Pork Quality and Porcine Stress Syndrom in Estonia

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Abstract

The effect of breed combinations and HAL gene to pork quality were analysed. In all 193 pigs (purebred Estonian Landrace (EL), Estonian Large White (ELW), Finnish Yorkshire (FY) and crossbred Hampshire (H)♂xELW♀, H/EL/ELW♂xEL♀) were investigated. Following traits were recorded with ultrasonic equipments (Piglog 105, A-Scan Plus and Ultra FOM 100): sidefat thickness at last and 11...12th rib, and diameter of loin eye. Lean meat percentage was calculated. Carcass length, weight, sidefat thickness measurements in 4 points and pH (24 h) were collected. Loin eye area was measured by planimeter, pH and boiling loss were found 48 hours after slaughtering. Blood samples were collected from 101 pigs. DNA tests were carried out by PCR-RFLP method. Higher sidefat thickness and lower lean meat % was found in ELW and FY breed. EL had significantly longer carcasses than FY and crossbred pigs. EL and H breed gave best influence to meat quality. EL had significantly longer carcasses, than FY and crossbred pigs. According to DNA test, 84.2% of tested pigs were stress negative (NN) and 15.8% heterozygous (Nn). The HAL homozygous mutant (nn) animals were not found. Significant relationship between testing weight and HAL gene ($p<0.05$) was found, when breed effect was skipped.

Key words: pigs, carcass measurements, pork quality, PSS, HAL gene

Introduction

For many years the main goal in pig breeding has been to improve growth rate, fertility, feed conversion and carcass composition. The meat quality improving has considered reduction of PSE meat (pale, soft and exudative). Porcine Stress Syndrome (PSS) is a well documented genetic disorder, transmitted by a single autosomal recessive gene, causing major economic loss in pig industry - sudden death of pigs and the low quality of pork received after slaughter (PSE-meat) (Fujii et al., 1991; Santoro and Faucitano, 1996). This gene has been variously called the stress gene, halothane gene and PSS gene. The researches have showed the effect of halothane carrier and non-susceptible genotype on growth of pigs, eating quality of pork and carcass composition (Murray et al., 1989; Dovc et al., 1996). Fujii et al., (1991) showed that point mutation at nucleotide 1843 (cytosine to thymine transition) within gene of calcium release channel of sceletal muscle sacroplasmic reticulum, also called ryanodine receptor gene (RYR1 locus), is responsible for stress-induced malignant hyperthermia.

The previous halothane test picks out only those animals that have two copies of the mutant gene. Those with only one copy, but which are still carriers of the gene, can not be separated from normal animals. The DNA probe has considerably higher accuracy than it was by previous halothane testing method. The new method identifies both reactors and those pigs that do not react but are carriers of the mutant gene which leads to Porcine Stress Syndrome (PSS). The DNA method allows very precise manipulation of the halothane gene in selected lines in order to achieve improved growth performance and carcass quality without the risk of increasing stress problems and associated effects on meat quality.

During the last decade the Estonian meat market has changed considerably. Consumers have started to require quality meat and meat products based on environmental, ethic and welfare concerns. Whether the acceptable pig carcass is fat or lean depends more on national predilection. As industrialisation develops, the desire for lean meat appears to

dominate the definition of carcass quality (Whittemore, 1996). Different methods to estimate meat content and pork quality have been used during the times (Kempster and Evans, 1979).

The aim of this study was to estimate the meat quality of live pigs and their carcasses, to investigate the effect of breed combinations and stress susceptibility on the pork quality.

Material and Methods

Hundred ninety-three pigs were tested ultrasonically between 1998...1999 in Kehtna Swine Testing Station. Animals originated from 22 different farms over Estonia. All pigs were kept according to the rules of control fattening, where two pigs were kept in the pen during testing time (at 25 to 100 kg) in stable feeding conditions. Five groups of purebred and crossbred pigs were under observation - purebred Estonian Landrace (EL), Estonian Large White (ELW), Finnish Yorkshire (FY) and crossbred Hampshire ♂ x ELW ♀ (H/ELW), H/EL/ELW ♂ x EL ♀ (H/EL/ELW x EL).

Live Animal Measurements. Ultrasonic measurements, backfat thickness and diameter of loin eye, were made with Piglog 105 and A-Scan Plus. Pigs were tested one day before slaughter. The following traits were recorded: backfat thickness at last (*x1*) and 11...12th (*x3*) rib, 7 cm from midline (mm), and diameter of loin eye (*x2*), 7 cm from midline (mm). Lean meat percentage (*y*) was calculated using the formula: $y = 64.39 - 0.28x1 + 0.14x2 - 0.55x3$ (Piglog 105…, 1991).

