peripheral investigations included the influence of stress susceptibility in South African Landrace gilts on certain growth, carcass and meat characteristics, and the effect of stress susceptibility on the percentage muscle fibre types.

The investigations have been published as journal articles, or are in the process of being published. Also, parts of this thesis have been used in short papers delivered at congresses and seminars.

Publications

*The results on the growth, carcass and meat characteristics of herd X in Chapter 3 has been published as:

- * Heine, P.H., & Mitchell, G., 1988. Growth, carcass and meat characteristics of stress susceptible and stress resistant South African Landrace gilts. *South African Journal of Animal Science* 18, 42-46.

- *The major part of Chapter 5 on the influence of stress on the blood variables has been published as:

Heinze, P.H., & Mitchell, G., 1989. Stress resistant and stress susceptible Landrace pigs: comparison of blood variables after exposure to halothane or exercise on a treadmill. *The Veterinary Record* 124, 163-168.

- *The major part of Chapter 6 on the muscle metabolite changes as a result of the exposure of the pigs to halothane or treadmill exercise has been submitted as an article, titled:

Heinze, P.H., & Mitchell, G. A comparison of some muscle metabolites in stress susceptible and stress resistant Landrace gilts after halothane exposure or exercise stress. *British Veterinary Journal*.

Short papers

- *The results on the growth, carcass and meat characteristics of herd X in Chapter 3 have been used for a short paper, titled:

Heinze, P.H., & Mitchell, G., 1986. Die invloed van maligne hipertermiese sindroom op verskeie groei-, karkas- en vleis eienskappe van S.A. Landrassoggies. South African Society for Animal Production Congress, Wild Coast, Transkei.

- *The results on the effect of halothane exposure on SS and SR pigs (Chapter 5) have been presented as:

Heinze, P.H., & Mitchell, G., 1987. Vergelyking van bloedveranderlikes tussen spanningsweerstandbiedende en -gevoelige Suid-Afrikaanse Landrasjongsde. South African Society for Animal Production Congress, Pretoria.

- *Heinze, P.H., 1988. Research needs and trends: biological - muscle and fat. In: *Proc. 4th Meat Symposium* - 10 September 1987. Technical communication no. 213, Department of Agriculture and Water Supply, Republic of South Africa.

The thesis has been constructed as follows:

- *In Chapter 1 a review of the relevant literature on PSE and porcine stress syndromes is given.
- *The methodology followed in this study is explained in Chapter 2.
- *The use of the halothane test, blood variables and muscle metabolites in predicting SS pigs as was found in this study, is analysed in Chapter 3.
- *The results on the various growth, carcass and meat characteristics are given in Chapter 4.
- *The results on the influence of halothane and exercise stress on blood variables are presented in Chapter 5.
- *In Chapter 6 the results on the influence of halothane exposure or treadmill exercise on muscle metabolites were analysed and presented.
- *In Chapter 7 the results on the muscle fibre characterisation are given.

*The influence of the halothane exposure and treadmill exercise on the rectal temperatures of the SS and SR pigs are given in Chapter 8.

*The conclusions and recommendations are given in Chapter 9.

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
ADA	Adenosine deaminase
ADG	Average daily gain
ALT	Alanine transaminase
AST	Aspartate transaminase
ATP	Adenosine triphosphate
CK	Creatine kinase
c-AMP	Adenosine 3',5'-cyclic monophosphate
DFD	Dark, firm, dry
FCR	Feed conversion ratio
IU/l	International units per litre
LDH	Lactate dehydrogenase
min.	minute
MHS	Malignant Hyperthermia Syndrome
mM	milli Molar
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
nmol/l	nano mole per litre
NS	Not significant $P > 0,05$
5-PGD	6-Phosphogluconate dehydrogenase
PHI	Phosphohexose isomerase
pmol/l	pico mole per litre
PSE	Pale, soft, exudative
PSES	Pale, Soft, Exudative Syndrome
PSS	Porcine Stress Syndrome
sec.	second
SR	Stress resistant
SS	Stress susceptible
TBA	2-Thio-barbituric acid
UK	United Kingdom
μmol/l	micro mole per litre
*	Significant $P \leq 0,05$
**	Highly significant $P \leq 0,01$

CHAPTER 1

Porcine stress syndromes and PSE musculature

1.1. Introduction

PSE musculature is a phenomenon usually associated with pig carcasses after death or slaughter of the animal. The muscle of PSE carcasses is very pale in colour, soft to the touch and exudes fluid, therefore a serious meat quality defect for the pig industry. These characteristics are associated with a combination of a rapid pH drop *post mortem* due to stimulation of glycogenolysis and glycolysis, and a carcass temperature above 38°C (Bendall & Wismer-Pederson, 1962; Briskey, 1964). Differences in the definition of PSE as determined by pH value in muscles occur between centres and researchers. These range from a pH value < 6,00 60 minutes *post mortem* (Berman, Conradie & Kench, 1972; Mitchell & Heffron, 1980a), < 6,00 45 minutes *post mortem* (McGloughlin & McLoughlin, 1975) and < 5,8 45 minutes *post mortem* (Schicfer, 1977). Nevertheless, it is clearly characterised by a rapid rate of *post mortem* pH decline.

PSE musculature is not a new phenomenon. As early as 1914 it has been mentioned as being a problem in the manufacture of quality products by German butchers, who called the meat "pale, watery, tasteless and leathery" (Herter & Wilsdorf, 1914, as cited by Scheper, 1980).

PSE musculature results as a consequence of stress of the animal and animals need have no genetic predisposition for developing PSE musculature. It must be recognised that any excessive stress may cause death and PSE type musculature (Mitchell & Heffron, 1982). However, pigs having a genetic disposition for the production of PSE musculature, are affected by the inherited genetic defect of stress susceptibility, commonly classified as porcine stress syndrome (Mitchell & Heffron, 1982). Therefore, the development of PSE musculature and the factors affecting its development need some discussion.

1.1.1. Pale colour and exudative nature of PSE musculature

Honikel & Kim (1985) showed in their experiments that about 20% of the sarcoplasmic and myofibrillar proteins denature as a result of the rapid drop in pH while the carcass is still hot. This

denaturation explained the pale colour of the muscle in that the white denatured sarcoplasmic proteins precipitated onto the redder myoglobin and thereby hiding it. The myoglobin of PSE prone pigs is also more unstable and prone to denaturation by heat (Bemmers & Satterlee, 1975). Both of these factors result in a loss of the red colour (Bemmers & Satterlee, 1975), aiding in the development of the paleness of PSE muscle.

The wateriness of the PSE muscles is explained by Lawrie (1979) as the result of the general denaturation of sarcoplasmic proteins. The proteins lose their water holding capacity, and consequently fluid exudates from the muscle. Electrophoresis separation of muscle proteins 24 hours *post mortem* seems to substantiate this hypothesis (Usunov & Zolova, 1974). The myofibrillar proteins of PSE producing pigs have also been found to be more susceptible to denaturation than the myofibrillar proteins of pigs producing normal meat (Sung, Ito & Fukazawa, 1976), therefore also contributing to the wateriness of the meat. However, Honkel & Kim (1985) maintain that only about 20% of the sarcoplasmic and myofibrillar proteins denature as a result of the high *post mortem* carcass temperature and quick drop in pH. According to these authors, the wateriness of PSE muscle is a result of permeability changes in muscle cell membranes, probably resulting from breaks in the membranes.

1.1.2. Practices and factors influencing the production of PSE pork

It has been recognised that various practices and factors influence the occurrence and development of PSE pork. The main causes are stunning techniques and a genetic predisposition for *post mortem* PSE musculature.

1.1.2.1. Stunning technique

The stunning of slaughter animals must be regarded as stressful (Scheper, 1977). Captive bolt stunning (Naudé & Klingbiel, 1977) and CO₂ (McLoughlin, 1971) have been recognised to promote the development of PSE musculature as they stimulate the rate of *post mortem* pH decline and glycolysis (Scheper, 1977) to the extent that a pH level of 5.58 develops within 45 minutes *post mortem* (Yang, Hawrysh, Price & Aherne, 1984). The influence of the different stunning methods on the production of PSE is illustrated in Table 1.1.

Table 1.1: Influence of stunning method on *post mortem* pH values in porcine *M. longissimus dorsi* (Naudé & Klingbiel, 1973)

Stunning method	n	pH 45 min <i>post mortem</i>	% PSE carcasses with pH ≤ 6.00
Captive bolt	10	5.79	80
CO ₂	10	6.31	20
Electrical stunning	10	6.48	0
Exsanguination	10	6.24	30

Differences in the reaction to captive bolt and electrical stunning have been observed. With captive bolt stunning, excessive struggling of the pig resulted, a phenomenon not encountered after electrical stunning (Naudé & Klingbiel, 1977). The severe muscle contractions associated with stunning cause accelerated *post mortem* glycolysis (McLoughlin & Tarrant, 1968) and PSE musculature.

Traditionally pigs have been classified as being stress resistant (SR) or stress susceptible (SS), usually by using the halothane test (see 1.4.1.). Proneness of the pig towards stress susceptibility has been indicated by an allele notated as *Hal*. Three genotypes are found, *Hal^NHal^N* (commonly referred to as *NN*) and *Hal^NHalⁿ* (commonly referred to as *Nn*), both being regarded as SR, and *HalⁿHalⁿ* (commonly referred to as *nn*) regarded as SS. The SS pigs react violently on exposure to halothane and on prolonged exposure die. Short duration pre-slaughter stress results in the increase of PSE occurrence in SR pigs. However, SS pigs (*nn*) were found to be insensitive to pre-slaughter treatment, and highly prone to PSE development, whereas SR pigs (*NN* and *Nn*) respond to pre-slaughter treatment as measured by the incidence of PSE muscle in these two groups (Barton-Gade, 1984).

It is therefore possible to reduce the incidence of PSE in SR pigs by changing some of the management practices at the abattoir. Nevertheless, these changes have no influence on the development of PSE musculature in SS pigs. It is therefore of importance to study the SS pig in comparison to the SR pig.

1.1.2.2. Porcine stress syndromes

Although the term porcine stress syndromes is used to describe the three commonly occurring conditions in SS pigs, which are death occurring in stressful situations, malignant hyperthermia and PSE production, it has been proposed that the term "Acidosis Proneness" might be a more appropriate term than porcine stress syndromes (Gregory, 1981). For the purpose of my study, the term porcine stress syndrome will be used as a general term for the different types of stress syndromes encountered in the literature. For the purpose of this study, SS pigs is also synonymous with halothane positive as indicated in some of the literature. The three commonly found porcine stress syndromes therefore needs some explanation.

1.1.2.2.1. Porcine Stress Syndrome

Porcine stress syndrome (PSS) is an inherited genetic defect which predisposes the affected pig towards the development of PSE musculature. PSS is characterised by sudden death of the pig as a result of natural stressors such as herding, servicing, parturition, fighting and exercise (Patterson & Allen, 1972; Nelson, Jones, Henrickson, Falk & Kerr, 1974). Symptoms characteristic for PSS under stressful situations are muscle and tail tremors, and with the continuation of the stress, dyspnoea, cyanosis, hyperthermia, metabolic and respiratory acidosis, muscle contraction and rigidity, and a fatal outcome (Waginger, Baumgartner, Schmid & Mayr, 1981). Also, electrical neur-

omuscular stimulation of SS-pigs precipitates the development of malignant hyperthermia (Ahern, Milde & Gronert, 1985).

In an effort to reduce the development of PSE musculature, the use of various premedications have been researched. Premedication with magnesium sulphate removed differences in muscle metabolites, although the muscles of SS pigs developed rigor mortis quicker (Schmidt, Cassens & Briskey, 1970a; Schmidt, Cassens & Briskey, 1970b). Premedication with magnesium elevates the initial pH value, and slows the rate of pH fall *post mortem* whereas calcium pretreatment resulted in a more rapid pH fall, especially in PSE prone pigs like Poland China (Campion, Marsh, Schmidt, Cassens, Kaufman & Briskey, 1971). Thus, calcium seems to be involved in the development of malignant hyperthermia in the PSS pigs.

Higher AST, LDH, CK and aldolase activities have been found in SS pigs after running 1100m at 3.3 km *min*⁻¹, than in SR pigs (Schmitzen, Schepers, Wagner & Trappmann, 1981b). The higher blood CK and LDH activities as a result of treadmill exercise in SS than SR pigs are ascribed to the greater disturbances of muscle cell permeability in SS pigs (Schmidt & Kallweit, 1980).

Muscle glucose 6-phosphate, glucose and lactic acid are elevated, and ATP and glycogen reduced at slaughter and at one hour *post mortem* in SS pigs compared to SR pigs. These lower energy levels result in the quicker development of rigor mortis in the musculature of SS pigs (Schmidt *et al.*, 1970a; Monin, Sellier, Ollivier, Goutefonges & Girard, 1981). As glycolysis and glycogenolysis are stimulated by catecholamines, it is assumed that it plays a pivotal rôle in the development of PSE musculature. However, "glucitic cores" which is the sum of the glycogen, glucose, glucose 6-phosphate and lactic acid and represent the major components transformable to lactic acid by glycolysis and glycogenolysis, are similar (Monin, *et al.*, 1981), suggesting that just prior to slaughter the SS and SR pigs have the same level of muscle energy in terms of carbohydrates. The slaughter of SS pigs after captive bolt stunning results in the significant reduction of phosphocreatine and ATP, and an increase in lactic acid in the *M. longissimus dorsi* compared to SR pigs. This trend is similar in the *M. vastus lateralis*, with the exception that the phosphocreatine concentrations in SS and SR pigs are similar (Schmidt *et al.*, 1970a).

Hence, although SS pigs might have the same amount of muscle energy reserves as SR pigs, these pigs have an inherent higher rate of post mortem pH decline and PSE muscle production. Therefore, stunning with a captive bolt stunner would only aggravates an already serious problem.

1.1.2.2. Malignant Hyperthermia Syndrome

The malignant hyperthermia syndrome (MHS) is identified in pigs resulting from severe reactions to various pharmacologic agents, notably gaseous anaesthetics and muscle relaxants, such as halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), methoxyflurane, diethyl ether, chloroform, suxamethonium and succinylcholine (Harrison, Saunders, Fiebigel, Hickman, Dent, Weaver & Terblanche, 1969; Hall, Trim & Woolf, 1972; Georck & Thoye, 1976; Hall, Lucke & Lister, 1980a;

McGrath, Rempel, Jessen, Addis & Crimi, 1981). Only animals with a genetic defect (*hm*) (Hradecky, Hruban, Pazdera & Klauudy, 1980) will develop malignant hyperthermia on exposure to the triggering agents. It may therefore be called a pharmacogenetic disease (Mitchell & Heffron, 1982).

The clinical manifestation of the disease on exposure to the drugs comprises (Harrison *et al.*, 1969):

- *Tachycardia of 200-300 beats/min, although arterial blood pressure is normal until a decrease in cardiac output occurs terminally
- *Muscle stiffness occurs rapidly, especially obvious in limbs, with the extending of the limbs as in rigor
- *Tachypnoea and hyperventilation, which rapidly progresses to apnoea
- *Blotchy cyanosis of the skin as a result of vasoconstriction (PO_2 normal), also becoming hot to the touch
- *A rapid sustained rise in body temperature to above $45^{\circ}C$, rising at a rate of $1^{\circ}C$ every 5 to 7 minutes
- *Acidosis with both metabolic and respiratory components.

The first clinical signs of malignant hyperthermia is the increase in heart rate and respiratory rate, with tonic contraction of the limbs and back muscles. Later the skin becomes blotchy cyanotic and body temperature increases rapidly (Lucke, 1981). Gross metabolic changes precede the change in temperature and is associated with a severe metabolic and respiratory acidosis and haemocoagulation (Lucke, Hall & Lister, 1977). After establishment of malignant hyperthermia, the discontinuation of the triggering agent, cooling of the body, treatment of the acidosis etc., still result in a poor prognosis, and death of the animal is common (Harrison *et al.*, 1969).

Changes in plasma and serum composition have been noticed. During malignant hyperthermia, significant differences are found in various plasma metabolites; lactate, glucose and pyruvate concentrations increase, whereas a decrease is found in the free fatty acid concentration (Lucke, Hall & Lister, 1976). A general increase in the serum electrolytes potassium, magnesium, phosphate, calcium, sodium and chloride is found, as well as haemocoagulation (Lucke *et al.*, 1976). Increases in cortisol (Mitchell & Heffron, 1981a) and catecholamines, serum creatine kinase (CK) and lactate dehydrogenase (LDH) (Van der Heide, Lister, Myllic, Ooms & Oyaert, 1976) and glutamic oxaloacetic transaminase (GOT or AST) (Meredith & Williams, 1980) are also common.

Several muscle changes occur during malignant hyperthermia. Concentrations of ATP and phosphocreatine decrease whereas the concentration of lactate and glucose 6-phosphate increase (Harrison *et al.*, 1969; Nelson *et al.*, 1974; Mitchell, Heffron & Van Reusburg, 1980). Even without triggering malignant hyperthermia, MHS pigs have significantly lower calcium and phosphate concentrations at slaughter in the *M. longissimus dorsi* than SR pigs (Nelson *et al.*, 1974).

Muscle fibre typing show that MHS pigs either have a significantly higher percentage intermediate (Cooper, Cassens & Briskey, 1969; Swatland & Cassens, 1973) or higher percentage white, and lower red muscle fibres, and thus a greater potential for anaerobic metabolism (Sair, Kasrouschmidt, Cassens & Benkey, 1972; Nelson *et al.*, 1974). A higher light to dark muscle fibre ratio was found in PSE than normal muscle, with the PSE muscle having greater light, but smaller dark muscle fibre diameters (Dixey, Aberle, Forbes & Judge, 1970). However, Hefron, Mitchell & Dreyer (1982) were unable to show any differences in percentage fibre types between pigs with various types of stress syndromes.

A consequence of MHS is the production of PSE musculature, even as a result of conventional slaughtering practices without any form of malignant hyperthermia triggering (Nelson *et al.*, 1974).

1.1.2.2.3. PSE Syndrome

The PSE syndrome (PSES) has been defined as being a stress syndrome (Cheah & Cheah, 1979). These pigs are linked to the efficient production of PSE meat, which is associated with the rapid rate of glycolysis (Lawrie, 1979). Although they are not susceptible to malignant hyperthermia and do not develop the symptoms of MHS on halothane exposure, these pigs produce PSE musculature with muscle pH values 60 minutes *post mortem* similar to those of MHS pigs (Mitchell & Hefron, 1980a). These pigs have a rapid rate of calcium efflux from the mitochondria, and although they are identified SR on halothane exposure, they have a rapid rate of lactate formation, thus stimulated glycolysis, and a high level of drip loss, indicative of PSE meat (Cheah & Cheah, 1979). The CK values of these pigs are also indistinguishable from those of normal pigs (Mitchell & Hefron, 1980a).

As heterozygous pigs (*Nh*), have been found not to be susceptible to halothane exposure (Hradecky *et al.*, 1980), but result in carcasses being classified as being P₂E (Andresen, Jensen & Barton-Gade, 1981), these heterozygous pigs are generally classified as being PSES pigs. Although PSES pigs do not react to halothane in terms of muscle rigidity and malignant hyperthermia reactions, differences are, however, still found.

Although halothane has no effect on the resting membrane potential of SR pigs, it causes a progressive depolarisation of muscle from SS pigs (Giarrant, Gödt & Gronort, 1980). With the dose/response relationship found between muscle contractions or rigidity and intramuscular calcium concentration, this phenomenon has been studied in relationship to MHS and PSES. The calcium binding capacity of the sarcoplasmic reticulum of MHS pigs is less than that of PSES pigs, which is very similar to that of normal pigs within the temperature range of 25°C to 35°C. However, at temperatures of 37 and 39°C, the calcium binding capacity of the PSES pigs decreases compared to normal pig calcium binding capacity, and is intermediate to that of the normal and SS pigs (Nelson & Bee, 1979). These authors have also shown that the proposed sarcoplasmic reticulum membrane abnormalities of the MHS and PSES pigs are similar at temperatures higher than 35°C.

1.2. Characteristics of stress susceptible pigs

1.2.1. Reproduction and growth characteristics

Several negative factors regarding the reproduction quality of SS pigs have been found. SS boars possess lower sperm qualities as measured by ejaculation volume, number of normal sperms and total number of sperms than SR boars (Schlenker, Jugert, Mudra, Pohle & Heinze, 1984). Also, litters of 100% SS progeny have fewer pigs and weigh less than litters from SR pigs (Webb & Jordan, 1978; Willeke, Amler & Fischer, 1984).

Regarding average daily gain (ADG), SS pigs have a lower ADG than the SR pigs (McGloughlin, Abern, Butler & McLoughlin, 1980; Schmitzen *et al.*, 1981b; De Wilde, 1984) and have a lower feed conversion ratio (FCR) (Eikelenboom, Minkema, Van Eldik & Sybesma, 1980b, generally take longer to reach marketing mass, and are more susceptible to developing PSE meat (Jons, Jones, Harrington & Judge, 1971). However, some researchers did not find differences between SS and SR pigs regarding ADG and feed efficiency (Webb & Jordan, 1978; Hanset, Luroy, Michaux & Kintaba, 1983). During growth trials using German Landrace pigs, it was shown that the mass gain rate of SS and SR pigs are indistinguishable from each other (Mitchell & Hellron, 1981b), which was also found in Sw. fish Landrace pigs (Lundström, Lundheim, Gahne, Sellei, André & Persson, 1983). Gender differences regarding growth characteristics were found within SS pigs. SS Dutch Landrace gilts have a lower ADG and feed efficiency than SR gilts, whereas no differences were found in these two growth characteristics between SS and SR barrows (Eikelenboom & Minkema, 1974).

1.2.2. Carcase characteristics

Generally, the carcasses of SS pigs have been found to have a higher slaughter-out percentage (Eikelenboom & Minkema, 1974; Eikelenboom, Minkema, Van Eldik & Sybesma, 1980a; Eikelenboom *et al.*, 1980b), and to be shorter (Webb & Jordan, 1978; Schmidt & Kallweit, 1980), and to have a higher muscle to fat ratio than carcasses from SR pigs (Monin *et al.*, 1981; Schmitzen *et al.*, 1981b). Webb & Jordan (1978) and Carlson, Christian, Kuhlers & Rasmussen (1980) also found the SS pigs to have a larger *M. longissimus dorsi* area. Also, SS pigs have lower backfat thicknesses than SR pig carcasses (Eikelenboom & Minkema, 1974; Eikelenboom *et al.*, 1980b; Schmidt & Kallweit, 1980; Schmitzen *et al.*, 1981b). This leads to SS pigs having generally higher carcass meat percentages than their SR counterparts (Eikelenboom *et al.*, 1980b).

Overall, these carcass characteristics shown by the SS pigs are sought after, and are therefore advantageous to particularly the producer. All these positive attributes should, however, also be examined in the light of the meat quality these carcasses produce.

1.2.3. Meat characteristics

Various negative meat quality factors involving SS pigs have been reported. Drip loss from the *M. longissimus dorsi* is greatest if the pH value 45 minutes *post mortem* is below 6.1, thus from PSE (type of saet (Warris, 1982)). The meat of SS pigs is also paler (Lundström *et al.*, 1983), although the muscle of SS pigs have the same concentration of pigment (haemic iron) than that of SR pigs (Monin *et al.*, 1981). General meat quality scores for SS gilt carcasses 24 hours *post mortem* are generally lower than those for SR gilts, with pH values 45 minutes *post mortem* and subjective scoring indicating a high percentage of PSE meat (Eikelenboom & Minkema, 1974).

No palatability differences were found by a trained taste panel between pork roasts of normal and PSE quality regarding factors such as tenderness, flavour and juiciness (Searcy, Harrison & Anderson, 1969). The pork roasts of normal and PSE quality were also found to be of equal tenderness by objective Warner-Bratzler tenderness measurement, with no differences in total moisture, as determined by Carver Press, and roasting losses. PSE meat was, however, found to be less tender by shear force measurement than meat of normal quality by Dilley *et al.*, (1970), whereas Fox, Wolfram, Kemp & Larsson (1980) found normal pork chops being less tender than PSE chops, with the PSE scoring lower on juiciness, raw meat colour, flavour, cooked aroma and general satisfaction. Thus, a high level of controversy still exists regarding the tenderness of PSE meat.

PSE meat has a definite influence on the processibility of meat into meat products. Meat from SS pigs, being PSE, has a lower curing ability (Monin *et al.*, 1981). Fermented sausages made of PSE meat have higher moisture diffusion rates, 2-thio-barbituric acid (TBA) values (which is associated with rancidity), and lactic acid content than sausages made of normal meat, whereas the colour of the PSE containing sausages have a paler red and more yellowish colour, with lower water holding capacity, water activity and shear force (Townsend, Davis, Lyon & Mescher, 1980), thus having a lower quality than sausages made from normal quality meat. Fatty acids of PSE meat are more susceptible to oxidation and rancidity. Retail displayed PSE chops also showed higher TBA values than their normal quality counterparts (Fox *et al.*, 1980).

Breed also influences meat quality between SS and SR pigs. Although the meat quality of SS Swiss Landrace pigs is lower than that of the SR pigs, no such differences were found in the Swiss Large White pigs (Schwöber, Blum & Rebsamen, 1980). The same type of results were found between the breeds Dutch Yorkshire and Dutch Landrace, with the meat quality of the SS Dutch Landrace being lower than that of the SR pigs, although no such differences were found between the SS and SR pigs of the Dutch Yorkshire breed (Eikelenboom, 1979).

1.3. Economic significance of PSE musculature

As no differences between the amino acid profiles of meat of normal and PSE quality exist, the differences between these two meat types is important in the economic and technological areas, and not in the nutritional-physiological sense (Freudenreich, Augustini, Schon & Schepfer, 1975). Consumer preference in terms of pork has been towards the reduction in fatness (Pedersen, 1978), which is an important factor in selecting breeding animals. The selection would be for the reduction in the fat to muscle ratio, usually without taking into account meat quality characteristics. As the area of the *M. longissimus dorsi* and carcass lean percentage of SS pigs have been found to be significantly greater than those of SR pigs, with the carcass fat percentage being lower and backfat thickness being thinner, it resulted in the selection of breeding stock with these traits without taking the proneness towards PSE musculature of these pigs into consideration (Carlson *et al.*, 1980; Eikelenboom *et al.*, 1980a). The higher probability towards PSE meat production has been correlated with the reduction of fat deposition, thus a thinner backfat, in the hypothesis by Müller (1983) (Figure 1.1).

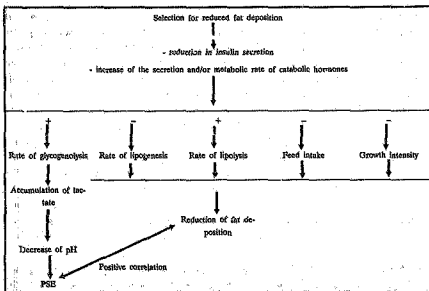


Figure 1.1: The implications of selection for reduced fat deposition (Müller, 1983)

PSE musculature in pork has been recognised as of importance in the meat industry as it results in great practical difficulties in the fresh meat trade, as well as the manufactured meat trade (Cansen, Marple & Eikelenboom, 1975). This prompted much research on the subject.

Pigs with a predisposition for producing PSE musculature have been found to have thinner backfats. Pork with thinner backfat, results in the splitting of the fat (Reid, 1983), and therefore in the difficulty in slicing sides, and leading to non-uniform end-products. Non-uniform end-products are a result of accumulation of brine in pockets formed with the use of new technology high pressure brine injection (Reid, 1984). The separation usually takes place between the different backfat layers, between backfat and muscle, and between intermuscular fat and muscle (Wood, 1983). Thinner backfat has a larger percentage unsaturated fatty acids which is correlated to the effect that PSE pork has higher TBA values, indicative of rancidity, even after frozen storage (Flynn & Bramblett, 1975).

Another quality defect in pork which causes severe problems in the meat trade, is the phenomenon of dark, firm and dry (DFD) meat. This is associated with pre-slaughter glycogen exhaustion. In a survey conducted at a South African bacon factory, it was found that the thinner the backfat, the higher the incidence of DFD pork (Heinoz, Gouws & Naudé, 1984). It would thus seem that pigs with a predisposition for developing PSE meat may also be more inclined to produce DFD meat under certain management conditions.

Fresh PSE meat also has a higher shrinkage rate (rate of mass loss) than normal meat. Economic consequences follow. Assuming that 5 billion kg of carcass pork was produced in the United States of America in 1977, and that the incidence of PSE was 5%, the loss as a result of excess shrinkage would be in the order of 1 to 2 million kg (Kauffman, Wachholz, Henderson & Lochner, 1978). Hall (1972) estimated that in the United States of America PSS, which results in the premature death of pigs during transport and other stressful situations, and PSE, resulted in a financial loss of between \$230 million and \$320 million per year. It is thus clear that PSE pork results in financial losses. In a more recent publication in the United Kingdom, the financial losses as a result of PSE were estimated to be 2,2% of the value of the mass of lean in all pigs slaughtered (Warris, 1982). Consumers prefer normal meat to PSE meat (Töpel, Miller, Berger, Rust, Parrish & Ono, 1976, Wachholz, Kauffman, Henderson & Lochner, 1978), with the result that PSE meat remains in display cabinets for longer periods. It has been calculated that the financial loss occurring as a result of PSE pork is as high as £8,74 per carcass, depending on the effort to ensure the early sale of PSE meat, whereas the curing of the meat for bacon results in a loss of £1,13 per carcass as a result of reduced bacon yield (Smith & Lesser, 1982).

The financial losses as a result of PSS pigs dying during or after transport to abattoirs in Sweden in 1979 were estimated to three million Swedish crowns per year (Fabiansson, Lundström & Hansson, 1979). The mortality rate amongst SS pigs during the fattening and transport periods are approximately 10 times higher than for the SR pigs (Eikelenboom *et al.*, 1980b). The negative financial influence of PSS pigs was also demonstrated by Köstleicher (1983) in Austria. Although the SS pig carcasses resulted in a better financial reward to the producer as a result of a higher meat to fat ratio, as well as thinner backfats, production losses such as slower growth rate, etc. of the SS pigs nullified the higher premium paid for the SS carcasses.

A survey in the Federal Republic of Germany amongst butchers and bacon factories indicated that the loss as a result of PSE pork ranges from 16 to 35 DM/100kg meat (Steinhilber, 1970). Although the financial losses incurred by the South African pork industry has not been estimated, it is probably substantial. Fifty to 60% of all carcasses at a South African commercial abattoir have been found to be PSE (Berman *et al.*, 1972).

The improvement in carcass characteristics in SS pigs is more than offset by negative meat-quality characteristics, smaller litter size and a higher incidence of sudden (Webb & Jordan, 1978; Carden, Hill & Webb, 1985). Therefore, it is in the interest of the pig industry to eliminate SS pigs from breeding stock as it results in serious financial losses.

1.4. Ante mortem identification of stress susceptibility

From the foregoing discussion it is clear that early identification of pigs likely to develop malignant hyperthermia or PSE is important. Several predictive tests have been proposed and are in use. The use of the halothane test, blood enzymes, blood typing and muscle variables will be discussed.

1.4.1. The halothane test

The development of the malignant hyperthermia reaction on the exposure of SS pigs to halothane, has led to the development of the halothane test. It is clear from Table 1.2 that no standard halothane test procedure has been set by countries or researchers, and this may complicate the comparison of results published between countries and even researchers. Nevertheless, it has been shown that the extension of the duration of the test beyond 3 min is impractical as most of the SS pigs react on halothane exposure within the first 3 minutes of exposure, with only 3% more SS pigs being detected with the test duration extended from 3 to 4 minutes (Webb & Jordan, 1978). However, with the increase in age and mass of the pigs, the time for the initiation of the positive reaction by SS pigs increases, and with the decrease in the halothane concentration, the time period for the appearance of the positive reaction increases (Schmidt & Kallweit, 1980).

The potential benefits resulting from the test has prompted the routine testing of Dutch Landrace pigs at the Dutch national testing stations (Eikelenboom *et al.*, 1980b). The halothane test is also used in the progeny testing of breeding herds affiliated with the Swedish Farmer's Marketing Association, and in this programme the sale of SS identified pigs is not permitted (Lundström *et al.*, 1983).

Limitations to the halothane test have been identified. It is not possible to detect N_n pigs with the test, whether using the test as a halothane vapor trough a face mask, or through intravenous administration (Gregory & Wilkins, 1984), although these pigs are also extremely prone to PSE development. Hence, it might only be possible with the application of the halothane test to reduce

the occurrence of SS pigs to between one and two percent (Eikelenboom *et al.*, 1980b). Using the halothane test as well as selective breeding trials, the occurrence of SS pigs could be prevented.

Table 1.2: The halothane test as used by different researchers for the identification of SR and SS pigs

Concentration	Flow rate	Duration	Country	Reference
2-4%		5 min	Netherlands	Eikelenboom & Minkema, 1974
5%		4 min	Australia	McPhee <i>et al.</i> , 1979
5%	2.5 l/min	5 min	Netherlands	Eikelenboom <i>et al.</i> , 1976
5%	2.5 l/min	4 min	Netherlands	Eikelenboom <i>et al.</i> , 1976a
6%		3 min	USA	Carlson <i>et al.</i> , 1980
5%	2 l/min	5 min	Ireland	McCloughlin <i>et al.</i> , 1980
4%	2.5 l/min	5 min	Federal Republic of Germany	Petri <i>et al.</i> , 1979
4-8% for 1 min; 3% thereafter	2-3 l/min	3 min	UK	Webb & Jordan, 1978
4%	6 l/min		Federal Republic of Germany	Schmitzen <i>et al.</i> , 1981a
4%	1.5 l/min	5 min	Switzerland	Schweizer <i>et al.</i> , 1980

Incorrect classification of pigs regarding stress susceptibility is calculated to be approximately 5%, with the assumption of equal error rates among SS and SR pigs. If this assumption is proved incorrect, the rate of error in classification may be higher (Webb & Jordan, 1978). Webb (1980a) found the disagreement between two repeated halothane tests to be 9%, and thus the incorrect classification on a single test roughly 1 in 20, thus 5%. Petri, Oster, Haberkorn, Gindele & Köppen (1979), however, found the percentage of animals being classified the same during two repeated halothane tests to be 72%, with 86% of the pigs being classified the same if pigs classified as being "uncertain" were ignored. With four repeated tests the repeatability of the halothane test was determined as 85% (Kallweit, Schmidt & Unshelm, 1980). The association of SS with PSS seems difficult to assess, with the association between PSE development and stress susceptibility not being complete (Webb & Jordan, 1978). Gender has been found not to influence stress susceptibility with the incidence of SS pigs being equal between the different sex types (Kallweit *et al.*, 1980).

The CO₂ concentration in the anaesthetic circuit also affects accuracy by diluting the halothane concentration. Therefore, SS pigs may not show muscle rigidity and are therefore misclassified as SR pigs. The halothane concentration may be 1-2% lower than the setting on the vaporiser (Kallweit *et al.*, 1980). The use of a CO₂ absorber with the use of semi-closed systems is thus advisable. The use of higher halothane concentrations and flow-rates are recommended for the more reliable detection of SS pigs (Kallweit *et al.*, 1980). Higher ambient temperatures (i.e. 36°C) result in the higher detection rate of SS pigs than at lower ambient temperatures (16 and 25°C) (Kallweit, Feuerhordt & Henning, 1981). Nutritional influences on the reaction time in SS pigs have been postulated (Jørgensen, 1982), possibly related to the protein levels in the feed (Jørgensen, 1983). Halothane concentrations lower than 3% increased the number of false negative pigs, and

increasing the concentration results in the more rapid onset of malignant hyperthermia symptoms, thus a *dose/response relationship* (McGrath, Lee & Rempel, 1984), a relationship also found by Gregory & Wilkins (1984) using an intravenous halothane test. The authors indicate that a concentration of 3% should be sufficient, which agrees with the conclusion of Webb & Jordan (1978).

1.4.1.1. Incidence of stress susceptibility in pig breeds

The selection of pigs with higher muscle to fat ratio's in breeding strategies seems to be implicated in the increase in the incidence of pork of lower meat quality, although the specific factors responsible for the deterioration in meat quality are still unclear (Hagemeister, 1969). However, pigs with a higher carcass meat percentage have lower ultimate meat quality scores (Hagemeister, 1969). Breed differences are evident in the pH values 45 minutes *post mortem*, with Landrace pigs having a lower value than Large White pigs, and this pH value is moderately heritable in both breeds. Taking the relationship pH value 45 minutes *post mortem* and certain economic important meat quality traits into account, the evidence indicates that breed differences exist (McGoughlin & McLoughlin, 1975), as is shown in Table 1.3 comparing the incidence of SS pigs in different breeds.

