EFFECT OF INTROGRESSION OF WILD BOAR INTO LITHUANIAN INDIGENOUS WATTLE PIGS ON FAT COMPOSITION IN PORK UNDER CONVENTIONAL REARING

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Summary. The animals used in the study were females and castrated male hybrids from Lithuanian indigenous wattle pigs and their backcross with wild boar, containing 1/4 of wild boar. The muscles of hybrid pigs had a higher content of dry matter (P<0.05) than the muscles of purebred Lithuanian indigenous wattle pigs. The introgression of wild boar into Lithuanian indigenous wattle pigs under conventional rearing conditions slightly decreased (P=0.072) the proportion of saturated fatty acids, including C18:0 (P<0.05) in intramuscular fat. Although the introgression of wild boar did not appear to affect significantly the proportions of MUFA, the concentration of the individual (C20:1) fatty acid was lower (P<0.05) in the meat from 1/4 WB genotype. The proportions of PUFA were insignificantly higher in the intramuscular fat of 1/4 WB genotype compared with purebred Lithuanian indigenous wattle pigs. The introgression of wild boar had a higher effect on the proportions of fatty acids in the subcutaneous tissue compared with the effect on the proportions of fatty acids in the intramuscular fat. The concentrations of SFA (P<0.001) were lower in the subcutaneous tissue of 1/4 WB genotype compared with Lithuanian indigenous wattle pigs. The hybrids had lower concentrations of SFA (P<0.001), including C16:0 (P=0.081), C18:0 (P<0.001) and C20:0 (P=0.052) acids and higher concentration of C16:1 (P<0.05), lower concentration of C20:1 (P<0.01) and higher concentrations of PUFA (P<0.05). Also, there was a more favourable PUFA/SFA ratio (P<0.01) in the subcutaneous tissue of hybrids compared with purebred pigs. Gender had a higher effect on the fatty acid composition in the intramuscular fat and subcutaneous tissue from Lithuanian indigenous wattle pigs compared with the gender effect on the fatty acid composition in 1/4 WB genotype hybrids.
Keywords: swine, wild boar, introgression, fatty acids, intramuscular fat, subcutaneous tissue

ĮTERPTO ŠERNO ĮTAKA LIETUVOS VIETINIŲ KIAULIŲ HIBRIDŲ, IŠAUGINTŲ ĮPRASTOMIS SĄLYGOMIS, MĖSOS RIEBALŲ SUDĖČIAI

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Santrauka. Tyrimams sudarytos Lietuvos vietinių kiaulių ir jų hibridų, kuriems įterpta 1/4 šerno, grupės (kiaulaitės ir kastratai). Hibridų raumenyse sausųjų medžiagų buvo daugiau (p<0,05), negu grynaveislių Lietuvos vietinių kiaulių raumenyse. Saikingas šerno dalies (1/4) įterpimas į Lietuvos vietines kiaules ir gautų hibridų auginimas įprastomis kiaulių auginimo sąlygomis nežymiai sumažino sočiųjų (p=0,072) ir padidino nesočiųjų (p>0,10) riebalų rūgščių kiekį raumenų riebaluose. Nors šerno įterpimas patikimai nepakeitė bendro mononesočiųjų riebalų rūgščių santykio, atskirų rūgščių, tokių kaip C20:1, hibridų mėsoje buvo mažiau (p<0,05), negu Lietuvos vietinių kiaulių mėsoje. Didesnę įtaką įterptas šernas turėjo poodinio riebalų audinio sudėčiai. 1/4 šerno genotipo hibridų riebaliniame audinyje buvo mažiau sočiųjų riebalų rūgščių (p<0,001) ir daugiau C16:1 (p<0,05), bet mažiau kitos C20:1 (p<0,01) iš mononesočiųjų riebalų rūgščių ir daugiau polinesočiųjų riebalų rūgščių (p<0,05), negu Lietuvos vietinių kiaulių poodiniuose riebaluose. Palyginti su grynaveislėmis kiaulėmis hibridų poodiniuose riebaluose taip pat buvo geresnis polinesočiųjų ir sočiųjų riebalų rūgščių santykis (p<0,01). Gyvūnų lytis didesnę įtaką turėjo Lietuvos vietinių kiaulių raumenų ir poodinio audinio riebalų rūgščių sudėčiai negu hibridų.
Raktažodžiai: kiaulės, šernai, įterpimas, riebalų rūgštys, raumenų riebalai, poodinis riebalinis audinys.