During ultrasonic testing weight, date and origin farm were registered, where testing weight was 93...112 kg. Testing year was divided into four parts: spring - March, April, May; summer - June, July, August; fall - September, October, November; and winter - December, January, February.

Carcass Measurements. All pigs were slaughtered on next day after ultrasonic testing in Valga Meat and Canning Factory. Ultrasonic measurements were made on slaughter day,

where carcasses were evaluated with an Ultra-FOM 100 in the same points as described above. Carcass data as carcass length, weight, backfat thickness by ruler (at scruff, at 6...7th rib, at middle and at lumbar) and pH (24 and 48 hours after slaughtering), were collected after slaughter. To measure pH and draw loin eye, half of the carcass was cut at last rib. Loin eye area was measured by planimeter, from same drawings, backfat and diameter of loin eye was measured. 48 hours after slaughtering, pH and boiling loss were found.

The General Linear Model (GLM) procedure (SAS Inst. Inc., 1991) was used for analysing the dataset by analyses of variance. The following statistical model was used: $Y_{ijkl}=\mu+Wt_{ijkl}+F_i+T_j+S_k+e_{ijkl}$, where Y - dependent variable; μ - general mean; Wt_{ijkl} - effect of pig weight at testing; F_{j-} - effect of farm 1...22; T_j - effect of breed 1...5; S_k - effect of season 1...4; e_{ijkl} - random error.

The results are given as least-square means Level of significances expressed conventionally: a, b, c – least square, within each effect with one letter in common do not differ significantly. As trait, HAL gene, has only two possible values here - 1 and 0 (HAL gene carrier or not), there are binomial distribution and suitable models to use are logistic regression and generalized linear models with appropriate link-funktions. More used link-funktion for binomial models is logit-transformation: $\text{logit}(\pi) = \ln\left(\frac{\pi}{1-\pi}\right)$, where π – probability of carrying HAL gene. Following model was used to analyse dataset: $\text{logit}(\pi_{ij}) = \eta + T_i + bX_{ij}$, where η - intercept, T_i - breed effect, X_{ij} - weight and b - regression coefficient.

Genetic Investigations. A total of 101 pigs were sampled for identification of HAL genotype. Genomic DNA was extracted from blood. PCR reaction mix with final reaction volume 28 µl contained 10xPCR Reaction Buffer (Pharmacia Biotech), 0.28 µl dNTP (20 mM DNA Polymerisation Mix, Pharmacia Biotech), 2.8 µl of each of the primers (primer 1: 5'-

GTG CTG GAT GTC CTG TGT TCC CT-3' and primer 2: 5'-CTG GTG ACA TAG TTG ATG AGG TTT G-3', Brening and Brem, 1992), 0.03 µl Taq DNA Polymerase (5U/µl, Pharmacia Biotech) and 8 µl of template DNA. After 3 min denaturation at 96˚ C, DNA was amplified for 40 cycles under the following conditions: denaturation at 94˚ C for 30 sec, annealing at 68˚ C for 30 sec and extension step at 72˚ C for 30 sec. The final extention was at 72˚ C for 10 min. The PCR product (134 bp) was digested with HhaI (Pharmacia Biotech) for 1 h at 37˚ C. The DNA fragments were separated on a 3% agarose (NuSieve 3:1) gel and stained with ethidium bromide.

Results

Measuring backfat by ultrasonic equipments and ruler, significantly thinner backfat (9.38...14.71) and higher lean meat percentage (61.17...61.95 %) were found in three breed cross (H/EL/ELW x EL), compared with other breed combinations (Table 1).

Very thick fat was measured only with Ultra-FOM 100 in H x ELW cross (Figure 1). Backfat of EL pigs differed significantly on ELW measured by A-Scan Plus and Ultra-FOM 100.

Diameter of loin eye did not differ significantly between breeds, measured by ultrasonic equipments. A significant difference, however, was found between ELW and H/EL/ELW x EL, where the diameter of loin eye (LD) of the cross was by 9.4 mm larger. As diameter of loin eye was quite equal, lean meat percentage was influenced more by differences between backfat measurements. Carcass weight did not differ between the breeds, being 70.21...72.40 kg and was lower in H/EL/ELW x EL cross and higher in FY breed (Table 3). Significantly longer carcass was observed in purebred pigs in EL (99.15 cm) and shorter in FY (93.43 cm). Crossbred pig carcasses were also significantly shorter than those of EL.