Table 1.3: Incidence of halothane reactors in different breeds

Breed	Year	% Reactors	Country	Reference
Australian Landrace		20,6%	Australia	McPhee et al., 1979
Irish Landrace	1976-78	4,7%	Ireland	McGoughlin et al., 1980
British Landrace	1977-79	11%	UK	Webb, 1980b
Norwegian Landrace		5%	UK	Webb & Jordan, 1978
Swiss Landrace	1977/78	23,2%	Switzerland	Schwörer et al., 1980
German Landrace		70%	Federal Republic of Germany	Schmitt et al., 1981b
Australian Large White		0%	Australia	McPhee et al., 1979
Irish Large White	1976-78	0%	Ireland	McGoughlin et al., 1980
Irish Large White	1977-79	0%	UK	Webb, 1980b
British Large White		0%	UK	Webb & Jordan, 1978
Swiss Large White	1977/78	3,4%	Switzerland	Schwörer et al., 1980
Irish Welsh	1976-78	6,3%	Ireland	McGoughlin et al., 1980
Hampshire		1%	UK	Webb & Jordan, 1978
Duroc		0%	UK	Webb & Jordan, 1978
Yorkshire		0%	UK	Webb & Jordan, 1978
Belgian Pietrain		90,8%	Belgium	Haeert et al., 1983

The general mode of inheritance of the gene has been indicated as a simple autosomal recessive gene (Eikelenboom, Min'na & Van Eldik, 1976; Smith & Bampton, 1977; Rempel, McGrath & Addis, 1979; McKay, Rempel, McGrath, Addis & Boylan, 1982) although with possible high or complete penetrance, but with variable expression of one recessive gene, (Hradecky et al., 1980; Pazdera, Hyaneck, Hruban, Hradecky & Gabne, 1983), or even with an added suppressor locus

(Imlah, 1984). The high or complete penetrance, but variable expression may explain the difference in the dose required to initiate the malignant hyperthermia reaction (Gregory & Wilkins, 1984).

1.4.2. Blood enzymes

Certain blood enzyme activities have been found to vary between SS and SR pigs. Serum activity of CK, LDH and aldolase are higher in the slaughter blood of pigs developing FSE musculature than pigs not developing the meat quality defect, and it has therefore been proposed that these enzymes might be of diagnostic value towards the identification of PSE prone pigs (Berman *et al.*, 1972; Eikelenboom & Minkema, 1974). Also the correlation between the activities of CK, LDH, aldolase and AST and meat quality have been found to be high (Schmitzer, Schepers, Wagner & Trappmann, 1981c). Exercise stress increases the total LDH, and LDH₅ activities in the blood (Kallweit, Mäder, Steinhilf & Weniger, 1975), thus giving rise to the assumption that the measurement of serum enzyme activities after stressful situations such as exercise, transport, or the injection of "Myostress", a preparation containing neostigmine and atropine, may result in more accurate prediction of stress susceptibility (Bickhardt & Richter, 1980).

The most promising results seem to indicate the use of serum CK activity 24 hours after a standardised stress imposition, such as a 100 m run (Bickhardt, 1981). The use of such a muscle specific enzyme as CK has some practical problems. During the fast growing period the serum CK activities of SS and SR pigs are indistinguishable (Mitchell & Heffron, 1975). The pre-blood taking history of the pig is generally unknown, with physical exercise and fighting resulting in elevated values. The method of blood taking may also have an adverse influence on the predictive value, as muscle is damaged and may lead to the leakage of CK into the blood. Vitamin E deficiency, injury and injections of aggressive drugs may also increase the CK activity in the blood of the animal (Bickhardt, 1979). It is therefore indicated that the use of CK activities cannot be used for the identification of all pigs likely to develop FSE (Mitchell & Heffron, 1980a), and that the use of CK activities as a routine measure for prediction is as yet not practical (Sönichsen, Ernst & Claus, 1981). However, it has been indicated that CK activities may still be of practical value in the detection of SS pigs, and that a more precise prediction can be made if:

- * only pigs above 60 kg are tested,
- * only sick animals are tested,
- * blood is taken from the ear rather than from the vena cava,
- * the animals be exposed to a standardised stress procedure 20 to 24 hours prior to CK determination,
- * blood is analysed immediately, is not frozen, and is analysed by an experienced laboratory (Bickhardt, 1979).

1.4.3. Blood typing

As a result of the halothane test only identifying homozygous SS pigs, the complete elimination of SS pigs requires more specific tests, which may include blood typing (Webb, 1980a). At the Swiss Institute of Animal Production Swiss Landrace pigs were selected as a result of a selection index which combined daily gain, ultrasonic backfat thickness, and the average performance of the animal's full and half sibs, without taking into consideration any parameter for stress susceptibility. Two lines were produced, one positive and one negative regarding the selection index. The results showed that the positive line had a significantly lower pH₁ value, greater percentage muscle in the carcass, and a paler type of meat, parameters very similar to that of SS pigs. Blood genetic markers for *Hal^a* and phosphohexose isomerase isoenzyme B (*PHI^B*) were positively correlated with the positive line. Adenosin deaminase (*ADA*) and 6-phosphogluconate dehydrogenase (*6-PGD*) differences between the two productive lines were also found. These markers thus give the opportunity for selection. No differences were obvious between the two lines regarding the H blood group (consisting of the factors *H^a*, *H^b* and *H^c*) *H^a* and *6-PGD^A*. The use of marker genes may therefore lead to the significant improvement of meat quality, and with the use of the markers H, S (A-O blood group) and *PHI* with or without halothane testing, meat quality could be improved and lead to the reduction of SS pigs in the herd (Lundström *et al.*, 1983; Schwärer, Vögeli, Blum & Rebsamen, 1983; Vögeli, Gerwig & Schneebeli, 1983). However, *6-PGD* is in general indicated to be of no use in the selection or prediction of SS pigs (Hanset *et al.*, 1983).

In Danish Landrace pigs the loci for *Hal* and *PHI* have been found to be closely linked, with a high association between *PHI* and meat quality (Andersen, 1979), and could therefore be important in the prediction of susceptibility type. In the Pietrain/Hampshire cross pigs a clear and predictable linkage between the halothane locus and the genotypes at H, *PHI* and *6-PGD* loci was found, although the association between the halothane locus (*Hal*) and the loci for H and *PHI* is not absolute, and may differ between different British Landrace herds (Allen, Cheah, Imlah, Lister, Steans & Webb, 1980). Thus, although the genetic markers may give an indication of the proneness of the pigs for producing PSE musculature, the prediction is still not unequivocal.

1.4.4. Muscle variables

Muscle biopsies have received attention in the research on SS pigs as a result of it being the source of the heat produced during malignant hyperthermia, and also as a result of the general stimulation of glycogenolysis and glycolysis during the development of malignant hyperthermia. The major obstacle in using muscle biopsies is that in order to obtain muscle biopsies, restraint and/or anaesthesia has to be used, resulting in stress of the animal. Anaesthesia inevitably causes several physiological reactions in the body that may change variables being investigated (Pfeiffer & Lengerken, 1984). Taking a muscle biopsy requires time, resulting in differences in metabolite concentrations *in vivo* and those determined in the biopsy. To minimise the problem of the time lapse

between taking the biopsy and extraction of the metabolites, and of the use of anaesthetics, the use of a "shoot biopsy" has been introduced using a modified captive bolt pistol (Pfeiffer, Lengerken & Hennebach, 1981). The application of this technique is complicated in that it is expensive, labourious, damages carcasses and may prove to be unacceptable to animal welfare organisations (Webb, Carden, Smith & Imlah, 1982). Despite these difficulties, several studies have been done using muscle biopsies.

As a result of the general stimulation of glycolysis and glycogenolysis, the high energy compounds such as ATP and phosphocreatine, as well as the metabolites of the glycogenolysis system have received attention. Muscle from SS pigs have lower glycogen and ATP, and higher glucose and lactate concentrations than SR pigs 60 minutes *post mortem* (Schmidt *et al.*, 1970a; Monin *et al.*, 1981), which is compatible with the idea of a general stimulation of glycogenolysis in SS pigs. Furthermore, after halothane exposure, muscle from SS pigs have also lower ATP and phosphocreatine, and higher lactate and glucose 6-phosphate concentrations compared to the muscles of SR pigs (Harrison *et al.*, 1969; Nelson *et al.*, 1974; Mitchell *et al.*, 1981b). These metabolites are therefore of potential value for the ante mortem identification of SS pigs. Also muscle LDH activity is higher in PSE *M. longissimus dorsi* than in the muscle of normal quality 24 hours *post mortem*, with an increase in the LDH₄ and especially the LDH₅ isoenzymes (Usunov & Zolova, 1974), and might also have potential diagnostic value. However, muscle biopsies are impractical in that the procedures are expensive, labourious and that its precision for diagnostic purposes is still unproven (Mitchell & Hefron, 1982).

The use of *in vitro* muscle contractions as a result of the exposure to malignant hyperthermia triggering drugs may also be used for the detection of SS pigs. On exposure of the muscle biopsy to the triggering agent, the contractions produced in SS pigs are greater than those in SR pigs. The use of only one agent is not advisable as some overlap may occur between the contractions of SR pigs and the wide scatter of values from SS pigs, which result from the high level of heterogeneity amongst SS pigs. Therefore the degree of susceptibility amongst SS pigs appears to vary. The use of multiple triggering agents reduces the chance of error. The use of 3% halothane, 2 mmol/l caffeine, 80 mmol/l KCl, 1 mmol/l succinylcholine and 100 μ mol/l thyrol have been suggested (Okumura, Crocker & Denborough, 1979).

The rate of calcium efflux from the mitochondria has been proposed as a very sensitive method for the detection of PSE prone pigs, and not only of SS pigs, but also of PSES pigs because of the close correlation between the calcium efflux rate and lactate formation in the muscle, and between the rate of calcium efflux and drip loss in the meat from pigs (Cheah & Cheah, 1979; Allen *et al.*, 1980). The mitochondria from SS pig *M. longissimus dorsi* also contains more endogenous calcium, release it more rapidly following the addition of an uncoupling agent under aerobic conditions than those of the SR pigs (Cheah & Cheah, 1981b; Cheah, Cheah, Crossland, Casey & Webb, 1984). Although these methods are promising, they are labourious and expensive. Interpretation of the results may not be unequivocal.

1.5. Development of malignant hyperthermia

From the literature it is evident that hormones, the neural system, the muscle and its subfractions ect. may all be involved in the development of malignant hyperthermia. Each of these will be discussed briefly, as well as the influence of the exposure of SS pigs to halothane and/or exercise.

1.5.1. Hormones

As the stress susceptibility of pigs and the production of PSE meat is associated with a general stimulation of glycogenolysis, hormones which stimulate glycogenolysis such as the catecholamines, corticosteroids and thyroid hormones, could be important in the development of malignant hyperthermia.

1.5.1.1. Thyroid hormones

Tissue metabolic rates are regulated by thyroid hormones. As stimulation of the metabolic rate in muscle leads to PSE musculature, thyroid hormones have indirectly been implicated. Hyperthyroidism, therefore should theoretically cause the production of PSE musculature.

Hyperthyroidism is, however, incompatible with the investigations into the effect of thyroid hormones on meat quality by Ludvigson (1954, 1955a, b, 1957a, 1960 as cited by Fischer, 1974). Thyroid hormone concentration changed with season, with higher concentrations during the colder winter months, and lower concentrations during the warmer summer months. The incidence of PSE has been found to correlate with these changes, with a higher incidence during the warmer months, and a lower occurrence during the colder months. As a result of this correlation, pigs with a predisposition for producing meat of a normal quality, were given methylthiouracil for 10 days prior to slaughter. These pigs subsequently produced PSE meat. Also, pigs with a predisposition for producing PSE meat were given iodine-casein for 9 to 14 days prior to slaughter, resulting in the enhancement of meat quality as indicated by the muscle pH values, although no differences were found in lactate concentration. No explanation for these findings could be found, but they imply that hypothyroidism is associated with PSE.

On the other hand, hyperthyroidism caused by supplementing pigs with thyroxine resulted in the stimulation of *post mortem* glycolysis, whereas hypothyroidism through thyroidectomy results in the retardation of *post mortem* glycolysis (Marple, Nachreiner, McGuire & Squires, 1975). Nevertheless, no significant differences were found between SS and SR pigs at rest, or after transport and subsequent slaughter, in T_2 or T_4 concentrations, with resting and after transport and slaughter-values also being similar (Kogdakis, Essinger & Faber, 1982). In a recent study, however, higher T_3 levels were found in SS pigs, although T_4 and thyroxin binding capacity between the SS and SR pigs did not differ (De Wilde, 1984), which is in accordance with the finding that SS pigs convert T_4 more rapidly to T_3 than SR pigs (Marple, Nachreiner, Pritchett, Miles, Brown & Noe, 1977).

These inconclusive findings suggest that the thyroid hormones are not the primary lesion for MHS, but may have a concurrent and contributing action once malignant hyperthermia has been triggered.

1.5.1.2. Corticosteroids

Although, it has been indicated by Rogdakis *et al.* (1982) that SS pigs have the same concentrations of cortisol and ACTH than SR pigs before and after stress, the consensus seems to be that SS pigs have lower cortisol levels than SR pigs (Mitchell & Heffron, 1981a). Even after stimulation by intravenous injection of Synacthen, a synthetic ACTH preparation, the concentration of cortisol in SS pigs was lower. The rate of cortisol increase in SS Landrace pigs was also lower compared to that of SR Landrace pigs (Mitchell & Heffron, 1981a). Halothane exposure stimulates the release of cortisol during the first 5 minutes of exposure, and the pattern was similar to the stimulation by Synacthen; the increase in SS Landrace pigs was lower than that in SR Landrace pigs (Mitchell & Heffron, 1981a).

Several reasons for the consistently lower cortisol levels in SS pigs have been given: exhaustion of the adrenal cortex and therefore reduced secretion, increased metabolism or utilisation of cortisol, impaired rate of biosynthesis or a reduced stimulation by ACTH of the adrenal cortex (Mitchell & Heffron, 1981a). It has been shown that SS pigs have a higher cortisol metabolic clearance rate (5x) and turn-over rate (3x) than SR pigs, and thus a higher level of cortisol utilisation, whereas the ACTH levels in SS pigs were approximately twice that of SR pigs (Marple & Cassens, 1973).

The results of Mitchell & Heffron (1981a) indicate a reduced interaction of adrenal cortex cell receptors with ACTH, thus explaining the higher ACTH levels in plasma of SS pigs, and the lower cortisol levels. However, the results of Marple & Cassens (1973) indicate a higher turn-over rate, and thus a higher rate of cortisol production. Halothane has been shown to have a stimulatory effect on the adrenal cortical functions of man (Oyama, Shibata, Matsumoto, Tsiguchi & Kudo, 1968), and could thus possibly promote halothane induced malignant hyperthermia.

As is the case for the thyroid hormones, the data on the corticosteroids is still inconclusive, but the hormones may be important during the development and sustainment of the syndrome, but are not likely to be the site of the primary lesion.

1.5.1.3. Catecholamines

The intravenous infusion of adrenaline has been found to stimulate a rapid post mortem pH drop in *M. longissimus dorsi* of Landrace pigs (Haid, Rogdakis & Feber, 1973), and could therefore be important in the development of PSE musculature. Adrenergic stimulation is known to function through α and β -adrenergic receptors. The actions stimulated by α and β -stimulators are given in Table 14.

Table 1.4: The actions stimulated by α and β -adrenergic stimulators

α -adrenergic stimulation	β -adrenergic stimulation
a) Vasoconstriction	a) Vasodilatation
b) Relaxation and inhibition of spontaneous insulin mobility	b) Relaxation and inhibition of spontaneous insulin mobility
c) Bronchoconstriction	c) Bronchodilatation
	d) Stimulation of heart work
	e) Glycogenolysis
	f) Lipolysis

Adrenergic stimulation can therefore explain some of the symptoms of malignant hyperthermia. In particular β -stimulation which functions through the activation of c-AMP has been studied in relationship to malignant hyperthermia development.

Also, in both SS type pigs (Pietrain) and SR type pigs (Large White), β -adrenergic receptors seem to be important in the mobilisation of fats from the fat cells (Wood, Gregory, Hall & Lister, 1977). Fat mobilisation is enhanced in the Pietrain relative to the Large White, which may be the result of a greater sensitivity to β -adrenergic action on body fat stores (Wood *et al.*, 1977), which is compatible with the hypothesis of Müller (1983) who showed a lower fat deposition rate in SS pigs, and a higher fat mobilisation rate.

Complete adrenergic blockade by bilateral adrenalectomy and bretylium prevented the hyperthermic reaction in SS pigs on halothane exposure (Lucke, Denny, Hall, Lovell & Lister, 1976), thus emphasising the importance of the adrenals in the establishment of the syndrome. The suggestion is that the action of the catecholamines is to stimulate muscle metabolism and malignant hyperthermia has been established (Lister, Hall & Lucke, 1976).

The results on various experiments have been reported reflecting the influence of halothane exposure on catecholamines. Blockade of β -receptors by propranolol, combined with the infusion of noradrenaline, which on its own triggers mild increases in temperature, caused fatal malignant hyperthermia (Hall, Lucke & Lister, 1977). However, no temperature rises during halothane exposure of propranolol premedicated SS pigs has been found (Groocert, Theye, Milde & Tinker, 1978). Hence, α -adrenergic stimulus seems important in producing heat during malignant hyperthermia. During malignant hyperthermia in SS pigs the concentration of noradrenaline increase approximately eight times, a result not found in SR pigs (Davis, Gehrke, Williams, Gehrke & Gerhardt, 1982).

The inhibition of increased heart rate, myocardial oxygen consumption and decrease in myocardial efficiency of propranolol premedicated SS pigs after halothane exposure indicate the importance of β -adrenergic stimulation during the malignant hyperthermia reaction (Gronert *et al.*, 1978).

The influence of management stress on catecholamine concentrations have been studied to a lesser extent than the influence of halothane exposure. The β -adrenergic receptor antagonists Wiskin (Haid *et al.*, 1973; Rogdaki & Haid, 1974) and Carazolol (using PSE prone Fletraia pigs) are effective in the *post-mortem* reduction of lactate concentrations in pigs exposed to normal management stress (Warriss & Lister, 1982), probably in reducing the activation of glycogenolysis, but is ineffective in the prevention of halothane induced lactacidemia (Gregory & Wilkins, 1984).

Thus, it seems from the results reported on in the literature that catecholamines may play an integral part in the development of malignant hyperthermia, especially β -adrenergic stimulation.

1.5.2. Neural system

It seems that various neuroleptic drugs may influence the development of malignant hyperthermia, but, the variation in effect on the various triggering agents and stressors show that the problem of malignant hyperthermia is multifaceted. Some drugs can prevent the onset of malignant hyperthermia by one triggering agent, slow down the onset by another triggering agent, or have no effect on the onset as a result of another triggering agent. This may indicate various different lesions responsible for the single phenomenon of malignant hyperthermia in pigs.

Although drugs such as propionylpromazine, azaperone and acepromazine are ineffective in preventing malignant hyperthermia as a result of halothane exposure, premedication with these drugs slow the onset of the malignant hyperthermia reaction on halothane exposure by three to four minutes (Eikelenboom, 1975; Somers & McLoughlin, 1982). Also, tubocurarine prevents the fatal response of SS pigs to succinylcholine, and large doses of pancuronium give some protection against halothane induced malignant hyperthermia (Hall, Lücke & Lister, 1976). These researchers suggested that the occurrence of malignant hyperthermia might be influenced by the state of muscle activity at the time the triggering agent is administered. It has been shown that exercised SS pigs react more violently on halothane exposure than well rested SS pigs (Van der Hende *et al.*, 1976). As a result of tubocurarine inhibiting succinylcholine induced malignant hyperthermia, but not halothane induced malignant hyperthermia, it can be concluded that the depolarisation of the motor end-plate is necessary in triggering succinylcholine induced malignant hyperthermia, and that halothane acts at a site beyond the motor end-plate (Hall *et al.*, 1976).

However, some research has also been done on the effect of different neuroleptic drugs in the prevention of PSE meat production and deaths as a result of PSS. Propionylpromazine, azaperone and acetyl propizine seem to reduce immediate *post-mortem* carcass temperature and PSE, as well as deaths during transport as a result of PSS.

1.5.3. Muscle subfractions

The development of PSE musculature has also been linked to the activity of the myosin ATPase, and calcium sequestration capability of the sarcoplasmic reticulum. With a drop in pH value below 5.9, an apparent damage of the sarcoplasmic reticulum takes place, and loses its ability to store calcium. Calcium accumulates in the sarcoplasm, increases the activity of the myosin ATPase and accelerates the fall in pH (Krzywicki, 1971).

Although both suxamethonium and acetylcholine act by depolarisation of the post-junctional membrane, suxamethonium induces muscle contraction in SS pigs, but acetylcholine does not. This might be the result of the slower metabolism of suxamethonium, resulting in a prolonged action. Also, tubocurarine which is able to block the transmitter action of acetylcholine, is not able to block the contractions resulting from suxamethonium exposure. The action of suxamethonium may therefore be via a depolarisation of the sarcolemma and the T-tubules resulting in the release of a small amount of calcium into the sarcoplasm, which subsequently stimulates the release of calcium from the sarcoplasmic reticulum (Okumura, Crocker & Denborough, 1980).

Support for this suggestion is given by Van der Hende, Myulle, Vlaminck & Oynert (1980) in that they found no differences in the calcium activated sarcoplasmic reticulum Mg-ATPase of SS and SR pigs, nor in the calcium accumulation capacity of the sarcoplasmic reticulum. A lower binding capacity was found at low pH values, probably because of membrane permeability changes. A change in permeability allows external calcium to move into the sarcoplasm after depolarisation of the membrane (Van der Hende *et al.*, 1980). However, lower myoplasmic concentrations of calcium and magnesium were found to exist in muscle of SS pigs relative to SR pigs (Nelson & Chausmer, 1981). These observations do not preclude the triggering of malignant hyperthermia in SS pigs as a result of the influx of small amounts of calcium into the sarcoplasm (Nelson & Chausmer, 1981). Cheah & Cheah (1981a) found a phospholipase A₂ in pig muscle mitochondria, with similar concentrations in the mitochondria of SS and SR pigs. However, the phospholipase of SS pigs is twice as active as that from SR pigs. The mitochondria membrane calcium activated phospholipase A₂, when stimulated by calcium, stimulate the hydrolysis of phospholipids and the liberation of unsaturated fatty acids, with a net increase in the content of saturated fatty acids in the membrane (Cheah & Cheah, 1981b). The release of the unsaturated fatty acids uncouples the mitochondria, and destabilises the mitochondrial membrane (Cheah & Cheah, 1981b), thus disrupting normal function of the organelle. The release of long chain unsaturated fatty acids by phospholipase A₂ of mitochondria membranes of SS pig muscle, inhibits the uptake of calcium by sarcoplasmic reticulum and induces the release of calcium from the sarcoplasmic reticulum (Cheah & Cheah, 1981a). In addition, dantrolene has been shown to slow the onset of malignant hyperthermia in SS pigs, but does not function through an influence on the cross membrane movement of calcium across the sarcoplasmic reticulum membrane (White, Collins & Denborough, 1983).

SS and SR pigs have the same number of mitochondria in *M. longissimus dorsi*, have similar responses during the anaerobic oxidation of succinate and have similar rates of calcium uptake (Cheah & Cheah, 1976). Under anaerobiosis, however, the efflux of calcium from mitochondria from SS pigs was twice as high as that of SR pigs (Cheah *et al.*, 1984). This resulted because halothane exposure stimulates the efflux of calcium from SS mitochondria only, an effect that can be counteracted by the addition of magnesium (Cheah & Cheah, 1976).

1.5.4. Malignant hyperthermia and muscle metabolism

The earliest biochemical muscle variable to be detected in SS pigs with the onset of malignant hyperthermia on halothane exposure is the loss of phosphocreatine, which remains quite constant during pentobarbital anaesthesia. This loss in phosphocreatine is accompanied by a stimulation in glycolysis, the accumulation of lactate, and a rise in glucose 6-phosphate (Ahern, Somers, Wilson & McLaughlin, 1980). ATP concentration remains steady during pentobarbital anaesthesia, but decreases on exposure to halothane (Ahern *et al.*, 1980; Hall & Lucke, 1983).

The heat produced by SS pigs during malignant hyperthermia triggered by halothane has been indicated to result from anaerobic metabolism, and a decreased ability to transfer heat (Nijland, Mitchell & Mitchell, 1985). The rise in body temperature usually starts late in the malignant hyperthermia reaction, and although muscle from SS and SR pigs have the same concentrations of glyceraldehyde 3-phosphate and aldolase, a consequence of malignant hyperthermia is the reduction of glyceraldehyde 3-phosphatase and aldolase activity (Lorkin & Lehmann, 1983). This might explain the temperature rise as a result of the susceptibility to halothane exposure. This entails the production of fructose 1,6-diphosphate by glycolysis which is hydrolysed by fructose 1,6-diphosphatase. With the loss of aldolase and glyceraldehyde-3-phosphatase on exposing the SS pig to halothane, fructose 1,6-diphosphate is diverted to the futile cycle, generating heat (Lorkin & Lehmann, 1983).

A link between MHS and PSES has therefore been established. Nelson & Bee (1979) also indicated that abnormal halothane induced muscle contraction occurs in MHS pigs at 31°C, becoming more severe with an increase in temperature. They suggested that this might be the result of conformational changes in the sarcoplasmic reticulum membrane structure/function relationships (Nelson & Bee, 1979). These results are interesting in that it is the high *post mortem* temperature of the carcass with the concurrent rapid rate of glycolysis which result in the formation of PSE musculature. The initial release of calcium from the sarcoplasmic reticulum of SS pigs induced *in vitro* by halothane was at least 70% higher than that of SR pigs, and the calcium release from sarcoplasmic reticulum of SS pigs was higher after induced membrane depolarisation (Kim, Sreter, Ohnishi, Ryan, Roberts, Allen, Meszaros, Antoniu & Ikemoto, 1984). A good correlation therefore exists between the establishment of malignant hyperthermia and muscle rigidity. The sarcoplasmic calcium concentration in the *M. longissimus-dorsi post mortem* has been found to be higher in SS pigs than in SR pigs and has been correlated with a faster rate of glycolysis in the SS pigs as measured by the muscle pH at 45 minutes *post mortem* (Cheah *et al.*, 1984). Furthermore, the MgCa-ATPase activity in skeletal muscle of SS pigs is higher than that of SR pigs, although

the Mg-ATPase activities of skeletal muscle of SS and SR pigs are similar (Campion, Topel & Christian, 1976). With the elevated calcium concentrations in the sarcoplasm, glycogenolysis is stimulated (Hollmeyer, Meyer, Haschke & Fisher, 1970). The higher calcium concentrations in the sarcoplasm may explain the characteristic heat production, lacticidosis and muscle rigidity of malignant hyperthermia (Hall, Lucke, Lovell & Lister, 1980).

Pietrain pigs with *post mortem* lower meat quality characteristics, had lower respiratory control rates than Dutch Landrace pigs due to lower State 3 respiratory rates, and not as a result of uncoupling of oxidative phosphorylation (Eikelenboom & Van den Bergh, 1973). As the respiration rate increased in the Pietrains as a result of uncoupling oxidative phosphorylation, although it was unaffected in the Landrace pigs, it would seem that the rate of oxidation in the Pietrains was limited by the capacity of the phosphorylating system (Eikelenboom & Van den Bergh, 1973). Thus, if this indicated a decreased rate of ATP synthesis through oxidative phosphorylation in pigs with a predisposition for PSE musculature development, these pigs would have to rely on additional ATP producing systems during periods of stress, such as phosphocreatine and through glycolysis and glycogenolysis. A consequence of this feature is that the aerobic pathway would be unable to oxidise the excess NADPH formed through glycolysis, thus forcing ATP production towards anaerobic glycolysis with the resultant accumulation of lactate (Eikelenboom & Van den Bergh, 1973). This could explain the lower phosphocreatine and higher lactate concentrations of PSE prone pigs, even immediately before slaughter (Sair, Lister, Moody, Cassons, Hookstra & Briske, 1970).

Cheah (1973), however, was unable to show the same difference, with the State 3 rates of fresh mitochondria from Pietrains and Large White pigs being similar. He ascribed the differences found by Eikelenboom & Van den Bergh (1973) to pH differences in the *post mortem* muscle from which the mitochondria has been isolated. A low pH value results in the loss of cytochrome c. Similar *in vitro* mitochondrial respiratory traits (State 3, State 4, respiratory control index and ratio of adenosine diphosphate to oxygen) was found in SS and SR pigs (Campion *et al.*, 1976). Therefore the mitochondrial respiration rates measured were not involved in the etiology of stress susceptibility. The activity of mitochondrial ATPase between SS and SR pigs was also found to be similar (Campion *et al.*, 1976). Therefore mitochondrial malfunction seems not to be an important cause of PSE musculature (Cheah, 1973).

With the exposure of SS pigs to halothane, the already problematic situation in which the SS pigs find themselves, is aggravated. Halothane activates calcium release from SS sarcoplasmic reticulum which also shows a calcium induced calcium release. This phenomenon is not found in SR sarcoplasmic reticulum. Halothane also disorders the lipid bilayer of SS sarcoplasmic reticulum to a greater extent than that of SR sarcoplasmic reticulum, and the release of the calcium through open calcium release channels could result in the activation of calcium induced calcium release of the sarcoplasmic reticulum, and cause malignant hyperthermia (Ohnishi, Waring, Fang, Horvath, Flick, Sadanaga & Ohnishi, 1986).

Halothane inhibits NADH dehydrogenase (Nahrholdt, Lecky & Cohen, 1974), and this increase in NADH in the mitochondria decreases the rate of pyruvate oxidation to CO_2 (Soling, Williams, Kleinecke & Gehlhoff, 1970), and the energy production in terms of ATP production could therefore be shifted to a more anaerobic glycolysis and glycogenolysis. Thus, although no mitochondrial malfunction has been found to be important in the production of PSE musculature under normal conditions (Cheah, 1973), it may still be important during the exposure of SS pigs to certain drugs (Mitchell & Heffron, 1980b).

Thus, the halothane exposure of SS pigs have been researched to a high degree with the general conclusion that halothane exposure of SS pigs results in major differences in muscle variables. However, the exposure of SS pigs to physical exercise and its relation to muscle variables has received very little attention.

1.5.5. Malignant hyperthermia and blood variables

With the development of malignant hyperthermia in SS pigs on exposure to halothane, haemococoncentration develops (Froystein, Gronseth, Nostvold & Standal, 1984) and a rise in the lactate and glucose concentrations in the blood are measured (Ahern *et al.*, 1980). Already in 1970 Berman, Harrison, Bull & Kench reported on the haemococoncentration during malignant hyperthermia. They suggested that the water shifts out of the blood into the inter or intra cellular spaces. The haemococoncentration is accompanied by the reduction in muscle density as measured by computerised tomography, and a consequent swelling of muscle cells (Froystein *et al.*, 1984). The increase of extravascular fluid volume accelerate the post mortem rate of glycolysis (Kolarik & Kraeling, 1986) which is found in SS pigs post mortem.

After the treadmill exercise of pigs, the activity of serum CK and LDH are generally higher in SS pigs compared to SR pigs (Schmidt & Kallweit, 1980). Using the hematocrit value as an indicator of haemococoncentration, it is evident that the treadmill exercise of SS and SR pigs do not lead to differences in haemococoncentration between the two types of pigs (Schmidt, 1980). Bicarbonate levels between SS and SR pigs as a result of exercise are not obvious (Schmidt, 1980), although general physical stress in pigs do decrease bicarbonate levels in pigs, and lead to an increase in blood lactate concentration (Van der Wal, Eisgel, Van Esten & Hulshof, 1986). (See also Section 1.1.3).

1.6. Other species susceptible to stress

One of the main reasons for the research on porcine stress syndromes other than its economic implications of this syndromes, is the similarity of porcine and human MHS (Mitchell & Heffron, 1982). The research on porcine stress syndromes may therefore be a model for the syndrome in humans (Mitchell & Heffron, 1982). Stress susceptibility has also been identified in other species,

such as the horse after halothane exposure (Waldron-Mease & Rosenberg, 1979; Waldron-Mease, Klein, Rosenberg & Leitch, 1981; Manley, Kelly & Hodgson, 1983). Although halothane exposure alone did not result in malignant hyperthermia in rabbits, the simultaneous administration of caffeine resulted in symptoms resembling those of malignant hyperthermia (Darbin & Rosenberg, 1979). Malignant hyperthermia as a result of halothane exposure has also been demonstrated in dogs (McGrath, Crimi & Ruff, 1982; O'Brian, Chubb, White, Offert & Steis, 1983), whereas succinylcholine administered to ponies anaesthetised with halothane also resulted in malignant hyperthermia symptoms in ponies (Hildebrand & Howitt, 1983).

1.7. MHS and PSS, are they identical?

As a result of the production of PSE muscle by PSS, MHS and PSES pigs, with common characteristics of the syndrome and the association with uncontrolled muscle glycogenolysis (Lucas *et al.*, 1978), it has been suggested that these syndromes may be identical, possibly expressions of the same myopathy (Harrison, 1972; Nelson *et al.*, 1974). Webb (1980a) argued therefore that with halothane exposure PSS pigs could be identified. According to Lucas (1981), PSS pigs may produce PSE musculature, and may develop malignant hyperthermia as a result of exposure to certain anaesthetics. However, Mitchell & Hedron (1980a) argued that the three stress syndromes, PSS, MHS and PSES are not necessarily identical.

Nevertheless, the mechanism by which halothane precipitates malignant hyperthermia is still unknown (Kallweit *et al.*, 1980) and the existence of possible different stress mechanisms operating, one associated with exercise, and one related to the stress of slaughter have been mentioned before (Kallweit, 1982).

Thus, it would seem that it is generally assumed that PSS and MHS are identical. Therefore it is also assumed that the response of SS pigs to exercise stress or halothane exposure would be similar, although it has never received any attention.

1.8. Conclusion

From the foregoing literature review, it is clear that much research is still needed. For this study, the following has been identified as areas in which research is warranted, and which will be addressed in this study:

- * The influence of stress sensitivity (SS vs SR) on certain growth, carcass and meat characteristics of South African Landrace pigs.

- * The use of the halothane test, blood variables and muscle metabolites for the identification of SS pigs of the South African Landrace breed.
- * The possible differences in muscle fibre types in the South African Landrace breed.
- * To establish whether the halothane induced syndrome is the same as the exercise induced syndrome in the South African Landrace breed.

CHAPTER 2

Methodology: materials and methods

2.1. Animals

For the purpose of this experiment South African Landrace pigs were chosen as a result of the higher incidence of SS pigs found in the breed relative to the South African Large White (Rosouw, 1982), as well as the general higher occurrence of SS pigs within the Landrace type of breeds (McGloughlin & McLoughlin, 1975). Also, only gilts were selected so as to prevent any influences of gender. Although it was planned to buy gilts only from one producer, namely producer X, he changed his breeding practices to such an extent that gilts also had to be bought from producer Y. The gilts were selected on the basis of a halothane screening test carried out between the ages of 7 and 14 weeks to ensure adequate numbers of SS and SR pigs. Animals from producer X were used for Phase 1, and animals from producer Y for Phase 2. All animals were housed in separate pens at the Animal and Dairy Science, Research Institute at Irene, Republic of South Africa. Feed was available *ad libitum*, and water was available at all times.

2.1.1. Halothane screening test

The standard halothane test used by personnel of the Pig Performance Testing Stations in the Republic of South Africa was used. This standard test was performed using a semi-closed Fluotek MK 11 vapouriser system with a close-fitting face mask. After the pig was restrained on its back, the pig was exposed to a concentration of 4% halothane (Fluothane, ICI) in oxygen at a flow-rate of 2.5 l/min for three minutes. If the pig showed signs of the malignant hyperthermia reaction, especially muscle rigidity within the three minutes, the halothane exposure was immediately terminated, and the pig classified as SS. Should the pig not show any signs of malignant hyperthermia or muscle rigidity within the three minutes of halothane exposure, the pig was designated SR.

2.2. The experiment

The experiment was conducted in two phases, Phase 1 during which the pigs were exposed to halothane at the age of 21 weeks, and Phase 2 in which animals were subjected to treadmill exercise instead of halothane exposure.

2.2.1. Phase 1

Forty-seven South African Landrace gilts were used during this phase.

2.2.1.1. Blood variables at the age of 11 weeks

At the age of 11 weeks blood was obtained by jugular venopuncture from the animals during manual restraint. The following variables were determined:

- a) blood lactate (Gutmann & Wahlefeld 1974). 500 μ l of blood was deproteinated in 1ml 0.6M perchloric acid, and centrifuged, and the supernatant used.
- b) blood glucose (Glucose-ate, General Diagnostics). Four ml of blood was collected in a tube containing sodium fluoride and oxalate.
- c) enzyme activities (CK, LDH, aldolase, ALT, AST). Ten ml of blood was left at room temperature for three hours to clot, after which it was centrifuged, and the serum used. The activities of the enzymes were determined at 37°C using commercial Boehringer Mannheim kits; CK (CK NAC-activated), LDH (LDH optimized), AST (GOT optimized), ALT (GPT optimized), and aldolase (aldolase test combination).
- d) electrolytes, urea, total protein, albumin, magnesium, calcium, inorganic phosphate, and bicarbonate in the serum collected for (c). The concentrations of albumin, urea, sodium, potassium, chloride, magnesium, calcium, creatinine and inorganic phosphate were determined on a Technicon SMA II according to the methods described in the Technicon SMA II manual (1977). Magnesium concentration was determined spectrophotometrically (Lancer Magnesium Rapid Stat Diagnostic kit), and total protein by the Biuret method.
- e) hormones. Six ml blood was collected in a heparinised tube, and the plasma collected after centrifugation and frozen for the determination of cortisol and ACTH concentrations. The cortisol and ACTH concentrations were determined using CIS commercially available RIA kits (SB-Cort, Sorin Biomedica, Italy, and ACTH-PR, Compagnie Oris Industrie SA, France). The globulin concentration was calculated as the difference between the total protein concentration and the albumin concentration. Osmolality was estimated using the equation: $\text{osmolality} = (2[\text{sodium}]) + [\text{urea}] + [\text{glucose}]$, and the anion gap by the equation: $\text{anion gap value} = ([\text{sodium}] + [\text{potassium}]) - ([\text{chloride}] + [\text{bicarbonate}])$.

