PREFACE

The aim of the third Scientific International Conference "Application of Scientific Achievements in the Field of Genetics, Reproduction, Nutrition, Carcass and Meat Quality in Modern Pig Production" was to present the actual carried out research studies conducted by different native and foreign scientific units. The spectrum covered different fields of animal breeding and production which are a challenge for both science and practice. The main research trends focus on animal production quality, factors determining a high degree of safe food, association of gene polymorphism with some swine reproductive and production traits.

The subject matter of papers deals many aspects of animal biology along with genomics and provides ground for animal biotechnology development. Presented in that issue publications are only a part of papers discussed at the Conference, the rest were published in other journals: Animal Science and Reports, vol. 24 Supplement 3 (2006); Annals of Animal Science, Supplement 2/1 (2006).

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Chairman of the Conference

Arch. Tierz., Dummerstorf **50** (2007) Special Issue, 102-106

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Survival time and chromatin damage of boar semen stored in different diluents

Abstract

Sperm motility and survival time are commonly applied for the evaluation of semen extender quality. However, a new quality parameter – sperm chromatin structure – is increasingly often used for this purpose in many mammalian species. The aim of the study was to find a relationship between the level of sperm chromatin abnormalities and semen survival time. A total of 11 ejaculates collected from 4 boars and stored in 5 popular, commercially available extenders were used. The semen stored in PBS was used as a control sample. Samples were stored at 15-17°C. Sperm motility was examined every day until motility decreased to 30%. Sperm chromatin abnormalities were examined flow cytometrically according to the Sperm Chromatin Structure Assay (SCSA) method on days 1 and 14 of storage. The correlation between survival time (T) and: a) % of spermatozoa with abnormal chromatin (DFI) on the day of semen collection or: b) the increase of DFI after 14-day storage (Δ DFI) was calculated. The shortest survival time (0.5 day) and the highest increase in spermatozoa with damaged chromatin (6.57%) were observed in the control group (PBS). In all the analysed diluents the average survival time ranged from 6.2 to 10.0 days, DFI ranged from 2.40% to 3.14% and Δ DFI from 0.94% to 2.55%. A negative correlation between DFI and survival time was found. It was statistically significant ($p \leq 0,05$) for two diluents. The correlation between T and Δ DFI was differential, possibly resulting from the effect of diluent.

Key Words: boar, semen, survival time, sperm chromatin

Zusammenfassung

Titel der Arbeit: **Überlebensdauer von Ebersperma und Beschädigungsgrad des Spermienchromatins in ausgewählten Verdünnern**

Die allgemein angewendeten Kriterien in den Labortests zur Beurteilung der Spermaqualität und der Verdünner, die zu seiner Konservierung verwendet wurden, sind die Beweglichkeit und das Überleben. Derzeit wird zur Bewertung der Qualität der Fruchtbarkeit des Spermas immer häufiger ein Chromatintest verwendet. Das Ziel der Untersuchungen war den Einfluss von verschiedenen Verdünnern für Ebersperma auf die Überlebenszeit und die Struktur des Spermienchromatins zu untersuchen sowie die Abhängigkeit zwischen dem Beschädigungsgrad des Spermienchromatins und der Überlebensdauer der Spermien zu definieren. Für die Untersuchung wurden 11 Frischejakulate verwendet, das von 4 verschiedenen Ebern gewonnen wurde. Die Ejakulate wurden jeweils in 6 Teile eingeteilt und mit PBS (Kontrolle) sowie mit den fünf am häufigsten verwendeten Verdünnern gemischt, und bei einer Temperatur von 15-17°C aufbewahrt. Die Beweglichkeit der Spermien wurde jeden Tag solange beurteilt, bis eine Beweglichkeit von unter 30% eintrat. Die Chromatinstruktur der Spermien (gemäß SCSA-Protokoll) wurde am Tag der Gewinnung des Ejakulates sowie nach 14 Tagen Aufbewahrung untersucht. Es wurden die Korrelations-Koeffizienten zwischen der Überlebenszeit des Spermas (T) und dem prozentualen Anteil der Spermien mit beschädigtem Chromatin am Tag der Entnahme (DFI) sowie die Erhöhung des prozentualen Anteil der Spermien mit beschädigtem Chromatin am Tag 14 der Aufbewahrung (Δ DFI) berechnet. Die kürzeste Zeit T (0,5 Tage) ergab sich in der Kontrollgruppe (PBS). In allen Verdünnern schwankte die durchschnittliche Zeit T zwischen 6,2 und 10,0 Tagen. Der durchschnittliche DFI lag in einem Intervall zwischen 2,40 und 3,14%. Nach 14 Tagen Aufbewahrung wurde der höchste Δ DFI (signifikanter Unterschied) (um 6,57%) in der Kontrollgruppe (PBS) festgestellt. In allen Verdünnern lag dieser dagegen zwischen 0,94 und 2,55%. Signifikante Unterschiede ($p \leq 0,01$) wurden zwischen T in der Flüssigkeit (PBS) und in allen untersuchten Verdünnern beobachtet sowie zwischen T und den Tagen 6,2 und 10,0. Eine negative Korrelation wurde zwischen DFI und T beobachtet, diese Korrelation ist jedoch nur im Falle von zwei Verdünnern signifikant ($p \leq 0,05$).