Higher fat for carcasses were measured in scruff and thinner in middle (Figure 2). As

in Ultrasonic test, thicker fat was found in ELW pig carcasses by ruler. They had significantly higher fat compared with EL and crossbred pig carcasses. Backfat was quite equal measured at 6...7 rib, middle and lumbar.

No significant differences were found between breed combinations for meat pH and boiling loss. But it should be noted, that 24 hours after slaughtering meat pH from crossbred pigs was lower than in 48 hours, while in purebred pigs this trait was higher. Meat from crossbred pigs had slightly higher boiling loss.

Genetic Analysis. On the basis of DNA test the pigs were assigned into two groups HAL normal homozygous (NN, n=85) and HAL hetrozygous (Nn, n=16). The HAL homozygous mutant (nn) animals among investigated pigs were not found (Table 3). The frequency of HAL gene carriers was 0.158.

There was significant relationship between testing weight and HAL gene ($p<0.05$), when breed effect was skipped (Table 4). To predict probability of carrying HAL gene according to testing weight, it was found, that possibility to carry HAL gene is lower in heavier pigs of the same age (Figure 3).

Discussion

Most scientists have found, that meat traits are hardly influenced by crossbreeding, as they are average or highly heritable (Skarman, 1965; Andersson, 1980). Meat traits are heredity as intermediate in crossbreeding. As Hampshire is well known in world by its thin fat and high lean meat percentage (Whittemore, 1996), it has a significant influence on crossbred pig meat quality. Thicker backfat of ELW caused thicker backfat in H x ELW cross, compared with H/EL/ELW x EL cross. Quite surprising was thick fat of purebred FY pigs, which was not, however, significantly different compared with other purebred pigs.

To compare between two points of ultrasound measurement, equal fat was found by

A-Scan (difference 1.16...1.91). In case of using Piglog 105 the backfat thickness varied between 0.66...4.19 mm in different places of measurement. Inversely to other equipments, thinner fat in x1, than x2 was found by using Ultra-FOM 100, except for three breed crosses.

As market demands more and more quality lean meat and pig selection for breeding considers more about meat quality traits, the different possibilities to measure live pig meat quality and enhance measuring accuracy must be investigated. Moreover, the ability of local and imported breeds to produce quality meat by crossing must be estimated. According to trial results, crossing Estonian sows with Hampshire boar gave thin fat and high lean meat percentage. From local breeds, Estonian Landrace breed gave better results for producing fattening pigs.

According to DNA test, 84.2% of tested pigs were stress negative (NN) and 15.8% heterozygous (Nn). The HAL homozygous mutant (nn) animals among investigated pigs were not found. In Finnish Yorkshire and crossbreed groups animals with mutant n allele were not determined. The frequency of n allele was 0.075 and 0.125 of investigated EL and ELW pigs, respectively. According to previous investigation in Estonia was found that 77% of EL pigs in one population were stress negative (NN) and 23% heterozygous, the frequency of n was 0.115 (Birkenfeld and Viinalass, 1999).

At present, several breeds are used in Estonia for improving local breeds, including Hampshire and Piertain breeds, which are characterised by high frequency of halothane sensitivity gene. It has been shown by Sellier (1998) that the frequency of HAL gene is varying from 0 to 0.97 among the world's breeds, being with the highest frequency in Pietrain breed. To investigate effect of breed combinations to pork quality, more extensive screening of pigs for PSS is necessary, to identify the HAL sensitivity gene carrier boars in order to compose breeding schemes. The main reason, why there was no influence between breed and HAL gene, was the different number of animals in pure and crossbreed groups.

Table 1. *Least-square means of meat traits measured by ultrasonic equipments in different pig breed crossing combinations*