2.2.1.2. Muscle metabolites at the age of 13 weeks

The pigs were manually restrained, whereafter they were anaesthetised using intravenous barbiturate (Intraval - Maybaker) at a rate of 14.90 ± 3.57 mg/kg live mass. A 3 g muscle biopsy was taken from the *M. semitendinosus* of the left side, and frozen in liquid nitrogen until analysis. The preparation of the muscle biopsy for the determination of the muscle metabolites took place on the same day the biopsy was taken. The muscle sample was used to determine the concentrations of ATP, glucose 6-phosphate, lactate, phosphocreatine, glycogen and glucose. The extraction from the frozen sample was done according to Dalrymple & Hamm (1973). The glycogen concentration was determined as glycosyl units after hydrolysis with α -amylglycosidase according to Keppler & Decker (1974). The glucose in the perchloric acid extract filtrate was also determined (Keppler & Decker, 1974), and the glycogen concentration corrected. ATP, glucose 6-phosphate and phosphocreatine were determined in the perchloric acid extract according to the method of Lausprecht, Stein, Heinz & Weisser (1974), and the lactate concentration according to the method of Gutmann & Wahlefeld (1974).

2.2.1.3. Determinations carried out at the age of 21 weeks.

At the age of 21 weeks, the pigs were manually restrained, after which they were exposed to halothane using a close fitting mask and Floetek Mk II vaporiser. The initial halothane concentration was 8% in oxygen at a flow-rate of 2.5 l/min for 30 seconds, after which the concentration was regulated at 5% (3-7%). The pigs were exposed to the halothane for ten minutes.

After 6 minutes of halothane exposure, blood was obtained as described in section 2.2.1.1. The blood variables determined and the methods used are described in section 2.2.1.1.

After the blood was obtained, a muscle biopsy was taken from the *M. semitendinosus* of the right side for the same determinations as in section 2.2.1.2. The methods are described in section 2.2.1.2.

Pigs that died as a consequence of halothane exposure were classified as SS. The carcasses of these animals were immediately taken to an abattoir where they were exsanguinated, scalded, de-haired and eviscerated. Certain carcass and meat characteristics were determined, as is described in section 2.2.1.6. Survivors were classified as SR. These pigs were allowed two weeks to recuperate, after which they were transported to the abattoir (distance of 2 km) and slaughtered. The pigs were electrically stunned (90 V), exsanguinated, scalded, de-haired and eviscerated. Carcass and meat characteristics were determined as described in section 2.2.1.6.

2.2.1.4. Muscle histochemistry

A pencil shaped biopsy from the *M. semitendinosus* from the right side was also taken at the age of 21 weeks for the histological determination of the percentage red, intermediate and white muscle fibres. The samples were sectioned on a cryostat (-20°C) to a thickness of 12 µm. The sections were stained according to the succinic dehydrogenase method of Barka & Anderson (1963). A projection microscope (140x) was used, and the sample slides subjectively scored for fibre type by technicians not familiar with the classification of the pigs, i.e. whether they were classified as being SS or SR. The number of red, intermediate and white muscle fibres were counted at 4 randomly selected areas, and the values expressed as a percentage of the total fibres counted.

2.2.1.5. Growth studies

From the age of 12 weeks until the end of the trial at the age of 21 weeks the pigs were weighed weekly. The amount of feed consumed was recorded. This facilitated the calculation of the average daily gain (ADG) and feed conversion ratio (FCR).

2.2.1.6. Carcass and meat characteristics

In both the SR and SS groups of animals a muscle sample was taken from the *M. longissimus lumborum* immediately after death or slaughter for determination of pH. Muscle samples were incubated in a moist nitrogen atmosphere at 37°C and pH determined at 15, 30, 45 and 60 minutes, and 24 hours post mortem. At each of these times 2 g of muscle was homogenised in 10ml of 5mM Iodoacetate (pH 7.0) (McLoughlin & Tarrant, 1988) and the pH measured using a Labion 17 pH meter (Labotec).

The carcasses were weighed after slaughter and chilled overnight in a chiller at 0°C after which they were again weighed. Twenty-four hours after death or slaughter the carcasses were split. Carcass length was measured (length 1: between first cervical vertebrae and the *symphysis pubis*; length 2: between first thoracic vertebrae and the *symphysis pubis*), and backfat thickness was measured 60 mm from the midline between the tenth and eleventh thoracic vertebrae. The outline of the *M. longissimus thoracis* was traced on graph paper, and the area determined. The *M. longissimus thoracis* of the right side was dissected between the tenth and the last thoracic vertebrae and vacuum packaged after the mass was recorded. The vacuum packaged sample was stored at 0°C for three days after which it was opened and weighed to determine the amount of fluid lost during vacuum packaged storage. The cut was then used for the determination of cooking loss at 60, 70 and 80°C respectively. Six pieces (each about 25 mm thick) from each muscle sample were put into separate plastic bags, cooked for 60 minutes at the respective temperatures (two sample pieces per temperature) without the addition of any fluid, and fluid loss as a result of cooking determined by mass. The anterior samples were subjected to 60°C, the posterior samples to 80°C and the middle to 70°C. The water holding capacity was determined from the fluid lost from a

small sample of cooked muscle placed between filter papers, and subjected to a pressure of 1 metric ton in a Carver Press for one minute. The difference between the original and subsequent masses was calculated and expressed as a percentage of the initial mass. The water holding capacity was determined in quadruplicate for each sample. The "shear" characteristic of samples were determined on cooked samples. Cooked samples were allowed to cool to room temperature, after which samples were taken parallel to the fibre direction with a cork borer (12.5 mm diameter). The force necessary to shear the meat, perpendicular to the fibre direction, was determined using an Instron Materials Testing Machine, fitted with a Warner-Bratzler measuring device.

2.2.2. Phase 2

Nineteen pigs were used in this phase. The pigs were kept under the same husbandry conditions as pigs in the first phase.

2.2.2.1. Blood variables at the age of 11 weeks

The collection of the blood and the determination of the blood variables were carried out as set out in section 2.2.1.1.

2.2.2.2. Muscle metabolites at the age of 13 weeks

A muscle biopsy of the *M. semitendinosus* was taken at the age of 13 weeks from the left side as described in section 2.2.1.2. The muscle metabolites determined and the methodology used is also described in section 2.2.1.2.

2.2.2.3. Determinations carried out at the age of 21 weeks

At 21 weeks of age the untrained pigs were subjected to exercise stress at room temperature (16-18°C) on a treadmill (Whispermill, Squibb) at 0.19 m per second for about 10 minutes, or until a rectal temperature of 40°C was recorded. Temperature was recorded with a Rustrak Ranger with steel shaft thermo-res. (or Gulton Industries). On average exercise lasted 10.34 ± 3.36 minutes. On reaching either end-point, the pigs were anaesthetized using intravenous thiopentone (Intraval sodium, Maybaker, 10.86 ± 2.48 mg/kg).

A blood sample was taken according to the method used in section 2.2.1.1. The blood was analysed using the same methods as in section 2.2.1.1.

Muscle samples were obtained from the *M. semitendinosus* of the right side for determination of muscle metabolites (see section 2.2.1.4.) and muscle fibre classification as set out in section

Four pigs died as a result of the exercise. One week after the exercise-stress, all survivors were subjected to halothane exposure according to the method followed in Phase 1, section 2.2.1.3. On exposure to halothane more pigs died. The animals that died during the treadmill exercise or during the halothane exposure were classified as SS, and the survivors of the treadmill exercise and halothane test as SR. The animals were slaughtered and/or dressed as set out in section 2.2.1.3.

2.2.2.4. Growth studies

Growth studies were carried out as described in section 2.2.1.5.

2.2.2.5. Carcass characteristics

The carcass characteristics were determined as described in section 2.2.1.6.

2.2.2.6. Metabolic characteristics

Metabolic characteristics were determined as set out in section 2.2.1.6. In addition this part of the study the measurement of muscle reflectance values by the Mk 11 Fibre Optic Probe (FOP) (TBL Fibre Optics Group Ltd.) and NEI Smoke/Steam Refractometer (Diffusion Systems Ltd.) were included as well as the muscle reflectance test after the vacuum storage period. The reflectance value of the muscle indicates the amount of white light reflected from the surface of the muscle, indicating the amount of the muscle that is indirectly the amount of denaturation. The FOP reflectance value was determined in the *M. longissimus thoracis* between the 10th and 11th thoracic vertebrae 74 days after slaughter. After cutting the carcass through between the 10th and 11th vertebrae, the reflectance value of the cut surface of the *M. longissimus thoracis* was determined at three different locations: dorsal, ventral and in the middle of the muscle, 15 minutes after cutting through the muscle. The average of the three measurements was calculated. The volume of the exudate after the vacuum packaged period was determined by measuring the volume in a measuring cylinder.

2.2.3. Statistical analysis

The results of the halothane and/or exercise exposure in Phase 1 and 2 at 71 weeks of age were used for the classification of SR (survived) and SS (died) in the statistical analysis, as it was regarded as being more accurate than the initial halothane test which was a test to assure adequate numbers of pigs in either category. Data was analysed using analysis of variance (and level of differences, unpaired t-tests and regression analyses) of the commercially available micro computer programme Statgraphics (version 2.1.5) (Statistical Graphics Corporation). Differences of $P < 0.05$ were considered to be significant. Although the actual P values are given in the relevant tables, the following symbols

have also been included in the tables for the convenience of the reader: NS = not significant; $P > 0,05$; * = significant $P \leq 0,05$; ** = highly significant $P \leq 0,01$.

CHAPTER 3

The predictive value of the halothane test, blood variables and muscle metabolites

3.1. Introduction

As a result of the negative influence SS pigs have on meat quality (Eikelenboom & Minkema, 1974) and therefore the severe economic disadvantages for the pig industry (Hall, 1972; Webb & Jordaa, 1978; Carden *et al.*, 1985), it is important to identify SS pigs at an early age. Several possible tests have been suggested and/or have been introduced. These are, for example, the halothane test (Eikelenboom & Minkema, 1974), use of serum CK, LDH and aldolase activities (Berman *et al.*, 1972; Eikelenboom & Minkema, 1974) as well as muscle contractures (Schmitzen *et al.*, 1981c). This experiment was conducted to evaluate some of these suggested tests as identifiers of SS pigs amongst the South African Landrace breed, as well as the possible use of muscle metabolites (Okumura *et al.*, 1979). Differences in various muscle metabolites have been found between SS and SR pigs (Harrison *et al.*, 1969; Nelson *et al.*, 1974; Mitchell *et al.*, 1980a). In this chapter the results of the experiment evaluating the predictive value of the halothane test, blood variables and muscle metabolites are given.

3.2. Results

3.2.1. The halothane test as predictive test for SS pigs

3.2.1.1. The halothane test and the pigs to Phase 1

Using the halothane test (4% halothane in oxygen for 3 minutes) as described to select pigs for my study, 30 pigs from herd X were initially classified as SR, and 17 pigs as SS because they displayed muscle rigidity. At the age of 21 weeks the pigs were again exposed to halothane, although at a higher initial concentration and for a longer time period of 10 minutes. Again 30 pigs were

classified as being SR on the absence of any muscle rigidity during the 10 minutes of halothane exposure. Also, 17 pigs were classified SS as a result of muscle rigidity and subsequent death. However, although the number of pigs classified as SR and SS remained the same as a result of the two halothane exposures, three of the pigs initially classified as SR died as a result of the halothane exposure, showing signs of muscle rigidity, and three pigs initially classified as SS were reclassified SR as a result of surviving the second halothane exposure, and also showing no signs of malignant hyperthermia and muscle rigidity. These pigs were subsequently reclassified according to the results obtained during the halothane exposure at 21 weeks of age. Therefore, the two halothane tests resulted in an intercorrelation of 10% for the SR pigs, and 18% for the SS pigs.

3.2.1.2. The halothane test and the pigs in Phase 2

The pigs were classified SR as a result of the initial halothane test (4% halothane in oxygen for 3 minutes), and 9 were classified SS. As a result of the treadmill exercise, four pigs initially classified SS died, and it was therefore assumed that these four pigs would have died on exposure to the higher concentration of halothane and extended exposure period as was applied during the halothane exposure at the age of 21 weeks. During this latter halothane exposure, the 10 initially SR classified pigs survived the halothane exposure, whereas the five remaining initially SS classified pigs all succumbed to the halothane exposure, showing signs of muscle rigidity. In this herd the accuracy of the halothane test was therefore 100%.

3.2.1.3. The halothane test and the total number of pigs

Combining these results of the two halothane exposures and treadmill exercise, 60 pigs out of 66 were classified the same during the two exposures, thus a repeatability of 91% on two tests and a disagreement of 9%.

3.2.2. Blood variables at 11 weeks of age

The analysis of blood variables at the age of 11 weeks were used to determine whether any differences exist between the SR and SS pigs under the same conditions of blood taking, namely manual restraint, and whether it would be possible to predict SS and SR pigs from these differences.

The results were analysed according to:

- * the influence of stress sensitivity and herd on the blood variables of all the pigs
- * the influence of stress sensitivity on the blood variables of pigs from herd X
- * the influence of stress sensitivity on the blood variables of pigs from herd Y
- * the influence of herd on the blood variables of the SR pigs

* the influence of herd on the blood variables of the SS pigs.

These analyses were repeated using initial halothane test to classify SS and SR pigs as a result of the differences found between the initial and final classification of the South African Landrace pigs. The tables containing the information of these analyses are given in Appendix A, Tables A.1 to A.5.

3.2.2.1. The influence of stress sensitivity and herd on blood variables of 11 week old pigs

The results of this analysis are illustrated in Table 3.1, with the mean values given in Table 3.2. Significant differences as a result of stress sensitivity (SR vs SS) were only recorded for the enzyme activities of LDH and aldolase, and for the concentrations of total protein, inorganic phosphate and cortisol (Column A, Table 3.1). The SS pigs had the higher LDH and aldolase activities, higher inorganic phosphate concentration, and the lower total protein and cortisol concentrations

Table 3.1: The results of 2-way analyses of variance on the blood variables of pigs as influenced by stress sensitivity (A: SR vs SS) and herd (B: herd X vs herd Y)

Variable	Stress sensitivity (A)			Herd (B)			AxB		
	F value	Significance level		F value	Significance level		F value	Significance level	
CK	0,003	0,9562	NS	0,379	0,5469	NS	1,436	0,2333	NS
LDH	11,421	0,0013	**	0,888	0,3596	NS	3,497	0,0662	NS
Aldolase	12,601	0,0038	**	45,853	<0,0001	**	5,808	0,0193	*
AST	0,243	0,6290	NS	0,236	0,6343	NS	5,735	0,0197	*
ALT	2,374	0,1285	NS	9,947	0,0025	**	1,159	0,2839	NS
Lactate	1,377	0,2453	NS	0,390	0,5414	NS	1,387	0,2435	NS
Total protein	9,442	0,0031	**	0,341	0,5678	NS	1,161	0,2854	NS
Albumin	2,303	0,1342	NS	3,170	0,0799	NS	1,607	0,2096	NS
Globulin	2,073	0,1549	NS	1,469	0,2270	NS	1,236	0,2533	NS
Urea	0,860	0,3671	NS	36,985	<0,0001	**	5,245	0,0294	*
Sodium	3,811	0,0554	NS	0,348	0,5635	NS	3,647	0,0608	NS
Potassium	0,463	0,5061	NS	3,643	0,0609	NS	0,328	0,5751	NS
Chloride	2,038	0,1584	NS	1,513	0,2233	NS	2,644	0,1090	NS
Magnesium	0,003	0,8051	NS	37,564	<0,0001	**	0,006	0,9372	NS
Calcium	0,101	0,7547	NS	4,349	0,0412	*	0,494	0,4924	NS
Creatinine	0,928	0,3495	NS	14,200	0,0004	**	6,45	0,0137	*
Glucose	0,167	0,6887	NS	18,756	0,0001	**	1,534	0,2201	NS
Inorganic phosphate	11,649	0,0011	**	0,247	0,6263	NS	33,273	<0,0001	**
Bicarbonate	2,980	0,0893	NS	5,855	0,0175	*	0,081	0,7800	NS
Cortisol	5,833	0,0187	*	11,716	0,0011	**	0,148	0,7059	NS
ACTH	0,847	0,3707	NS	2,053	0,1570	NS	0,091	0,9816	NS
Urea/creatinine ratio	0,604	0,4485	NS	61,496	<0,0001	**	0,277	0,6065	NS
Albumin/globulin ratio	0,006	0,9384	NS	3,145	0,0811	NS	0,039	0,8663	NS
Osmolality	3,473	0,0671	NS	0,127	0,8724	NS	2,599	0,1120	NS
Anion gap	0,829	0,8673	NS	7,635	0,0075	**	0,422	0,5253	NS

(Table 3.2). Classifying the pigs according to the initial halothane test, the SS pigs also had a lower ALT activity, albumin and urea concentrations (Tables A.1 and A.2).

Table 3.2: Mean values and standard deviations (sd) of blood variables as influenced by stress sensitivity and herd

Variable	SR pigs			SS pigs			Herd X			Herd Y			
	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n	
K	IU/l	2092	1823	40	2138	788	26	1634	47	2259	1146	19	
LDH	IU/l	1205	243	40	1474	384	26	286	311	47	1368	19	
Aldolase	IU/l	10.4	3.7	35	13.1	3.1	23	13.3	3.9	42	7.4	2.7	18
AST	IU/l	56	17	40	56	16	26	38	17	47	55	16	19
ALT	IU/l	52	13	40	-7	11	26	23	13	47	49	9	19
Lactate	mmol/l	7.85	2.26	39	7.12	2.33	25	7.71	2.47	45	7.23	1.82	19
Total protein	mmol/l	61	6	40	57	5	26	60	7	47	59	4	19
Albumin	mmol/l	34	3	40	33	6	26	33	4	47	35	6	19
Globulin	mmol/l	27	6	40	25	4	26	27	6	47	25	3	19
Urea	mmol/l	3.4	1.2	40	3.4	0.7	26	3.1	0.8	47	4.3	0.7	19
Sodium	mmol/l	149	6	40	152	4	26	150	3	47	150	8	19
Potassium	mmol/l	7.2	1.3	40	7.5	0.9	26	7.1	1.0	47	7.8	1.5	19
Chloride	mmol/l	101	5	40	102	2	26	101	2	47	100	6	19
Magnesium	mmol/l	1.07	0.17	40	1.08	0.17	25	1.00	0.16	46	1.25	0.10	19
Calcium	mmol/l	2.81	0.17	40	2.80	0.16	26	2.78	0.14	47	2.87	0.23	19
Creatinine	mmol/l	99	13	39	102	12	25	104	13	46	91	10	18
Glucose	mmol/l	6.1	0.5	40	6.2	0.6	26	5.9	0.6	47	6.7	0.7	19
Inorganic phosphate	mmol/l	3.18	0.33	39	3.44	0.33	26	3.26	0.34	46	3.33	0.17	19
Bicarbonate	mmol/l	23	1	40	24	3	26	24	3	47	22	3	19
Cortisol	nmol/l	28	16	40	22	6	26	23	11	47	33	16	19
ACTH	pmol/l	10	10	40	8	4	26	11	10	47	17	3	19
Urea/creatinine ratio		35	11	39	34	9	25	30	8	46	47	9	18
Albumin/globulin ratio		1.33	0.29	40	1.35	0.29	26	1.30	0.28	47	1.44	0.30	19
Osmolality	mmol/l	308	11	40	315	7	26	310	7	47	311	15	19
Anion gap	mmol/l	33	4	40	34	4	26	33	4	47	36	4	19

Several significant differences were found in blood variables between the two herds (Column B, Table 3.1). The pigs from herd Y had the higher urea, magnesium, calcium, glucose and cortisol concentrations, as well as a higher urea-to-creatinine ratio and anion gap value than the pigs from herd X. However, the pigs from herd Y also had the lower aldolase and ALT activities, and lower creatinine and bicarbonate concentrations (Table 3.2).

Interactions between herd and stress type were recorded for aldolase and AST activity, and the concentrations of urea, sodium, creatinine and inorganic phosphate.

3.2.2.2. The influence of stress sensitivity on the blood variables of pigs from herd X

The results of this analysis are given in Table 3.3. The results indicate that the aldolase activity and creatinine concentration of the SS pigs of herd X are higher than those of the SR pigs. However, the total protein, albumin and cortisol concentrations of the SS pigs were lower than those of the SR pigs. No other significant differences were found between the blood variables of SS and SR pigs of herd X at the age of 11 weeks. If the SS and SR pigs were classified according to the initial halothane test, the SS pigs also had lower AST and ALT activities than the SR pigs, although the creatinine concentrations of the SS and SR pigs were similar (Table A.3).

Table 3.3: Mean values, standard deviations (sd) and level of significance of blood variables from pigs in herd X as influenced by stress sensitivity

Variables	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
CK IU/l	2138	1996	30	1854	635	17	0,5722 NS
LDH IU/l	1220	267	30	1386	360	17	0,0776 NS
Aldolase IU/l	11,6	5,4	25	15,8	3,2	17	0,0002 **
AST IU/l	61	18	30	52	14	17	0,0947 NS
ALT IU/l	56	13	30	49	13	17	0,0852 NS
Lactate mmol/l	8,12	2,53	29	6,96	2,24	16	0,1340 NS
Total protein mmol/l	62	6	30	56	6	17	0,0361 **
Albumin mmol/l	34	3	30	32	4	17	0,0173 *
Globulin mmol/l	27	8	30	25	4	17	0,5030 NS
Urea mmol/l	3,0	0,7	30	3,1	0,8	17	0,6347 NS
Sodium mmol/l	150	3	30	151	4	17	0,3956 NS
Potassium mmol/l	7,1	1,1	30	7,2	0,8	17	0,7723 NS
Chloride mmol/l	101	3	30	102	2	17	0,6543 NS
Magnesium mmol/l	1,00	0,15	30	0,99	0,20	16	0,8192 NS
Calcium mmol/l	2,79	0,13	30	2,76	0,15	17	0,4258 NS
Creatinine μ mol/l	101	14	29	109	11	17	0,0455 *
Glucose mmol/l	5,9	0,6	30	6,1	0,6	17	0,2996 NS
Inorganic phosphate mmol/l	3,27	0,32	29	3,20	0,37	17	0,7566 NS
Bicarbonate mmol/l	23	4	30	24	2	17	0,2112 NS
Cortisol nmol/l	25	13	30	18	6	17	0,0474 *
ACTH pmol/l	11	12	30	9	5	17	0,5160 NS
Urea/creatinine ratio	30	8	29	29	8	17	0,7017 NS
Albumin/globulin ratio	1,25	0,30	30	1,31	0,25	17	0,8598 NS
Osmolality mmol/l	309	6	30	311	8	17	0,0286 NS
Anion gap mmol/l	33	5	30	32	4	17	0,6193 NS

3.2.2.3. The influence of stress sensitivity on the blood variables of pigs from herd Y

The influence of stress susceptibility on the various blood variables of the pigs from herd Y are given in Table 3.4. These results differ somewhat from those of herd X. The SS pigs from herd Y had a higher LDH activity than the SR pigs, as well as a higher concentration of inorganic phosphate. However, the SS pigs had a lower urea concentration than the SR pigs. No other significant differences were found.

Table 3.4: Mean values, standard deviations (sd) and level of significance of blood variables from pigs in herd Y as influenced by stress sensitivity

Variable	SR pigs			SS pigs			Significance level	
	Mean	sd	n	Mean	sd	n		
CK	IU/l	1952	1241	10	2675	1027	9	0,1876 NS
LDH	IU/l	1160	153	10	1642	428	9	0,0039 **
Aldolase	IU/l	7,4	2,6	10	7,4	2,9	8	0,9556 NS
AST	IU/l	49	11	10	62	20	9	0,0903 NS
ALT	IU/l	42	11	10	43	8	9	0,9517 NS
Lactate	mmol/l	7,08	0,90	10	7,41	2,48	9	0,6974 NS
Total protein	mmol/l	60	4	10	57	4	9	0,2564 NS
Albumin	mmol/l	35	3	10	35	8	9	0,8766 NS
Globulin	mmol/l	25	3	10	25	3	9	0,7816 NS
Urea	mmol/l	4,7	0,9	10	3,8	0,4	9	0,0197 *
Sodium	mmol/l	147	10	10	153	3	9	0,1065 NS
Potassium	mmol/l	7,6	1,8	10	8,0	1,1	9	0,5176 NS
Chloride	mmol/l	99	8	10	102	2	9	0,1942 NS
Magnesium	mmol/l	1,25	0,10	10	1,25	0,10	9	0,9138 NS
Calcium	mmol/l	2,86	0,27	10	2,90	0,17	9	0,7603 NS
Creatinine	μmol/l	95	7	10	86	13	8	0,0627 NS
Glucose	mmol/l	6,8	0,9	10	6,6	0,4	9	0,4571 NS
Inorganic phosphate	mmol/l	2,89	0,10	10	3,82	0,22	9	<0,0001 **
Bicarbonate	mmol/l	21	3	10	22	3	9	0,2359 NS
Cortisol	nmol/l	38	20	10	29	7	9	0,1055 NS
ACTH	pmol/l	8	3	10	6	3	9	0,1551 NS
Urea/creatinine ratio		49	9	10	46	10	8	0,4492 NS
Albumin/globulin ratio		1,44	0,25	10	1,43	0,35	9	0,9091 NS
Osmolality	mmol/l	306	20	10	316	7	9	0,1474 NS
Anion gap	mmol/l	35	4	10	36	5	9	0,6602 NS

3.2.2.4. The influence of herd on the blood variables of SR pigs

The results of this analysis are given in Table 3.5. The aldolase and ALT activities and the concentration of inorganic phosphate of the SR pigs from herd Y were lower than those of herd X. The concentrations of urea, magnesium, glucose, cortisol and the urea-to-creatinine ratio were higher in the SR pigs from herd Y than those of herd X. No significant differences were found

for the remaining blood variables determined. Classifying the SS and SR pigs according to the initial halothane test performed between the ages of 7 and 11 weeks, resulted in the SS pigs having a lower AST activity than the SR pigs (Table A.5).

Table 3.5: Mean values, standard deviations (sd) and level of significance of blood variables from SR pigs as influenced by herd

Variable		Herd X			Herd Y			Significance level
		Mean	sd	n	Mean	sd	n	
CK	IU/l	2138	1996	30	1952	1241	10	0,7940 NS
LDH	IU/l	1220	267	30	1160	153	10	0,5078 NS
Aldolase	IU/l	11,6	3,4	25	7,4	2,6	10	0,0015 **
AST	IU/l	61	18	30	49	11	10	0,0626 NS
ALT	IU/l	56	13	30	42	11	10	0,0050 **
Lactate	mmol/l	6,12	2,53	29	7,08	0,90	10	0,2138 NS
Total protein	mmol/l	72	6	30	60	4	10	0,2759 NS
Albumin	mmol/l	34	3	30	35	3	10	0,5030 NS
Globulin	mmol/l	27	8	30	25	3	10	0,1407 NS
Urea	mmol/l	3,0	0,7	30	4,7	0,9	10	<0,0001 **
Sodium	mmol/l	150	3	30	147	10	10	0,1398 NS
Potassium	mmol/l	7,1	1,1	30	7,6	1,8	10	0,3567 NS
Chloride	mmol/l	101	3	30	99	8	10	0,1004 NS
Magnesium	mmol/l	1,00	0,15	30	1,25	0,10	10	<0,0001 **
Calcium	mmol/l	2,79	0,13	30	2,86	0,27	10	0,2933 NS
Creatinine	μmol/l	101	14	29	95	7	10	0,2434 NS
Glucose	mmol/l	5,9	0,6	30	6,8	0,9	10	0,0095 **
Inorganic phosphate	mmol/l	3,27	0,52	29	2,89	0,10	10	0,0006 **
Bicarbonate	mmol/l	25	4	30	21	5	10	0,0758 NS
Cortisol	nmol/l	25	13	30	38	20	10	0,0252 *
ACTH	pmol/l	11	12	30	6	3	10	0,2911 NS
Urea/creatinine ratio		30	8	29	49	9	10	<0,0001 **
Albumin/globulin ratio		1,29	0,30	30	1,44	0,25	10	0,1531 NS
Osmolality	mmol/l	309	6	30	306	20	10	0,4055 NS
Anion gap	mmol/l	33	5	30	35	4	10	0,1126 NS

3.2.2.5. The influence of herd on the blood variables of SS pigs

The results are shown in Table 3.6, and show that the CK activity of the SS pigs from herd Y was higher than that of herd X, although the aldolase activity of the SS pigs from herd Y was lower. The SS pigs from herd Y had higher concentrations of urea, potassium, magnesium, glucose, inorganic phosphate and cortisol than the SS pigs from herd X, as well as a higher urea-to-creatinine ratio and anion gap value. A lower creatinine concentration was recorded for the SS pigs from herd Y. No other significant differences between the SS pigs from herd X and Y were found.

However, the SS pigs also had a higher calcium concentration and osmolality if the pigs were classified as SS and SR pigs according to the initial halothane test performed between the ages of 7 and 11 weeks (Table A.5).

Table 3.6: Mean values, standard deviations (sd) and level of significance of blood variables from SS pigs as influenced by herd

Variable		Herd X			Herd Y			Significance level
		Mean	sd	n	Mean	sd	n	
CK	IU/l	1854	635	17	2675	1027	9	0,0185 *
LDH	IU/l	1386	360	17	1642	428	9	0,1193 NS
Aldolase	IU/l	15,8	3,1	17	7,4	2,9	8	<0,0001 **
AST	IU/l	52	14	17	62	20	9	0,1433 NS
ALT	IU/l	49	13	17	43	8	9	0,1867 NS
Lactate	mmol/l	6,96	2,24	16	7,41	2,48	9	0,6490 NS
Total protein	mmol/l	56	6	17	57	4	9	0,6360 NS
Albumin	mmol/l	32	4	17	35	8	9	0,1200 NS
Globulin	mmol/l	25	4	17	25	3	9	0,9435 NS
Urea	mmol/l	3,1	0,8	17	3,8	0,4	9	0,0186 *
Sodium	mmol/l	151	4	17	153	3	9	0,1753 NS
Potassium	mmol/l	7,2	0,8	17	8,0	1,1	9	0,0359 *
Chloride	mmol/l	102	2	17	102	2	9	0,5033 NS
Magnesium	mmol/l	0,99	0,20	16	1,25	0,10	9	0,0013 **
Calcium	mmol/l	2,76	0,15	17	2,90	0,17	9	0,0570 NS
Creatinine	μ mol/l	109	11	17	86	13	8	0,0001 **
Glucose	mmol/l	6,1	0,6	17	6,6	0,8	9	0,0340 *
Inorganic phosphate	mmol/l	3,20	0,37	17	3,82	0,22	9	0,0001 **
Bicarbonate	mmol/l	24	2	17	22	3	9	0,0948 NS
Cortisol	nmol/l	18	6	17	29	7	9	0,0008 **
ACTH	pmol/l	9	5	17	6	3	9	0,0566 NS
Urea/creatinine ratio		29	8	17	46	10	8	0,0002 **
Albumin/globulin ratio		1,31	0,25	17	1,43	0,35	9	0,3137 NS
Osmolality	mmol/l	311	8	17	316	7	9	0,0553 NS
Anion gap	mmol/l	32	4	17	36	5	9	0,0273 *

3.2.3. Muscle metabolites

The muscle variables were also analysed according to:

- * the influence of stress sensitivity and herd on the muscle metabolites of all the pigs
- * the influence of stress sensitivity on the muscle metabolites of pigs from herd X
- * the influence of stress sensitivity on the muscle metabolites of pigs from herd Y

* the influence of herd on the SR pigs

* the influence of herd on muscle metabolites of the SS pigs.

These analyses were repeated using the initial classification of SS and SR pigs as was found using the halothane test between the ages of 7 and 11 weeks, because of the differences in classification found between the initial and final classification of SS and SR pigs. These results are shown in Appendix A, Tables A.6 to A.10.

3.2.3.1. The influence of stress sensitivity and herd on the muscle metabolites of 13 week old pigs

In relation to animal stress sensitivity (Column A, Table 3.7), the SS pigs had generally higher concentrations of lactate, glucose 6-phosphate and glucose than the SR pigs, with a concomitant lower phosphocreatine concentration. No significant difference were found between SS and SR pigs concerning the concentrations of ATP and glycogen (Table 3.8). However, using the initial halothane test to classify SS and SR pigs, the difference in the glucose concentrations between the SS and SR pigs was not significant (Tables A.6 and A.7).

Table 3.7: The results of 2-way analyses of variance on the muscle metabolites of pigs as influenced by stress sensitivity (A: SR vs SS) and herd (B: herd X vs herd Y)

Variable	Stress susceptibility (A)		Herd (B)		AxB	
	F value	Significance level	F value	Significance level	F value	Significance level
Lactate	41,891	<0,0001 **	54,997	<0,0001 **	0,128	0,7251 NS
ATP	3,096	0,0834 NS	7,140	0,0096 **	0,179	0,6778 NS
Glucose 6-phosphate	24,407	<0,0001 **	4,031	0,0490 **	16,013	0,0001 **
Phosphocreatine	21,683	<0,0001 **	26,811	<0,0001 **	0,917	0,3522 NS
Glucose	5,476	0,0225 *	0,112	0,7430 NS	6,666	0,0122 *
Glycogen	0,634	0,4375 NS	24,263	<0,0001 **	4,046	0,0486 *

Table 3.8: Mean values and standard deviations (sd) of muscle metabolites as influenced by stress sensitivity and herd

Variable	SR pigs			SS pigs			Herd X			Herd Y		
	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n
Lactate	11,21	7,94	40	22,55	8,73	27	11,76	7,77	47	25,32	7,91	19
ATP	5,61	1,70	40	4,87	1,00	27	5,64	1,60	47	4,53	0,85	19
Glucose 6-phosphate	1,29	1,11	40	2,71	1,45	27	1,64	1,60	47	2,40	0,3	19
Phosphocreatine	0,65	4,89	40	3,86	2,86	27	6,30	4,65	47	2,88	1,48	19
Glucose	0,63	0,39	40	0,85	0,47	27	0,70	0,42	47	0,77	0,36	19
Glycogen	62,48	13,11	40	57,21	24,46	27	66,62	17,85	47	45,13	6,25	19

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose $\mu\text{mol/g}$ muscle
Glycogen: $\mu\text{mol/g}$ glycogen unit/g muscle

As is illustrated in Tables 3.7, herd had a significant influence on the different muscle variables studied (Column B, Table 3.7). The concentrations of lactate and glucose 6-phosphate in the pigs from herd Y was higher than for the corresponding herd X, but the concentrations of ATP, phosphocreatine and glycogen were lower for the pigs from herd Y (Table 3.8).

Significant differences in the 2-way interactions of herd and stress sensitivity were found for glucose 6-phosphate and glucose concentrations.

3.2.3.2. The influence of stress sensitivity on the muscle metabolites of pigs from herd X

The results are shown in Table 3.9. The lactate and glucose 6-phosphate concentrations of the SS pigs from herd X were higher than those of the SR pigs, with a concomitant lower phosphocreatine concentration in the SR pigs. No difference was found between the ATP, glucose and glycogen concentrations of the SS and SR pigs. Similar results were found using the initial halothane test for classifying stress sensitivity (Table A.8).

Table 3.9: Mean values, standard deviations (sd) and level of significance of muscle metabolites of pigs from herd X as influenced by stress sensitivity

Variable	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
Lactate	7.97	5.29	30	18.47	5.54	17	< 0.0001 **
ATP	5.91	1.85	30	5.17	0.68	17	0.1340 NS
Glucose 6-phosphate	0.86	0.87	30	3.03	1.68	17	< 0.0001 **
Phosphocreatine	10.12	4.72	30	5.35	1.79	17	0.0003 **
Glucose	0.68	0.39	30	0.75	0.28	17	0.0125 NS
Glycogen	65.85	13.20	30	67.9	17.7	17	0.7004 NS

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose: $\mu\text{mol/g}$
 Glycogen: $\mu\text{mol/glycogen unit/g muscle}$

3.2.3.3. The influence of stress sensitivity on the muscle metabolites of pigs from herd Y

The results of this analysis are given in Table 3.10. The lactate and glucose concentrations of the SS pigs were higher than those of the SR pigs. However, the SS pigs had lower phosphocreatine and glycogen concentrations. No significant differences in the ATP and glucose 6-phosphate concentrations between the SS and SR pigs were found.

3.2.3.4. The influence of herd on the muscle metabolites of SR pigs

The results are given in Table 3.11. The SR pigs from herd Y had significantly higher lactate and glucose 6-phosphate concentrations than the SR pigs from herd X. The SR pigs from herd Y also had lower phosphocreatine and glycogen concentrations than the SR pigs of herd X. No significant differences were found between the ATP and glucose concentrations of the SR pigs

of herd X and Y. The results of the analysis using the initial halothane test to classify SS and SR pigs were similar (Table A.9).

