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PORK QUALITY AND PORCINE STRESS SYNDROME IN ESTONIA

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The effect of breed combinations and the HAL gene to pork quality were analysed. In total, 193 pigs (purebred Estonian Landrace (EL), Estonian Large White (ELW), Finnish Yorkshire (FY), and crossbred Hampshire (H)♂xELW♀, H♂x(ELW×EL)♀) were investigated. The following traits were recorded using ultrasonic equipment (Piglog 105, A-Scan Plus, and Ultra FOM 100): backfat thickness at last and 11–12th rib and the diameter of loin eye. The lean meat percentage was calculated. Carcass length, weight, and backfat thickness measurements in four points, and pH (24 h after slaughtering) were measured. Loin eye area was measured by planimeter; pH and boiling loss were determined 48 hours after slaughtering. Blood samples were collected from 101 pigs. DNA tests were carried out by restriction analysis (PCR–RFLP – polymerase chain reaction restricted fragment length polymorphism). Higher backfat thickness and a lower lean meat percentage was found in the ELW and FY breeds. EL pigs had significantly longer carcasses than FY and crossbred pigs. EL and H breeds had superior meat quality. DNA tests showed 84.2 % of the tested pigs to be stress negative (NN) and 15.8 % were heterozygous (Nn). HAL homozygous mutant (nn) animals were not found. A significant relationship between testing weight and the presence of HAL gene (P<0.05) was found, when breed effect was not considered.

Key words: *pigs, carcass measurements, pork quality, Porcine Stress Syndrome, 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 has been improved by reduction of pale, soft, and exudative (PSE) meat. Porcine Stress Syndrome (PSS) is a well documented genetic disorder that is transmitted by a single autosomal recessive gene. It causes major economic loses in the pig industry by sudden death of pigs and 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. Researchers have shown that the halothane carrier and non-susceptible genotype can affect the growth of pigs, the eating quality of pork, and carcass composition (Murray *et al.*, 1989; Dovc *et al.*, 1996). Fujii *et al.* (1991) showed that a point mutation at nucleotide 1843 (cytosine to thymine transition) within the gene for the calcium release channel of skeletal muscle sacroplasmic reticulum, also called the ryanodine receptor gene (RYR1 locus), is responsible for stress-induced malignant hyperthermia.

The halothane test used earlier identifies only those animals that have two copies of the mutant gene. Those with only one copy, but which are still carriers of the gene, cannot be separated from normal animals. The DNA probe has considerably higher accuracy than the halothane testing method. The new method identifies both reactors and those pigs that do not react but are carriers of the mutant gene causing 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 the associated effects on meat quality.

During the last decade, the Estonian meat market has changed considerably. Consumers have started to demand improved quality of 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 over time (Kempster and Evans, 1979).

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

MATERIALS AND METHODS

Pigs. A total of 193 pigs at the age of six months were tested ultrasonically between 1998 and 1999 in the Kehtna Swine Testing Station. The animals had been raised from birth till weaning in 22 different farms over Estonia. All pigs were raised according to the rules of controlled fattening, where two pigs were housed in a pen during the testing period (at 25 to 100 kg) in stable feeding conditions. Five groups of purebred and crossbred pigs were studied – three purebred: Estonian Landrace (EL; n=137), Estonian Large White (ELW; n=38), Finnish Yorkshire (FY; n=7) and two crossbred: Hampshire (H) ♂ x ELW ♀ (H×ELW; n=7), and H ♂ x (ELW×EL) ♀ (H x (ELW×EL); n=4).

Live Animal Measurements. Ultrasonic measurements of backfat thickness and diameter of loin eye were made using a Piglog 105 (SFK Technology A/S, Denmark) and A-Scan Plus (Sonic Industries, Inc., USA). 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 the midline (mm), and diameter of the loin eye (*x2*) 7 cm from the midline (mm). Lean meat percentage (*y*) was calculated using the formula: $y = 64.39–0.28x1+0.14x2–0.55x3$ (Anonymous, 1991a).

During ultrasonic testing, weight (93–112 kg), date, and farm of origin were recorded.