Trait		EL	ELW	FY	H x ELW	H/EL/ELW x EL
		n=137	38	7	7	4
A-Scan	x1 *(mm)*	16.14^{b}	20.76^{c}	19.85^{bc}	18.10^{bc}	11.15^{a}
	x2 *(mm)*	54.21^{a}	55.03^{a}	58.86^{a}	56.65^{a}	53.84^{a}
	x3 *(mm)*	14.43^{b}	19.25^{c}	18.69^{bc}	16.19^{bc}	9.38^{a}
	y *(%)*	59.52^{b}	55.69^{c}	56.79^{bc}	58.35^{bc}	63.65^{a}
Piglog 105	x1 *(mm)*	18.52^{bc}	21.75^{c}	21.07^{bc}	18.00^{b}	11.02^{a}
	x2 *(mm)*	47.72^{a}	46.57^{a}	46.11^{a}	47.11^{a}	46.52^{a}
	x3 *(mm)*	17.60^{bc}	18.85^{c}	19.63^{bc}	13.81^{ab}	10.36^{a}
	y *(%)*	56.06^{bc}	54.27^{c}	53.91^{bc}	57.85^{ab}	61.95^{a}
Ultra FOM	x1 *(mm)*	17.32^{ac}	24.02^{b}	23.09^{bc}	23.54^{bc}	14.71^{a}
	x2 *(mm)*	50.50^{a}	50.74^{a}	50.69^{a}	45.10^{a}	54.26^{a}
	x3 *(mm)*	17.41^{a}	25.79^{bc}	22.03^{ac}	25.30^{bc}	13.94^{a}
	y *(%)*	57.32^{b}	50.88^{c}	53.29^{bc}	52.17^{bc}	61.17^{a}
By ruler	backfat (x1) *(mm)*	13.78^{ab}	18.67^{b}	21.09^{b}	13.88^{ab}	8.59^{a}
	diameter of LD *(mm)*	58.25^{ab}	52.96^{b}	54.47^{ab}	57.26^{ab}	62.36^{a}
	area of LD *(cm^2)*	37.99^{a}	33.42^{b}	36.01^{ab}	39.96^{a}	41.97^{a}

x1 - backfat thickness at last

x3 - backfat thickness at $11...12^{th}$ rib, 7 cm from midline

x2 - diameter of loin eye, 7 cm from midline

y - lean meat percentage

EL - purebred Estonian Landrace , ELW - purebred Estonian Large White, FY - purebred Finnish Yorkshire, H/ELW - crossbred Hampshire ♂ x Estonian Large White ♀,

a, b, c – level of significances, least squares, within each effect with one letter in common do not differ significantly

Table 2. *Least-square means of meat traits in different pig breed combinations after slaughter*

Trait		EL	ELW	FY	H x ELW	H/EL/ELW x EL
Carcass	weight *(kg)*	71.45[a]	71.79[a]	72.40[a]	71.58[a]	70.21[a]
	length *(cm)*	99.15[b]	97.07[ab]	93.43[a]	95.07[a]	94.24[a]
Backfat at *(by ruler)* scruff *(mm)*		35.39[a]	44.12[b]	39.93[ab]	36.10[a]	30.89[a]
	6...7th rib *(mm)*	23.29[a]	25.15[a]	22.29[a]	20.51[a]	18.23[a]
	middle *(mm)*	19.01[a]	21.54[a]	19.60[a]	18.40[a]	15.32[a]
	lumbar *(mm)*	32.33[a]	31.34[a]	27.71[a]	29.79[a]	27.48[a]
Average		27.70[b]	30.91[b]	28.35[ab]	27.31[ab]	22.78[a]
pH 24		5.56[a]	5.57[a]	5.51[a]	5.57[a]	5.41[a]
pH 48		5.51[a]	5.54[a]	5.35[a]	5.60[a]	5.49[a]
pH difference		0.05	0.03	0.16	-0.03	-0.08
Boiling loss *(%)*		44.46[a]	43.04[a]	43.19[a]	45.12[a]	45.29[a]

a, b, c – level of significances, least squares, within each effect with one letter in common do not differ significantly

Table 3. Descriptive statistics of genetic analysis

	Breed					Total
	EL	ELW	FY	H x ELW	H/EL/ELW x EL	
No. of animals	73	20	2	3	3	101
Frequency of HAL gene carriers	0.151	0.25	0	0	0	0.158

Table 4. *Difference between testing weight according to HAL gene (0 - not carrier, 1 -carrier)*

	HAL gene		Total
	0	1	
No. of animals	85	16	101
Average testing weight	100.87	99.06	100.58