Table 3.10: Mean values, standard deviations (sd) and level of significance of muscle metabolites of pigs from herd Y as influenced by stress sensitivity

Variable	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
Lactate	20.93	6.55	10	20.21	9.20	9	0.0206 *
ATP	4.73	0.61	10	4.32	1.06	9	0.3198 NS
Glucose 6-phosphate	2.60	0.62	10	2.19	0.83	9	0.2303 NS
Phosphocreatine	4.25	1.77	10	1.36	1.05	9	0.0005 **
Glucose	0.46	0.38	10	1.11	0.35	9	0.0013 **
Glycogen	52.37	5.59	10	37.10	6.92	9	<0.0001 **

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose : $\mu\text{mol/g}$ muscle
Glycogen : $\mu\text{mol glycogen/unit/g}$ muscle

Table 3.11: Mean values, standard deviations (sd) and level of significance of muscle metabolites from SR pigs as influenced by herd

Variable	Herd X			Herd Y			Significance level
	Mean	sd	n	Mean	sd	n	
Lactate	7.97	5.29	30	20.93	6.55	10	<0.0001 **
ATP	5.91	1.85	30	4.73	0.61	10	0.0570 NS
Glucose 6-phosphate	0.86	0.87	30	2.60	0.62	10	<0.0001 **
Phosphocreatine	10.12	4.72	30	4.25	1.77	10	0.0004 **
Glucose	0.68	0.39	30	0.46	0.38	10	0.1330 NS
Glycogen	65.85	13.20	30	52.37	5.59	10	0.0034 **

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose : $\mu\text{mol/g}$ muscle
Glycogen : $\mu\text{mol glycogen/unit/g}$ muscle

3.2.3.5. The influence of herd on the muscle metabolites of SS pigs

Table 3.12 gives the results of the analysis of the influence of herd on the muscle variables of SS pigs. The analysis shows that the SS pigs from herd Y had a significantly higher lactate concentration than the SS pigs from herd X, whereas the concentrations of ATP, phosphocreatine and glycogen were lower in the SS pigs of herd Y than the SS pigs from herd X. No significant differences were found between the glucose 6-phosphate and glucose concentrations of the SS pigs as a result of herd influence. However, using the initial halothane test to classify pigs as SS or SR, it was found that the SS pigs from herd Y had a higher glucose concentration than the SS pigs from herd X (Table A.10).

Table 3.12. Mean values, standard deviations (sd) and level of significance of muscle metabolites from SS pigs as influenced by herd

Variable	Herd X			Herd Y			Significance level
	Mean	sd	n	Mean	sd	n	
Lactate	18.47	5.54	17	30.21	9.20	9	0.0004 **
ATP	5.17	0.89	17	4.32	1.06	9	0.0408 *
Glucose 6-phosphate	5.03	1.66	17	2.19	0.20	9	0.1774 NS
Phosphocreatine	5.35	2.53	17	1.56	1.05	9	0.0001 **
Glucose	0.75	0.47	17	1.11	0.35	9	0.0359 NS
Glycogen	67.97 ^a	24.44	17	37.10	6.92	9	0.0012 **

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose : $\mu\text{mol/g}$ muscle
 Glycogen : $\mu\text{mol glycoyl units/g}$ muscle

3.3. Discussion

Differences in classification of pigs as being SR or SS by using the halothane test has been reported previously (Webb & Jordan, 1978; Petri *et al.*, 1979; Webb, 1980a). Webb & Jordan (1978) found a 9% disagreement between repeated halothane tests after a 20 day interval, in which pigs initially classified SS, were reclassified as being SR during the second halothane test. This is in close agreement with the results obtained in my study. Using an intravenous halothane test, a dose/response relationship was found in pigs after a certain threshold was exceeded, and this threshold has been found to vary between individual pigs (Gregory & Wilkins, 1984), which is in agreement with the hypothesis of a simple autosomal recessive gene with possible high or complete penetrance, but with variable expression of one of the recessive genes (Hradecky *et al.*, 1980; Pazdera *et al.*, 1983). Gregory & Wilkins (1984) subsequently speculate that, by using the conventional halothane test, SS pigs could produce a mild acidosis response, without it being expressed towards muscle contracture and limb rigidity. The pig is thus being classified as SR. Differences in classification may thus occur. Variations in classification may also result due to factors such as the variation in exertional, nutritional and/or health status of the pig (Marby, Christian & Kuhlers, 1981). Also the age at which the pig is halothane tested, the halothane concentration (Kallweit *et al.*, 1980; McGrath *et al.*, 1984), duration of the test (Webb & Jordan, 1978) and ambient temperature (Kallweit *et al.*, 1981) influence the outcome of the halothane test. Another reason for misclassification is the experience of the operator, and it is thought that this might be the obvious reason for differences in my study in the classification of the pigs as SS and SR between the two halothane tests. Unfortunately, the halothane test does not identify stress susceptibility carrier (*Nn*) pigs (Gregory & Wilkins, 1984).

Although the classification of SS pigs could be done according to the initial halothane test performed between the ages of 7 and 11 weeks of age, or the final test classifying the SS pigs as those that died as a result of treadmill exercise or halothane exposure, and although differences were found in blood variables and muscle metabolites as a result of the two different classification

methods, the discussion will centre around the classification using the final test. The reason for this is that the final test leaves little room for misclassification of pigs as a result of operator inexperience, which might have been the case during the initial halothane test. Also, the differences in blood variables and muscle metabolites between the initial and final classification are of a minor nature.

In the literature the use of CK, LDH and aldolase activities have already been indicated as being useful in the identification of SS and SR pigs (Berman *et al.*, 1970; Berman *et al.*, 1972; Eikelenboom & Minkema, 1974). However, in my study no difference was found in CK activity between the SS and SR pigs, although differences were recorded between the SS and SR pigs regarding LDH and aldolase activities. On further investigation it was found that herd had an influence on the possible use of LDH and aldolase activities for the identification of SS and SR pigs. The SS pigs from herd X had a higher aldolase activity than the SR pigs, without any difference in their LDH activities. Still, the SS pigs from herd Y had a higher LDH activity than the SR pigs, with the aldolase activities being similar. Therefore, the use of these two enzyme activities for classification purposes are influenced by herd effects, and accordingly not unequivocally. This finding could thus explain some of the differences being reported in the literature using LDH and aldolase activities as identifiers of SS pigs.

A possible explanation why the CK activities between the SS and SR pigs were not significantly different is given by Mitchell & Heffron (1975). According to their results CK activities during the rapid growth phase do not significantly differ between SS and SR pigs, and has therefore no diagnostic value during this period. It is suggested that the CK values should only be used before the age of 11 weeks, and after 28 weeks for possible classification of stress susceptibility purposes (Mitchell & Heffron, 1975). The use of CK activities might still be of importance, especially if the pigs are stressed before a blood sample is taken, as has been proposed by Richardt (1979). In this regard, Richardt (1979) has indicated that CK activity can only be used with relative accuracy under certain standardised conditions, such as a standardised stressing procedure applied before CK is determined, in the prediction of SS pigs which was not part of the methodology followed in this study.

Although the analysis over the total number of pigs also indicated significant differences in total protein, inorganic phosphate and cortisol, these differences between SS and SR pigs were also subject to differences between the different herds. Therefore these variables would also be prone to herd differences and could therefore not be used for unequivocally identifying SS and SR pigs.

The use of muscle metabolites of the *M. semitendinosus* at 13 weeks of age was also investigated for the possible use in the identification of SS pigs. Possible metabolites were found to be lactate, glucose 6-phosphate, phosphocreatine and glucose. In both the herds the SS pigs had the higher lactate and lower phosphocreatine which is similar to the findings of Hall & Lucke (1983). However, although lactate and phosphocreatine showed the same type of differences in both the herds, it must be noted that differences were also found for the SR pigs between the two herds.

as well as for the SS pigs. Although the SS pigs in herd X had a higher glucose 6-phosphate concentration than the SR pigs, this difference was not found between the SS and SR pigs of herd Y. Glucose was significantly different between the SS and SR pigs of herd Y, but not between the pigs of herd X. Clearly the use of the muscle metabolites are subject to herd differences, and therefore of limited value, in identifying SS pigs.

Hence, the use of either the blood profiles of pigs at the age of 11 weeks and the muscle metabolites at the age of 13 weeks have limited value in the prediction or identification of SS pigs. These values are subject to herd differences.

Several blood variables differences were found between the two herds. Although the higher serum aldolase and ALT activities of the pigs from herd Y were lower, indicative of a possible lower level of stress susceptibility (Schmittet *et al.*, 1981c), the higher potassium, magnesium, calcium, glucose and cortisol concentrations would indicate a higher level of stress susceptibility amongst the pigs from herd Y in general, or a higher level of perceived stress (Berman *et al.*, 1972), which is also borne out by the lower bicarbonate concentrations. These indications are also found in the muscle metabolites, with the pigs from herd Y having the higher lactate, but lower ATP, phosphocreatine and glycogen concentrations.

The glycogen concentrations of the SR and SS pigs from herd Y were significantly lower than that of the corresponding pigs from herd X, thus further evidence to the idea that the pigs from herd Y have either a higher general level of stress susceptibility or perceived a higher level of stress during the taking of the blood and muscle biopsies.

One of the major constraints in using blood or muscle variables for predicting SS pigs has been the issue that to obtain the blood or muscle, the animal has to be restrained or anaesthetised, both procedures that may influence the psychological and physiological status of the animal (Pfeiffer & Lengerken, 1984).

The idea of a higher general level of stress as perceived by the pigs from herd Y seems to be more appropriate than a higher level of stress susceptibility for the differences between the two herds. It was noted at 11 as well as 13 weeks of age that the pigs from herd X seemed "tamer" as they did not shy away from personnel, whereas the pigs from herd Y seemed to be "less tame" and did not allow touching by personnel. These differences might be a result of the type of handling the animals, and also the differences in the type of housing used by the different producers. The housing used by the producer of herd X was very similar to that used during the study, but very different from that used by the producer of herd Y. Also, the housing pens at producer X and at ADSRI were cleaned every day, while those at producer Y only once a week. Therefore, it may be assumed that the pigs from herd X would react less to handling than pigs from producer Y. Further it might be assumed that the three weeks allowed for the pigs of herd Y for getting used to the different environment was not long enough, and the pigs would therefore react more negatively on handling during the manual restraint during blood taking, or the taking of the muscle

biopsy. A higher level of stress was thus perceived by the pigs of herd X, and therefore the differences between the two herds.

These blood and muscle variable differences were also found in the SR pigs between of the two herd, but the differences were exacerbated in the case of the SS pigs between the two herds. The SS pigs of herd Y had a higher CK activity, hypertalemia, higher magnesium, calcium, glucose and inorganic phosphate concentrations, which are all associated with stress susceptibility or MHS (Berman *et al.*, 1970). The higher cortisol concentration of the SS pigs from herd Y might indicate that these pigs, and in general the pigs from herd Y, perceived the stress as of a higher level than the pigs from herd X. The higher muscle lactate, and lower ATP, phosphocreatine and glycogen concentrations of the SS pigs from herd Y support this idea.

3.4. Conclusion

Differences in blood variables and muscle metabolites between herds complicate and negate the predictive values these variables and metabolites might have. These differences might even be ascribed to the different husbandry management practises followed by the different producers. The general use of blood variables and muscle metabolites to identify SR and SS pigs is thus not recommended.

Although some misclassification of SR and SS pigs during the use of the halothane test do occur, the results show that this method is still the most reliable, and the most simple method, but this might only be true if the operator is experienced in the management of the halothane test. Unfortunately it only identifies SS pigs (*nn*), and not stress susceptibility carrier pigs (*Nn*). However, the application of the halothane test in breeding strategies still produces positive results in that the incidence of SS pigs decreases.

CHAPTER 4

Growth, carcass and meat characteristics

4.1. Introduction

It has been suggested that stress susceptibility in pigs is the result of selection for, and is associated with, heavily muscled pigs, that have a high growth rate, improved feed efficiency, and lean carcasses (Nelson, 1973). Also, the carcasses of SS pigs have a higher slaughter-out percentage (Eikelenboom & Minkema, 1974) and have a higher muscle to fat ratio than carcasses from SR pigs (Monin *et al.*, 1981; Schmitt *et al.*, 1981b). Carcasses from SS pigs also have a thinner backfat thickness (Eikelenboom & Minkema, 1974; Schmidt & Kallweit, 1980). It would therefore seem to be advantageous for the producer to produce SS pigs. McGloughlin *et al.* (1980) on the other hand have indicated that stress susceptibility is associated with reduced daily gain, a result also found by Mitchell & Heffron (1981b) in a preliminary study, and which suggests that stress susceptible animals may be less economic to produce than stress resistant animals.

Nevertheless, SS pigs produce PSE musculature *post mortem* (Mitchell & Heffron, 1982), which is an accepted defect in the quality of meat. Regarding specific characteristics of PSE meat such as toughness, aroma and juiciness, no consistent results have been reported in the literature. The results regarding the tenderness of PSE meat range from being tougher than meat of normal quality (Dilday *et al.*, 1970) to PSE meat being more tender (Fox *et al.*, 1980). Conflicting results regarding moisture content of PSE meat has also been reported (Searcy *et al.*, 1969; Fox *et al.*, 1980).

These meat characteristics have been investigated in an extended study of growth, carcass and meat characteristics of SS and SR South African Landrace gilts, and the results of this investigation are reported here.

The results regarding growth, carcass and meat characteristics were analysed according to:

- * the influence of stress sensitivity and herd on the characteristics of the total number of pigs
- * the influence of stress sensitivity on the characteristics of the pigs from herd X

- * the influence of stress sensitivity on the characteristics of the pigs from herd Y
- * the influence of herd on the characteristics of SR pigs
- * the influence of herd on the characteristics of SS pigs.

4.2. Results

4.2.1. Growth characteristics

4.2.1.1. Average daily gain (ADG) and feed conversion ratio (FCR)

Average daily gain and FCR are two very important criteria for the producer for the evaluation of pig efficiency, and are therefore of economic consequence.

4.2.1.1.1. The influence of stress sensitivity and herd on ADG and FCR

The SS pigs had a lower FCR compared to that of the SR pigs, but herd had no significant influence on the ADG and FCR of the total number of pigs (Table 4.1). The average ADG and FCR values are given in Table 4.2.

Table 4.1: The results of 2-way analyses of variance on ADG and FCR of pigs as influenced by stress sensitivity (A: SR vs SS) and herd (B: herd X vs herd Y)

Variable	Stress sensitivity (A)			Herd (B)			F.B		
	F value	Significance level		F value	Significance level	F value	Significance level		
ADG	<0,001	0,9940	NS	1,729	0,1935	NS	0,084	0,7363	NS
FCR	8,681	0,0046	**	0,176	0,6808	NS	13,756	0,0004	**

Table 4.2: Mean values and standard deviations (sd) of ADG and FCR of pigs as influenced by stress sensitivity and herd

Variable	SR pigs			SS pigs			Herd X			Herd Y		
	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n
ADG kg/day	0,801	0,163	38	0,810	0,160	26	0,789	0,179	47	0,812	0,092	37
FCR	3,276	0,328	37	2,897	0,318	26	3,101	1,342	46	3,052	0,265	17

4.2.1.1.2. Influence of stress sensitivity on ADG and FCR of pigs from herd X.

Stress sensitivity had no significant influence on the ADG and FCR of the pigs from herd X (Table 4.3).

Table 4.3: Mean values, standard deviations (sd) and level of significance of ADG and FCR of pigs from herd X as influenced by stress sensitivity

Variable		SR pigs			SS pigs			Significance level
		Mean	sd	n	Mean	sd	n	
ADG	kg/day	0,791	0,178	30	0,784	0,181	17	0,8950 NS
FCR		3,171	0,344	29	2,982	0,339	17	0,0777 NS

4.2.1.1.3. Influence of stress sensitivity on ADG and FCR of pigs from herd Y

The SS pigs from herd Y had a significantly lower FCR than the SR pigs from the same herd, although the ADG between the two types of animals was not significant (Table 4.4).

Table 4.4: Mean values, standard deviations (sd) and level of significance of ADG and FCR of pigs from herd Y as influenced by stress sensitivity

Variable		SR pigs			SS pigs			Significance level
		Mean	sd	n	Mean	sd	n	
ADG	kg/day	0,839	0,076	8	0,859	0,105	9	0,6686 NS
FCR		3,656	0,258	8	2,477	0,270	9	<0,0001 **

4.2.1.1.4. The differences in ADG and FCR of SR pigs from the two herds

Although the difference in ADG of the SR pigs between the two herds was not significant, the SR pigs from herd Y had the higher FCR compared to the SR pigs from herd X (Table 4.5).

Table 4.5: Mean values, standard deviations (sd) and level of significance of ADG and FCR of SR pigs as influenced by herd

Variable		Herd X			Herd Y			Significance level
		Mean	sd	n	Mean	sd	n	
ADG	kg/day	0,791	0,178	30	0,839	0,076	8	0,4651 NS
FCR		3,171	0,344	29	3,656	0,258	8	0,0007 **

4.2.1.1.5. The differences in ADG and FCR of SS pigs from the two herds

The ADG of the SS pigs from the two herds was similar, but the SR pigs from herd Y had a lower FCR than the SS pigs from herd X (Table 4.6).

Table 4.6: Mean values, standard deviations (sd) and level of significance of ADG and FCR of SS pigs as influenced by herd

Variable		Herd X			Herd Y			Significance level
		Mean	sd	n	Mean	sd	n	
ADG	kg/day	0,784	0,181	17	0,859	0,105	9	0,2667 NS
FCR		2,982	0,309	17	2,477	0,270	9	0,0007 **

4.2.1.2. Live mass

4.2.1.2.1. The influence of stress sensitivity and herd on the live mass of all the pigs

The results of this analysis are illustrated in Tables 4.7 and 4.8. The mass differences between the SS and SR pigs were only significant at the ages of 12 and 13 weeks, with the SR pigs being heavier. The pigs from herd Y were heavier than the pigs from herd X throughout the growth period of 12 to 21 weeks of age.

Table 4.7: The results of 2-way analyses of variance on live mass from the age of 12 to 21 weeks as influenced by stress sensitivity (A: SR vs SS) and herd (B: herd X vs herd Y)

	Stress sensitivity (A)		Herd (B)		AxB	
	F value	Significance level	F value	Significance level	F value	Significance level
Week 12	10,921	0,0016 **	16,436	0,0022 **	0,109	0,7455 NS
Week 13	6,433	0,0139 *	22,899	<0,0001 **	0,009	0,9266 NS
Week 14	3,003	0,0882 NS	14,867	0,0003 **	0,169	0,6867 NS
Week 15	1,484	0,2279 NS	21,529	<0,0001 **	0,558	0,4659 NS
Week 16	2,004	0,1621 NS	23,314	<0,0001 **	0,326	0,5764 NS
Week 17	1,511	0,2237 NS	20,906	<0,0001 **	0,109	0,7464 NS
Week 18	1,814	0,1831 NS	25,425	<0,0001 **	0,831	0,3753 NS
Week 19	0,940	0,3464 NS	27,704	<0,0001 **	0,526	0,4789 NS
Week 20	0,127	0,7268 NS	23,651	<0,0001 **	0,197	0,6633 NS
Week 21	0,008	0,9301 NS	20,225	<0,0001 **	0,813	0,3809 NS

Table 4.8: Mean values and standard deviations (sd) of live mass as influenced by stress sensitivity and herd

Variable	SR pigs			SS pigs			Herd X			Herd Y		
	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n
Week 12	31,90	3,53	36	28,87	5,10	26	29,42	4,71	45	33,82	3,62	17
Week 13	36,51	4,16	36	34,25	5,23	26	33,94	4,98	45	39,85	4,13	17
Week 14	40,72	6,04	38	38,96	5,80	26	38,38	6,39	47	44,50	4,44	17
Week 15	45,41	6,37	38	44,26	6,36	26	42,90	6,78	47	51,03	4,78	17
Week 16	50,70	7,39	38	49,46	6,32	26	47,74	7,45	47	56,97	5,25	17
Week 17	56,36	7,73	38	55,31	7,24	26	55,41	8,13	47	62,88	5,23	17
Week 18	62,45	8,06	38	61,25	7,75	26	59,04	8,39	47	70,03	6,14	17
Week 19	66,86	8,35	38	66,50	7,33	26	63,62	8,37	47	75,27	6,25	17
Week 20	72,41	8,95	38	74,05	8,53	22	69,51	9,36	43	81,86	6,60	17
Week 21	77,99	8,54	38	81,19	8,97	18	75,44	8,82	39	87,23	8,05	17

4.2.1.2.2. The influence of stress sensitivity on the live mass of pigs from herd X

The average mass of the SS pigs was lower than that of SR pigs at 12 weeks of age. The differences in live mass from the ages of 13 weeks to 21 weeks were not significant (Table 4.9).

Table 4.9: Mean values, standard deviations (sd) and level of sig. of live mass of pigs from herd X as influenced by stress sensitivity

Variable	Age Weeks	SR pigs			SS pigs			Significance level
		Mean	sd	n	Mean	sd	n	
Live mass	12	30,71	3,58	28	27,20	5,81	17	0,0299 *
Live mass	13	35,13	4,22	28	32,00	5,61	17	0,0549 NS
Live mass	14	39,20	6,43	30	36,94	6,25	17	0,2449 NS
Live mass	15	43,35	6,78	30	42,12	6,90	17	0,5569 NS
Live mass	16	48,43	7,85	30	46,3	6,71	17	0,3848 NS
Live mass	17	54,13	8,34	30	52,15	7,84	17	0,4191 NS
Live mass	18	59,62	8,55	30	58,03	8,25	17	0,5348 NS
Live mass	19	64,00	8,84	30	62,94	7,70	17	0,6699 NS
Live mass	20	69,25	9,54	30	69,42	9,34	13	0,9578 NS
Live mass	21	75,07	8,90	30	76,67	8,96	9	0,6408 NS

4.2.1.2.3. The influence of stress sensitivity on the live mass of pigs from herd Y

As was found in herd X, the difference in mass between the SS and SR pigs was only significant at the age of 12 weeks, with the SS pigs being lighter than the SR pigs (Table 4.10).

Table 4.10: Mean values, standard deviations (sd) and level of significance of live mass of pigs from herd Y as influenced by stress sensitivity

Variable	Age Weeks	SR pigs			SS pigs			Significance level
		Mean	sd	n	Mean	sd	n	
Live mass	12	36,06	3,33	8	31,83	3,86	9	0,0296 *
Live mass	13	41,28	3,93	8	38,50	4,30	9	0,1725 NS
Live mass	14	46,44	4,00	8	43,78	4,79	9	0,1105 NS
Live mass	15	53,13	4,28	8	49,17	5,11	9	0,1054 NS
Live mass	16	59,19	4,99	8	55,00	5,47	9	0,1215 NS
Live mass	17	64,69	4,41	8	61,28	5,05	9	0,1995 NS
Live mass	18	73,06	5,58	8	67,33	6,58	9	0,0739 NS
Live mass	19	77,39	5,93	8	73,22	6,52	9	0,1713 NS
Live mass	20	83,15	5,92	8	80,72	7,13	9	0,4611 NS
Live mass	21	88,94	6,84	8	85,71	8,97	9	0,4224 NS

4.2.1.2.4. The live mass of SR pigs from the two herds

The results of this analysis are given in Table 4.11. Throughout the growth trial the SR pigs from herd Y were heavier than the SR pigs from herd X.

Table 4.11: Mean values, standard deviations (sd) and level of significance of live mass from SR pigs as influenced by herd

Variable	Age Weeks	Herd X			Herd Y			Significance level
		Mean	sd	n	Mean	sd	n	
Live mass	12	30,71	3,58	28	36,06	3,33	8	0,0006 **
Live mass	13	35,13	4,22	28	41,38	3,93	8	0,0007 **
Live mass	14	39,20	6,43	30	46,44	4,00	8	0,0037 **
Live mass	15	43,35	6,78	30	53,13	4,28	8	0,0005 **
Live mass	16	48,43	7,86	30	59,19	4,99	8	0,0068 **
Live mass	17	54,13	8,34	30	64,69	4,41	8	0,0015 **
Live mass	18	59,62	8,55	30	73,06	5,58	8	0,0002 **
Live mass	19	64,00	8,84	30	77,59	5,93	8	0,0002 **
Live mass	20	69,55	9,54	30	83,15	5,92	8	0,0005 **
Live mass	21	75,07	8,90	30	88,94	6,84	8	0,0002 **

4.2.1.2.5. The live mass of SS pigs from the two herds

The results of this analysis are similar to the results found in the SR pigs. The SS pigs of herd Y were heavier throughout the growth trail than the SS pigs from herd X (Table 4.12).

Table 4.12: Mean values, standard deviations (sd) and level of significance of live mass from SS pigs as influenced by herd

Variable	Age Weeks	Herd X			Herd Y			Significance level
		Mean	sd	n	Mean	sd	n	
Live mass	12	27,29	5,62	17	31,83	3,86	9	0,0412 *
Live mass	13	32,00	5,56	17	38,50	4,30	9	0,0060 **
Live mass	14	36,94	6,23	17	42,78	4,79	9	0,0224 *
Live mass	15	42,12	6,90	17	49,17	5,11	9	0,0129 *
Live mass	16	46,53	6,71	17	55,00	5,47	9	0,0034 **
Live mass	17	52,15	7,84	17	61,28	5,85	9	0,0054 **
Live mass	18	58,03	8,25	17	67,33	6,58	9	0,0075 **
Live mass	19	62,94	7,70	17	73,22	6,52	9	0,0023 **
Live mass	20	69,42	9,34	13	80,72	7,15	9	0,0063 **
Live mass	21	76,67	8,96	9	85,71	8,97	9	0,0482 *

4.2.2. Carcass characteristics

4.2.2.1. The influence of stress sensitivity and herd on carcass characteristics of all the pigs.

Several significant differences were found between carcass characteristics as being influenced by herd and stress sensitivity (Table 4.13). Stress sensitivity resulted in significant differences in all the carcass characteristics measured (Table 4.13). The average values are reported in Table 4.15.

SS pigs had a significantly higher slaughter-out percentage, whether calculated using the hot or cold mass. The mass loss during chilling was less for the SS pigs than for the SR pigs. The backfat thickness of the SS pigs was thinner than that of the SR pigs, whereas the SS pig carcasses were significantly shorter than the SR pig carcasses.

Table 4.13: The results of 2-way analyses of variance on carcass characteristics as influenced by stress sensitivity (A: SR vs SS) and herd (B: herd X vs herd Y)

Variable	Stress sensitivity (A)		Herd (B)		AxB	
	F value	Significance level	F value	Significance level	F value	Significance level
Slaughter-out %						
warm mass	10,044	0,0024 **	1,017	0,3173 NS	0,460	0,5075 NS
cold mass	18,451	0,0001 **	3,147	0,0810 NS	0,317	0,5817 NS
Chilling loss	6,097	0,0165 *	5,086	0,0278 *	0,017	0,8979 NS
Backfat thickness	16,072	0,0001 **	62,581	<0,0001 **	0,707	0,4125 NS
Length 1	13,697	0,0005 **	1,804	0,1841 NS	5,634	0,0612 NS
Length 2	8,371	0,0053 **	1,754	0,1903 NS	4,453	0,0389 *

Pigs from herd X had smaller difference between the slaughter-out percentages as calculated using the hot and cold carcass masses than from herd Y. Thus, the pigs from herd X had a smaller evaporative loss during chilling than pigs from herd Y. No significant differences were found between pigs of herd X and herd Y on slaughter-out percentage as calculated using the hot or cold carcass mass, and no significant differences in the lengths of the carcasses (Table 4.15).

Table 4.14: Mean values and standard deviations (sd) of carcass characteristics as influenced by stress sensitivity and herd

Variable	SR pigs			SS pigs			Herd X			Herd Y		
	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n
Slaughter-out %												
warm mass	79,69	2,34	40	81,55	2,34	23	80,46	2,66	44	80,11	2,11	19
cold mass	77,34	2,30	40	79,70	2,27	26	78,51	2,67	47	77,69	2,19	19
Chilling loss (%)	2,89	0,95	49	2,43	0,6	23	2,59	0,97	44	3,03	0,45	19
Backfat thickness (cm)	2,43	0,81	40	1,94	0,72	26	1,91	0,60	47	3,04	0,68	19
Length 1 (cm)	98,47	4,05	40	94,59	4,93	26	96,61	4,95	47	97,74	4,33	19
Length 2 (cm)	82,12	3,62	40	77,60	3,88	26	78,84	4,07	47	79,85	3,42	19

4.2.2.2. The influence of stress sensitivity on the carcass characteristics of pigs from herd X

Although the difference in slaughter-out percentage using the hot carcass mass was not significantly different, the slaughter-out percentage using the cold carcass mass was (Table 4.13). The backfat thickness of the SS pigs was smaller than that of the SR pigs. No significant differences, however, were determined between the SS and SR pigs of herd X regarding the carcass characteristics, slaughter-out percentages as calculated using the hot or cold carcass mass, or the lengths of the carcasses (Table 4.15).

Table 4.15: Mean value, standard deviations (sd) and level of significance of carcass characteristics of pigs from herd X as influenced by stress sensitivity

Variable	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
Slaughter-out %							
warm mass	79,93	2,53	30	81,60	2,65	14	0,0542 NS
cold mass	77,70	2,44	30	79,95	2,51	17	0,0646 **
Chilling loss (%)	2,76	1,05	30	2,24	0,68	14	0,0567 NS
Backfat thickness (cm)	2,10	0,58	30	1,58	0,48	17	0,0020 **
Length 1 (cm)	97,58	4,04	30	94,92	6,01	17	0,1107 NS
Length 2 (cm)	79,23	3,68	30	77,96	4,68	17	0,3047 NS

4.2.2.3. The influence of stress sensitivity on the carcass characteristics of pigs from herd Y

The SS pigs of herd Y had a higher slaughter-out percentage using either the hot or cold mass, with the SR pigs showing the higher chilling loss (Tables 4.16). Also, the SS pigs had a significant thinner backfat than the SR pigs. The SS pigs also had shorter carcasses.

Table 4.16: Mean values, standard deviations (sd) and level of significance of carcass characteristics of pigs from herd Y as influenced by stress sensitivity

Variable	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
Slaughter-out %							
warm mass	78,89	1,47	10	80,74	1,45	7	0,0216 *
cold mass	76,29	1,69	10	78,55	1,21	7	0,0047 **
Chilling loss (%)	3,30	0,34	10	2,71	0,40	7	0,0649 **
Backfat thickness (cm)	3,41	0,58	10	2,64	0,56	9	0,0088 **
Length 1 (cm)	101,14	2,82	10	93,97	1,69	9	<0,0001 **
Length 2 (cm)	82,48	2,20	10	76,92	1,58	9	<0,0001 **

4.2.2.4. Herd differences in the SR pigs regarding carcass characteristics

The results of the analysis of the influence of the two herds on the carcass characteristics of SR pigs are given in Table 4.17. No significant differences were found between SR pigs of herd X and herd Y regarding slaughter-out percentages or the chilling loss. The SR pigs from herd Y was significantly fatter as indicated by the thicker backfat thickness. The SR pigs from herd Y were also longer than those of herd X.

4.2.2.5. Herd differences in the SS pigs regarding carcass characteristics

The results of this analysis are illustrated in Table 4.18. The SS pigs from herd Y were fatter than those of herd X as determined by the thicker backfat thickness. Significant differences between the other determined carcass characteristics were found.

Table 4.17: Mean values, standard deviations (sd) and level of significance of carcass characteristics of SR pigs as influenced by herd

Variable	Herd X			Herd Y			Significance level
	Mean	sd	n	Mean	sd	n	
Slaughter-out %							
warm mass	79,93	2,53	30	78,89	1,47	10	0,2294 NS
cold mass	77,70	2,44	30	76,29	1,49	10	0,0949 NS
Chilling loss (%)	2,76	1,05	30	3,30	0,34	10	0,1201 NS
Backfat thickness (cm)	2,10	0,58	30	3,41	0,58	10	<0,0001 **
Length 1 (cm)	97,58	4,04	30	101,14	2,82	10	0,0140 *
Length 2 (cm)	79,33	3,68	30	82,48	2,20	10	0,0151 *

Table 4.18: Mean values, standard deviations (sd) and level of significance of carcass characteristics of SS pigs as influenced by herd

Variable	Herd X			Herd Y			Significance level
	Mean	sd	n	Mean	sd	n	
Slaughter-out %							
warm mass	81,60	2,65	14	80,74	1,45	7	0,8925 NS
cold mass	79,95	2,51	17	78,55	1,21	7	0,4623 NS
Chilling loss (%)	2,24	0,68	14	2,71	0,40	7	0,0652 NS
Backfat thickness (cm)	1,58	0,48	17	2,64	0,36	9	<0,0001 **
Length 1 (cm)	94,92	6,01	17	93,97	1,69	9	0,6490 NS
Length 2 (cm)	77,96	4,68	17	76,92	1,58	9	0,5237 NS

4.2.3. Meat characteristics

4.2.3.1. The influence of stress sensitivity and herd on meat characteristics

The results of the 2-way analyses of variance are given in Table 4.19, with the average values in Table 4.20. The pH values of the SS pigs at 15, 30, 45 and 60 minutes *post mortem* were lower than those of the SR pigs. In general, no other significant differences in meat characteristics as a result of stress type was found, except for shear force values at cooking temperatures of 70°C and 80°C. At these temperatures the meat from the SS pigs had the lower shear force value. The same results were found on the exclusion of the DFD carcasses (carcasses with a pH value 6,00 24 hours *post mortem*) (Appendix B, Tables B.1 and B.2). The only exception was that the meat cooked at 70°C from the SS pigs had a lower water holding capacity than the meat from SR pigs.

Although no significant differences were found between the two herds for the pH values 15, 30, 45 and 60 minutes *post mortem*, the pigs from herd Y had a lower pH value 24 hours *post mortem* than the pigs from herd X. The results indicate that the pigs from herd X had greater cooking losses during the cooking of the meat at 60°C and 70°C. However, no difference in cooking loss

was found for cooking the meat at 80°C. Simultaneously, the water holding capacity of the pigs from herd X was found to be lower at 60°C, 70°C and 80°C. Although no significant difference in shear force was determined between pigs from herds X and Y for meat cooked at 60°C, at cooking temperatures of 70°C and 80°C the meat from herd X was found to have a lower shear force. No significant differences in the percentage drip loss during vacuum packaged storage, or the area of the *M. longissimus thoracis* was found between the two herds. Excluding the DFD carcasses (Tables B.1 and B.2) which could influence meat characteristics, the same results were found, except that the pH value 60 minutes *post mortem* of the pigs from herd Y was higher than the pH value of the pigs from herd X, although the water holding capacity between the two herds of meat cooked at 80°C was not significant.

Table 4.19: The results of 2-way analyses of variance on meat characteristics of pigs as influenced by stress sensitivity (A: SR vs SS) and herd (B: herd X vs herd Y)

Variable	Stress sensitivity (A)		Herd (B)		AxB	
	F value	Significance level	F value	Significance level	F value	Significance level
pH value						
15 min p.m.	21,952	<0,0001 **	3,840	0,0545 NS	7,138	0,0096 **
30 min p.m.	25,360	<0,0001 **	2,111	0,1513 NS	4,077	0,0478 *
45 min p.m.	31,059	<0,0001 **	0,024	0,8794 NS	1,044	0,3109 NS
60 min p.m.	29,902	<0,0001 **	0,651	0,4331 NS	0,052	0,8235 NS
24 h.p.m.	1,928	0,1699 NS	10,339	0,0021 **	11,150	0,0014 **
Drip loss	1,111	0,2959 NS	0,794	0,3858 NS	5,943	0,0177 **
Cooking loss						
60°C	0,052	0,8227 NS	18,386	0,0001 **	0,005	0,9459 NS
70°C	0,541	0,4728 NS	24,135	<0,0001 **	0,115	0,7398 NS
80°C	0,001	0,9893 NS	2,835	0,0975 NS	0,072	0,7924 NS
Water holding capacity						
60°C	0,020	0,8886 NS	9,089	0,0037 **	2,459	0,122 NS
70°C	1,663	0,2020 NS	10,225	0,0002 **	1,748	0,1910 NS
80°C	0,031	0,8617 NS	9,899	0,0025 **	0,154	0,7006 NS
Shear force						
60°C	1,200	0,2776 NS	2,253	0,1384 NS	1,908	0,1722 NS
70°C	6,924	0,0107 *	6,509	0,0132 *	0,399	0,5365 NS
80°C	6,365	0,0142 *	23,070	<0,0001 **	1,943	0,1683 NS
<i>M. longissimus thoracis</i> area	0,114	0,7399 NS	1,892	0,1739 NS	3,415	0,0694 NS

4.2.3.2. The influence of stress sensitivity on meat characteristics of pigs from herd X

The pH values of the SS pigs were lower than those of the SR pigs 15, 30, 45 and 60 minutes *post mortem*, as well as at 24 hours *post mortem*. No other significant differences in meat characteristics have been found in herd X as a result of stress sensitivity, except that at a cooking temperature of 70°C, the meat from the SS pigs had a lower shear force (Tables 4.21). In excluding the meat from DFD carcasses, no difference in pH value was found at 24 hours *post mortem* between

the two stress sensitivities, although the SS pigs also had a lower shear force value of meat cooked at 60°C (Table B.3).