Introduction. Meat plays a significant role in human nutrition. In recent years awareness of the diet importance for human health has increased. Many authorities have recommended that the contributions of fat and especially saturated fatty acids to dietary energy intake should be reduced (Wood and Enser, 1997). However, since fat contributes succulence to pork cuts and manufactured pork products, total removal of carcass fat is undesirable (Darling et al., 1998). There are also a few vitamins (A, D, E, F) which, being fat soluble, require a certain amount of fat in the diet to enable the supply and absorption of these substances. An alternative to reducing the amount of carcass fat is to modify the fatty acid profiles so that they are better aligned with nutritional goals. Therefore, fatty acid composition in pork has received considerable interest in view of its implications for consumer health and for meat quality characteristics. The meat quality concept has become dynamic and includes not only eating and technological quality but also diversity, meat functionality, nutritional value and safety (Oksbjerg et al., 2005). Scientific evidence is accumulating that meat itself is not a risk factor for western lifestyle diseases such as cardiovascular disease, but rather the risk stems from the excessive fat and particularly saturated fat associated with the meat of

modern domesticated animals (Mann, 2000; Li et al., 2005). During long time, evolutionary selection was in action, adapting our genetic make up and hence our physiological features to the diet high in lean meat (Mann, 2000). This meat was wild game meat, low in total and saturated fat and relatively rich in polyunsaturated fatty acids (Mann, 2000; Cordain et al., 2002; Li et al., 2005). In recent years wild mammals represent only 2% of the herbivore biomass (Gorman and Raffaelli, 2008), therefore an alternative to increase the amount of relatively "wild" meat could be captive rearing of wild species or their crossbreeding with domestic animals. Studies describing the quality of wild boar meat have shown its advantage over pork (Koizumi et al., 1991; Meyer et al., 1998; Źmijewski et al., 2001; Marchiori et al., 2003), and therefore introgression of wild boar into domestic pig genotype should be used for food diversification. In Lithuania, with a growing interest in captive wild boar breeding, there is an interest in the domestic pig and wild boar crossbreeding. Wild boar and their hybrids have a slower growth rate than domestic pigs, therefore, it is very important to estimate expedient introgression level into domestic pigs. The rearing of entire male pigs is avoided in Lithuania because of its association with boar taint. Therefore, this study was set up to evaluate the fatty acid composition in intramuscular fat and subcutaneous tissue from females and castrated males of Lithuanian indigenous wattle pigs and their hybrids with wild boar introgression (1/4 WB genotype).

Material and methods. Forty-three animals used were females and castrated male hybrids from Lithuanian indigenous wattle pigs and their backcross with wild boar (Lithuanian indigenous wattle x wild boar) x Lithuanian indigenous wattle containing 1/4 of wild boar. All animals were born at the Institute of Animal Science of the Lithuanian Veterinary Academy and reared indoors under similar conditions from birth to slaughter consuming twice a day the same standard concentrate feed, containing 12.2 MJ metabolisable energy and 14.5% crude protein balanced with lysine (0.78%/kg feed). When the pigs reached the mean final body weight of about 90 kg, the slaughtering was conducted with minimum handling stress in the abattoir for control slaughtering of the state pig breeding station after 5 km transportation immediately prior to slaughter in accordance with the technological standards adopted in meat processing plants. The samples were removed from the *longissimus dorsi* (LD) and subcutaneous fat at the level of the 1-2 lumbar vertebra of the left sides of carcasses after chilling for 24 h at $+2^0$C - $+4^0$C temperature. The samples of *M. longissimus dorsi* were analysed in duplicate for meat chemical content. Crude protein was analysed by the Kjeldahl method using the Kjeltec system 1002 apparatus (Foss-Tecator AB); crude ash and ether extract after hydrolysis of intramuscular fat were determined according to the standard methods described in the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 1990). Extraction of lipids for fatty acid analysis was performed with chloroform/methanol (2:1 v/v) as described by Folch et al. (1957). Fatty acid methyl esters (FAME) were prepared using the procedure of Christopherson and Glass (1969). The FAMEs were analysed using a gas liquid chromatograph (GC – 2010 SHIMADZU) fitted with a flame ionization detector. The separation of methyl esters of fatty acids was effected on a ALLTECH capillary column AT Silar, 30 m x 0.32 mm x 0.25µm, by temperature programming from 100°C to 240°C. The rate of flow of carrier gas (nitrogen) through column was 0.33 ml/min. The column was operated at 100°C for 4 min, then temperature was increased to 240°C at 3°C/min and held for 10 min. The temperature of the injector and detector were held, respectively, at 225° and 250°C. The peaks were identified by comparison with the retention times of the standard fatty acids methyl esters FAME MIX (SUPELCO, USA). The relative proportion of each fatty acid was expressed as the relative percentage of the sum of the total fatty acids.