Carcass measurements. All pigs were slaughtered on the day after ultrasonic testing of live animals, in the Valga Meat and Canning Factory. Ultrasonic measurements on carcasses were made immediatly after slaughtering, using an Ultra-FOM 100 (SFK Technology A/S, Denmark), at the same points as described above for live animals. Carcass data regarding 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. A portable MP120 pH Meter (Mettler Toledo, Switzerland) was used for measuring the pH. Half of the carcass was cut at last rib to measure pH and to draw the loin eye area onto tracing-paper. Loin eye area was measured by planimeter; and from the same drawings, backfat and diameter of loin eye were measured. 48 hours after slaughtering, pH and boiling loss were measured in the laboratory of Estonian Agricultural University.

The General Linear Model (GLM) procedure (Anonymous, 1991b) was used for analysis of variance. The following statistical model was used:

$Y_{ijkl}=\mu+Wt_{ijkl}+F_i+T_j+S_k+e_{ijkl}$, where Y was 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. For season, the testing year was divided into four periods: spring (March–May); summer (June–August); fall (September–November); and winter (December–February).

The results are given as least-square means. As a trait, the HAL gene has only two possible values: 1 and 0 (HAL gene carrier or not). As the distribution for HAL is binomial, suitable models to use are logistic regression and generalised linear models with the appropriate link-functions. A common link-function used for binomial models is logit-transformation: $\text{logit}(\pi) = \ln\left(\frac{\pi}{1-\pi}\right)$, where π is the probability of carrying a HAL gene. The following model was used to analyse the dataset: $\text{logit}(\pi_{ij}) = \eta + T_i + bX_{ij}$, where η was intercept; T_i, breed effect; X_{ij}, weight; and b, regression coefficient.

Genetic investigations. A total of 101 pigs were tested for the presence of the HAL genotype. Genomic DNA was extracted from blood. A PCR reaction mixture 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 (5 U·µl^{-1}, Pharmacia Biotech), and 8 µl of template DNA. After 3 min denaturation at 96 ^{o}C, DNA was amplified for 40 cycles under the following conditions: denaturation at 94 ^{o}C for 30 s, annealing at 68 ^{o}C for 30 s, and extension at 72 ^{o}C for 30 seconds. The final extention was at 72 ^{o}C for 10 minutes. The PCR product (134 bp) was digested with HhaI (Pharmacia Biotech) for 1 h at 37 ^{o}C. Digestion of this product by HhaI yields two fragments of 84 and 50 bp for normal animals (NN), three fragments of 134, 84, and 50 bp for heterozygotes (Nn) and only the 134 bp DNA fragment for mutant homozygous (nn) individuals. The DNA fragments were separated on a 3% agarose (NuSieve 3:1) gel and stained with ethidium bromide.

RESULTS

Significantly thinner backfat (9.38–14.71) and a higher lean meat percentage (61.17–61.95 %) were found in a cross of three breeds [H x (ELW×EL)], compared with other breed combinations (Table 1).

Very thick fat was found only in the H x ELW cross when measured with an Ultra-FOM 100 (Figure 1). Backfat thickness of EL pigs differed significantly from that of ELW pigs (using an A-Scan Plus and Ultra-FOM 100).

Thicker backfat for carcasses was observed when measured by ruler at the scruff and thinner in the middle (Figure 2). As in the ultrasonic test, thicker fat was found in ELW pig carcasses when measured by ruler. They had significantly thicker backfat compared with EL and crossbred pig carcasses. Backfat thickness was similar between breeds when measured at 6–7 rib, middle, and lumbar.