Table 4.2b: Mean values and standard deviations (sd) of meat characteristics of pigs as influenced by stress sensitivity and herd

Variable	SR pigs			SS pigs			Herd X			Herd Y		
	Mean	sd	n	Mean	sd	n	Mean	sd		Mean	sd	n
pH value												
15 min p.m.	6.48	0.20	40	6.21	0.32	26	6.35	0.25	47	6.44	0.25	19
30 min p.m.	6.42	0.19	40	6.11	0.34	26	6.28	0.26	47	6.34	0.30	19
45 min p.m.	6.34	0.18	40	5.99	0.33	26	6.21	0.27	47	6.18	0.30	19
60 min p.m.	6.34	0.23	22	5.83	0.37	23	6.10	0.36	26	6.06	0.40	19
24 h.p.m.	5.78	0.36	40	5.64	0.40	26	5.81	0.34	47	5.51	0.36	19
Drip loss (%)	5.33	3.15	40	6.00	2.23	26	5.76	2.87	47	5.18	2.75	19
Cooking loss (%)												
60°C	17.00	4.35	40	16.80	3.54	26	18.20	4.09	47	13.80	2.34	19
70°C	28.10	5.32	40	28.40	4.43	26	29.80	4.81	47	24.20	2.31	19
80°C	34.40	4.42	40	34.30	3.58	26	34.90	4.56	45	33.00	2.11	19
Water holding capacity (%)												
60°C	49.30	4.97	40	49.50	4.26	26	48.30	4.32	47	51.90	4.61	19
70°C	43.80	5.54	40	42.70	5.14	26	41.90	4.61	47	47.00	5.49	19
80°C	38.40	4.30	40	38.60	4.79	26	37.40	4.16	47	41.00	4.21	19
Shear force (N/2.5 cm dia.)												
60°C	77.70	23.84	40	72.90	14.01	26	73.60	21.67	47	81.30	16.76	19
70°C	90.60	33.01	40	74.40	17.70	26	79.40	30.90	47	95.20	19.29	19
80°C	108.50	31.21	40	96.40	19.90	26	95.30	24.66	47	124.50	24.39	19
M. longissimus thoracis area (cm ²)	28.10	4.27	40	28.34	5.57	26	28.69	5.09	47	26.97	3.75	19

4.2.3.3: The influence of stress sensitivity on meat characteristics of pigs from herd Y

The results of the influence of stress sensitivity on the pigs of herd Y are given in Tables 4.22. The pH values of the SS pigs were lower than those of the SR pigs 15, 30, 45 and 60 minutes post mortem, but not at 24 hours post mortem. The vacuum packaged meat of the SS pigs showed a higher drip loss during the storage period than the meat from the SR pigs. No significant differences were found between the meat from SS and SR pigs regarding cooking loss and water holding capacity at the three cooking temperatures of 60, 70 and 80°C. The shear force values between the meat from SS and SR pigs cooked at 60 and 70°C were also not significant. The meat of the SS pigs cooked at 80°C was significantly lower than the corresponding meat from the SR pigs.

Table 4.2: Mean values, standard deviations (sd) and level of significance of meat characteristics of pigs from herd X as influenced by stress sensitivity

Variable	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
pH values:							
15 min p.m.	6.41	0.17	30	6.23	0.32	17	0.0403 **
30 min p.m.	6.37	0.17	30	6.13	0.31	17	0.0066 **
45 min p.m.	6.32	0.17	30	6.01	0.31	17	0.0004 **
60 min p.m.	6.39	0.16	12	5.86	0.28	17	0.0001 **
24 h p.m.	5.92	0.28	30	5.62	0.37	17	0.0057 **
Drip loss (%)	5.90	3.23	30	5.52	2.17	17	0.3076 NS
Cooking loss (%)							
60°C	18.12	4.33	30	18.29	3.76	17	0.8469 NS
70°C	29.64	5.17	30	30.20	4.22	17	0.3153 NS
80°C	34.82	4.88	30	35.02	4.12	17	0.1395 NS
Water holding capacity (%)							
60°C	47.94	4.86	30	48.93	3.20	17	0.5435 NS
70°C	42.05	5.24	30	41.54	3.33	17	0.9124 NS
80°C	37.57	4.54	30	37.11	3.51	17	0.7063 NS
Shear force (N/2.5 cm ² dia.)							
60°C	77.31	24.73	30	67.02	13.02	17	0.0859 NS
70°C	86.92	34.86	30	66.05	15.75	17	0.0081 **
80°C	98.79	26.83	30	89.27	19.52	17	0.1694 NS
M. longissimus thoracis area (cm ²)	29.07	3.94	30	28.01	6.74	17	0.5600 NS

Also, the muscle area of the SS pigs was larger than that of the SR pigs. Although no difference was found in the FOP readings between the SS and SR pigs, the reflectance values at the different positions, as well as the average value of the three positions, as measured with the EEL reflectometer, were higher for the meat from SS pigs than for the meat from SR pigs. The volume of drip formed during the storage period was higher for the meat from SS pigs than from SR pigs. Similar results were obtained with the exclusion of DFD carcasses (Table A.4), with the exception that with the exclusion of the DFD carcasses, the cooking loss at 70°C of the SS pigs was higher, the water holding capacity and shear force lower. No significant differences were found for shear force, except at a cooking temperature of 80°C and muscle area (Table 4.22).

The pH values (including DFD carcasses) of the *M. longissimus thoracis* at 15, 30, 45 and 60 minutes post mortem were significantly lower in the carcasses of SS pigs than of SR pigs, with the mean pH value of the SS pig carcasses below 6.00. However, the pH value 24 hours post mortem between the SS and SR pigs was not significant (Table 4.22). Similar results were obtained after the exclusion of DFD carcasses (Table B.4).

Table 22: Mean values, standard deviations (sd) and level of significance of meat characteristics of SR pigs from herd Y as influenced by sire sensitivity

Variable	SR pigs			SS pigs			Significance level
	Mean	G. sd	n	Mean	G. sd	n	
pH values							
15 min. p.m.	6.69	0.14	10	6.17	0.33	9	0.0003 **
30 min. p.m.	6.59	0.16	10	6.07	0.40	9	0.0013 **
45 min. p.m.	6.69	0.21	10	5.94	0.38	9	0.0014 **
60 min. p.m.	6.22	0.30	10	5.80	0.50	9	0.0169 *
24 h p.m.	5.37	0.21	10	5.67	0.48	9	0.0563 NS
Drip loss (%)	3.65	2.36	10	5.90	2.16	9	0.0010 **
Cooking loss (%)							
60°C	13.67	2.28	10	13.94	2.51	9	0.7784 NS
70°C	20.31	2.03	10	24.87	2.22	9	0.2084 NS
80°C	25.21	2.39	10	32.81	1.54	9	0.8909 NS
Water holding capacity (%)							
60°C	53.25	2.7	10	50.42	5.88	9	0.1951 NS
70°C	48.20	2.31	10	44.85	7.22	9	0.1130, NS
80°C	40.82	2.21	10	41.26	5.85	9	0.8250 NS
Shear force (N/25mm dia.)							
60°C	21.7	22.16	10	83.94	8.00	9	0.5234 NS
70°C	21.51	25.01	10	90.17	7.45	9	0.2045 NS
80°C	25.47	25.47	10	109.98	12.65	9	0.0093 **
M. longissimus thoracis area (cm ²)	4.05	4.05	10	28.93	2.24	9	0.0239 **
IBEL values							
top	36	4	10	42	7	9	<0.0001 **
middle	37	3	10	39	10	9	0.0019 **
bottom	3	3	10	35	7	9	0.0038 **
average	21.5	3	10	39	8	9	0.0003 **
POP	141	12	10	150	22	9	0.4921 NS
Drip volume (ml)	14.24	15.89	10	29.44	11.60	9	0.0069 **

4.2.3.4. The influence of the two herds on the meat characteristics of SR pigs

The results of the analysis on the influence of the different herds on the meat characteristics of SR pigs are given in Table 4.23. Although the SR pigs from herd Y had higher pH values 15 and 30 minutes *post mortem*, the values 45 and 60 minutes *post mortem* were not significantly different from the values of the SR pigs from herd X. However, 24 hours *post mortem* the pigs from herd Y had a lower pH value than the pigs from herd X. The drip loss of vacuum packaged meat from the Y herd was lower than that of the X herd. Also, the cooking loss at the temperatures of 60 and 70°C were lower, although no significant difference was found at a cooking temperature of 80°C.

Regarding the water holding capacity, the SR pigs from herd Y had higher water holding capacities at all three of the cooking temperatures than did the SR pigs from herd X. Although the differences in shear force of meat cooked at 60 and 70°C were not significant, the meat from SR pigs of herd Y had higher shear force values than the cooked meat of the SR pigs from herd X. The SR pigs from herd Y also had a larger *M. longissimus thoracis* area than the corresponding pigs from herd X. In excluding the meat from DFD carcasses from the analysis, similar results were obtained, although the water holding capacity of the SR pigs between the two herds was found not to be significant (Table B.5).

Table 4.23 Mean values, standard deviations (sd) and level of significance of meat characteristics of SR pigs as influenced by herd

Variable	Herd X			Herd Y			Significance level
	Mean	sd	n	Mean	sd	n	
Loss							
15 min. p.m.	6.41	0.17	30	6.69	0.14	10	<0.0001 **
30 min. p.m.	6.37	0.17	30	6.59	0.16	10	0.0009 **
45 min. p.m.	6.32	0.17	30	6.40	0.21	10	0.2766 NS
60 min. p.m.	6.39	0.16	12	6.29	0.30	10	0.3319 NS
24 h p.m.	5.92	0.28	30	5.21	0.21	10	<0.0001 **
Drip loss (%)	-5.90	3.23	30	3.63	2.26	10	0.0467 *
Cooking loss (%)							
60°C	18.12	4.33	30	13.67	2.28	10	0.0037 **
70°C	29.64	5.17	30	23.51	2.31	10	0.0008 **
80°C	34.82	4.88	30	33.21	2.59	10	0.3279 NS
Water holding capacity (%)							
60°C	47.94	4.86	30	53.22	2.78	10	0.0024 **
70°C	42.05	5.24	30	48.90	2.31	10	0.0003 **
80°C	37.57	4.34	30	40.82	2.21	10	0.0571 *
Shear force (N/2.5cm dia.)							
60°C	77.31	9.73	30	78.84	22.16	10	0.8627 NS
70°C	86.92	34.86	30	101.63	25.01	10	0.2268 NS
80°C	98.79	26.85	30	137.50	25.47	10	0.0002 **
<i>M. longissimus thoracis</i> area (cm ²)	29.07	3.94	30	25.20	4.05	10	0.0111 *

4.2.3.5. The influence of the two herds on the meat characteristics of SS pigs

The results of this analysis are given in Table 4.24. No difference in pH values, and in the drip loss between vacuum packaged meat samples of SS pigs from herds X and Y were found. However, the cooking losses at cooking temperatures of 60 and 70°C were significantly lower for the samples from herd Y than for herd X, although no significant difference was found at a cooking temperature of 80°C. The water holding capacity of meat from the SS pigs of herd Y was higher than that of the X herd at a cooking temperature of 80°C. No significant differences were found at cooking

temperatures of 60 and 70°C. The shear force values of meat from the SS pigs of herd Y were higher than those of the X herd at all three of the cooking temperatures. No difference between the SS pigs of the two breeds was found for *M. longissimus thoracis* area. After excluding the meat from DFD carcasses, similar results were obtained in the analysis including meat from DFD carcasses (Table B.6).

Table 4.24: Mean values, standard deviations (sd) and level of significance of meat characteristics of SS pigs as influenced by herd

Variable	Herd X			Herd Y			Significance level
	Mean	sd	n	Mean	sd	n	
pH values							
15 min. p.m.	6.23	0.32	17	6.17	0.33	9	0.6105 NS
30 min. p.m.	6.13	0.31	17	6.07	0.40	9	0.6919 NS
45 min. p.m.	6.01	0.31	17	5.94	0.38	9	0.6227 NS
60 min. p.m.	5.86	0.28	14	5.80	0.50	9	0.7357 NS
24 h p.m.	5.62	0.37	17	5.67	0.46	9	0.7688 NS
Drip loss (%)	5.52	2.17	17	6.90	2.16	9	0.1330 NS
Cooling loss (%)							
60°C	18.29	3.78	17	13.98	2.52	9	0.0033 **
70°C	30.20	4.21	17	24.87	2.22	9	0.0017 **
80°C	35.02	4.12	17	32.81	1.54	9	0.1355 NS
Water holding capacity (%)							
10°C	48.93	3.20	17	50.42	5.88	9	0.4064 NS
70°C	41.54	3.33	17	44.88	7.22	9	0.1368 NS
80°C	37.11	3.51	17	41.26	5.85	9	0.0321 *
Shear force (N/2.5cm dia.)							
60°C	67.02	13.02	17	83.94	8.00	9	0.0016 **
70°C	66.05	15.75	17	90.17	7.45	9	0.0002 **
80°C	89.27	19.52	17	109.98	12.65	9	0.0085 **
<i>M. longissimus thoracis</i> area (cm ²)	28.02	6.74	17	28.93	2.24	9	0.6992 NS

4.3. Discussion

4.3.1. Growth characteristics

For the producer of pigs, it is important to increase ADG and lower FCR of his pigs, as these two variables are of economic importance. Webb & Jordan (1978) have shown that ADG and FCR are similar in SS and SR Pietrain/Hampshire pigs. A similar finding was obtained by Eijkelboom, Minkema, Van Eldik & Sybesma (1978b) and Eijkelboom *et al.* (1980a) using Dutch Yorkshire and Dutch Landrace gilts, thus suggesting that at least in respect of ADG and FCR stress susceptibility is not a disadvantage.

However, Eikelenboom *et al.* in an earlier study (1976), McGloughlin *et al.* (1980) and Mitchell & Heffron (1981b) found a lower ADG and FCR in stress susceptible Dutch and German Landrace pigs. The results obtained in my study using South African Landrace gilts indicated no significant difference in ADG between SR and SS pigs. However, the FCR of the SS pigs was lower which was as a result of the difference in FCR between the SS and SR pigs from herd Y, similar to the finding of Eikelenboom *et al.* (1980b), suggesting that the results of at least the FCR between SR and SS pigs may vary between different herds within the same breed. The live mass differences between the two herds seem to be more substantial than the differences within stress sensitivity, i.e. SR and SS, with the live masses of the SR and SS pigs being very similar.

These results show that, although ADG did not differ between SR and SS pigs in general, FCR may differ between SR and SS pigs, in general, but that these differences are dependant on differences between herds. Therefore, it would seem from a growth point of view, taking into account ADG, FCR and live mass, that the production of SS pigs is not disadvantageous compared to SR pigs.

4.3.2. Carcass characteristics

SS Landrace pigs seem to have economically advantageous carcass characteristics. They have a significantly lower backfat thickness and higher slaughter-out percentage compared to SR pigs (Eikelenboom *et al.*, 1976; Webb & Jordan, 1978; Eikelenboom *et al.*, 1980a; Jensen & Andresen, 1980; Schmidt, 1980). These characteristics also occurred in the South African Landrace gilts used in my study. These differences were, however, not found in SS Pietrain/Hampshire crosses (Webb & Jordan, 1978) or Dutch Yorkshire pigs (Eikelenboom *et al.*, 1978b). It would therefore seem that breed may influence the differences in slaughter-out percentages between SS and SR pigs. In my study it was shown using the cold carcass mass after 24 hours of chilling, that the SS pigs had the higher slaughter-out percentage, with no herd influences regarding stress sensitivity. This is a sought after carcass characteristic which is to the advantage of the producer, especially as it was found that ADG and FCR were not negatively influenced by stress sensitivity. Although the lower backfat thickness is regarded as a desirable carcass characteristic for pork production, which is desired by the producer and to his advantage, it might not be to the advantage of the pig industry in general. It has been shown that pigs with a lower backfat thickness are more prone to produce undesirable DFD pork (Heinze *et al.*, 1984) which is extremely susceptible to microbial spoilage, and leads to a short shelf life (Newton & Gill, 1981). Also, thinner backfat thicknesses lead to processing difficulties (Reid, 1983; Wood, 1983).

The SS pigs also had shorter carcasses than the SR pigs, a finding similar to that found by various other researchers (Webb & Jordan, 1978; Schmidt & Kallweit, 1980; Monia *et al.*, 1981; Schmitten *et al.*, 1981b). Furthermore, SS Dutch Landrace pigs (Eikelenboom *et al.*, 1978b) had significantly less carcass mass loss during chilling, a finding supported by my study, while SS Yorkshire and Pietrain/Hampshire pigs did not (Eikelenboom *et al.*, 1978b; Webb & Jordan, 1978). Thus, it seems that differences between breeds may be found. However, in my study, it was also found that within

breeds differences may be found for carcass characteristics such as backfat thickness and chilling loss. Overall, it can be concluded that the SS pigs in general have advantageous carcass characteristics. However, these positive carcass characteristics of SS pigs have to be evaluated in the light of the meat these pigs produce.

4.3.3. Meat characteristics

Although it has been found that meat from SS pigs is less tender as measured by shear force (Dilley *et al.*, 1970), Fox *et al.* (1980) found PSE meat to be more tender, a finding similar to the results in my study. In my study, meat from SS pigs was generally more tender, and significantly so at cooking temperatures of 70 and 80°C. No significant differences were found between SS and SR pigs in general regarding drip loss, cooking loss, water holding capacity and *M. longissimus thoracis* area. These were also the findings regarding the influence of stress sensitivity within the two different herds. However, differences were found between the meat characteristics of the pigs between the two herds regarding the meat characteristics cooking loss, water holding capacity and shear force. The meat from pigs of herd Y generally had a lower cooking loss (significantly so at cooking temperatures of 60 and 70°C). These differences were also reflected in the water holding capacity, with the meat from pigs of herd Y having a higher water holding capacity than the meat from pigs of herd X. However, the meat from pigs of herd Y was less tender than the meat from pigs of herd X.

The advantageous carcass qualities of SS Landrace pigs were, however, offset by the rapid fall in muscle pH which occurred *post mortem* in these pigs, and which is characteristic of SS animals (Mitchell & Heffron, 1981b; Lundström *et al.*, 1983). The rapid fall in muscle pH *post mortem* leads to the development of PSE pork (Honikel & Kim, 1985) and a resultant loss in mass during processing (Klingbeil, Naudé & Van Esten, 1976), undesirable pale colour, and an accumulation of undesirable fluid (Lister, Gregory & Warwick, 1981). The results of my study using the pigs from herd Y, support these findings in that SS pigs had an undesirable colour and greater volume of drip (herd Y).

4.3.4. General

Selective breeding has the aim of exploiting certain animal-traits to satisfy certain needs, which usually have economic advantages. Therefore, the pig producer aims to increase ADG, FCR and muscle to fat ratio, whereas the pig industry also wants a reduced incidence of PSE meat. From the results obtained in my study, the large differences between herds in the same breed is obvious. Also, these differences are found between SR pigs of the different herds. It should therefore be possible to satisfy the needs of the pig producer and pig industry by selecting only SR pigs and excluding all SS pigs from breeding stock. Selection criteria should therefore also include the identification of SS pigs if the selection criteria include such characteristics as daily gain, backfat thickness *ect.* (Vögeli *et al.*, 1983).

4.4. Conclusion

The results of my study support previous data collected on the growth and carcass characteristics of SS Landrace pigs. In general, SS pigs have a similar ADG to that of SR pigs, but a lower FCR, whereas the live masses of the two types of pigs are similar for the ages of 13 to 21 weeks. Thus, although the ADG of the SR and SS pigs are similar, the SS pigs have a lower FCR. Certain carcass and meat quality traits of SS animals are superior to those of SR animals. These characteristics included a higher slaughter-out percentage, lower chilling losses, thinner backfat, more compact carcasses, and more tender meat. However, these positive characteristics are offset by the lower pH values of the meat up to 60 minutes *post mortem*, and *pH* colour, indicative of PSE meat. This lower muscle pH values and resultant PSE meat has a negative influence on the pig industry, as it is the quality of the end-product, in this case meat, that determines whether the influence of SS pigs on overall production is positive or negative. The low pH value and resultant PSE meat neutralises therefore the advantageous carcass and meat characteristics SS pigs possess. Thus, although the production of SS pigs have positive advantages for the producer in terms of leaner pigs, slaughter-out percentages etc., it leads to a loss for the pig industry in general as a result of the production of PSE meat by these pigs, consequently to a lower quality of meat.

Nevertheless, several herd differences within the South African Landrace breed was found, which may effect the growth (as measured by live mass), carcass and meat characteristics to a varying degree. These differences between the SR pigs of the two herds did not effect the pH value of the meat negatively, i.e. towards meat of PSE quality. Thus, the advantageous carcass and meat characteristics which may result from the use of SS pigs, may also result from selective breeding using only SR pigs, thus eliminating the negative influence of breeding with, or producing SS pigs, such as PSE meat. It is therefore, in the interests of the pig industry to reduce the incidence of stress susceptibility.

CHAPTER 5

The effect of stress on various blood variables of pigs

5.1. Introduction

In Chapter 3 differences between the two herds X and Y were observed in blood variables. However, it has been assumed in Chapter 3 that these differences in the 11 week of age blood variables were the result of the different husbandry management procedures followed by the different producers, for example, in one herd pigs were used to personnel regularly entering their pens, and in the other they were not. After a minimum of 12 weeks at Ireno, all pigs seemed to have adapted to their new environment, as well as to handling by personnel. It is therefore assumed that the differences which resulted at 11 weeks of age would have disappeared, and that the results measured at 21 weeks after halothane exposure or treadmill exercise are the consequence of the stress procedure and not as a result of herd differences.

Pigs susceptible to stress often die when subjected to natural stress such as exercise, service, transport and heat, or exposed to drugs such as halothane and suxamethonium (Patterson & Allen, 1972). The signs of a stress reaction are high body temperature, tachycardia, rigidity of muscles, blotchy cyanosis and hypercapnia (Harrison *et al.*, 1969).

Although the physiology and biochemical changes which occur in SS pigs after exposure to halothane have been extensively investigated (Mitchell & Hoffron, 1962), the effects of exercise have not, nor have similarities and differences between halothane induced and exercise induced stress been assessed. Such information would be of importance to determine whether the reactions which follow exposure to halothane are the same as those which follow physical stress. It has been reported that both exercise and halothane result in the death of stress susceptible pigs, and it is thought that a common feature is diversion of glucose metabolism from aerobic to anaerobic pathways (Patterson & Allen, 1972). This idea was investigated in this study, and the results are presented here as a comparison of several blood variables to illustrate differences between the reactions of SR and SS pigs to halothane exposure and physical stress resulting from treadmill exercise.

The blood variable data obtained was analysed as follow:

- * 2-way analysis of variance for the factors stress procedure (halothane exposure vs treadmill exercise) and stress sensitivity
- * the influence of halothane exposure on the blood variables
- * the influence of treadmill exercise on the blood variables
- * the influence of halothane exposure or treadmill exercise on the blood variables of SR pigs
- * the influence of halothane exposure or treadmill exercise on the blood variables of SS pigs.

5.2. Results

5.2.1. The effects of halothane exposure, treadmill exercise and stress sensitivity on blood variables of pigs

The results of the 2-way analyses of variance with the type of stress procedure (halothane exposure and treadmill exercise) and stress sensitivity (SS or SR) are given in Table 5.1, and the mean values found within each of types of stress in Table 5.2. Comparing treadmill exercise to halothane exposure in all pigs (Column A, Table 5.1) treadmill exercise resulted in significantly elevated CK, LDH, aldolase, AST and ALT activities, significantly higher lactate, urea, creatinine, glucose, cortisol and ACTH concentrations, a higher urea-to-creatinine ratio and anion gap value compared to halothane exposure. Treadmill exercise also resulted in significantly lower albumin, sodium, chloride, inorganic phosphate and bicarbonate concentrations compared to halothane exposure. No significant differences were found between the concentrations of total protein, globulin, potassium, magnesium and calcium, osmolality and the albumin-to-globulin ratio as the result of halothane exposure and treadmill exercise. The same results were obtained after excluding the exercised SS pigs that survived the exercise, except that the difference in sodium concentrations was not significant. However, the magnesium concentration of the halothane exposed pigs was higher than that of the treadmill exercised pigs (Appendix C, Tables C.1 and C.2)

Comparing SS and SR pigs (Column B, Table 5.1), the two types of stress used caused in SS pigs significantly higher CK, LDH, aldolase, AST and ALT activities, and higher concentrations of lactate, total protein, albumin, sodium, potassium, magnesium, calcium, creatinine, inorganic phosphate, bicarbonate and ACTH. In addition the osmolality was significantly higher, and the anion gap greater in SS pigs than SR pigs. Stress also resulted in a significantly lower urea concentration and urea-to-creatinine ratio in SS pigs. No significant differences were recorded between the SS and SR pigs for globulin, glucose and cortisol concentrations, and albumin-to-globulin ratio as a result of the stresses applied in this study. In excluding the surviving treadmill exercised SS

pigs, the same results were found, except that the difference in bicarbonate concentrations between the SR and SS pigs was not significant (Tables C.1 and C.2).

Table 5.1: The results of 2-way analyses of variance on blood variables as influenced by stress procedure (A: halothane exposure vs treadmill exercise) and stress sensitivity (B: SR vs SS)

Variable	Stress procedure (A)		Stress sensitivity (B)		AxB	
	F value	Significance level	F value	Significance level	F value	Significance level
CK	22,027	<0,0001 **	28,877	<0,0001 **	33,452	<0,0001 **
LDH	12,260	0,0009 **	25,625	<0,0001 **	19,400	<0,0001 **
Aldolase	4,389	0,0403 *	150,810	<0,0001 **	0,110	0,7446 NS
AST	26,491	<0,0001 **	15,276	0,0002 **	9,533	0,0028 **
ALT	13,887	0,0004 **	8,759	0,0044 **	0,056	0,8161 NS
Lactate	17,590	<0,0001 **	20,593	<0,0001 **	9,635	0,0029 **
Total protein	0,834	0,3744 NS	10,792	0,0017 **	0,061	0,8091 NS
Albumin	4,699	0,0340 *	15,884	0,0002 **	7,334	0,0087 **
Globulin	0,078	0,7833 NS	1,927	0,1689 NS	1,969	0,1655 NS
Urea	32,991	<0,0001 **	13,030	0,0006 **	12,821	0,0007 **
Sodium	12,232	0,0009 **	70,949	<0,0001 **	31,253	<0,0001 **
Potassium	2,208	0,1423 NS	21,941	<0,0001 **	3,472	0,0672 NS
Chloride	64,420	<0,0001 **	3,055	0,0854 NS	39,571	<0,0001 **
Magnesium	2,536	0,1154 NS	13,107	0,0006 **	35,440	<0,0001 **
Calcium	5,997	0,0897 NS	36,670	<0,0001 **	3,198	0,0786 NS
Creatinine	4,794	0,0325 *	6,661	0,0122 *	3,926	0,0526 NS
Glucose	15,141	0,0002 **	0,779	0,3902 NS	0,505	0,4877 NS
Inorganic phosphate	10,231	0,0022 **	22,293	<0,0001 **	0,906	0,9369 NS
Bicarbonate	114,969	<0,0001 **	6,313	0,0146 *	4,428	0,0394 *
Cortisol	201,020	<0,0001 **	0,788	0,3875 NS	6,851	0,0111 *
ACTH	38,974	<0,0001 **	4,332	0,0415 *	2,546	0,1156 NS
Urea/creatinine ratio	19,224	<0,0001 **	27,722	<0,0001 **	4,367	0,0408 *
Albumin/globulin ratio	0,423	0,5250 NS	0,024	0,8791 NS	7,517	0,0060 **
Osmolality	1,948	0,1678 NS	52,831	<0,0001 **	38,918	<0,0001 **
Anion gap	49,978	<0,0001 **	38,177	<0,0001 **	1,288	0,2608 NS

5.2.2. Effects of halothane exposure on blood variables of SS and SR pigs

Table 5.3 shows the differences in the concentrations of blood variables in the 30 SR and 17 SS pigs. Compared to SR pigs, the SS pigs had significantly elevated activities of the enzymes CK, LDH, aldolase, AST and ALT activities. The SS pigs also had significantly higher concentrations of lactate, total protein, albumin, sodium, potassium, chloride, magnesium, calcium, creatinine, glucose, inorganic phosphate and ACTH. Plasma osmolality and the anion gap after exposure to halothane was higher in SS pigs than in SR pigs. The cortisol concentration of the SS pigs was significantly lower, as was the urea-to-creatinine ratio. No significant differences between the two

groups were found for globulin, urea and bicarbonate concentrations, nor in the albumin-to-globulin ratio.

Table 5.2: Mean values and standard deviations (sd) of the influence of stress procedure and stress sensitivity on blood variables

Variable		Halothane exposure			Treadmill exercise			SR pigs			SS pigs		
		Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n
CK	IU/l	2007	3283	47	13221	17334	19	1673	1019	40	12341	14846	26
LDH	IU/l	1196	370	47	1976	1622	19	1037	273	40	2011	1327	26
Aldolase	IU/l	14,6	7,2	47	18,4	7,9	19	10,7	3,5	40	23,5	5,0	26
AST	IU/l	46	16	47	78	40	19	46	13	40	70	59	26
ALT	IU/l	50	14	47	65	16	19	50	14	40	61	17	26
Lactate	mmol/l	7,84	4,75	46	12,24	2,22	19	7,39	3,57	39	11,73	4,81	26
Total protein	mmol/l	72	6	47	71	8	19	69	6	40	74	6	26
Albumin	mmol/l	40	4	47	39	3	19	39	3	40	42	3	26
Globulin	mmol/l	31	4	47	32	7	19	31	5	40	32	4	26
Urea	mmol/l	5,8	1,3	47	7,6	1,8	19	6,6	1,7	40	5,7	1,5	26
Sodium	mmol/l	251	6	47	149	4	19	148	3	40	155	6	26
Potassium	mmol/l	5,2	0,9	47	5	1,3	19	4,8	0,5	40	5,8	1,3	26
Chloride	mmol/l	102	3	47	96	5	19	101	2	40	99	7	26
Magnesium	mmol/l	0,90	0,27	47	1,00	0,24	19	0,85	0,25	40	1,04	0,25	26
Calcium	mmol/l	2,95	0,42	47	2,84	0,42	19	2,72	0,31	40	3,22	0,39	26
Creatinine	mmol/l	144	20	47	156	13	19	143	20	40	155	15	26
Glucose	mmol/l	5,4	0,8	47	7,2	2,8	19	5,7	1,6	40	6,3	2,1	26
Inorganic phosphate	mmol/l	3,29	0,45	47	3,00	0,43	19	3,04	0,35	40	3,47	0,49	26
Bicarbonate	mmol/l	25	2	47	17	4	19	22	4	40	23	4	26
Cortisol	nmol/l	23	11	47	84	26	19	40	27	40	42	40	26
ACTH	pmol/l	20	11	47	26	6	19	12	13	40	18	11	26
Urea/creatinine ratio		40	9	47	49	12	19	47	10	40	37	9	26
Albumin/globulin ratio		1,32	0,21	47	1,28	0,28	19	1,31	0,25	40	1,31	0,21	26
Osmolality	mmol/l	313	12	47	312	10	19	308	8	40	321	11	26
Arterial gap	mmol/l	30	6	47	40	7	19	29	6	40	38	7	26

5.2.3. Effects of treadmill exercise on blood variables of SS and SR pigs

The differences in blood variables of SS and SR pigs after treadmill exercise are summarised in Table 5.4: Compared to exercised SR pigs, exercised SS pigs had significantly elevated CK, LDH, aldolase and AST activities, and elevated inorganic phosphate and bicarbonate concentrations. The exercised SS pigs had significantly lower urea, chloride and magnesium concentrations than did exercised SR pigs, and a lower urea-to-creatinine ratio. No significant differences were found between SS and SR pigs for ALT activity, lactate, total protein, albumin, globulin, sodium, potassium, calcium, creatinine, glucose concentration, osmolality, the 'anion gap' or for the albumin-to-globulin ratio. In exercising treadmill SS pigs, similar results were obtained, except that the

SS pigs that died as a result of exercise had a higher ALT activity. The differences in magnesium and bicarbonate concentrations between the SR and SS pigs were not significant (Table C.3).

Table 5.3: Mean values, standard deviations (sd) and level of significance of blood variables during halothane exposure as influenced by stress sensitivity

Variable	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
CK IU/l	1475	984	30	5432	4315	17	<0,0001 **
LDH IU/l	1057	302	30	1443	358	17	0,0003 **
Aldolase IU/l	10,3	3,6	30	22,5	4,7	17	<0,0001 **
AST IU/l	42	12	30	52	20	17	0,0317 *
ALT IU/l	46	13	30	56	14	17	0,0167 *
Lactate mmol/l	5,69	2,26	29	11,50	5,61	17	<0,0001 **
Total protein mmol/l	70	4	30	75	6	17	0,0012 **
Albumin mmol/l	39	3	30	43	4	17	<0,0001 **
Globulin mmol/l	31	4	30	32	4	17	0,6263 NS
Urea mmol/l	5,9	1,0	30	5,5	1,6	17	0,3160 NS
Sodium mmol/l	147	3	30	158	2	17	<0,0001 **
Potassium mmol/l	4,7	0,5	30	6,1	0,9	17	<0,0001 **
Chloride mmol/l	101	2	30	104	3	17	0,0063 **
Magnesium mmol/l	0,76	0,19	30	1,34	0,22	17	<0,0001 **
Calcium mmol/l	2,73	0,28	30	3,34	0,33	17	<0,0001 **
Creatinine μ mol/l	138	19	30	153	17	17	0,0041 **
Glucose mmol/l	5,2	0,8	30	5,8	0,7	17	0,0225 *
Inorganic phosphate mmol/l	3,13	0,37	30	3,58	0,44	17	0,0004 **
Bicarbonate mmol/l	25	2	30	25	3	17	0,2725 NS
Cortisol nmol/l	27	11	30	16	8	17	0,0012 **
ACTH pmol/l	7	10	30	15	11	17	0,0212 *
Urea/creatinine ratio	43	8	30	35	8	17	0,0022 **
Albumin/globulin ratio	1,28	0,23	30	1,39	0,17	17	0,0847 NS
Osmolality mOsmol/l	306	7	30	327	6	17	<0,0001 **
Anion gap mmol/l	26	4	30	35	4	17	<0,0001 **

5.2.4. Comparison of halothane exposure and treadmill exercise on blood variables of SR pigs

The effect of halothane exposure and treadmill exercise on SR pigs are illustrated in Table 5.5. Compared to halothane exposure, exercise produced a significant increase in CK, AST and ALT activities. Exercise also resulted in a higher lactate, urea, magnesium, creatinine, glucose, cortisol, and ACTH concentrations, urea-to-creatinine ratio, anion gap and osmolality. Exercised SR pigs also had significantly lower inorganic phosphate and bicarbonate concentrations. No significant differences were found between the two types of stresses for the activities of LDH and aldolase,

between concentrations of total protein, albumin, globulin, sodium, potassium, chloride and calcium, and in the albumin-to-globulin ratio.

Table 5.4: Mean values, standard deviations (sd) and level of significance of blood variables after treadmill exercise as influenced by stress sensitivity

Variable		SR pigs			SS pigs			Significance level
		Mean	sd	n	Mean	sd	n	
CK	IU/l	2268	926	10	25390	18934	9	0.0012 **
LDH	IU/l	979	158	10	3083	1869	9	0.0019 **
Aldolase	IU/l	12.3	2.6	10	25.3	5.4	9	<0.0001 **
AST	IU/l	56	13	10	103	46	9	0.0066 **
ALT	IU/l	60	12	10	71	19	9	0.1276 NS
Lactate	mmol/l	12.51	1.37	10	12.17	2.99	9	0.8989 NS
Total protein	mmol/l	69	9	10	73	7	9	0.2482 NS
Albumin	mmol/l	39	2	10	39	3	9	0.9293 NS
Globulin	mmol/l	30	8	10	34	5	9	0.1875 NS
Urea	mmol/l	8.9	1.1	10	6.1	1.2	9	<0.0001 **
Sodium	mmol/l	149	3	10	149	5	9	0.9866 NS
Potassium	mmol/l	4.8	0.5	10	5.2	1.9	9	0.5061 NS
Chloride	mmol/l	100	2	10	91	4	9	<0.0001 **
Magnesium	mmol/l	1.13	0.20	10	0.86	0.19	9	0.0099 **
Calcium	mmol/l	2.71	0.40	10	2.99	0.42	9	0.1517 NS
Creatinine	μmol/l	157	14	10	155	12	9	0.7695 NS
Glucose	mmol/l	7.3	2.4	10	7.2	3.3	9	0.9522 NS
Inorganic phosphate	mmol/l	2.78	0.06	10	3.25	0.52	9	0.0104 *
Bicarbonate	mmol/l	16	2	10	19	4	9	0.0286 *
Cortisol	nmol/l	78	25	10	91	27	9	0.3379 NS
ACTH	pmol/l	27	8	10	26	4	9	0.7909 NS
Urea/creatinine ratio		57	7	10	40	9	9	0.0003 **
Albumin/globulin ratio		1.39	0.30	10	1.16	0.21	9	0.0756 NS
Osmolality	mmol/l	314	7	10	311	12	9	0.5269 NS
Anion gap	mmol/l	37	4	10	43	9	9	0.0258 NS

5.2.5. Comparison of halothane exposure and treadmill exercise on blood variables of SS pigs

In Table 5.6 the differences of the effects of exercise stress and halothane exposure on SS pigs are given. In comparison to halothane exposure, the exercised SS pigs had significantly raised CK, LDH, AST and ALT activities, and an increased anion gap. On the other hand, exercise resulted in significantly lower concentrations of albumin, sodium, chloride, magnesium, calcium and bicarbonate, as well as a lower albumin-to-globulin ratio and osmolality. Both the cortisol and ACTH concentrations were increased significantly after exercise stress in comparison to halothane exposure. In excluding the SS pigs that survived the treadmill exercise, similar results were obtained.

except that the differences in the albumin, magnesium, calcium and cortisol concentrations were not significant (Table C.4)

Table 5.5: Mean values, standard deviations (sd) and level of significance of blood variables from SR pigs as influenced by stress procedure

Variable	Halothane exposure			Treadmill exercise			Significance level
	Mean	sd	n	Mean	sd	n	
CK IU/l	1475	984	30	2268	926	10	0,0312 *
LDH ¹ IU/l	1057	302	30	979	158	10	0,4452 NS
Aldolase IU/l	10,3	5,6	30	12,2	2,6	10	0,1144 NS
AST IU/l	42	12	30	56	13	10	0,0030 **
ALT IU/l	46	132	30	60	12	10	0,0056 **
Lactate mmol/l	5,69	2,26	29	12,31	1,37	10	<0,0001 **
Total protein mmol/l	70	4	30	69	9	10	0,5891 NS
Albumin mmol/l	39	3	30	39	2	10	0,8501 NS
Globulin mmol/l	31	4	30	30	8	10	0,5087 NS
Urea mmol/l	5,9	1,0	30	8,9	1,1	10	<0,0001 **
Sodium mmol/l	147	3	30	149	3	10	0,2598 NS
Potassium mmol/l	4,7	0,5	30	4,8	0,5	10	0,8182 NS
Chloride mmol/l	101	2	30	100	2	10	0,2579 NS
Magnesium mmol/l	0,76	0,19	30	1,13	0,20	10	<0,0001 **
Calcium mmol/l	2,73	0,28	30	2,71	0,40	10	0,8804 NS
Creatinine μ mol/l	138	19	30	157	14	10	0,0030 **
Glucose mmol/l	5,2	0,8	30	7,3	2,6	10	0,0002 **
Inorganic phosphate mmol/l	3,13	0,57	30	2,78	0,06	10	0,02,7 **
Bicarbonate mmol/l	25	2	30	16	2	10	<0,0001 **
Cortisol nmol/l	27	11	30	78	25	10	<0,0001 **
ACTH pmol/l	7	10	30	27	8	10	<0,0001 **
Urea/creatinine ratio	43	8	30	57	7	10	<0,0001 **
Aspartate/albumin ratio	1,28	0,23	30	1,30	0,30	10	0,2341 NS
Osmolality mmol/l	306	7	30	314	7	10	0,0040 **
Anion gap mmol/l	26	4	30	37	4	10	<0,0001 **

5.3. Discussion

Previous experiments show that a common consequence of both halothane and exercise in SS pigs is the development of PSE pork after death. Data has indicated that PSE is caused by a stimulation of glycolysis before or at the death (Mitchell & Heffron, 1982). However, the idea that stimulation of glycolysis is the major or single cause of the stress reaction in pigs can only be supported if changes in a variety of biochemical variables are similar for different types of stress.