Statistical analysis was performed with the General Linear Model (GLM) procedure in MINITAB release 15. The model included genotype (purebred Lithuanian indigenous wattle pigs and 1/4WB genotype hybrids) and gender (gilts and castrates) as fixed factors for the muscle chemical composition, fat composition of intramuscular fat and subcutaneous tissue. Tukey`s HSD significance tests were used to ascertain the existence of significant differences between the traits. Significance was determined at $P<0.05$, but differences of $0.05 \leq P<0.10$ would be considered as trends.

Results. The muscles of hybrid pigs had a relatively 1.53% higher content of dry matter ($P<0.05$) than the muscles of purebred Lithuanian indigenous wattle pigs (Table 1).

There were no significant differences in protein, fat and ash contents between the genotypes. However, within both genotypes (purebred Lithuanian indigenous wattle pigs and 1/4 WB) castrated males had relatively 75.7% ($P<0.001$) and 34.1% ($P<0.01$), respectively, higher content of intramuscular fat (Table 2).

Table 1. **Least square means and standard errors of differences for chemical composition of longissimus dorsi muscle from Lithuanian indigenous pigs and 1/4 WB genotype hybrids**

Variables	Genotype		SED
	LIW (n=19)	1/4 WB genotype (n=24)	
Dry matter, %	24.89	25.27	0.17*
Protein, %	22.44	22.76	0.18
Fat, %	1.34	1.43	0.09
Ash, %	1.04	1.04	0.01

*P<0.05

Table 2. **Least square means and standard errors of differences for chemical composition of longissimus dorsi muscle by gender**

Variables	LIW			1/4 WB genotype		
	Castrates (n=9)	Gilts (n=10)	SED	Castrates (n=13)	Gilts (n=11)	SED
Dry matter, %	24.75	25.05	0.37	25.31	25.49	0.57
Protein, %	21.89	22.97	0.30**	22.54	23.13	0.57
Fat, %	1.74	0.99	0.08***	1.69	1.26	0.14**
Ash, %	1.05	1.03	0.02	1.06	1.02	0.02

**P<0.01; ***P<0.001

Table 3. **Least square means and standard errors of differences for fatty acids in intramuscular fat of longissimus dorsi muscle and subcutaneous tissue from Lithuanian indigenous pigs and 1/4 WB genotype hybrids**