Diameter of loin eye (ultrasonic measurement) did not differ significantly between breeds. However, the loin eye diameter was larger by 6–4 mm in H x (ELW×EL) compared to that in ELW. As the diameter of loin eye was not very variable, the lean meat percentage was influenced more by

Table 1

LEAST-SQUARE MEANS OF MEAT TRAITS (MEASURED BY ULTRASONIC EQUIPMENT AND BY RULER) IN DIFFERENT PIG BREED CROSSING COMBINATIONS

Measured by	Trait	EL	ELW	FY	H x ELW	H x ELW/EL
		n=137	n=38	n=7	n=7	n=4
A-Scan Plus	*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 100	*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}
Ruler	*x1*, mm	13.78^{ab}	18.67^{b}	21.09^{b}	13.88^{ab}	8.59^{a}
	diameter of LE, mm	58.25^{ab}	52.96^{b}	54.47^{ab}	57.26^{ab}	62.36^{a}
	area of LE, cm2	37.99^{a}	33.42^{b}	36.01^{ab}	39.96^{a}	41.97^{a}

x1, backfat thickness at last rib; *x2,* diameter of loin eye, 7 cm from midline; *x3,* backfat thickness at 11–12th rib, 7 cm from midline; *y,* lean meat percentage; LE, loin eye; EL, purebred Estonian Landrace; ELW, purebred Estonian Large White; FY, purebred Finnish Yorkshire; H×ELW, crossbred Hampshire ♂ x Estonian Large White ♀; H x (ELW×EL), crossbred Hampshire ♂ x Estonian Large White / Estonian Landrace ♀; $^{a, b, c}$, level of significances, least squares, within each effect one letter in common indicates no significant differences

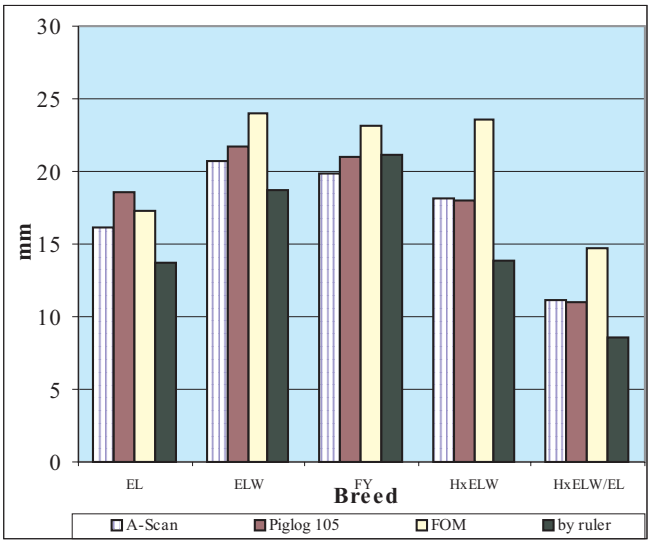

Fig. 1. Backfat thickness (*x1*) measured by ultrasonic equipment and by ruler in different pig combinations. EL, purebred Estonian Landrace; ELW, purebred Estonian Large White; FY, purebred Finnish Yorkshire; H x ELW, crossbred Hampshire ♂ x Estonian Large White ♀; H x ELW/EL, Hampshire ♂ x Estonian Large White / Estonian Landrace ♀.

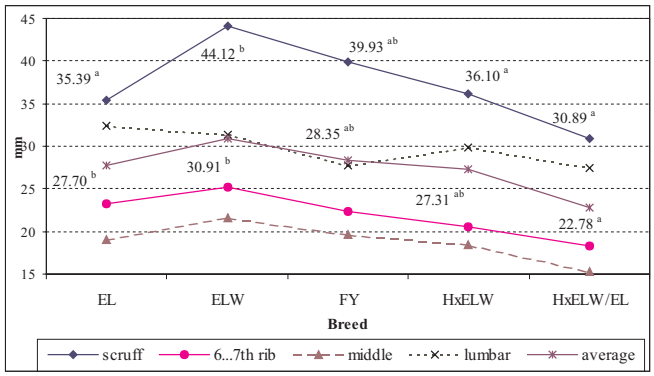

Fig. 2. Carcass backfat thickness measured by ruler. EL, purebred Estonian Landrace; ELW, purebred Estonian Large White; FY, purebred Finnish Yorkshire; H x ELW, crossbred Hampshire ♂ x Estonian Large White ♀; H x (ELW×EL), Hampshire ♂ x Estonian Large White / Estonian Landrace ♀; $^{a, b, c}$, level of significances, least squares, one letter in common indicates no significant differences.

differences between backfat thickness. Carcass weight did not differ between the breeds, varying between 70.21 and 72.40 kg and being lower in the H x (ELW×EL) cross and higher in the FY breed (Table 2). A significantly longer carcass was observed in EL purebred pigs (99.15 cm) and a shorter carcass in FY (93.43 cm). Crossbred pig carcasses were also significantly shorter than those of EL.