The results show, however, that in general exercising pigs have a different blood variable profile than do pigs exposed to halothane. The treadmill exercise of untrained pigs resulted in significantly

more tissue damage as indicated by the elevated serum enzyme activities (Table 5.1 (Column A) and 5.2). Lactacidosis was also increased in the exercised pigs, as was the blood glucose concentration, and a metabolic acidosis developed as indicated by the lower bicarbonate concentration and larger anion gap. The urea-to-creatinine ratio was also diminished, as were the concentrations of albumin and sodium. The osmolalities, however, were similar.

Table 5.6: Mean values, standard deviations (sd) and level of significance of blood variables from SS pigs as influenced by stress procedure

Variable		Halothane exposure			Treadmill exercise			Significance level
		Mean	sd	n	Mean	sd	n	
CK	IU/l	5432	4315	17	25390	18934	9	0.0003 **
LDH	IU/l	1443	358	17	3083	1809	9	0.0012 **
Aldolase	IU/l	22.5	4.7	17	25.3	5.4	9	0.1911 NS
AST	IU/l	52	20	17	103	45	9	0.0005 **
ALT	IU/l	56	14	17	71	19	9	0.0295 *
Lactate	mmol/l	11.5	5.61	17	12.17	2.09	9	0.7427 NS
Total protein	mmol/l	75	6	17	73	7	9	0.4667 NS
Albumin	mmol/l	43	4	17	39	3	9	0.0053 **
Globulin	mmol/l	32	4	17	34	5	9	0.5024 NS
Urea	mmol/l	5.5	1.6	17	6.1	1.2	9	0.3593 NS
Sodium	mmol/l	158	2	17	149	5	9	<0.0001 **
Potassium	mmol/l	4.1	0.9	17	5.2	1.9	9	0.1164 NS
Chloride	mmol/l	105	3	17	91	4	9	<0.0001 **
Magnesium	mmol/l	1.14	0.22	17	0.86	0.19	9	0.0037 **
Calcium	mmol/l	2.34	0.33	17	2.99	0.42	9	0.0302 *
Creatinine	μmol/l	155	17	17	153	12	9	0.9728 NS
Glucose	mmol/l	5.8	0.7	17	7.2	3.3	9	0.1063 NS
Inorganic phosphate	mmol/l	3.58	0.44	17	5.25	0.52	9	0.1029 NS
Bicarbonate	mmol/l	22	3	17	19	4	9	0.0002 **
Cortisol	nmol/l	16	8	17	91	27	9	<0.0001 **
ACTH	pmol/l	15	11	17	26	4	9	0.0062 **
Urea/creatinine ratio		35	8	17	40	9	9	0.2224 NS
Albumin/globulin ratio		1.39	0.17	17	1.16	0.21	9	0.0055 **
Osmolality	mmol/l	327	6	17	311	12	9	<0.0001 **
Anion gap	mmol/l	35	4	17	43	9	9	0.0040 **

In addition, the data confirms that the response of SS pigs to stress in general differs significantly from that of SR pigs (Tables 5.1 (Column B) and 5.2) (Mitchell & Heffron, 1982). The enzyme activities were significantly higher in the stressed SS pigs than in the stressed SR pigs, as were the concentrations and ratios of the other variables determined in this study. The exceptions to this general finding were that the urea concentration and urea-to-creatinine ratio were lower in SS than SR pigs, and the globulin, chloride, glucose, cortisol concentrations, and albumin-to-globulin ratio were not significantly different. The results suggest that stress precipitates a membrane defect in the SS pigs resulting in the leakage of cell enzymes and higher enzyme activities in the serum,

as has already been suggested by Allen, Berrett, Harding & Patterson (1970). Moreover, a shift of fluid from the plasma to the inter- and intracellular spaces (total protein and sodium increase, and also osmolality) occurs, as was suggested by Berman *et al.* (1970). These changes have been characterised best in the many experiments in which pigs have been exposed to halothane, and in general the results confirm previous data. On exposing SS and SR pigs to halothane, an increase was found in the activities of the enzymes CK, LDH, aldolase, AST and ALT, as well as in concentrations of the various metabolites and electrolytes result. As suggested by Berman *et al.* (1970), the increased values are caused by a shift of water from the plasma to intra- and intercellular spaces, thus causing haemoconcentration. My results support this idea. In my study both total serum protein concentration and osmolality increased. This movement of fluid is possibly the result of accumulation of lactate in the muscles of the SS pigs (Hall *et al.*, 1976; Van der Hende *et al.*, 1976), and consequently a higher tissue osmolality.

The mechanism of fluid shift can account for a 7% increase in the blood variables. However, the enzyme activities, potassium, magnesium, calcium, lactate, creatinine, glucose, inorganic phosphate and ACTH all increased by more than 7%. The increase in the enzyme activities, potassium, inorganic phosphate, calcium and magnesium can be attributed to changes in cell membrane permeability caused by halothane (Mitchell & Heffron, 1982), whereas the increase in glucose and lactate concentrations reflects the expected stimulation in the SS pigs of glycolysis (Patterson & Allen, 1972). An elevation of ACTH levels can be expected during stress, although it has been found that SS pigs have a higher ACTH concentration even if they are not stressed (Marple & Cassens, 1973).

The bicarbonate concentrations of the SS and SR pigs were not significantly different during halothane exposure, and remained within the normal range, indicating that a state of metabolic acidosis in this experiment was not reached. This result differs from results of other experiments. SS pigs have been shown to develop a metabolic acidosis as a consequence of exposure to halothane (Allen *et al.*, 1970; Van der Hende *et al.*, 1976). Another finding in this study was that SS pigs had a lower urea-to-creatinine ratio caused by an increase in creatinine concentration. This finding suggests that during exposure to halothane SS pigs have a reduced ability to secrete creatinine into tubular filtrate. In general therefore, halothane exposure of SS pigs stimulates anaerobic glycolysis, causes muscle damage and haemoconcentration.

Few experiments have assessed the effects of exercise on blood variables of SS and SR pigs. One study (Schmidt, 1980) found no significant differences in bicarbonate, lactate, glucose and cortisol concentrations up to 30 minutes after treadmill exercise, although CK and LDH (and notably EDHs) activities were significantly higher in the serum of SS pigs. This is in accordance with the results found in this experiment after treadmill exercise for the same blood variables, except that the SS pigs had a higher bicarbonate concentration than the SR pigs. Although treadmill exercise was expected to result in muscle damage of the untrained pigs, the results indicate a much higher level of muscle damage in the SS pigs as indicated by the higher CK, LDH and AST activities in the SS pigs. A surprising finding, however, was that the lactate concentrations

of the SS and SR pigs were not significantly different, thus indicating the same level of anaerobic glycolysis under these experimental conditions. It was expected that the SS pigs would have a greater tendency to anaerobic glycolysis under stressful situations as suggested by Patterson & Allen (1972), with a concomitant development of lacticidosis and a metabolic acidosis, especially as was shown that the SS pigs have a larger percentage white muscle fibres than the SR pigs (See Chapter 7). In my experiment it seems that the SR pigs developed a more significant metabolic acidosis than did SS pigs: the bicarbonate concentration of the SR pigs was lower than that of SS pigs. The anion gap of SR pigs was, however, not significantly different to that of SS pigs. This result is in contrast to that of Judge, Eikelenboom, Zuidam & Sybesma (1973) who found lower bicarbonate concentrations in SS pigs, but is similar to that of Schmidt (1980) who found no significant differences in bicarbonate concentrations between SS and SR pigs after treadmill stress. In addition, no significant difference in osmolality was found after exercise stress in this experiment, indicating that no haemoconcentration developed. In general therefore, the results suggest that the level of exercise as used in this experiment stimulated glycolysis and caused leakage of intracellular enzymes into the plasma.

The data strongly suggest therefore, that both halothane and exercise in general produce similar changes and that the changes produced are similar in both SS and SR pigs. Moreover, because the *post mortem* characteristics of pork from pigs which die of natural stress or from exposure to halothane are similar (Mitchell & Heffron, 1982), the idea that the stress reaction induced by exercise and halothane is linked by a common causative mechanism (Koleczak & Kraeling, 1986), seems to be supported.

The data, however, show significant differences between the effects of exercise and halothane exposure. In SR pigs, when compared to halothane exposure, exercise causes more severe tissue damage, a metabolic acidosis, secretion of A.C.T.H and cortisol, and an increased plasma osmolality. In SS pigs, in general, these changes are exacerbated (Table 5.3 and 5.4).

In SS pigs exercise results in significantly greater tissue damage as measured by the activity of CK, ALT and AST. On the other hand haemoconcentration is a feature of exposure to halothane, but not exercise. Indeed, exercise resulted in significantly lower bicarbonate, chloride, magnesium and albumin concentrations, which suggests that haemodilution is a consequence of exercise. Sodium concentration, however, was not affected by exercise. Thus, it is likely that albumin, magnesium and chloride moved out of the plasma rather than being moved into the plasma. In addition the decrease in bicarbonate concentration and the associated increase in the anion gap which occurs in SS pigs during exercise suggests that they develop a severe metabolic acidosis. As lactate level are similar in both halothane exposure and exercise, the acidosis of exercise is caused by other acids, possibly free fatty acids and ketones. The greater propensity to metabolise fats has been demonstrated in SS pigs by Wood *et al.* (1977), and is also compatible with the hypothesis of Müller (1983) that pigs selected for reduced fat deposition, have a higher fat metabolism rate.

Another significant difference between exercise and halothane exposure in SS pigs is that in exercise both ACTH and cortisol concentrations are higher than during exposure to halothane. The implications of this finding are that either halothane depresses ACTH secretion and hence cortisol secretion, or that exercise is a more severe physiological stress than exposure to halothane. Other experiments have shown that ACTH secretion is normal or enhanced in SS pigs while cortisol clearance is enhanced (Marple & Cassens, 1973), and that secretion of cortisol is depressed after artificial stimulation of secretion (Mitchell & Heffron, 1981a). Nevertheless, it must therefore be concluded that exercise is a greater physiological stress than is exposure to halothane, especially in the light of the higher CK, L.F. of, aldolase and AST activities and higher lactate, urea, creatinine, cortisol and ACTH concentrations, and greater urea to creatinine ratio and anion gap, with lower chloride and bicarbonate concentrations (Tables 5.1 and 5.2).

The idea that exercise and halothane induced stress may differ, as indicated by the significant interactions (Column AxB, Table 5.1), is not new. For example, Gregory & Wilkins (1984) showed that Carazolol, a β -adrenergic receptor antagonist could not prevent halothane induced lactacidemia while it did reduce generation of lactate in pigs exposed to normal management stress (Warriss & Lister, 1982). This finding, when taken with other experiments which have assessed the effects of injected catecholamines (Mitchell & Heffron, 1982) indicate that activation of a β -adrenergic receptor is important in the generation of exercise induced stress, while in halothane induced stress this is less important. The two mechanisms can nevertheless be synergistic. Van der Hende *et al.* (1976) and Gregory & Wilkins (1984) have shown that when SS pigs were exposed to natural stressors before being exposed to halothane, the pigs reacted more violently to halothane exposure.

5.4. Conclusion

The data shows that in both exercise and halothane induced stress, glycolysis is stimulated. However, exercise induced stress differs from halothane induced stress in that there is more severe muscle damage and greater secretion of ACTH and cortisol. Exercise caused severe muscle damage, metabolic acidosis, secretion of cortisol and ACTH, and increased plasma osmolality compared to halothane exposure. These changes were also found in SS pigs between the two types of stress procedures, but the differences were exacerbated on the exposure of SS pigs to the stressors. The data supports the idea, therefore, that although both stresses induce glycolysis, and that both stressors produce PSE in SS pigs, the mechanisms involved are different.

CHAPTER 6

The effect of stress on various muscle metabolites of pigs

6.1. Introduction

In Chapter 3 differences between the two herds X and Y were observed regarding the various muscle metabolites. As has been indicated in Chapter 3, it is assumed that the differences in the various muscle metabolites which resulted at 13 weeks of age occurred as a result of the difference in the level of perceived stress by the pigs from the different herds, and would not occur at 21 weeks of age as a result of the pigs being used to human and personnel involvement. The results measured at 21 weeks after halothane exposure or treadmill exercise therefore are the consequence of the stress procedure and not as a result of herd differences.

The production of PSE pork has severe negative economic implications (Hall, 1972). As a result of these economic implications, much research has been done to try and prevent the production of PSE pork (Cassens *et al.*, 1975). It has been found that pigs suffering from PSS produce PSE pork (Webb *et al.*, 1982). These pigs are characterised by death as a result of natural stresses such as transport, servicing *ect.*, during which malignant hyperthermia develops. It was, however, found that some pigs exposed to the gaseous anaesthetic halothane, would develop the same characteristics, and would also produce PSE meat. Pigs reacting to the halothane were classified as suffering from MHS (Mitchell & Holton, 1982). Moreover, as both these syndromes resulted in PSE meat, and showed the same characteristics (Eikelenboom & Sybesma, 1969), the exposure of pigs to halothane, the so-called halothane test, was introduced to identify SS pigs (Eikelenboom, Minkem & Sybesma, 1978a), thus MHS pigs, and SR pigs. As it was recognised that the PSE meat resulting from PSS or MHS pigs were the result of a stimulation of glycogenolysis and lactic acid production (Gregory, 1981; Hall *et al.*, 1970a), it was suggested that PSS and MHS are similar syndromes, and may be an identity and the expression of the same myopathy (Cassens *et al.*, 1975; Sybesma & Eikelenboom, 1978; Harrison, 1972). This assumption has as to yet not been established unequivocally (Ahern, Soeters, Wilson & McLoughlin, 1979; Patters *e.* & Allen, 1972).

As a result of the introduction of the halothane test, the incidence of PSE pork has declined (Vigoll, Schwörer, Kühne & Wysshaar, 1965). However, the question still exists whether the MHS and PSS syndromes are in fact synonymous. The results in Chapter 5 on the blood profiles of SS and SR pigs exposed to halothane or treadmill exercise show that glycolysis and glycogenolysis are stimulated, but probably by different mechanisms. Much research has also been done on the effect of halothane exposure on SS and SR pigs, but relatively few on the effect of physical stress on the SS and SR pigs. This study was done in an effort to determine whether the exposure of SS and SR pigs to halothane or exercise stress would result in similarities or differences in various muscle metabolites.

The results were analysed according to the following:

- * the influence of stress procedure and stress sensitivity on the muscle metabolites of all the pigs
- * the influence of halothane exposure on SR and SS pigs
- * the influence of treadmill exercise on SR and SS pigs
- * the influence of halothane exposure and treadmill exercise on SR pigs
- * the influence of halothane exposure and treadmill exercise on SS pigs.

6.2. Results

6.2.1. Effect of halothane exposure, treadmill exercise and stress sensitivity on muscle metabolites of pigs

The results of the 2-way analysis of variance on the muscle metabolites of all pigs are given in Table 6.1. The main factors in the analysis of variance were stress procedure (halothane exposure or treadmill exercise) and stress sensitivity (SR or SS). The mean values are shown in Table 6.2.

Analysis of the effect of halothane exposure and treadmill exercise showed that there were no significant differences in the muscle lactate, ATP, glucose 6-phosphate and phosphocreatine concentrations (Column A, Table 6.1). The glucose concentration of the muscle from treadmill exercised pigs was, however, significantly higher, and the glycogen concentration significantly lower than the respective concentrations in the halothane exposed pigs. After excluding the data of SS pigs that survived exercise, the same results were recorded, with the single difference that the ATP concentration of the pigs that died after exercise was significantly lower than that of the halothane exposed pigs (Appendix A, Table D.1 Column 1).

The results of the comparison of the response of all SR and SS pigs to stress (halothane exposure and treadmill exercise combined), indicate that SS pigs had significantly higher lactate, glucose

6-phosphate and glucose concentrations than did SR pigs, and significantly lower ATP, phosphocreatine and glycogen levels than did SR pigs (Column B, Table 6.1).

Table 6.1: The results of 2-way analyses of variance on muscle metabolites as influenced by stress procedure (A: halothane exposure vs treadmill exercise) and stress susceptibility (B: SR vs SS)

Variable	Stress procedure (A)			Stress susceptibility (B)			AxB		
	F value	Significance level		F value	Significance level		F value	Significance level	
Lactate	0,664	0,4271	NS	58,918	<0,0001	**	5,294	0,0248	*
ATP	2,479	0,1205	NS	22,412	<0,0001	**	6,003	0,0166	*
Glucose 6-phosphate	0,542	0,4722	NS	28,662	<0,0001	**	1,494	0,2202	NS
Phosphocreatine	1,814	0,1829	NS	39,614	<0,0001	**	4,944	0,0298	*
Glucose	21,271	<0,0001	**	6,136	0,0160	*	4,247	0,0435	*
Glycogen	12,308	0,0008	**	7,625	0,0076	**	0,373	0,4599	NS

Table 6.2: Mean values and standard deviations (sd) of muscle metabolites as influenced by stress procedure and stress susceptibility

Variable	Halothane exposure			Treadmill exercise			SR pigs			SS pigs		
	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n
Lactate	20,40	12,18	47	20,36	8,76	19	14,28	7,04	40	29,78	9,96	26
ATP	4,64	1,57	47	3,12	1,37	19	5,05	1,37	40	3,47	1,29	26
Glucose 6-phosphate	2,35	2,23	47	2,27	1,54	19	1,43	1,43	40	3,72	2,09	26
Phosphocreatine	5,41	4,50	47	3,60	3,68	19	7,02	4,26	40	1,61	1,64	26
Glucose	0,05	0,64	47	1,81	0,79	19	1,01	0,66	40	1,49	0,86	26
Glycogen	49,66	16,79	47	39,19	11,17	19	46,66	16,59	40	38,01	14,27	26

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose - $\mu\text{mol/g}$ muscle
Glycogen - $\mu\text{mol glycol units/g}$ muscle

6.2.2. Effect of halothane exposure on muscle metabolites of SR and SS pigs

The differences in muscle metabolites between SR and SS pigs under halothane exposure are shown in Table 6.3. Compared to SR pigs, SS pigs had significantly higher lactate, glucose 6-phosphate

Table 6.3: Mean values, standard deviations (sd) and level of significance of muscle metabolites of halothane exposed pigs as influenced by stress susceptibility

Variable	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
Lactate	13,60	6,72	30	32,39	10,28	17	<0,0001 **
ATP	5,38	1,11	30	3,33	1,40	17	<0,0001 **
Glucose 6-phosphate	1,39	1,55	30	4,06	2,25	17	<0,0001 **
Phosphocreatine	7,78	3,93	30	7,24	1,13	17	<0,0001 **
Glucose	0,72	0,37	30	1,55	0,81	17	<0,0001 **
Glycogen	53,86	16,58	30	41,70	14,55	17	0,0153 *

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose - $\mu\text{mol/g}$ muscle
Glycogen - $\mu\text{mol glycol units/g}$ muscle

and glucose concentrations, and significantly lower ATP, phosphocreatine and glycogen concentrations. These results confirm the results obtained in analysis 6.2.1. above.

6.2.3. Effect of treadmill exercise on muscle metabolites of SR and SS pigs

Table 6.4 summarises the differences found in muscle metabolites of SR and SS pigs after treadmill exercise. SS pigs had significantly higher lactate and glucose 6-phosphate concentrations than the SR pigs. However, no significant differences in the concentrations of muscle ATP, phosphocreatine, glucose and glycogen were found between SR and SS pigs (Table 6.4). SS pigs that died after exercise had a significantly higher lactate concentration, glucose 6-phosphate concentration, and a significantly lower glycogen concentration than those of the SR pigs (Table D.3).

Table 6.4. Mean values, standard deviations (sd) and level of significance of muscle metabolites of treadmill exercised pigs as influenced by stress sensitivity

Variable	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
Lactate	16.30	7.94	10	24.86	7.63	9	0.0285 *
ATP	4.07	1.64	10	3.75	1.07	9	0.6198 NS
Glucose 6-phosphate	1.55	1.93	10	3.07	1.67	9	0.0271 *
Phosphocreatine	4.77	4.59	10	2.30	1.76	9	0.1496 NS
Glucose	1.86	0.63	10	1.75	0.98	9	0.7823 NS
Glycogen	37.05	10.78	10	31.02	11.54	9	0.2517 NS

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose - $\mu\text{mol/g}$ muscle
Glycogen - $\mu\text{mol glycoyl units/g}$ muscle

6.2.4. The influence of halothane exposure and treadmill exercise on muscle metabolites of SR pigs

The effects of halothane exposure and treadmill exercise on the SR pigs are illustrated in Table 6.5. Treadmill exercise resulted in significantly higher glucose, but lower ATP and glycogen concentrations when compared to the halothane exposed SR pigs. The differences found in lactate,

Table 6.5. Mean values, standard deviations (sd) and level of significance of muscle metabolites from SR pigs as influenced by stress procedure

Variable	Halothane exposure			Exercise exposure			Significance level
	Mean	sd	n	Mean	sd	n	
Lactate	13.60	6.72	30	16.30	7.94	10	0.2098 NS
ATP	5.38	1.11	30	4.07	1.64	10	0.0070 **
Glucose 6-phosphate	1.35	1.55	30	1.55	1.03	10	0.7597 NS
Phosphocreatine	7.78	3.93	30	4.77	4.59	10	0.0514 NS
Glucose	0.72	0.37	30	1.86	0.63	10	<0.0001 **
Glycogen	53.86	16.58	30	37.05	10.78	10	0.0049 **

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose - $\mu\text{mol/g}$ muscle
Glycogen - $\mu\text{mol glycoyl units/g}$ muscle

glucose 6-phosphate and phosphocreatine in the SR pigs as a result of the two types of stresses were not significant.

6.2.5. The influence of halothane exposure and treadmill exercise on muscle metabolites of SS pigs

The results of the effects of the halothane exposure and treadmill exercise on SS pigs are given in Table 6.6. No significant differences were found in any of the muscle metabolites determined in this experiment as a result of the exposure of SS pigs to the two different types of stresses, although the SS pigs that died as a result of treadmill exercise had a significantly lower glycogen concentration than did the SS halothane exposed pigs (Table D.4).

Table 6.6: Mean values, standard deviations (sd) and level of significance of muscle metabolites from SS pigs as influenced by stress procedure

Variable	Halothane exposure			Exercise exposure			Significance level
	Mean	sd	n	Mean	sd	n	
Lactate	32.39	10.28	17	34.86	7.63	9	0.0662 NS
ATP	3.33	1.40	17	3.75	1.07	9	0.4416 NS
Glucose 6-phosphate	4.06	2.25	17	3.07	1.67	9	0.2570 NS
Phosphocreatine	1.24	1.13	17	2.50	1.76	9	0.0745 NS
Glycose	1.35	0.81	17	1.75	0.98	9	0.2740 NS
Glycogen	41.70	14.55	17	31.02	11.34	9	0.0662 NS
Lactate, ATP, glucose 6-phosphate, phosphocreatine - $\mu\text{mol/g}$ muscle Glycogen - $\mu\text{mol glycogen unit/g}$ muscle							

6.3. Discussion

It has been shown in previous experiments that the exposure of SS pigs to halothane or exercise results in the development of PSE meat (Schmidt, 1980; Mitchell & Heffron, 1981b). This development of the PSE meat has been thought to be a consequence of the stimulation of glycolysis just prior to, or after death or slaughter in which pigs producing PSE meat have low levels of muscle glycogen, ATP and phosphocreatine, but high levels of lactate (Lahucky, Fierstein & Augustin, 1982). The low pH value of the muscle after death or slaughter, at a time when the carcass is still warm, results in the denaturation of various sarcoplasmic proteins, giving rise to the pale colour of the meat. Also, the denatured proteins lose a lot of their water binding capacity, which results in a high level of free water in the muscle cell. The high lactate level in the muscle cell, and thus low pH value, makes muscle cell membranes more permeable, and fluid exudes from the muscle cell (Hogskel & Kim, 1985).

As a result of the findings that the stimulation of glycolysis results in PSE, it has been assumed that PSE and MHS are similar, if not identical (Casper *et al.*, 1975; Sybesma & Eikenboom, 1978). However, the idea that PSS and MHS are identical or similar can only be supported if

metabolic changes in muscle of exercised or halothane exposed pigs are similar. Measurements of blood variables seem to indicate that this might not be the case (Chapter 5).

The results suggest that both halothane exposure and exercise stimulate glycogenolysis and glycolysis in muscle of pigs. The evidence for this is that muscle glycogen and ATP concentrations after halothane exposure and exercise at 21 weeks of age was lower than under barbiturate anaesthesia at 13 weeks of age, while glucose and lactate concentrations were higher (See Tables 6.2 and 3.8).

The results show, however, that the degree of stimulation in pigs exposed to halothane or exercise is different. The muscle concentration of glucose was higher after exercise than after halothane exposure, while the concentration of ATP and glycogen were lower. This result suggests that exercise is more glycogenolytic and more anaerobic than is exposure to halothane. These conclusions are based on analysis of muscle samples taken from both SS and SR pigs. As SS pigs are known to be more anaerobic than SR pigs under stress (Mitchell & Heffron, 1982) the data may be reflecting a difference in type of pig rather than type of stress. When the response of SS and SR pigs to each of the stressors were compared, it was found that in general SS pigs had higher muscle lactate, glucose 6-phosphate and glucose, and lower ATP, phosphocreatine and glycogen which confirms previous work (Lahucky *et al.*, 1982; Mitchell & Heffron, 1982). These data indicate that SS pigs show greater stimulation of glycogenolysis and glycolysis than do SR pigs, a finding supported by Hall & Lucke (1983), and that their metabolism is more anaerobic under conditions of stress. Under halothane exposure it has been calculated that the heat produced as a result of malignant hyperthermia in SS pigs is a consequence of a shift of metabolism towards anaerobic metabolism to an extent of approximately 70% (Nijland *et al.*, 1986). Malignant hyperthermia in SS pigs as a result of halothane exposure is known to reduce blood flow in the muscles, to which the muscles respond by shifting to a more anaerobic metabolism (Hall, Lucke, Orchard, Lovell & Lister, 1982). In general it has been shown that SS pigs have a lower aerobic capacity than do SR pigs (Essén-Gustavsson & Lindholm, 1984).

When the effects of stress on SR pig muscle metabolites were assessed, however, we found that exercised SR pigs had lower phosphocreatine, ATP and glycogen, and higher glucose than SR pigs exposed to halothane, suggesting that exercise in SR pigs stimulates glycogenolysis, inhibits glycolysis and produces anaerobic metabolism. This result implies that the metabolic response to exercise in SR pigs is different to the metabolic response to halothane.

In analysing the responses of SS pigs to halothane and exercise stress, no significant differences were found in muscle metabolites. However, the data strongly suggests that during exercise SS pigs are less anaerobic and more glycogenolytic than SS pigs exposed to halothane. The evidence for this is that exercised SS pigs have lower lactate ($P=0.0562$), lower glycogen ($P=0.0682$) and higher phosphocreatine ($P=0.0745$) concentrations than do pigs exposed to halothane, although these differences do not reach statistical significance.

This conclusion is supported if the changes in SS pigs during halothane exposure and treadmill exercise are compared to changes in SR pigs. During exercise SS pigs develop a higher lactate and higher glucose 6-phosphate concentration than do SR pigs. During halothane exposure SS pigs develop a higher lactate, glucose 6-phosphate and glucose and lower ATP, phosphocreatine and glycogen concentrations. This comparison indicates that exercise produces a less severe stimulation of anaerobic metabolism in SS pigs.

The results in general, therefore, indicate several differences between the effects of halothane exposure and treadmill exercise. The indication that the effects of exercise stress and exposure to halothane are different, is borne out by the significant interactions (Column AxB, Table 6.1). The same indication has been found in the various blood variables of halothane exposed and treadmill exercised SR and SS pigs (Chapter 5). This finding is supported by Gregory & Wilkins (1984) who have shown that Carazolol, a β -adrenergic receptor antagonist could not prevent halothane induced lactacidemia, and Warris & Lister (1982) who showed that it reduced the generation of lactate in pigs exposed to normal management stress, and thus prevented PSE muscle. It appears, therefore, that the activation of an α -adrenergic receptor is important in halothane induced stress, as β -adrenergic receptor is important in exercise induced stress.

Thus it would seem that the different metabolic responses to halothane exposure and treadmill exercise induced stress may be a consequence of different mechanisms of activation. These mechanisms result in the activation of glycogenolysis and induction of anaerobic metabolism of varying degrees. It nevertheless follows that the two mechanisms may be synergistic. Indeed, as Van der Hendt *et al.* (1976) and Gregory & Wilkins (1984) have shown, SS pigs exposed to natural stresses like exercise prior to halothane exposure, react more violently on subsequent halothane exposure than SS pigs not exposed to the natural stress prior to halothane exposure.

6.4. Conclusion

Halothane exposure and treadmill exercise result in the stimulation of glycogenolysis and glycolysis, which is more severe in the SS pigs than the SR pigs. This leads to a higher accumulation of lactate in the muscle of the SS pigs, and consequent low pH value, and therefore the formation of PSE musculature.

Compared to SR pigs, SS pigs responded to halothane exposure in a dramatic way, with a severe stimulation of glycogenolysis and glycolysis, and with fatal consequence. On exposure to treadmill exercise, the SR and SS pigs responded in a very similar way to the stress as measured by muscle metabolites, and the stress was severe enough to result in the death of some of the SS pigs. However, the results show that the halothane exposure resulted in a more severe anaerobic metabolism in the muscle of the SS pigs. Thus, although both halothane exposure and treadmill exercise resulted

in the stimulation of glycogenolysis and glycolysis, it would seem that the mechanisms involved are different, but possibly synergistic.

CHAPTER 7

Muscle fibre type

7.1. Introduction

From the results on the blood variables (Chapter 5), but especially the muscle metabolites (Chapter 6), it is clear that during stressful situations such as during halothane exposure, the SS pigs have a higher rate of muscle glycolysis and glycogenolysis. It has also been shown that SS pig muscle has a higher glycogenolysis rate than SR pig muscle in the white muscle fibres (Essén-Gustavsson & Lindholm, 1984). Although Heffron *et al.* (1982) were unable to find any differences in the *M. longissimus dorsi* muscle fibre type ratios between SS and SR pigs, Sair *et al.* (1972) found the SS pigs to have a higher white, and lower red muscle fibre percentages compared to SR pigs, and would therefore be more inclined to anaerobic metabolism, which would be compatible with the results obtained for the blood variables (Chapter 5) and muscle metabolites (Chapter 6). Thus, it was decided to investigate the muscle fibre ratios in the *M. semitendinosus* of the South African Landrace gilts used in this study, in an effort to gain more knowledge regarding the muscle fibre composition of these pigs.

The results of the muscle fibre type characterisation were analysed as follows:

- *2-way analysis of variance for the factors stress sensitivity and herd
- *influence of stress sensitivity on the characterisation of muscle fibres type from pigs of herd X
- *influence of stress sensitivity on the characterisation of muscle fibres type from pigs of herd Y
- *influence of herd on the muscle fibre type characterisation of SR pigs
- *influence of herd on the muscle fibre type characterisation of SS pigs.

7.2. Results

7.2.1. The influence of stress sensitivity and herd on the muscle fibre type percentages of pigs

The results of the 2-way analyses of variance with the factors stress sensitivity and herd are given in Table 7.1, with the means and standard deviations in Table 7.2. The SS pigs had lower percentages red and intermediate muscle fibres, but a higher percentage white muscle fibres than the SR pigs. The pigs from herd Y had a higher percentage red, but a lower percentage intermediate muscle fibres than the pigs from herd X, although the percentage white muscle fibres were similar for the pigs of the two herds.

Table 7.1: The results of 2-way analyses of variance on the muscle fibre type percentages of pig as influenced by stress sensitivity and herd

Variable	Stress susceptibility (A)		Herd (B)		AxB	
	F value	Significance level	F value	Significance level	F value	Significance level
Muscle fibre %						
red	45,604	<0,0001 **	8,160	0,0056 **	7,241	0,0092 **
intermediate	27,970	<0,0001 **	14,302	0,0004 **	0,305	0,5885 NS
white	76,954	<0,0001 **	0,454	0,5100 NS	4,457	0,0389 *

Table 7.2: Mean values and standard deviations (sd) of pig muscle fibre type percentages as influenced by stress sensitivity and herd

Variable	SR pigs			SS pigs			Herd X			Herd Y		
	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n
Muscle fibre %												
red	20,83	3,72	39	15,83	2,76	26	18,25	3,62	47	20,07	2,91	18
intermediate	26,46	3,27	39	21,94	3,51	26	25,71	3,83	47	19	2,00	18
white	52,55	4,26	39	62,37	4,50	26	55,88	5,97	47	58,04	3,91	18

7.2.2. Differences in muscle fibre type percentages of SR and SS pigs from herd X

The results are shown in Table 7.3. The SS pigs from herd X had lower percentages red and intermediate muscle fibres, but a higher white muscle fibre percentage than the SR pigs.

7.2.3. Differences in muscle fibre type percentages of SR and SS pigs from herd Y

The SS pigs had a lower percentage red and intermediate muscle fibres, but a higher percentage white muscle fibres than the SR pigs (Table 7.4).

Table 7.3: Mean values, standard deviations (sd) and level of significance of muscle fibre type percentages from pigs of herd X as influenced by stress sensitivity

Variable	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
Muscle fibre %							
red	19.77	3.34	30	15.85	2.67	17	0.0001 **
intermediate	27.11	3.35	30	23.24	3.41	17	0.0005 **
white	52.90	4.51	30	61.12	4.46	17	<0.0001 **

Table 7.4: Mean values, standard deviations (sd) and level of significance of muscle fibre type percentages from pigs of herd Y as influenced by stress sensitivity

Variable	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
Muscle fibre %							
red	20.24	2.71	9	15.60	3.10	9	<0.0001 **
intermediate	24.40	3.11	9	19.98	2.19	9	<0.0001 **
white	51.36	4.18	9	64.42	3.93	9	<0.0001 ***

7.2.4. The influence of the two herds on the muscle fibre type percentages of the SR pigs

SR pigs from herd X had a higher percentage red, but a lower percentage intermediate muscle fibres than the SR pigs from herd Y (Table 7.5).

Table 7.5: Mean values, standard deviations (sd) and level of significance of muscle fibre type percentages of SR pigs from herd X and herd Y

Variable	Herd X			Herd Y			Significance level
	Mean	sd	n	Mean	sd	n	
Muscle fibre %							
red	19.77	3.34	30	21.11	3.11	30	0.0006 **
intermediate	27.11	3.35	30	24.30	3.66	30	0.0216 *
white	52.90	4.51	30	51.59	3.83	30	0.3583 NS

7.2.5. The influence of the two herds on the muscle fibre type percentages of the SS pigs

The only significant difference between the SS pigs from herd Y and herd X was that the SS pigs from herd Y had a lower percentage intermediate muscle fibres than the SS pigs from herd X (Table 7.6).