Fatty acids	Intramuscular fat of longissimus dorsi			Subcutaneous tissue		
	Genotype		SED	Genotype		SED
	LIW (n=17)	1/4 WB (n=18)		LIW (n=18)	1/4 WB (n=23)	
C14:0	1.23	1.25	0.38	1.15	1.15	0.04
C16:0	26.52	26.18	0.38	26.66	25.86	0.45^{t}
C17:0	0.14	0.15	0.01	0.26	0.26	0.02
C18:0	12.48	11.67	0.34*	16.00	14.09	0.41***
C20:0	0.15	0.15	0.01	0.19	0.17	0.01^{t}
SFA	40.52	39.42	0.59^{t}	44.28	41.54	0.65***
C16:1	3.67	3.80	0.11	1.69	1.89	0.09*
C17:1	0.15	0.17	0.01^{t}	0.20	0.22	0.01
C18:1	47.38	47.55	0.51	39.25	40.23	0.66
C20:1	0.57	0.51	0.03^{t}	0.86	0.75	0.04*
MUFA	51.77	52.03	0.55	42.02	43.10	0.69
C18:2n-6	6.33	7.02	0.44	12.28	13.86	0.57**
C18:3n-3	0.24	0.26	0.01	0.63	0.68	0.05
C20:3n-3	0.24	0.25	0.02	0.60	0.60	0.03
C20:4n-6	0.72	0.79	0.12	0.21	0.20	0.02
C22:5n-3	0.12	0.18	0.04	nd	nd	
C22:6n-3	0.03	0.01	0.03	nd	nd	
PUFA	7.69	8.52	0.59	13.71	15.35	0.64*
P/S	0.19	0.22	0.02	0.31	0.37	0.02**

*P<0.05; **P<0.01; ***P<0.001; ^{t}0.05≤P<0.10

The introgression of wild boar into Lithuanian indigenous wattle pigs under conventional rearing conditions slightly affected the proportion of saturated fatty acids in the intramuscular fat (P=0.072). The predominant saturated fatty acids in the intramuscular fat were hexadecanoic acid (C16:0) and octadecanoic acid (C18:0) (Table 3). The proportions of both these acids were lower in 1/4 WB genotype.

However, significant difference was found only in the concentration of C18:0 (P<0.05). Although the introgression of wild boar did not appear to affect significantly the proportions of MUFA, the concentration of individual eicosenoic (C20:1) fatty acid was relatively 10.53% lower (0.05≤P<0.10) in the meat from 1/4 WB genotype. The proportions of PUFAs were insignificantly higher in the intramuscular fat of 1/4 WB genotype compared with purebred Lithuanian indigenous wattle pigs. The introgression of wild boar had a higher effect on the proportions of fatty acids in the subcutaneous tissue compared with the effect on the proportions of fatty acids in the intramuscular fat (Table 3). The concentrations of SFA (P<0.001), including hexadecanoic C16:0 (P=0.081), octadecanoic C18:0 (P<0.001) and eicosanoic C20:0 (P=0.052) acids, were relatively 3%, 11.9% and 10.5%, respectively, lower in the subcutaneous tissue of 1/4 WB genotype compared with Lithuanian indigenous wattle pigs. The hybrids had a relatively11.8% higher concentration of monounsaturated hexadecenoic C16:1 (P<0.05), 12.8% lower concentration of C20:1 (P<0.05) and 11.9% higher concentration of PUFA (P<0.05). However, only relatively 12.9% higher concentration of octadecenoic C18:2n-6 in the subcutaneous tissue of 1/4 WB genotype was statistically significant (P<0.01). Also, there was a more favourable PUFA/SFA ratio in the intramuscular fat (P>0.10) and in the subcutaneous tissue (P<0.01 of hybrids compared with purebred pigs.

Table 4. **Least square means and differences standard errors of differences for fatty acids in intramuscular fat of longissimus dorsi muscle by gender**