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

Genetic analysis. On the basis of the DNA test, the pigs were assigned into two groups: HAL normal homozygous (NN, n=85) and HAL hetrozygous (Nn, n=16). HAL homozygous mutant (nn) animals were not found among the in-

Table 2

LEAST-SQUARE MEANS OF MEAT TRAITS IN DIFFERENT PIG BREED COMBINATIONS AFTER SLAUGHTER

Trait		EL	ELW	FY	H x ELW	H x (ELW×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 scruff (by ruler, 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]

EL, purebred Estonian Landrace; ELW, purebred Estonian Large White; FY, purebred Finnish Yorkshire; H×ELW, crossbred Hampshire ♂ x Estonian Large White ♀; H x (ELW×EL), crossbred Hampshire ♂ x (Estonian Large White × Estonian Landrace) ♀; [a, b, c], level of significances, least squares, one letter in common indicates no significant differences.

Table 3

DESCRIPTIVE STATISTICS OF GENETIC ANALYSIS OF DIFFERENT PIG BREEDS

	EL	ELW	FY	H x ELW	H x (ELW×EL)	Total
N of animals	73	20	2	3	3	101
Frequency of HAL gene carriers	0.151	0.25	0	0	0	0.158

EL, purebred Estonian Landrace; ELW, purebred Estonian Large White; FY, purebred Finnish Yorkshire; H×ELW, crossbred Hampshire ♂ x Estonian Large White ♀; H x (ELW×EL), crossbred Hampshire ♂ x (Estonian Large White × Estonian Landrace) ♀

vestigated pigs (Table 3). The frequency of HAL gene carriers was 0.158.

There was significant relationship between testing weight and the HAL gene (P<0.05) when breed effect was ignored (Table 4). Investigation of the HAL gene carriers showed that the probability of carrying the HAL gene was lower in heavier pigs of the same age (Figure 3).

Table 4

DIFFERENCE BETWEEN TESTING WEIGHT ACCORDING TO PRESENCE OF THE HAL GENE (0, not carrier, 1, carrier)

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

DISCUSSION

Many studies have shown that meat traits are hardly influenced by crossbreeding, as they are average or highly heritable (Skarman, 1965; Andersson, 1980). Meat traits are inherited as intermediate in crossbreeds. The Hampshire breed is well known by its thin fat and high lean meat percentage (Whittemore, 1996), and it has a significant influence on crossbred pig meat quality. Thicker backfat of ELW caused thicker backfat in the HxELW cross, compared with the H x (ELW×EL) cross. The thick fat of purebred FY pigs was surprising as it was not significantly different when compared with other purebred pigs.

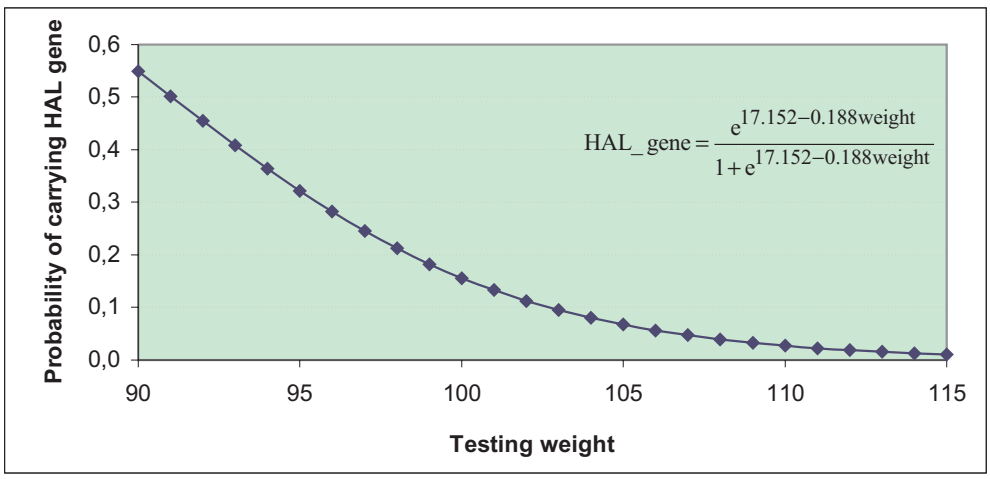

Fig. 3. Probability of carrying the HAL gene according to testing weight (at the same age). e, constant (e=2.718)