Table 7.6: Mean values, standard deviations (sd) and level of significance of muscle fibre type percentages of SS pigs as influenced by herd

Variable	Herd A			Herd Y			Significance level
	Mean	sd	n	Mean	sd	n	
Muscle fibre %							
red	15.85	3.67	17	15.00	3.10	9	0.9038 NS
intermediate	23.24	3.41	17	19.48	2.19	9	0.0065 **
white	61.12	4.46	17	64.72	3.98	9	0.0539 NS

7.3. Discussion

The lower percentage red and intermediate muscle fibres and higher percentage white muscle fibres found in the SS pigs compared to the SR pigs, may influence the response of SS pigs to stressful situations such as treadmill exercise and halothane exposure. The SS pigs, because of the high percentage white muscle fibres have a lower capacity for aerobic metabolism (Sair *et al.*, 1972; Nelson *et al.*, 1974), which is supported by the results of my study. This undoubtedly contribute to the higher rate of glycolysis found in SS pigs as measured by the drop in pH value in SS pigs post mortem (Chapter 4). Cooper *et al.* (1969) attributed the response of SS pigs to anaemia to the higher percentages of intermediate and lower percentage red muscle fibres they found in the *M. longissimus dorsi*. However, in my study, it was found that the SS pigs had consistently a lower percentage intermediate muscle fibres than the SR pigs. The result of a higher white muscle fibre percentage and lower red muscle fibre percentage is similar to the findings of Sair *et al.* (1972) and Nelson *et al.* (1974). On the other hand, Swatland & Cassens (1973) found a higher percentage intermediate muscle fibres in SS pigs, although Heffron *et al.* (1932) were unable to measure any differences in the muscle fibre ratios in the *M. longissimus dorsi* between SS and SR pure bred Landrace pigs. The difference in the findings between the different studies may be the result of the use of different muscles and pig breeds (SS Poland China and SR Chester White pigs, and *M. longissimus dorsi* by Sair *et al.*, 1972; various breeds (Yorkshire, Hampshire, Duroc, Poland China and cross bred pigs, and *M. longissimus dorsi* by Swatland & Cassens, 1973; *M. longissimus dorsi* by Nelson *et al.*, 1974). Nevertheless, Gallant (1960) found no differences in the percentage muscle fibre composition using *M. semitendinosus* and *M. longissimus dorsi*, and argued therefore that the possible differences in muscle fibre composition between SS and SR pigs could therefore not be the basis for malignant hyperthermia. Although it may not be the basis for malignant hyperthermia, the results of my study indicate that it may still play a role in the development of malignant hyperthermia, albeit it be a secondary role.

Although differences in the red and intermediate muscle fibres were found between the pigs from the two herds, these differences were in the percentage red and intermediate muscle fibres, but not in the percentage white muscle fibres. Both the red and the intermediate muscle fibres possess the capability for oxidative respiration. These differences between the two herds were also found in analysing only the effect of the two herds on the muscle fibre percentages of SR and SS pigs.

In none of the two analyses did the percentage white muscle fibres differ between the two herds. The red and/or intermediate muscle fibres, however, differed significantly in the SR and SS groups between the two herds.

In both the herds, the SS pigs had the lower percentage red and intermediate muscle fibres, but the higher percentage of white muscle fibres, which is in agreement with the work of Sair *et al.* (1972) and Nelson *et al.* (1974). These differences in my results of the percentages of the different muscle fibres were in general consistent over the two herds. These differences could therefore possibly be used for the identification of SS and SR pigs in the South African Landrace breed. However, this must still be established whether it holds true for the pig at a young age. Nevertheless, this method is labour intensive and expensive, and might not be practical for breeding selection purposes.

7.4. Conclusion

The *M. semitendinosus* of the SS pigs have a higher percentage white muscle fibres, and lower percentages of red and intermediate muscle fibres. These differences may contribute to the higher rate of glycolysis found in the SS pigs as measured by the lower *post mortem* pH levels reached 60 minutes post mortem compared to that of the SR pigs. The differences between the SS and SR pigs were relatively constant over the two herds, and may therefore be important in the identification of SS and SR pigs, especially for experimental purposes.

CHAPTER 8

Rectal temperatures of pigs exposed to halothane and treadmill exercise

8.1. Introduction

The term "malignant hyperthermia" indicates that a high temperature is one of the consequences of the syndrome, and therefore temperature is of importance in the study of the porcine stress syndromes. The primary site of this heat production is the skeletal muscles (Britt & Kalow, 1970) and this rise in body temperature is one of the early signs of malignant hyperthermia (Lucke *et al.*, 1976). This sustained rise in temperature up to as high as 45°C, increasing at a rate of 1°C every 7 minutes (Harrison *et al.*, 1969). However, this rise in temperature is only recorded late in the event of malignant hyperthermia, even 6 minutes after exposing an SS pig to halothane (Harrison *et al.*, 1969). After a 5 minute treadmill exercise, SS pigs have also been found to have higher rectal temperatures 5 to 10 minutes after the exercise, and it would therefore seem that the rectal temperatures shown by SS pigs relative to the SR pigs, might be similar whether as a result of treadmill exercise or halothane exposure. To test this assumption, this experiment was conducted, and the results obtained in this experiment are reported here.

The data on the rectal temperatures were analysed as follows:

- * influence of stress procedure and stress sensitivity
- * influence of stress sensitivity on rectal temperatures of pigs during halothane exposure
- * influence of stress sensitivity on rectal temperatures of pigs during treadmill exercise
- * influence of stress procedure on rectal temperatures of SR pigs
- * influence of stress procedure on rectal temperatures of SS pigs.

8.2. Results

8.2.1. The influence of halothane exposure, treadmill exercise and stress sensitivity on rectal temperature of pigs

The results of this analysis are illustrated in Tables 8.1 and 8.2. The two types of stress procedures resulted in significantly different rectal temperature responses of the pigs. The halothane exposure of the pigs resulted in lower mean temperatures than did the treadmill exercise from 90 to 630 seconds after initiation of the stress procedure. The temperatures, however, were similar 660 and 690 seconds after initiation of the stress procedure (Column A, Table 8.1).

The halothane exposure and treadmill exercise did not result in any significant differences in the rectal temperatures between the SR and SS pigs (Column B, Table 8.1). All interactions were also not significant.

8.2.2. The response in rectal temperature of SR and SS pigs on exposure to halothane

The results are given in Table 8.3. No significant differences were found between the rectal temperatures of SR and SS pigs on exposure to halothane.

Table 8.1: Results of 2-way analyses of variance on rectal temperatures of pigs as influenced by stress procedure (A: halothane exposure vs treadmill exercise) and stress sensitivity (B: SR vs SS) from 90 to 690 seconds after initiating the stress procedure

Time (sec)	Stress procedure (A)		Stress sensitivity (B)		AxB	
	F value	Significance level	F value	Significance level	F value	Significance level
90	10,203	0,0037 **	2,478	0,2350 NS	0,505	0,4913 NS
120	8,874	0,0060 **	0,325	0,5794 NS	0,050	0,8275 NS
150	7,694	0,0099 **	0,146	0,7097 NS	0,000	0,9263 NS
180	9,173	0,0054 **	0,078	0,7855 NS	0,007	0,9364 NS
210	2,985	0,0994 NS	0,403	0,5376 NS	0,214	0,6521 NS
240	14,770	0,0006 **	0,272	0,6118 NS	0,001	0,9976 NS
270	18,312	0,0002 **	0,035	0,8539 NS	0,060	0,8112 NS
300	15,510	0,0005 **	0,361	0,5589 NS	0,001	0,9730 NS
330	12,863	0,0014 **	0,217	0,6500 NS	0,044	0,8379 NS
360	13,883	0,0010 **	0,374	0,5526 NS	0,055	0,8193 NS
390	9,854	0,0044 **	1,394	0,2492 NS	0,605	0,4324 NS
420	5,574	0,0051 **	0,684	0,4254 NS	0,914	0,3599 NS
450	6,576	0,0185 *	1,611	0,2189 NS	0,340	0,5709 NS
480	6,358	0,0198 *	2,162	0,1563 NS	0,206	0,5595 NS
510	8,152	0,0095 **	2,138	0,1585 NS	0,506	0,4921 NS
540	6,273	0,0215 *	2,158	0,1582 NS	0,639	0,4435 NS
570	5,508	0,0298 *	2,344	0,1515 NS	0,758	0,4299 NS
600	6,260	0,0228 *	2,750	0,1256 NS	0,676	0,3723 NS
630	4,933	0,0432 *	3,068	0,1095 NS	0,818	0,3885 NS
660	3,359	0,0767 NS	3,373	0,0961 NS	0,741	0,4185 NS
690	0,345	0,5553 NS	0,897	0,3968 NS	0,706	0,4475 NS

Table 8.2: Means and standard deviations (SD) of rectal temperatures of pigs as influenced by halothane exposure, treadmill exercise and stress sensitivity from 90 to 690 seconds after initiating the stress procedure

Time (sec)	Halothane exposure			Treadmill exercise			SR pigs			SS pigs		
	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n
90	38.64	0.53	14	39.12	0.25	16	38.82	0.47	19	39.02	0.29	11
120	38.81	0.47	15	39.22	0.26	16	38.99	0.42	20	39.08	0.26	11
150	38.90	0.46	15	39.79	0.30	16	39.07	0.44	20	38.15	0.23	11
180	38.95	0.46	15	39.36	0.28	16	39.14	0.42	20	39.19	0.25	11
210	38.98	0.47	15	39.47	0.30	15	39.21	0.39	19	39.25	0.29	11
240	39.01	0.46	15	39.49	0.22	17	39.22	0.39	20	39.33	0.28	12
270	38.97	0.51	15	39.56	0.25	17	39.27	0.40	20	39.30	0.37	12
300	39.03	0.49	15	39.57	0.24	17	39.27	0.40	20	39.39	0.34	12
330	39.05	0.53	14	39.61	0.23	15	39.27	0.42	18	39.45	0.37	11
360	39.06	0.54	14	39.66	0.25	15	39.29	0.42	18	39.50	0.39	11
390	39.11	0.54	13	39.64	0.28	15	39.30	0.42	19	39.60	0.40	9
420	39.11	0.54	13	39.67	0.35	14	39.33	0.45	19	39.57	0.44	8
450	39.12	0.56	13	39.62	0.23	11	39.25	0.42	17	39.58	0.50	7
480	39.11	0.58	13	39.76	0.28	12	39.25	0.44	18	39.61	0.53	7
510	39.14	0.58	13	39.69	0.24	12	39.30	0.42	18	39.66	0.54	7
540	39.12	0.63	12	39.71	0.25	11	39.28	0.44	17	39.76	0.63	6
570	39.11	0.63	12	39.68	0.25	10	39.26	0.43	17	39.74	0.75	5
600	39.08	0.65	11	39.69	0.25	10	39.24	0.42	16	39.77	0.78	5
630	39.07	0.72	10	39.70	0.25	9	39.21	0.56	14	39.81	0.83	5
660	39.14	0.71	7	39.75	0.28	7	39.22	0.46	9	39.86	0.88	5
690	39.39	0.52	3	39.85	0.33	6	39.46	0.68	4	39.88	0.94	5

8.2.3. The response in rectal temperature of SR and SS pigs on exposure to treadmill exercise

The results are illustrated in Table 8.4. As was found during the exposure of the pigs to halothane, the exposure of the pigs to treadmill exercise resulted in no significant differences in the rectal temperatures of SR and SS pigs.

8.2.4. The response in rectal temperatures of SR pigs as a result of exposure to halothane or treadmill exercise

The results of the analysis are given in Table 8.5. In general, the rectal temperatures of the SR pigs during treadmill exercise were higher than those of the SR pigs during the halothane exposure. No significant differences, however, were found between the rectal temperatures of SR pigs as a result of halothane exposure or treadmill exercise 150 seconds after initiation of the stress procedure.

Table 8.3: Mean values, standard deviations (sd) and level of significance of rectal temperatures of halothane exposed pigs as influenced by stress sensitivity

Time (sec)	SR pigs			SS pigs			Significance level	
	Mean	sd	n	Mean	sd	n		
90	38.53	0.62	9	38.84	0.35	5	0.3272	NS
120	38.77	0.52	10	38.89	0.32	5	0.6653	NS
150	38.89	0.51	10	38.95	0.30	5	0.8739	NS
180	38.93	0.50	10	38.98	0.35	5	0.8405	NS
210	38.97	0.49	10	39.00	0.42	5	0.9086	NS
240	38.98	0.48	10	39.05	0.45	5	0.7947	NS
270	38.99	0.48	10	38.92	0.57	5	0.8176	NS
300	39.00	0.47	10	39.08	0.52	5	0.7765	NS
330	39.02	0.50	10	39.13	0.63	4	0.7296	NS
360	39.02	0.49	10	39.16	0.65	4	0.6649	NS
390	39.02	0.49	10	39.39	0.71	3	0.3193	NS
420	39.02	0.48	10	39.40	0.73	3	0.3127	NS
450	39.03	0.49	10	39.41	0.78	3	0.3352	NS
480	39.02	0.51	10	39.42	0.82	3	0.3114	NS
510	39.03	0.50	10	39.49	0.85	3	0.2638	NS
540	39.03	0.52	10	39.61	1.25	2	0.2605	NS
570	39.01	0.51	10	39.63	1.29	2	0.2318	NS
600	38.95	0.50	9	39.66	1.34	2	0.1972	NS
630	38.90	0.54	8	39.71	1.43	2	0.1944	NS
660	38.90	0.25	5	39.74	1.50	2	0.2144	NS

Table 8.4: Mean values, standard deviations (sd) and level of significance of rectal temperatures of treadmill exercised pigs as influenced by stress sensitivity

Time (sec)	SR pigs			SS pigs			Significance level	
	Mean	sd	n	Mean	sd	n		
90	39.09	0.26	10	39.18	0.23	6	0.5094	NS
120	39.20	0.29	10	39.25	0.19	6	0.7105	NS
150	39.26	0.36	10	39.33	0.15	6	0.6667	NS
180	39.34	0.33	10	39.37	0.12	6	0.8434	NS
210	39.48	0.25	9	39.47	0.08	6	0.9265	NS
240	39.47	0.27	10	39.53	0.12	7	0.5432	NS
270	39.56	0.31	10	39.56	0.10	7	0.9685	NS
300	39.53	0.29	10	39.62	0.13	7	0.4729	NS
330	39.59	0.30	8	39.64	0.11	7	0.7200	NS
360	39.63	0.31	8	39.70	0.14	7	0.6153	NS
390	39.60	0.33	9	39.70	0.14	6	0.5222	NS
420	39.67	0.42	9	39.67	0.13	5	0.9871	NS
450	39.57	0.27	7	39.71	0.13	4	0.3538	NS
480	39.54	0.32	8	39.76	0.13	4	0.2308	NS
510	39.63	0.27	8	39.80	0.11	4	0.2900	NS
540	39.64	0.29	7	39.84	0.12	4	0.2562	NS
570	39.62	0.28	7	39.81	0.12	3	0.2846	NS
600	39.62	0.28	7	39.84	0.13	3	0.2462	NS
630	39.61	0.29	6	39.88	0.14	3	0.1714	NS
660	39.61	0.33	4	39.93	0.15	3	0.1901	NS
690	39.73	0.45	3	39.95	0.19	3	0.5027	NS

Table 8.5: Mean values, standard deviations (sd) and level of significance of rectal temperatures of SR pigs as influenced by halothane exposure and treadmill exercise

Time (sec)	Halothane exposure			Treadmill exercise			Significance level
	Mean	sd	n	Mean	sd	n	
90	38.53	0.62	9	39.69	0.26	10	0.0172 *
120	38.77	0.52	10	39.20	0.29	10	0.0376 *
150	38.89	0.51	10	39.26	0.26	10	0.0749 NS
180	38.93	0.50	10	39.34	0.33	10	0.0416 *
210	38.97	0.49	10	39.48	0.25	9	0.0118 *
240	38.98	0.48	10	39.47	0.27	10	0.0121 *
270	38.99	0.48	10	39.56	0.31	10	0.0053 **
300	39.00	0.48	10	39.53	0.29	10	0.0085 **
330	39.02	0.50	10	39.39	0.30	8	0.0113 *
360	39.02	0.49	10	39.63	0.31	8	0.0079 **
390	39.02	0.49	10	39.60	0.33	9	0.0039 **
420	39.02	0.48	10	39.67	0.42	9	0.0066 **
450	39.03	0.49	10	39.57	0.27	7	0.0200 **
480	39.02	0.51	10	39.54	0.32	8	0.0236 *
510	39.03	0.50	10	39.63	0.27	8	0.0061 **
540	39.03	0.52	10	39.64	0.29	7	0.0123 *
570	39.11	0.51	10	39.62	0.28	7	0.0122 **
600	38.95	0.50	9	39.62	0.28	7	0.0071 **
630	38.90	0.54	8	39.61	0.29	6	0.0140 **
660	38.90	0.25	5	39.61	0.33	4	0.0074 **

Table 8.6: Mean values, standard deviations (sd) and level of significance of rectal temperatures of SS pigs as influenced by halothane exposure and treadmill exercise

Time (sec)	Halothane exposure			Treadmill exercise			Significance level
	Mean	sd	n	Mean	sd	n	
90	38.84	0.35	5	39.18	0.23	6	0.0829 NS
120	38.89	0.32	5	39.25	0.19	6	0.0442 *
150	38.93	0.30	5	39.33	0.15	6	0.0176 *
180	38.98	0.35	5	39.27	0.12	6	0.0296 *
210	39.00	0.42	5	39.47	0.08	6	0.0237 *
240	39.05	0.43	5	39.53	0.12	7	0.0156 *
270	39.01	0.57	5	39.56	0.12	7	0.0150 *
300	39.08	0.52	5	39.62	0.13	7	0.0325 *
330	39.13	0.63	4	39.64	0.11	7	0.0576 NS
360	39.16	0.65	4	39.70	0.14	7	0.0588 NS
390	39.39	0.71	3	39.70	0.14	6	0.3073 NS
420	39.48	0.73	3	39.67	0.13	5	0.4200 NS
450	39.41	0.78	3	39.71	0.13	4	0.4654 NS
480	39.42	0.82	3	39.76	0.11	4	0.4450 NS
510	39.49	0.85	3	39.80	0.11	4	0.4872 NS
540	39.61	1.25	2	39.84	0.12	4	0.6946 NS
570	39.63	1.29	2	39.81	0.12	3	0.8084 NS
600	39.66	1.34	2	39.84	0.13	3	0.8169 NS
630	39.71	1.43	2	39.88	0.14	3	0.8304 NS
660	39.74	1.50	2	39.90	0.15	3	0.8260 NS
690	39.78	1.61	2	39.95	0.19	3	0.8530 NS

8.2.5. The response in rectal temperatures of SS pigs as a result of exposure to halothane exposure or treadmill exercise

The results are shown in Table 8.6. The rectal temperatures of the SS pigs were higher during treadmill exercise than during halothane exposure between 120 and 300 seconds after initiation of the stress procedure. Before 120 seconds, and after 300 seconds, the rectal temperatures between the SS pigs were similar for the other time periods.

8.2.6. Correlation between the time of exposure to the stress of each of the different animal stress sensitivity types

Linear regression equations were calculated for each of the stress sensitivity groups regarding halothane exposure and treadmill exercise. With these equations it was determined that the temperature would be after 5 and 10 minutes after initiating the stress procedure, as well as the time it would take to increase the rectal temperature by 1°C. These values are given in Table 8.8. The linear regression equation was also determined for each individual pig during treadmill exercise and/or halothane exposure, and the time required to raise rectal temperature 1°C under these conditions, determined. No significant differences were found either for the different stress procedures, nor for stress sensitivity in a 2-way analysis of variance using these latter values.

Table 8.7: Simple regression analysis between the stress time period and rectal temperatures

	Regression equation	r	R ²
Halothane exposure:			
SR pigs	$y = 38,8632 + 5,3614 \times 10^{-4}(x)$	0,18	3,33%
SS pigs	$y = 38,5823 + 1,7970 \times 10^{-4}(x)$	0,49	24,19%
Treadmill exercise:			
SR pigs	$y = 39,0383 + 1,1921 \times 10^{-4}(x)$	0,35	17,69%
SS pigs	$y = 39,1101 + 1,3614 \times 10^{-4}(x)$	0,77	59,30%

Table 8.8: The calculated rectal temperatures of the different stress sensitivity types as a result of halothane exposure or treadmill exercise

	Temperature after		Time, required to increase temperature 1°C
	5 min	10 min	
Halothane exposure			
SR pigs	39,02	39,18	31 min.
SS pigs	39,12	39,66	9 min.
Treadmill exercise			
SR pigs	39,40	39,75	14 min.
SS pigs	39,52	39,93	12 min.

8.3. Discussion

The pigs responded to the treadmill exercise compared to the halothane exposure in that during treadmill exercise the pigs had higher rectal temperatures than during halothane exposure (Column A, Tables 8.1 and 8.2). During the treadmill exercise of about 10 minutes, which corresponded to about 80 meters of walking during the 10 minute period, the heat produced in the muscles, resulted in higher rectal temperatures. During halothane anaesthesia, the general response to anaesthesia is a relaxation of muscles, thus an assumed lower energy requirement, and therefore lower rectal temperature.

Although the SS pigs develop malignant hyperthermia as a result of exercise (Patterson & Allen, 1972) or halothane exposure (Harrison *et al.*, 1969), no differences in rectal temperatures were found between SR and SS pigs as a result of the stress imposed on the pigs (Column B, Table 8.1). This might be the result of an increase in temperature as a response to the stress, which takes place relatively late after initiating of the stress procedure (Harrison *et al.*, 1969), compared to changes in plasma variables and muscle metabolites. The same result was found in analysing the response of the SR and SS pigs to either halothane exposure or treadmill exercise. In neither of the cases did the rectal temperatures between the SR and SS pigs differ. This differs somewhat from the results obtained by Schmidt (1980). After treadmill exercise of 5 minutes, the SS pigs had a higher rectal temperature than the SR pigs, as well as 5 and 15 minutes after the 5 minutes running, but not at 10 minutes, or at 20 minutes and thereafter. The treadmill speed as used by Schmidt (1980) was 1 m/sec for 5 minutes, compared to the 0.19 m/sec for approximately 10 minutes used in my experiment. It is thus further evidence that during stress as was applied in this study, the rise in temperature as a result of malignant hyperthermia is perhaps a rather late consequence of the syndrome, although the treadmill stress was stressful enough to kill four out of the nine SS pigs, showing other typical symptoms of malignant hyperthermia.

As was found with all the pigs, the SR pigs exposed to halothane had lower rectal temperatures than the SR exercised pigs. The results found in the SS pigs were similar for the time period 120 to 270 seconds, after which the temperatures of the SS pigs, whether exposed to halothane or treadmill exercise, were similar, and not significantly different. Thus, the difference in the temperatures due to stress procedure (Column A, Table 8.1) is therefore more a consequence of the differences in rectal temperatures of the SR pigs than of the SS pigs. These differences are also evident from the time period necessary under the different stress procedures to increase the rectal temperature by 1°C. The time necessary under halothane anaesthesia for the SR pigs would be 31 minutes (although the correlation coefficient was very low) compared to the 9 minutes necessary for the SS pigs, which correlates well with the finding of Harrison *et al.* (1969). During treadmill exercise, the time period needed for the SR and SS pigs were very similar, namely 14 and 12 minutes, which did not differ much from the 9 minutes necessary for the SS pigs under halothane anaesthesia, compared to the 31 minutes needed by SR pigs. Therefore, it seems that the malignant hyperthermia of rectal temperatures of SS pigs as a consequence of halothane exposure and treadmill exercise are similar, or even identical. However, comparing the results of the rectal tempera-

tures with the results obtained for blood variables (Chapter 5) and muscle metabolites (Chapter 6), the physiological response is not the same, although both lead to a stimulation of glycolysis and glycogenolysis. The end results in terms of malignant hyperthermia, death of the pig and PSE musculature are the same, although the mechanisms of activation might be different.

8.4. Conclusion

Treadmill exercise resulted in higher rectal temperatures than did halothane exposure. This difference was more a consequence of the differences in rectal temperatures of the SR pigs than the SS pigs.

During the 10 minutes exposure to the stress (whether halothane exposure or treadmill exercise), the rectal temperatures of the SR pigs and SS pigs were similar. It was calculated that during halothane exposure the rectal temperatures of the SS pigs would rise 1°C every 9 minutes compared to the 31 minutes of the SR pigs, yet, during the treadmill exercise, the times calculated for a rise of 1°C for the SR and SS pigs were 14 and 12 minutes.

Although the rectal temperatures did not differ in SS pigs between the halothane exposure and treadmill exercise, these results compared with the results on the blood variables and muscle metabolites indicate differences in the mechanisms of activation, albeit the end results in terms of rectal temperatures, death of the animal and PSE are similar.

CHAPTER 9

Conclusions and recommendations

9.1. Conclusions

The aim of this study was to determine the possible use of blood variables and muscle metabolites in classifying SS pigs, relative to the use of the halothane test, and to determine the influence of stress susceptibility in the Landrace breed on:

- * growth characteristics
- * carcass characteristics
- * meat characteristics
- * muscle fibre type characterisation

Also, this study was undertaken to determine the response of SR and SS pigs to halothane exposure and treadmill exercise in terms of blood variables and muscle metabolites in an effort to ascertain if the mechanisms for the onset of malignant hyperthermia, and stimulation of glycolysis and glycogenolysis in the PSS and MHS are the same.

As a result of the methodology followed, pigs from two herds were used. The results show that the use of blood variables and muscle metabolites in identifying SS pigs is complicated as a result of the between herd differences. Husbandry practices may also influence the accuracy of prediction of SS pigs by the use of blood variables or muscle metabolites, rendering the use of these variables and metabolites useless as predictive tests in general. The between herd differences are in various cases larger than the differences between SR and SS pigs. Also, the influence of restraint and anaesthesia used in taking the blood or obtaining the muscle biopsy complicate the use of blood variables and muscle metabolites for predicting SS pigs. The halothane test, although prone to misclassification, results in a better predictive test than the blood variables or muscle metabolites, although it is impossible to detect any stress susceptible carrier (N_h) pigs. However, the test is only to be handled by an experienced operator to maximise accuracy.

Generally, the SS pigs had advantageous carcass and meat characteristics relative to that of the SR pigs. These advantageous characteristics included a higher slaughter-out percentage, lower chilling losses, thinner backfat thicknesses, shorter, thus more compact carcasses, and more tender meat. These positive characteristics are nevertheless negated by the single factor of low muscle pH values within 60 minutes *post mortem*, indicative of PSE meat by the SS pigs. Although the ADG of the SJ and SR pigs were similar, the SS pigs had a lower FCR, which is another economic advantageous characteristic. Thus, from a product quality point of view, the production of SS pigs is economically negative.

Significant herd differences were found regarding live mass, backfat thickness, cooking loss, water holding capacity and tenderness between the pigs of the two herds, although no significant difference was found in the muscle pH values 60 minutes *post mortem*. It is therefore possible in selective breeding to improve certain growth, carcass and meat characteristics towards the advantageous characteristics SS pigs possess, by only using SR pigs. Such a scheme would not negatively influence meat quality, and would therefore be an economic advantage.

The differences found in muscle fibre type indicate a higher anaerobic capacity in SS pigs. White muscle fibre percentage was higher in SS pigs, with a concomitant lower red and intermediate muscle fibre percentages. These differences between the muscle fibre percentages of the SR and SS pigs may contribute to the higher rate of anaerobic glycolysis and glycogenolysis characteristic of SS pigs during exposure to stress.

Regarding the influence of stress on the blood variables of pigs, the data show that both halothane exposure and treadmill exercise stimulate glycolysis and glycogenolysis. The treadmill exercise resulted in more severe muscle damage as indicated by the rise in muscle specific enzyme activities, and the greater secretion of ACTH and cortisol. These differences were also found in SS pigs as a result of the two types of stress, but were, compared to the changes in SR pigs, exacerbated. Nevertheless, the results indicate the possibility of different mechanisms of activation of malignant hyperthermia as a consequence of halothane exposure and treadmill exercise. This conclusion is also made on analysing the effect of the two types of stressors on the change in muscle metabolites of the SR and SS pig during the stress procedure.

The muscle metabolites indicated that, although halothane exposure and treadmill exercise both stimulated glycolysis and glycogenolysis in both the SR and SS pigs, the halothane exposure resulted in the stimulation of a more anaerobic metabolism in the SS pigs as indicated by the higher lactate concentrations. The difference in the muscle metabolites between SR and SS pigs were greater during halothane exposure than during the treadmill exercise, thus indicating the difference in response to the two types of stressors, and therefore the possibility of two different mechanisms of activation of malignant hyperthermia as a result of either halothane exposure or physical exertion.

The response between the two types of stress in rectal temperature was as a result of the difference in the response of the SR pigs rather than a difference in response by the SS pigs. The treadmill

exercise resulted overall in the higher rectal temperatures, which was significantly different in the SR pigs throughout the time period of temperature measurement, but only significant in the SS pigs early during the stress periods, whereafter the temperatures were similar during the remaining experimental time period. The calculated time period needed for the SR pigs to increase rectal temperature by 1°C was 31 minutes compared to the 9 minutes needed by the SS pigs during the halothane exposure. During the treadmill exercise the same difference was expected, yet, the times calculated were very similar, namely 14 and 12 minutes respectively. This finding also supports the possibility of different mechanisms involved in the stimulation of glycolysis and glycogenolysis, resulting in the rectal temperature, which is an end result, being similar between SS pigs as a result of halothane exposure and treadmill exercise.

It is concluded therefore, that the possibility exists for two different mechanisms involved in the activation of malignant hyperthermia and the concomitant activation of glycolysis and glycogenolysis. The activation of malignant hyperthermia as a result of PSS and MHS are thus not identical, and may therefore not be the same syndrome, although certain aspects of the syndrome are similar.

9.2. Recommendations

It is recommended that the use of the halothane test in identifying SS pigs be maintained. Guidelines on the optimal use of the halothane test must be drawn up, and distributed amongst the interested parties. The use of the halothane test in identifying SS pigs for the exclusion of such pigs from breeding herds should be encouraged in an effort to decrease the incidence of SS pigs, and ultimately the production of PSE meat. New methods should be developed to identify the carrier pigs unequivocally, as the tests to date are ineffective in doing so. This should lead to the measurement of the influence of this type of pig on growth carcass and meat characteristics, and whether this type of pig should be excluded from breeding herds as well.

The results of this study indicate the possibility of different mechanisms of activation of malignant hyperthermia and the concomitant stimulation of glycolysis and glycogenolysis. This aspect should receive attention as it could give valuable information on the stimulation of glycolysis and glycogenolysis, and the etiology of malignant hyperthermia.

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APPENDIX A

Results of the statistical analyses on the blood variables and muscle metabolites as influenced by stress sensitivity (according to the classification using the halothane screening test between the ages of 7 and 11 weeks) and herd

Table A.1: Results of 2-way analyses of variance on blood variables of pigs at 11 weeks of age as influenced by stress sensitivity (A: SR vs SS) and herd (B: herd X vs Herd Y)

Variable	Stress sensitivity (A)		Herd (B)		AxB	
	F value	Significance level	F value	Significance level	F value	Significance level
CK	0,313	0,8616 NS	0,412	0,5301 NS	1,841	0,1797 NS
LDH	10,312	0,0021 **	0,909	0,3542 NS	3,845	0,0544 NS
Aldolase	6,220	0,0156 *	39,732	<0,0001 **	2,913	0,0934 NS
AST	0,555	0,4671 NS	0,215	0,6492 NS	6,656	0,0123 *
ALT	5,990	0,0172 *	10,179	0,0022 **	2,825	0,0984 NS
Lactate	2,249	0,1389 NS	0,356	0,5591 NS	1,97	0,1656 NS
Total protein	14,075	0,0004 **	0,300	0,5916 NS	2,219	0,1414 NS
Albumin	5,991	0,0172 *	3,784	0,0563 NS	3,585	0,0630 NS
Globulin	1,783	0,1864 NS	1,504	0,2347 NS	1,180	0,2815 NS
Urea	5,512	0,0221 *	39,516	<0,0001 **	1,903	0,1727 NS
Sodium	1,347	0,2503 NS	0,257	0,6191 NS	5,894	0,0181 *
Potassium	0,067	0,7989 NS	3,612	0,0554 NS	0,067	0,7989 NS
Chloride	0,482	0,4977 NS	1,337	0,2521 NS	4,477	0,0384 *
Magnesium	0,374	0,5496 NS	38,337	<0,0001 **	0,104	0,7515 NS
Calcium	0,728	0,4059 NS	4,672	0,0345 **	1,126	0,2928 NS
Creatinine	0,205	0,6569 NS	13,436	0,0005 **	4,735	0,0335 *
Glucose	0,047	0,8307 NS	18,802	0,0001 **	1,231	0,2715 NS
Inorganic phosphate	8,705	0,0045 **	0,299	0,5924 NS	37,514	<0,0001 **
Bicarbonate	2,339	0,1313 NS	5,609	0,0189 *	0,167	0,6886 NS
Cortisol	8,891	0,0041 **	12,593	0,0007 **	0,003	0,9591 NS
ACTH	3,170	0,0799 NS	1,890	0,1741 NS	0,283	0,6034 NS
Urea/creatinin ratio	3,344	0,0724 NS	65,130	<0,0001 **	0,017	0,8979 NS
Albumin/globulin ratio	0,057	0,8151 NS	3,263	0,0757 NS	<0,001	0,9907 NS
Osmolality	0,945	0,3452 NS	0,065	0,8111 NS	4,817	0,0319 *
Anion gap	0,145	0,7086 NS	7,795	0,0070 **	0,734	0,4408 NS

Table A.2: Mean values and standard deviations (sd) of blood variables as influenced by stress sensitivity and herd

Variable		SR pigs			SS pigs			Herd X			Herd Y		
		Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n
CK	IU/l	2127	1826	40	2084	832	26	2035	1640	47	2294	1146	19
LDH	IU/l	1209	241	40	1467	35	26	1280	305	47	1388	314	19
Albumin	IU/l	10,7	3,30	35	12,7	3,5	25	13,3	3,6	42	7,4	2,7	18
AST	IU/l	58	17	40	55	16	26	58	17	47	55	16	19
ALT	IU/l	53	12	40	45	11	26	53	12	47	43	9	19
Lactate	mmol/l	7,92	2,3	39	7,01	2,17	25	7,71	3,40	45	7,23	1,82	19
Total protein	mmol/l	62	5	40			26	60	6	47	59	4	19
Albumin	mmol/l	35	3	40	32	5	26	35	3	47	35	6	19
Globulin	mmol/l	27	5	40	25	5	26	27	6	47	25	3	19
Urea	mmol/l	3,5	0,8	40	3,2	0,7	26	3,1	0,8	47	1,3	0,7	19
Sodium	mmol/l	150	5	40	151	4	26	150	3	47	150	8	19
Potassium	mmol/l	7,3	1,3	40	7,4	0,9	26	7,1	1,0	47	7,8	1,5	19
Chloride	mmol/l	101	4	40	102	2	26	101	2	47	100	6	19
Magnesium	mmol/l	1,07	0,14	40	1,08	0,16	25	1,00	0,16	46	1,25	0,10	19
Calcium	mmol/l	2,81	0,17	40	2,79	0,16	26	2,78	0,13	47	2,87	0,23	19
Creatinine	μmol/l	100	12	39	101	13	25	104	13	46	91	10	18
Glucose	mmol/l	6,1	0,7	40	6,2	0,5	26	5,9	0,6	47	6,7	0,7	19
Inorganic phosphate	mmol/l	3,19	0,27	39	3,42	0,34	26	3,26	0,34	46	3,33	0,17	19
Bicarbonate	mmol/l	23	4	40	24	3	26	24	3	47	22	3	19
Cortisol	μmol/l	29	15	40	21	7	26	23	11	47	33	16	19
ACTH	pmol/l	11	10	40	7	4	26	11	10	47	9	3	19
Urea/cortisol ratio		36	7	39	33	9	25	30	8	46	47	9	18
Albumin/globulin ratio		1,34	0,28	40	1,33	0,31	26	1,30	0,28	47	1,44	0,30	19
Osmolality	mmol/l	309	11	40	312	7	26	310	7	47	311	15	19
Anion gap	mmol/l	34	4	40	35	4	26	35	4	47	36	4	19

Table A.3: Mean values, standard deviations (sd) and level of significance of blood variables from pigs of herd X as influenced by stress sensitivity

Variable		SR pigs			SS pigs			Significance level
		Mean	sd	n	Mean	sd	n	
CK	IU/l	2185	1973	30	1772	714	17	0,4165 NS
LDH	IU/l	2226	263	30	1378	370	17	0,1343 NS
Aldolase	IU/l	12,0	3,5	25	15,2	3,7	17	0,0074 **
AST	IU/l	61	18	30	51	14	17	0,0492 *
ALT	IU/l	57	12	30	46	12	17	0,0074 **
Lactate	mmol/l	8,21	2,29	29	6,79	1,99	16	0,0637 NS
Total protein	mmol/l	62	6	30	56	6	17	0,0074 **
Albumin	mmol/l	35	3	30	31	3	17	0,0002 **
Globulin	mmol/l	28	6	30	25	6	17	0,1309 NS
Urea	mmol/l	3,2	0,7	30	2,9	0,8	17	0,2455 NS
Sodium	mmol/l	151	3	30	150	4	17	0,5856 NS
Potassium	mmol/l	7,2	1,1	30	7,1	0,8	17	0,7719 NS
Chloride	mmol/l	102	3	30	101	2	17	0,3715 NS
Magnesium	mmol/l	1,01	0,15	30	0,98	0,13	16	0,5352 NS
Calcium	mmol/l	2,80	0,13	30	2,73	0,14	17	0,1135 NS
Creatinine	μ mol/l	102	13	29	108	13	17	0,1506 NS
Glucose	mmol/l	5,9	0,7	30	6	0,5	17	0,4211 NS
Inorganic phosphate	mmol/l	3,29	0,31	29	3,21	0,39	17	0,4111 NS
Bicarbonate	mmol/l	23	4	30	24	2	17	0,3054 NS
Cortisol	nmol/l	26	12	30	17	6	17	0,0070 **
ACTH	pmol/l	12	12	30	7	4	17	0,1289 NS
Urea/creatinine ratio		31	7	29	27	8	17	0,8963 NS
Albumin/globulin ratio		1,20	0,29	30	1,28	0,28	17	0,8353 **
Osmolality	mmol/l	310	6	30	309	8	17	0,5389 **
Anion gap	mmol/l	33	5	30	32	4	17	0,4244 **