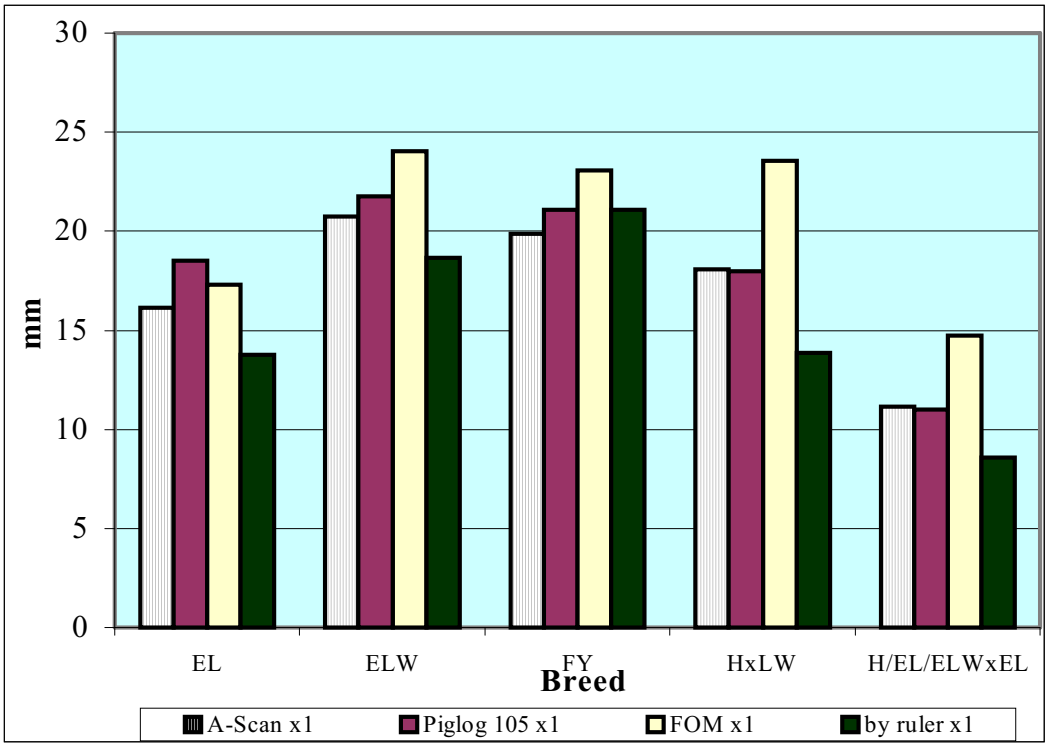

Figure 1. *Backfat thickness measured by ultrasonic equipments in different pig combinations*