Backfat thickness was comparable between two points of ultrasound measurement (using A-Scan, difference 1.16–1.91). Measurements using a Piglog 105 varied between 0.66–4.19 mm in different locations. In contrast to the other equipments, thinner fat in *x1*, than in *x2* was found when using an Ultra-FOM 100, except for three breed crosses.

As the market demands more and more quality lean meat, and since pig selection for breeding now considers these traits, efforts should be made to improve the accuracy and precision of measurement of live pig meat quality. Moreover, the ability of local and imported breeds to produce quality meat by crossing must be estimated. According to the trial results, crossing Estonian sows with Hampshire boar gave thin fat and a high lean meat percentage. From local breeds, the Estonian Landrace breed gave better results for producing fattening pigs.

According to the DNA test, 84.2 % of the tested pigs were stress negative (NN) and 15.8 % were heterozygous (Nn). The HAL homozygous mutant (nn) animals was not found among the investigated pigs. In Finnish Yorkshire and crossbreed groups, animals with a mutant n allele were not found. The frequency of the n allele was 0.075 and 0.125 among the investigated EL and ELW pigs, respectively. According to a previous investigation in Estonia, 77 % of EL pigs in one population were stress negative (NN), 23 % were heterozygous, and 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 Pietrain breeds which are characterised by a high frequency of the halothane sensitivity gene. It has been shown by Sellier (1998) that the frequency of the HAL gene varies from 0 to 0.97 among the world's breeds, with the highest frequency in the Pietrain breed. To investigate the effect of breed combinations on 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 correlation between breed and HAL gene was the different number of animals in pure and crossbred groups.

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CŪKGAĻAS KVALITĀTE UN STRESA SINDROMS CŪKĀM IGAUNIJĀ

Analizēja cūku šķirņu krustojumu un HAL gēna ietekmi uz cūkgaļas kvalitāti. Kopā pētīja 193 sivēnus no trim šķirnēm: Igaunijas Landrases (EL), Igaunijas Lielās Baltās (ELW) un Somu Jorkšīras (FY), kā arī no diviem Hempšīras šķirnes krustojumiem – (H)♂xELW♀ un H♂x(ELWxEL)♀. Ar ultraskaņas iekārtām (Piglog 105, A-Scan Plus un Ultra FOM 100) reģistrēja muguras zemādas tauku slāņa biezumu pēdējās un 11.–12. ribas līmenī, kā arī muguras garā muskuļa diametru. Aprēķināja liesās gaļas īpatsvaru. 24 stundas pēc nokaušanas mērīja kautķermeņa garumu, svaru, muguras zemādas tauku slāņa biezumu četrās vietās un pH. "Muskuļacs" laukumu izmērīja ar planimetru; vielu zudumu vārīšanās temperatūrā un pH noteica 48 stundas pēc nokaušanas. Asins paraugus ievāca no 101 sivēna. DNS testu veica, izmantojot PCR-RFLP metodi. Biezāks zemādas tauku slānis un zemāks liesās gaļas īpatsvars bija ELW un FY šķirņu cūkām. EL šķirnes cūkām bija statistiski ticami garāks ķermenis nekā, FY un hibrīdām cūkām. EL un H šķirnēm bija labāka gaļas kvalitāte. DNS tests parādīja, ka 84,2 procenti pētīto cūku ir stresa negatīvas (NN) un 15.8 % – heterozigotiska (Nn). HAL homozigotie mutanti (nn) dzīvnieku starpā netika atrasti. Izslēdzot šķirnes efektu, konstatēta būtiska sakarība starp kautsvaru un HAL gēna klātbūtni (P<0.05).