Fatty acids	LIW			1/4 WB genotype		
	Gender		SED	Gender		SED
	Castrates (n=7)	Gilts (n=10)		Castrates (n=8)	Gilts (n=1)	
C14:0	1.24	1.16	0.08	1.29	1.18	0.09
C16:0	26.53	26.73	0.84	26.54	25.30	0.78
C17:0	0.13	0.14	0.01	0.14	0.15	0.03
C18:0	13.03	10.94	0.90*	11.27	11.24	0.51
C20:0	0.16	0.14	0.01	0.22	0.11	0.05*
SFA	41.08	38.38	1.38^{t}	39.46	37.98	1.09
C16:1	3.67	3.77	0.27	4.03	3.65	0.25
C17:1	0.15	0.16	0.03	0.16	0.19	0.03
C18:1	48.14	48.18	1.37	47.72	49.42	1.04
C20:1	0.63	0.51	0.05*	0.49	0.55	0.06
MUFA	52.58	52.62	1.56	52.41	53.81	1.11
C18:2n-6	5.32	7.14	1.09	6.76	6.68	0.86
C18:3n-3	0.22	0.24	0.03	0.22	0.24	0.03
C20:3n-3	0.22	0.24	0.03	0.22	0.27	0.06
C20:4n-6	0.51	0.62	0.22*	0.73	0.79	0.26
C22:5n-3	0.12	0.20	0.05	0.17	0.19	0.10
C22:6n-3	0.06	0.18	0.05***	0.03	0.04	0.06
PUFA	6.33	7.74	1.39^{t}	8.14	8.21	0.95
P/S	0.15	0.22	0.04^{t}	0.20	0.22	0.03

*P<0.05; **P<0.01; ***P<0.001

Table 5. **Least square means and standard errors of differences for fatty acids in subcutaneous tissue by gender**

Fatty acids	LIW			1/4 WB genotype		
	Gender		SED	Gender		SED
	Castrates (n=9)	Gilts (n=9)		Castrates (n=11)	Gilts (n=12)	
C14:0	1.17	1.15	0.06	1.14	1.16	0.06
C16:0	26.81	26.59	0.80	25.94	25.82	0.61
C17:0	0.26	0.24	0.01	0.28	0.23	0.03^{t}
C18:0	15.77	15.98	0.53	14.46	13.90	0.55
C20:0	0.19	0.20	0.01	0.18	0.17	0.01
SFA	44.21	44.16	1.04	42.01	41.28	0.86
C16:1	1.76	1.67	0.11	1.87	1.90	0.14
C17:1	0.22	0.18	0.01*	0.25	0.20	0.02^{t}
C18:1	40.20	38.65	0.87^{t}	40.48	39.97	0.95
C20:1	0.87	0.84	0.07	0.79	0.73	0.07
MUFA	43.05	41.35	0.88^{t}	43.38	42.80	1.01
C18:2n-6	11.42	13.03	0.64*	13.16	14.41	0.94
C18:3n-3	0.58	0.63	0.05	0.64	0.69	0.07
C20:3n-3	0.56	0.62	0.05	0.59	0.62	0.04
C20:4n-6	0.19	0.21	0.03	0.19	0.21	0.02
PUFA	12.75	14.49	0.72*	14.60	15.93	1.05
P/S	0.29	0.33	0.02^{t}	0.35	0.39	0.03

*P<0.05; ^{t}0.05≤P<0.10

Gender had a higher effect on the fatty acid composition in the intramuscular fat from Lithuanian indigenous wattle pigs compared with the fatty acid composition in the intramuscular fat from 1/4 WB genotype hybrids (Ta-

ble 4). The proportion of C18:0, which caused an increase of total SFA (P=0.072), was relatively 19.1% higher (P<0.05) in Lithuanian indigenous wattle castrated males than in females. There were lower proportions of C20:1 (P<0.05) and higher proportions of PUFA (P=0.077), including relatively 21.6% higher eicosatetraenoic C20:4n-6 (P<0.05) and treble higher docosahexaenoic C22:6n-3 P<0.001) proportions in Lithuanian indigenous wattle females compared with Lithuanian indigenous wattle castrated males, whereas gender had a significant effect only on the portion of C20:0 fatty acid for 1/4 WB hybrids. The proportion of this fatty acid in the intramuscular fat was twice lower (P<0.05) in females compared with the castrated males of the same genotype. The results for fatty acid composition of the subcutaneous tissue within the studied genotypes according to gender are shown in Table 5.

The proportions of C17:1 (P<0.05), C18:1 (P=0.099) and MUFA (P=0.075) were relatively by 18.2%, 3.9% and 3.95%, respectively, lower in Lithuanian indigenous females than in castrated males. The proportions of PUFA were lower in the subcutaneous tissue of the castrates compared with the gilts within the same genotype.