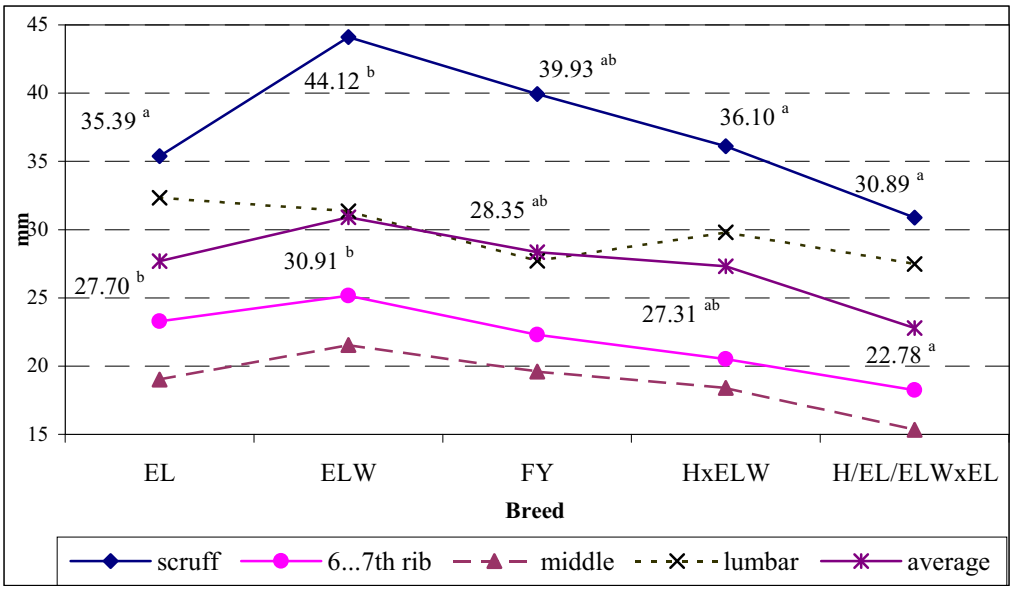

Figure 2. *Carcass backfat depth measured by ruler*

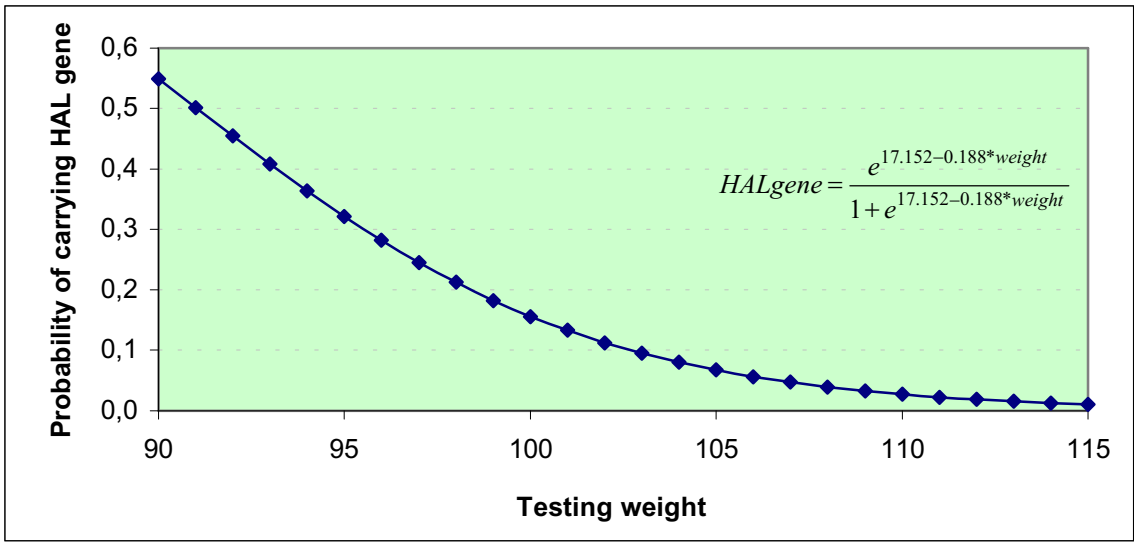

Figure 3. *Probability of carrying HAL gene according to testing weight (at the same age)*

References

Andersson, K. (1980) *Studies on Crossbreeding and Carcass Evaluation in Pigs*. Swedish University of Agricultural Sciences. Report 46. Uppsala. 126 pp.

Brening, B., Brem, G. (1992) Molecular cloning and analysis of the porcine "halothane" gene. *Archiv für Tierzucht*, Dummerstorf, **35** (1/2), 129-135.

Birkenfeldt, M., Viinalass, H. (1999) Sigade stressisündroom [Porcine Stress Syndrome]. *Eesti Loomaarstlik Ringvaade,* **XXV** (1), 7-10. (in Estonian).

Dovc P., Šalehar A., Kovac M., Kastelic M. (1996) Frequency of the RYR1 n allele and its influence on fattening traits. *Archiv für Tierzucht*, Dummerstorf **39**, (4), 441-446.

Fujii J., Otsu K., Zorzato F., De Leon S., Khanna V.K., Weiler J., O'Brien P. J., MacLennan D.H. (1991) Identification of a Mutation in Porcine Ryanodine Receptor Associated with Malignant Hyperthermia. *Science* **253**, 448-451.

Kempster, A.J., Evans, D.G. (1979) A comparison of different predictors of the lean content of pig carcasses. 1. Predictors for use in commercial classification and grading. Animal Production **28**, 87-96.

Murray A.C., Jones S.D., Sather A.P. (1989) The effect of preslaughter feed restriction and genotype for stress susceptibility on pork lean quality and composition. *Canadian Journal of Animal Science* **69**, 83-91.

Piglog *105* (1991) *Piglog 105 User's Guide.* Soborg, Denmark: SFK - Technology, 14 pp

Santoro P., Faucitano L. (1996) Stress in pig production. *Pig News and Information* **17**, (2), 49N-52N.

SAS. (1991) *SAS User's guide: Statistics*. SAS Inst. Inc., GARY, NC. 305 pp.

Sellier, P. (1998) Genetics of Meat and Carcass Traits. In: *The Genetic of Pig*. Rotshchild, F. M., Ruvinsky, A. (ed.). CAB International, pp. 463-510.

Skarman, S. (1965) Crossbreeding Experiment with Swine. *Lantbrukshögskolans Annaler*. Uppsala, 31, 3-92.

Whittemore, C. (1996) In: *The Science and Practice of Pig Production*. Longman Scientific & Technical. Longman Group UK Limited, England, pp. 5-82.

Acknowledgments

The investigations were supported by the Estonian Science Foundation (Grant No 0171417S99 and Grant No 3153). The authors wish to express their gratitude to Kehtna Swine Testing Station and Valga Meat and Canning Factory.