Table A.4: Mean values, standard deviations (sd) and level of significance of blood variables from SR pigs as influenced by herd

Variable		Herd X			Herd Y			Significance level
		Mean	sd	n	Mean	sd	n	
CK	IU/l	2185	1973	30	1932	1241	10	0,7290 NS
LDH	IU/l	1236	263	30	1160	153	10	0,4592 NS
Alkalase	IU/l	12,0	3,5	25	7,4	2,6	10	0,0038 **
AST	IU/l	61	18	30	49	11	10	0,0496 **
ALT	IU/l	57	12	30	42	11	10	0,0016 **
Lactate	mmol/l	8,21	2,59	29	7,08	0,90	10	0,1860 NS
Total protein	mmol/l	82	6	30	6	4	10	0,1627 NS
Albumin	mmol/l	35	3	30	35	3	10	0,7863 NS
Globulin	mmol/l	28	6	30	25	3	10	0,1139 NS
Urea	mmol/l	3,2	0,7	30	4,7	0,9	10	<0,0001 **
Sodium	mmol/l	151	3	30	147	10	10	0,0887 NS
Potassium	mmol/l	7,2	1,1	30	7,6	1,8	10	0,4260 NS
Chloride	mmol/l	102	3	30	99	8	10	0,0623 NS
Magnesium	mmol/l	1,01	0,15	30	1,25	0,10	10	<0,0001 **
Calcium	mmol/l	2,80	0,13	30	2,86	0,27	10	0,3823 NS
Creatinine	mmol/l	103	13	29	32	7	10	0,1651 NS
Glucose	mmol/l	5,9	0,7	30	6,8	0,9	10	0,0011 **
Inorganic phosphate	mmol/l	3,29	0,31	29	2,89	0,10	10	0,0003 **
Bicarbonate	mmol/l	23	4	30	21	3	10	0,0660 NS
Cortisol	nmol/l	26	12	30	38	20	10	0,0311 *
ACTH	pmol/l	12	12	30	8	3	10	0,2650 NS
Urea/creatinine ratio		31	7	29	49	9	10	<0,0001 **
Albumin/globulin ratio		1,30	0,29	30	1,44	0,25	10	0,2707 **
Osmolality	mmol/l	310	6	30	306	20	10	0,2714 **
Anion gap	mmol/l	33	5	30	35	4	10	0,1289 **

Table A.5: Mean values, standard deviations (sd) and level of significance of blood variables from SS pigs as influenced by herd

Variable		Herd X			Herd Y			Sign. level
		Mean	sd	n	Mean	sd	n	
CK	IU/l	1771	714	17	2675	1027	9	0,0145 *
LDH	IU/l	1375	370	17	1642	428	9	0,1109 NS
Aldolase	IU/l	15,2	3,7	17	7,4	2,9	9	<0,0001 **
AST	IU/l	51	14	17	62	20	9	0,1094 NS
ALT	IU/l	46	12	17	43	8	9	0,3752 NS
Lactate	mmol/l	6,79	1,99	16	7,41	2,48	9	0,5017 NS
Total protein	mmol/l	56	6	17	57	4	9	0,4717
Albumin	mmol/l	31	3	17	25	8	9	0,0505 NS
Globulin	mmol/l	25	6	17	25	3	9	1,0000 NS
Urea	mmol/l	2,9	0,8	17	3,8	0,4	9	0,0027 **
Sodium	mmol/l	150	4	17	153	3	9	0,0530 NS
Potassium	mmol/l	7,1	0,8	17	8,0	1,1	9	0,0212 *
Chloride	mmol/l	101	2	17	102	2	9	0,1948 NS
Magnesium	mmol/l	0,98	0,18	16	1,25	0,10	9	0,0005 **
Calcium	mmol/l	2,73	0,14	17	2,90	0,17	9	0,0249 *
Creatinine	μ mol/l	108	13	17	86	13	8	0,0008 **
Glucose	mmol/l	6,0	0,5	17	6,6	0,4	9	0,0119 *
Inorganic phosphate	mmol/l	3,21	0,39	17	3,82	0,22	9	0,0002 **
Bicarbonate	mmol/l	24	2	17	22	3	9	0,1270 NS
Cortisol	nmol/l	17	6	17	29	7	9	0,0003 **
ACTH	pmol/l	7	4	17	6	3	9	0,2861 NS
Urea/creatinine ratio		27	8	17	46	10	8	<0,0001 **
Albumin/globulin ratio		1,28	0,28	17	1,43	0,35	9	0,2646 **
Osmolality	mmol/l	309	8	17	316	7	9	0,0225 *
Anion gap	mmol/l	32	4	17	36	5	9	0,0206 *

Table A.6: Results of 2-way analyses of variance on muscle metabolites from pigs as influenced by stress sensitivity (A: SR vs SS) and herd (B: herd X vs herd Y)

Variable	Stress sensitivity (A)		Herd (B)		AxB	
	F value	Significance level	F value	Significance level	F value	Significance level
Lactate	19,264	<0,0001 **	44,267	<0,0001 **	0,325	0,7251 NS
ATP	1,487	<0,2223 NS	7,230	0,0092 **	0,006	0,9397 NS
Glucose 6-phosphate	15,089	0,0003 **	3,708	0,0588 NS	12,055	0,0009 **
Phosphocreatine	13,252	0,0006 **	24,673	<0,0001 **	0,164	0,6915 NS
Glucose	2,857	0,1221 NS	0,171	0,6849 NS	9,538	0,0030 **
Glycogen	0,133	0,7024 NS	24,955	<0,0001 **	5,262	0,0252 *

Table A.7: Mean values and standard deviations (sd) of muscle metabolites as influenced by stress sensitivity and herd

Variable	SR pigs			SS pigs			Herd X		Herd Y			
	Mean	sd	n	Mean	sd	n	Mean	sd	n	n		
Lactate	12,13	6,54	40	21,11	7,61	26	11,76	6,59	47	25,32	7,91	19
ATP	5,54	1,67	40	4,99	0,96	26	5,64	1,60	47	4,53	0,85	19
Glucose 6-phosphate	1,37	0,91	40	2,61	1,55	26	1,64	1,33	47	2,40	0,73	19
Phosphocreatine	8,37	4,16	40	4,40	3,05	26	8,39	4,33	47	2,88	1,48	19
Glucose	0,66	0,45	40	0,82	0,33	26	0,70	0,42	47	0,77	0,36	19
Glycogen	62,85	12,40	40	58,26	19,65	26	66,62	17,92	47	45,13	6,25	19

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose : $\mu\text{mol/g}$ muscle
Glycogen : $\mu\text{mol glycerol units/g}$ muscle

Table A.8: Mean values, standard deviations (sd) and level of significance of muscle metabolites from pigs of herd X as influenced by stress sensitivity

Variable	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
Lactate	9,20	6,54	30	16,29	6,68	17	<0,0009 **
ATP	5,81	1,88	30	5,35	0,90	17	0,3472 NS
Glucose 6-phosphate	0,96	0,98	30	2,84	1,81	17	<0,0001 **
Phosphocreatine	9,74	4,96	30	6,01	3,66	17	0,0068 **
Glucose	0,72	0,47	30	0,67	0,32	17	0,7055 NS
Glycogen	65,01	13,85	30	69,46	23,56	17	0,4173 NS

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose : $\mu\text{mol/g}$ muscle
Glycogen : $\mu\text{mol glycerol units/g}$ muscle

Table A.9: Mean values, standard deviations (sd) and level of significance of muscle metabolites from SR pigs as influenced by herd

Variable	Herd X			Herd Y			Significance level
	Mean	sd	n	Mean	sd	n	
Lactate	9.20	6.54	30	20.99	6.55	16	<0.0001 **
ATP	5.81	1.88	30	4.73	0.61	10	0.0840 NS
Glucose 6-phosphate	0.96	0.98	30	2.60	0.62	10	<0.0001 **
Phosphocreatine	9.74	4.66	30	4.25	1.77	10	0.0009 **
Glucose	0.72	0.47	30	0.46	0.38	10	0.1238 NS
Glycogen	65.01	13.85	30	32.37	5.99	10	0.0081 **

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose : $\mu\text{mol/g}$ muscle
 Glycogen : $\mu\text{mol glycogen units/g}$ muscle

Table A.10: Mean values, standard deviations (sd) and level of significance of muscle metabolites from SR pigs as influenced by herd

Variable	Herd X			Herd Y			Significance level
	Mean	sd	n	Mean	sd	n	
Lactate	10.79	6.68	17	30.21	9.20	9	0.0002 **
ATP	5.53	0.90	17	4.32	1.06	9	0.0159 *
Glucose 6-phosphate	2.84	1.81	17	2.19	0.83	9	0.3171 NS
Phosphocreatine	6.01	3.66	17	1.36	1.05	9	0.0011 **
Glucose	0.67	0.32	17	1.11	0.35	9	0.0038 **
Glycogen	69.46	23.56	17	37.10	6.92	9	0.0005 **

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose : $\mu\text{mol/g}$ muscle
 Glycogen : $\mu\text{mol glycogen units/g}$ muscle

APPENDIX B

The results of the statistical analyses on meat characteristics as influenced by stress sensitivity and herd, in which carcasses with DFD characteristics were excluded

Table B.1: Results of 2-way analyses of variance on meat characteristics as influenced by stress sensitivity (A: SR vs SS) and herd (B: herd X vs herd Y)

Variable	Stress sensitivity (A)		Herd (B)		AxB	
	F value	Significance level	F value	Significance level	F value	Significance level
pH value						
15 min p.m.	28,506	<0,0001 **	1,675	0,262 NS	10,272	0,0025 **
20 min p.m.	33,943	<0,0001 **	0,586	0,4559 NS	8,235	0,0059 **
45 min p.m.	35,469	<0,0001 **	0,195	0,6659 NS	2,711	0,1065 NS
60 min p.m.	68,945	<0,0001 **	4,193	0,0484 *	0,426	0,5255 NS
24 h p.m.	0,986	0,3366 NS	11,636	0,0014 **	3,033	0,0883 NS
Drop loss	0,014	0,9081 NS	3,247	0,0781 NS	6,252	0,0160 *
Cooking loss						
60°C	1,385	0,2453 NS	8,972	0,0044 **	0,003	0,9595 NS
70°C	3,046	0,0874 NS	10,372	0,0023 **	0,003	0,9542 NS
80°C	1,646	0,2062 NS	0,139	0,7152 NS	0,884	0,3622 NS
Water holding capacity						
60°C	0,044	0,8375 NS	6,096	0,0173 *	6,145	0,0178 *
70°C	6,449	0,0145 *	9,251	0,0039 **	4,444	0,0405 *
80°C	2,105	0,1536 NS	3,280	0,0767 NS	0,026	0,8754 NS
Shear force						
60°C	2,215	0,1435 NS	1,191	0,2809 NS	3,988	0,0518 NS
70°C	9,092	0,0042 **	6,998	0,0111 *	1,208	0,2775 NS
80°C	7,691	0,0080 **	20,672	<0,0001 **	0,528	0,4789 NS

Table B.2: Mean values and standard deviations (sd) of meat characteristics of pigs as influenced by stress sensitivity and herd

Variable	SR pigs			SS pigs			Herd X			Herd Y		
	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n
pH value												
15 min. p.m.	6.51	0.23	27	6.15	0.26	23	6.31	0.27	33	6.42	0.18	17
30 min. p.m.	6.44	0.22	27	6.04	0.29	23	6.23	0.26	33	6.31	0.21	17
45 min. p.m.	6.35	0.21	27	5.93	0.30	23	6.16	0.30	33	6.15	0.25	17
60 min. p.m.	6.34	0.26	18	5.72	0.23	20	6.30	0.35	21	6.00	0.26	17
24 h.p.m.	5.59	0.26	27	5.54	0.28	23	5.65	0.25	33	5.41	0.22	17
Drip loss (%)	5.72	3.27	27	5.73	2.05	23	6.23	3.68	33	4.74	2.37	17
Cooking loss (%)												
60°C	15.74	3.79	27	17.16	3.40	23	17.53	4.15	33	14.20	2.13	17
70°C	26.25	24.50	27	28.75	3.70	23	28.91	5.24	33	24.46	1.97	17
80°C	32.85	4.62	25	34.46	3.69	23	33.84	4.92	31	33.21	2.23	17
Water holding capacity (%)												
60°C	49.62	4.30	27	49.18	3.89	23	48.38	4.09	33	51.44	4.17	17
70°C	45.10	4.08	27	41.88	4.16	23	42.27	4.19	33	46.24	3.97	17
80°C	39.52	4.28	27	37.67	3.93	23	37.85	4.01	33	40.22	3.48	17
Shear force (N/2.5 cm dia)												
60°C	81.12	21.99	27	73.04	12.06	23	75.20	18.25	33	51.44	4.17	17
70°C	94.66	27.09	27	74.6	13.81	23	79.03	23.1	33	46.24	3.97	17
80°C	116.19	24.52	27	97.03	18.06	23	96.86	22.18	33	40.22	3.48	17

Table B.3: Mean values, standard deviations (sd) and level of significance of meat characteristics of pigs from herd X as influenced by stress sensitivity

Variable	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
pH value							
15 min p.m.	6.41	0.21	17	6.20	0.30	16	0.0293 *
30 min p.m.	6.35	0.21	17	6.10	0.29	16	0.0064 **
45 min p.m.	6.32	0.21	17	5.98	0.29	16	0.0005 **
60 min p.m.	6.40	0.20	8	5.80	0.19	13	<0.0001 **
24 h.p.m.	5.72	0.18	17	5.57	0.30	16	0.0878 NS
Drip loss (%)	6.95	3.71	17	5.46	2.22	18	0.1757 NS
Cooking loss (%)							
60°C	16.96	4.41	17	18.13	3.84	16	0.4230 NS
70°C	27.87	5.99	17	30.02	4.30	16	0.2464 NS
80°C	52.60	5.55	15	35.01	4.26	16	0.1849 NS
Water holding capacity (%)							
60°C	47.51	4.95	17	49.30	2.90	16	0.2182 NS
70°C	42.86	4.80	17	41.44	3.42	16	0.4059 NS
80°C	38.75	5.09	17	36.92	3.54	16	0.2408 NS
Shear force (N/2.5 cm dia)							
60°C	82.45	21.90	17	67.49	13.30	16	0.0251 *
70°C	90.56	28.19	17	66.78	15.97	16	0.0059 **
80°C	103.66	23.97	17	89.64	20.10	16	0.0793 NS

Table B.4: Mean values, standard deviations (sd) and level of significance of meat characteristics of pigs from herd Y as influenced by stress sensitivity

Variable	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
pH values							
15 min. p.m.	6.69	0.14	10	6.04	0.22	7	< 0.0001 **
30 min. p.m.	6.59	0.16	10	5.92	0.27	7	< 0.0001 **
45 min. p.m.	6.40	0.21	10	5.81	0.29	7	0.0002 **
60 min. p.m.	6.29	0.30	10	5.59	0.25	7	0.0001 **
24 h p.m.	5.37	0.21	10	5.47	0.24	7	0.3789 NS
Drip loss (%)	3.63	2.26	10	6.33	1.52	7	0.0150 *
Cooking loss (%)							
60°C	13.67	2.28	10	14.95	1.89	7	0.2406 NS
70°C	23.51	2.51	10	25.82	1.32	7	0.0310 *
80°C	33.21	2.59	10	33.20	1.54	7	0.9946 NS
Water holding capacity (%)							
60°C	53.22	2.78	10	48.90	5.66	7	0.0531 NS
70°C	48.90	2.51	10	42.34	5.60	7	0.0047 **
80°C	40.82	2.21	10	39.38	4.78	7	0.4145 NS
Shear force (N/2.5 cm dia)							
60°C	78.84	22.16	10	85.74	8.30	7	0.4470 NS
70°C	101.63	25.01	10	92.4	5.44	7	0.3600 NS
80°C	137.50	25.47	10	113.5	1.45	7	0.0379 *
EBL values							
top	26	4	10	44	9	7	< 0.0001 **
middle	27	3	10	43	8	7	< 0.0001 **
bottom	27	3	10	37	5	7	0.0002 **
average	27	3	10	41	7	7	< 0.0001 **
SDP	141	32	10	157	21	7	0.2878 NS
Drip volume (ml)	14.65	15.89	10	32.71	12.52	7	0.0243 *

Table B.5. Mean values, standard deviations (sd) and level of significance of meat characteristics of SR pigs as influenced by herd

Variable	Herd X			Herd Y			Significance level
	Mean	sd	n	Mean	sd	n	
pH values							
15 min. p.m.	6.41	0.21	17	6.09	0.14	10	0.0008 **
30 min. p.m.	6.35	0.21	17	6.39	0.16	10	0.0056 **
45 min. p.m.	6.32	0.21	17	6.40	0.21	10	0.3740 NS
60 min. p.m.	6.40	0.20	8	6.29	0.30	10	0.3869 NS
24 h p.m.	5.72	0.18	17	5.37	0.21	10	0.0001 **
Drip loss (%)							
60°C	6.95	3.71	17	3.63	2.26	10	0.0171 *
Cooking loss (%)							
60°C	16.96	4.41	17	13.67	2.38	10	0.0390 *
70°C	27.87	5.99	17	23.51	2.31	10	0.0381 *
80°C	32.60	5.53	15	33.21	2.59	10	0.7515 NS
Water holding capacity (%)							
60°C	47.51	4.95	17	53.22	2.78	10	0.0026 **
70°C	42.86	4.80	17	48.90	2.31	10	0.0010 **
80°C	36.75	5.09	17	40.82	2.21	10	0.2381 NS
Shear force (N/2.5 cm dia)							
60°C	82.45	21.90	17	78.84	22.16	10	0.6838 NS
70°C	90.56	28.19	17	101.63	25.01	10	0.3148 NS
80°C	103.66	23.97	17	137.50	25.47	10	0.0019 **

Table B.6. Mean values, standard deviations (sd) and level of significance of meat characteristics of SS pigs as influenced by herd

Variable	Herd X			Herd Y			Significance level
	Mean	sd	n	Mean	sd	n	
pH values							
15 min. p.m.	6.20	0.30	16	6.04	0.22	7	0.2053 NS
30 min. p.m.	6.10	0.29	16	5.92	0.27	7	0.1798 NS
45 min. p.m.	5.98	0.28	16	5.81	0.29	7	0.2044 NS
60 min. p.m.	5.80	0.19	13	5.59	0.25	7	0.0486 *
24 h p.m.	5.57	0.30	16	5.47	0.24	7	0.4358 NS
Drip loss (%)							
60°C	5.46	2.71	18	6.33	1.52	7	0.3616 NS
Cooking loss (%)							
60°C	18.13	3.84	16	14.95	1.89	7	0.0516 NS
70°C	30.02	4.30	16	25.82	1.32	7	0.0206 *
80°C	35.01	4.26	16	33.20	1.54	7	0.2924 NS
Water holding capacity (%)							
60°C	49.30	5.90	16	48.90	5.66	7	0.8217 NS
70°C	41.64	3.42	16	42.34	5.00	7	0.6778 NS
80°C	36.92	3.54	16	39.38	4.78	7	0.1818 NS
Shear force (N/2.5 cm dia)							
60°C	67.7	13.30	16	85.74	8.20	7	0.0031 **
70°C	64.78	15.97	16	92.48	5.44	7	0.0003 **
80°C	70.64	20.10	16	113.92	11.45	7	0.0273 **

APPENDIX C

The results of the statistical analyses on blood variables as influenced by stress procedure and stress sensitivity in which data from the SS pigs that survived the treadmill exercise was excluded

Table C.1: The results of 2-way analyses of variance on blood variables from pigs as influenced by stress procedure (A: halothane exposure vs treadmill exercise) and stress sensitivity (B: SR vs SS)

Variable	Stress procedure (A)		Stress sensitivity (B)		AxB	
	F value	Significance level	F value	Significance level	F value	Significance level
CK	23,433	<0,0001 **	26,352	<0,0001 **	44,369	<0,0001 **
LDH	18,084	0,0001 **	38,773	<0,0001 **	51,289	<0,0001 **
Aldolase	4,640	0,0285 **	146,102	<0,0001 **	0,543	0,4719 NS
AST	21,151	<0,0001 **	13,460	0,0006 **	12,580	0,0006 **
ALT	7,451	0,0001 **	11,785	0,0011 **	2,147	0,1484 NS
Lactate	20,397	<0,0001 **	22,941	<0,0001 **	5,328	0,0247 *
Total protein	0,596	0,4307 NS	9,066	0,0040 **	0,067	0,7997 NS
Albumin	0,280	0,6044 NS	23,115	<0,0001 NS	1,253	0,2676 NS
Globulin	0,358	0,5384 NS	9,389	0,5418 NS	0,145	0,7048 NS
Urea	30,708	<0,0001 **	8,731	0,0045 **	12,405	0,0009 **
Sodium	0,930	0,3344 NS	107,242	<0,0001 **	13,311	0,0006 **
Potassium	0,222	0,6440 NS	38,591	<0,0001 **	0,227	0,6406 NS
Chloride	25,638	<0,0001 **	0,017	0,8968 NS	36,471	<0,0001 **
Magnesium	10,828	0,0017 **	23,054	<0,0001 **	17,149	0,0001 **
Calcium	0,181	0,6769 NS	43,383	<0,0001 **	0,165	0,6900 NS
Creatinine	9,123	0,0038 **	10,480	0,0020 **	0,515	0,4834 NS
Glucose	14,612	0,0003 **	0,959	0,3547 NS	0,631	0,4388 NS
Inorganic phosphat	6,435	0,0149 *	23,151	<0,0001 **	0,469	0,5034 NS
Bicarbonate	119,673	<0,0001 **	3,863	0,0542 NS	2,717	0,1048 NS
Cortisol	166,163	<0,0001 **	1,454	0,2329 NS	7,106	0,0100 **
ACTH	33,944	<0,0001 **	4,910	0,0307 *	1,488	0,2275 NS
Urea/Creatinine ratio	14,197	0,0004 **	26,105	<0,0001 **	7,929	0,0067 **
Albumin/Globulin ratio	0,761	0,3960 NS	1,639	0,2056 NS	1,197	0,2784 NS
Osmolality	0,823	0,3778 NS	80,520	<0,0001 **	16,208	0,0001 **
Anion gap	56,597	<0,0001 **	45,5930	<0,0001 **	0,006	0,9275 NS

Table C2: Mean values and standard deviations (sd) of blood variables from pigs as influenced by halothane exposure, treadmill exercise, and stress sensitivity.

Variable	Halothane exposure			Treadmill exercise			SS pigs			SS pigs			
	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n	
CK	IUA	2907	2091	47	10993	11066	14	1673	970	40	10320	9624	21
LDH	IUA	1196	323	47	1832	2048	14	1037	275	40	1936	889	21
Alkalase	IUA	14.6	4.0	47	16.2	3.5	14	10.7	9.4	40	23.3	4.8	21
AST	IUA	4.6	1.5	47	7.9	3.3	14	4.6	1.2	40	6.4	3.1	21
ALT	IUA	5.0	1.5	47	6.6	1.5	14	3.0	1.2	40	6.1	1.6	21
Lactate	mmol/l	7.83	3.83	46	12.35	2.53	14	7.26	2.08	39	11.71	5.41	21
Total protein	mmol/l	72	5	47	70	9	14	69	6	40	75	7	21
Albumin	mmol/l	49	3	47	40	2	14	39	3	40	43	4	21
Globulin	mmol/l	31	4	47	30	8	14	31	5	40	32	4	21
Urea	mmol/l	5.8	1.3	47	7.9	1.2	14	6.6	1.0	40	5.3	1.6	21
Sodium	mmol/l	151	3	47	150	4	14	145	3	40	157	3	21
Potassium	mmol/l	5.2	0.6	47	5.3	1.3	14	4.8	0.5	40	6.1	1.3	21
Chloride	mmol/l	102	3	47	98	3	14	101	2	40	101	3	21
Magnesium	mmol/l	0.89	0.19	47	1.7	0.22	14	0.83	0.19	40	1.18	0.22	21
Calcium	mmol/l	2.94	0.29	47	2.86	0.44	14	2.72	0.31	40	3.33	0.36	21
Creatinine	mmol/l	144	18	47	159	13	14	143	18	40	157	16	21
Glucose	mmol/l	5.4	0.8	47	7.2	2.9	14	5.7	1.4	40	7.6	1.8	21
Inorganic phosphate	mmol/l	3.29	0.39	47	2.96	0	14	3.03	0.32	40	3.55	0.47	21
Bicarbonate	mmol/l	25	2	47	17	3	14	22	2	40	14	3	21
Cortisol	nmol/l	23	10	47	83	47	14	40	15	40	31	15	21
ACTH	pmol/l	10	10	47	27	7	14	12	9	40	17	11	21
Urea:creatinine ratio		40	8	47	59	8	14	47	8	40	35	9	21
Albumin:globulin ratio		1.33	0.2	47	1.37	0.27	14	1.30	0.24	40	1.38	0.16	21
Bun:creo													
Catechol	mmol/l	313	5	47	314	9	14	305	7	40	305	7	21
Amion gap	mmol/l	3	4	47	40	7	14	20	4	40	37	6	21

Table C.3: Mean values, standard deviations (sd) and level of significance of blood variables of treadmill exercised pigs as influenced by stress sensitivity

Variable		SR pigs			SS pigs			Significance level
		Mean	sd	n	Mean	sd	n	
CK	IU/l	2268	926	10	31090	22074	4	0,0008 **
LDH	IU/l	979	158	10	4032	2078	4	0,0003 **
Aldolase	IU/l	12,2	2,6	10	26,5	5,3	4	0,0002 **
AST	IU/l	56	13	10	114	62	4	0,0120 *
ALT	IU/l	60	12	10	83	23	4	0,0270 *
Lactate	mmol/l	12,31	1,37	10	12,62	4,23	4	0,8330 NS
Total protein	mmol/l	69	9	10	73	10	4	0,4660 NS
Albumin	mmol/l	39	2	10	41	4	4	0,1530 NS
Globulin	mmol/l	30	8	10	32	7	4	0,6990 NS
Urea	mmol/l	8,9	1,1	10	5,6	1,6	4	0,0066 **
Sodium	mmol/l	149	3*	10	152	5	4	0,2180 NS
Potassium	mmol/l	4,8	0,5	10	6,4	2,5	4	0,0630 NS
Chloride	mmol/l	100	2	10	92	4	4	0,0001 **
Magnesium	mmol/l	1,13	0,20	10	0,95	0,26	4	0,1960 NS
Calcium	mmol/l	3,71	0,40	10	3,23	0,52	4	0,0670 NS
Cholesterol	mmol/l	157	14	10	165	6	4	0,2720 NS
Glucose	mmol/l	7,3	2,4	10	7,0	4,3	4	0,8910 NS
Inorganic phosphate	mmol/l	2,78	0,06	10	3,41	0,60	4	0,0043 **
Bicarbonate	mmol/l	16	2	10	19	5	4	0,0020 NS
Cortisol	nmol/l	78	25	10	55	33	4	0,3230 NS
ACTH	pmol/l	27	8	10	26	4	4	0,9220 NS
Urea:creatinine ratio		57	7	10	34	11	4	0,0063 **
Albumin:globulin ratio		1,29	0,30	10	1,34	0,19	4	0,0757 NS
Osmolality	mmol/l	314	7	10	316	14	4	0,7180 NS
Anion gap	mmol/l	37	4	10	47	13	4	0,0540 NS

Table C.4: Mean values, standard deviations (sd) and level of significance of blood variables of SS pigs during halothane exposure or after treadmill exercise

Variable		Halothane exposure			Treadmill exercise			Significance level
		Mean	sd	n	Mean	sd	n	
CK	IU/l	5432	4315	17	31090	22074	4	0.001 **
LDH	IU/l	1443	398	17	4032	2078	4	<0.0001 **
Aldolase	IU/l	22.5	4.7	17	26.5	5.3	4	0.1390 NS
AST	IU/l	53	10	17	114	62	4	0.017 **
ALT	IU/l	56	14	17	83	23	4	0.0054 **
Lactate	mmol/l	11.50	5.61	17	12.62	4.23	4	0.7156 NS
Total protein	mmol/l	75	6	17	72	30	4	0.5790 NS
Albumin	mmol/l	43	4	17	41	4	4	0.3050 NS
Globulin	mmol/l	32	4	17	31	7	4	0.9910 NS
Urea	mmol/l	5.5	1.6	17	5.6	1.6	4	0.9390 NS
Sodium	mmol/l	158	2	17	152	5	4	0.0012 **
Potassium	mmol/l	6.1	0.9	17	6.4	2.5	4	0.6730 NS
Chloride	mmol/l	105	3	17	92	4	4	<0.0001 **
Magnesium	mmol/l	1.14	0.22	17	0.95	0.26	4	0.1530 NS
Calcium	mmol/l	3.34	0.33	17	3.23	0.52	4	0.6110 NS
Creatinine	μmol/l	153	17	17	165	6	4	0.2590 NS
Glucose	mmol/l	5.8	0.7	17	7.0	4.3	4	0.2410 NS
Inorganic phosphate	mmol/l	3.58	0.44	17	3.41	0.60	4	0.5270 NS
Bicarbonate	mmol/l	25	3	17	19	5	4	0.0015 **
Cortisol	nmol/l	16	8	17	95	33	4	<0.0001 **
ACTH	pmol/l	15	11	17	26	4	4	0.0600 NS
Urea/creatinine ratio		33	8	17	34	11	4	0.7950 NS
Albumin/globulin ratio		1.39	0.17	17	1.34	0.19	4	0.5740 NS
Osmolality	mmol/l	322	6	17	316	14	4	0.0120 *
Anion gap	mmol/l	35	4	17	44	13	4	0.0030 **

APPENDIX D

The results of the statistical analyses on muscle metabolites as influenced by stress procedure and stress sensitivity in which data from the SS pig, that survived the treadmill exercise was excluded

Table D.1: The results of 2-way analyses of variance on the muscle metabolites of pigs as influenced by stress procedure (A: halothane exposure vs treadmill exercise) and stress sensitivity (B: SR vs SS)

Variable	Stress procedure (A)			Stress sensitivity (B)		AxB			
	F value	Significance level	NS	F value	Significance level	F value	Significance level		
Lactate	0,002	0,9685	NS	58,067	<0,0001	**	2,494	0,1198	NS
ATP	4,481	0,0386	*	24,900	<0,0001	**	3,249	0,0767	NS
Glucose 6-phosphate	0,014	0,9091	NS	28,763	<0,0001	**	0,413	0,5301	NS
Phosphocreatine	2,543	0,1163	NS	36,274	<0,0001	**	3,716	0,058	NS
Glucose	30,028	<0,0001	**	11,911	0,0014	**	0,317	0,5814	NS
Glycogen	15,065	0,0003	**	10,915	0,0025	**	0,084	0,7760	NS

Table D.2: Mean values and standard deviations (sd) of muscle metabolites as influenced by halothane exposure, treadmill exercise and stress sensitivity

Variable	Halothane exposure			Treadmill exercise			SR pigs			SS pigs		
	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n
Lactate	20,40	12,18	47	19,21	9,31	14	14,28	7,04	40	33,26	10,17	21
ATP	4,64	1,57	47	3,93	1,51	14	5,05	1,37	40	3,37	1,35	21
Glucose 6-phosphate	2,35	2,23	47	2,10	1,59	14	1,43	1,45	40	3,95	2,17	21
Phosphocreatine	5,41	4,50	47	4,16	4,23	14	7,02	4,26	40	1,51	1,51	21
Glucose	0,95	0,54	47	1,20	0,83	14	1,01	0,66	40	1,52	0,95	21
Glycogen	49,46	15,79	47	32,73	12,37	14	49,65	16,90	40	37,85	15,72	21

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose : *muscle*
Glycogen : *antagonist of muscle*

Table D.3: Mean values, standard deviations (sd) and level of significance of muscle metabolites of treadmill exercised pigs as influenced by stress sensitivity

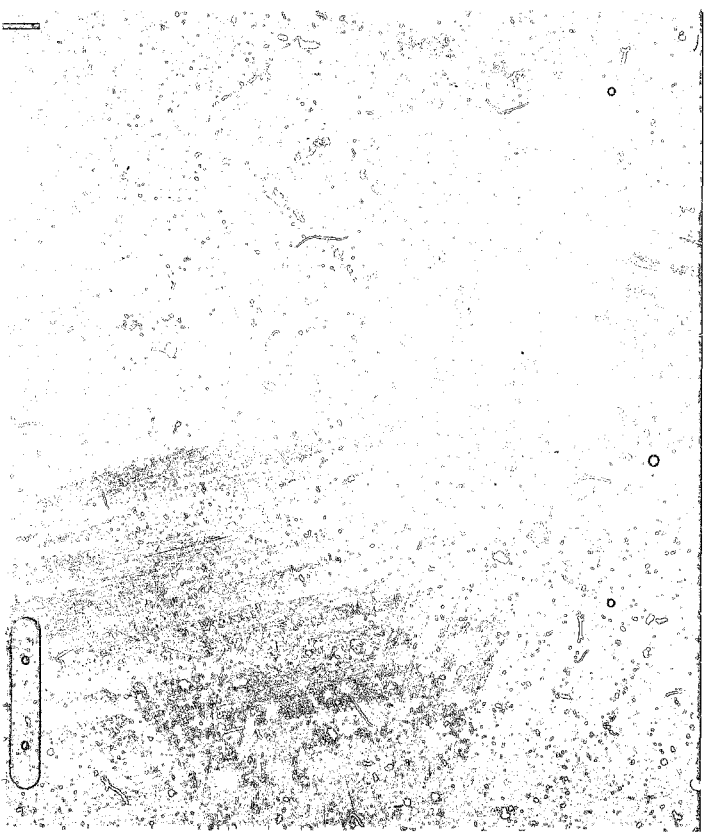
Variable	SR pigs			SS pigs			Significance level
	Mean	sd	n	Mean	sd	n	
Lactate	16.30	7.94	10	26.47	9.38	4	0.0611 NS
ATP	4.07	1.64	10	3.58	1.24	4	0.5973 NS
Glucose 6-phosphate	1.55	1.03	10	3.48	2.05	4	0.0335 *
Phosphocreatine	4.77	4.59	10	2.65	2.49	4	0.4068 NS
Glucose	1.86	0.63	10	2.25	1.28	4	0.4493 NS
Glycogen	37.65	10.78	10	22.01	9.96	4	0.0198 *

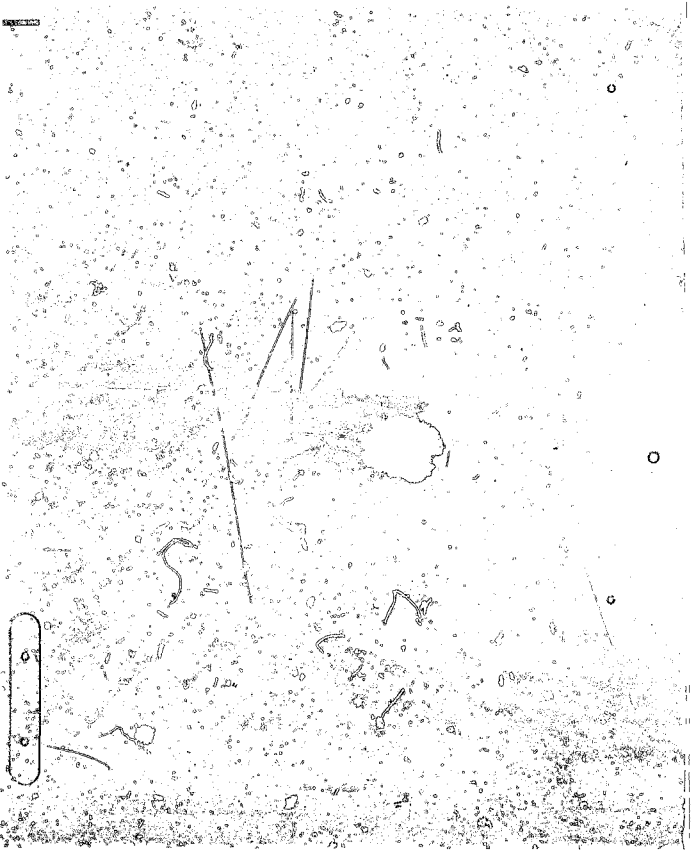
Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose: $\mu\text{mol/g}$ muscle
Glycogen: $\mu\text{mol/glycogen units/g}$ muscle

Table D.4: Mean values, standard deviations (sd) and level of significance of muscle metabolites of SS pigs as influenced by stress procedure

Variable	Halothane exposure			Treadmill exercise			Significance level
	Mean	sd	n	Mean	sd	n	
Lactate	32.29	10.28	17	26.47	9.38	4	0.3074 NS
ATP	3.33	1.40	17	3.58	1.24	4	0.7494 NS
Glucose 6-phosphate	4.06	2.25	17	3.48	2.05	4	0.6427 NS
Phosphocreatine	1.24	1.13	17	2.65	2.49	4	0.0933 NS
Glucose	1.35	0.81	17	2.25	1.28	4	0.0934 NS
Glycogen	41.70	14.55	17	22.01	9.96	4	0.0198 *

Lactate, ATP, glucose 6-phosphate, phosphocreatine and glucose: $\mu\text{mol/g}$ muscle
Glycogen: $\mu\text{mol/glycogen units/g}$ muscle





Author Heinze Paul Hermann

Name of thesis Porcine Stress Syndromes. 1989

PUBLISHER:

University of the Witwatersrand, Johannesburg

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