Discussion and conclusion. In recent years there has been an increased interest in ways to aimed at manipulation with the fatty acid composition. As most animal production traits, fatty acid composition is influenced by both genetic and environmental factors (De Smet et al., 2004). The majority of studies attempting to achieve fatty acid profiles in meat that correspond better to current human nutrition guidelines, have dealt with pig feeding based principally on fat-enriched diets (Wood and Enser, 1997; Kouba et al., 2003; Weber et al., 2006; Pascual et al., 2007) or on use of α-tocopherol enriched diets in order to increase the level of this antioxidant in muscles (Guo et al., 2006) and with pig rearing in an indoor or outdoor housing systems (Högberg et al. 2004; Gonzalez et al., 2006). Genetic factors have been investigated far less. However, several studies have reported pig breed differences for fatty acid composition (Cameron and Enser, 1991; Alfonso et al., 2005, Renaudeau et al., 2005, Alonso et al., 2009). Meyer et al. (1998) have reported that depot fat of wild boar showed by far the highest content of essential fatty acids compared with ruminant species, and that the diet affects the fat composition. The fatty acid composition of the intramuscular fat determined relative to breed and feeding regime suggested that the meat of pasture fed or wild animals is better for health than the meat from pen fed animals (Koizumi et al., 1991). The results in this study show that it is possible to increase the concentrations of PUFA and PUFA/ SFA ratio in the subcutaneous tissue by wild boar introgression (1/4 wild boar) under conventional hybrid rearing. The effects of the genotype, diet and the genotype with the diet interaction on fatty acid composition in the study of Cameron et al. (2000) showed that nutritional effects on intramuscular fat characteristics were greater than genetic effects. In relative terms lower breed differences than those induced by diet were reported also by Pascual et al. (2007). It is likely that pasture rearing for Lithuanian indigenous and wild boar hybrids should suggest a better fatty acid composition not only in the subcutaneous tissue but also in intramuscular fat for consumer health. Cameron et al. (2000) and De Smet et al. (2004) have reported that muscle phospholipids fatty acid composition does not seem to differ between gilts and barrows, but higher PUFA concentrations have repeatedly been found in total lipids for gilts. Higher SFA content in the intramuscular fat and subcutaneous tissue of Lithuanian indigenous castrated males compared with females in this study was also in agreement with the data of Högberg et al., 2003; Renaudeau et al., 2005, Zhang et al., 2007, Alonso et al., 2009. Lo Fiego et al. (2005) who have noted that an increase in backfat thickness was associated with a lower degree of lipid unsaturation. Backfat thickness of castrated males from both genotypes used in present study was higher compared with females (Razmaitė and Kerzienė, 2009). However, fatness of Lithuanian indigenous castrated males from 1/4 WB genotype was higher and that is why in this study gender had a higher effect on the fatty acid composition in the tissue from Lithuanian indigenous wattle pigs compared with the gender effect on the fatty acid composition in 1/4 WB genotype hybrids. The recommended ratio of PUFA to SFA (P/S) should be increased to above 0.4 but some meats naturally have a P/S ratio of around 0.1 (Wood et al., 2004). In this study the P/S ratio was higher in both studied genotypes.

It can be concluded that introgression of wild boar into Lithuanian indigenous wattle pigs under conventional rearing conditions slightly decreased (P=0.072) the proportion of saturated fatty acids in intramuscular fat but the effect on the proportions of fatty acids in the subcutaneous tissue compared with the effect on the proportions of fatty acids in the intramuscular fat was higher. The concentrations of SFA (P<0.001) were lower and concentrations of PUFA (P<0.05) were higher in the subcutaneous tissue of 1/4 WB genotype compared with Lithuanian indigenous wattle pigs. There was a more favourable PUFA/SFA ratio (P<0.01) in the subcutaneous tissue of hybrids compared with purebred pigs. Gender had a higher effect on the fatty acid composition in the intramuscular fat and subcutaneous tissue from Lithuanian indigenous wattle pigs compared with the gender effect on the fatty acid composition in 1/4 WB genotype hybrids.

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Received 28 March 2008
Accepted 5 March 2010