Schlüsselwörter: Eber, Sperma, Überlebensdauer, Spermienchromatin

Introduction

The application of artificial insemination resulted in a search for the most convenient evaluation methods and efforts to improve semen conservation. Although many laboratory tests evaluating the morphological or physiological state of spermatozoa have been developed to complement the basic evaluation of motility, no methods are available for accurate prediction of fertility (GRAHAM and MOCÉ, 2005; TARDIF et al., 1999). This concerns both fresh and conserved semen (DUBÉ et al., 2004; HRSTKOVA et al., 2001). Freezing is the best conservation method, but this method is not widely used in pig breeding because of its low efficiency. With a small number of insemination doses, fertility results are reduced and the freezing process is laborious (GILLMORE et al., 1998). Therefore, insemination is mainly based on semen stored at above-zero temperatures. Extenders used for semen conservation should provide spermatozoa with adequate environmental osmotic pressure, stable pH and energy components, prevent the growth of bacteria and protect against a thermal shock to maximize fertilizing capacity. The usefulness of extenders can be evaluated using the same tests as for fresh semen, although motility and survival of conserved semen are evaluated the most often.

An increasingly common method of semen fertility evaluation is the Sperm Chromatin Structure Assay (SCSA) (GRAHAM and MOCÉ, 2005; BOCHENEK et al., 2001) which evaluates fragmentation level of DNA. This fragmentation depends on the stage of cell growth and differentiation, disturbances in these processes or the action of toxic agents. Opinions on the correlation between the level of chromatin damage and other semen traits are similar and usually reflect the relationship between the state of sperm chromatin and fertility. Studies with a large number of frozen bull ejaculates (BALLACHEY et al., 1987) showed the presence of a high correlation between sperm sensitivity to denaturation and fertility obtained after insemination with this semen, with the level of this sensitivity reflecting even the smallest differences in fertility (BOCHENEK et al., 2001). KĄTSKA-KSIĄŻKIEWICZ et al. (2005) obtained better results of embryonic development after in vitro fertilization with frozen semen containing a lower percentage of spermatozoa with damaged chromatin. Studies with humans (ARAVINDAN et al., 1997; GOLAN et al., 1997) also showed a close relationship between the increased sensitivity of chromatin to denaturation and lower fertility.

The aim of the study was to evaluate the effect of different boar semen extenders on survival time and sperm chromatin structure and to determine a relationship between the level of sperm chromatin abnormalities and semen survival time.

Material and Methods

A total of 11 fresh semen ejaculates were collected from 4 crossbred boars. Whole ejaculates were collected by hand manipulation into water-jacketed vessels. After collection, removal of gelatinous material and evaluation of motility and concentration, semen was divided into 6 portions and extended using PBS (control) and the five most common extenders to a concentration of approx. $30x10^6$ sperm per mL, after which they were placed in a cold store at 15-17°C. To evaluate motility, 1 mL samples were taken every day from the stored semen, placed in a water bath at 37°C and evaluated after 30 min under a phase-contrast optical microscope (200x magnification). The day on which the proportion of normally motile sperm decreased

to 30% was considered to be the end of the survival period. Sperm chromatin structure was analysed on the day of collection and after 14 days of semen storage using a flow cytometer in accordance with the SCSA protocol (EVENSON, 1990). Differences between survival time, percentage and increase of sperm with damaged chromatin, stored in different extenders, was made using the T test. Correlation coefficients were calculated between semen survival time (T) and the percentage of sperm with damaged chromatin on the day of collection (DFI) and the increase in the percentage of sperm with damaged chromatin after 14 days of storage (Δ DFI).

Results

The shortest mean survival time of semen (0.5 day) was found in the control group (PBS). For all the extenders analyzed, mean survival time ranged from 6.2 to 10.0 days. The mean percentage of sperm with damaged chromatin on the day of dilution was very low in all the fluids and ranged from 2.40 to 3.14%. The highest increase in the percentage of sperm with damaged chromatin (by 6.57 %) was found in the control group (PBS), and in all the extenders used for boar semen conservation it ranged from 0.94 to 2.55%. Highly significant differences ($p \leq 0.01$) were found using the T test between survival time of semen stored in PBS (0.5 day) and in all the extenders analysed, and between survival time of semen conserved with diluents II and IV. Significant differences ($p \leq 0.05$) were found between survival time of semen diluted with extenders II and V and between the increase in the percentage of sperm with damaged chromatin, stored for 14 days in PBS and extender IV (Tab. 1).

Table 1
Average survival time (T), percentage of spermatozoa with damaged chromatin on the day of collection (DFI) and increase of DFI after 14 days of storage (Δ DFI) in different diluents (I-V and PBS).

Diluent	I	II	III	IV	V	PBS
T (no.of days) ±SD	8,5±4,3^{B}	10,0±3,5^{B,C,a}	8,4±4,3^{B}	6,2±2,6^{B,D}	6,8±3,0^{B,b}	0,5±0,9^{A}
DFI (%) ±SD	2,79±0,92	3,12±0,97	3,14±1,14	2,80±0,75	2,40±0,69	2,49±0,82
Δ DFI (%) ±SD	2,21±2,76	2,55±2,43	2,44±2,08	0,94±1,39^{b}	2,41±2,17	6,57±6,88^{a}

SD – standard deviation
A,B and C,D – statistically significant differences ($p \leq 0.01$)
a,b - statistically significant differences ($p \leq 0,05$)

A negative correlation was found between the percentage of sperm with damaged chromatin and semen survival time (Tab. 2), although this correlation was significant ($p \leq 0.05$) only for extenders I and III.

Table 2. Correlations between survival time (T) and percentage of spermatozoa with damaged chromatin on the day of collection (DFI) and increase of DFI after 14 days of storage (Δ DFI) in different diluents (I-V) and PBS.

Diluent	I	II	III	IV	V	PBS
T vs DFI correlation	-0,69*	-0,43	-0,64*	-0,004	-0,28	-0,54
T vs Δ DFI correlation	-0,03	-0,56	0,43	0,64*	0,09	-0,15

* - statistically significant correlation ($p \leq 0,05$)

The correlations calculated between semen survival time and the increase in the percentage of sperm with damaged chromatin are varied (Tab.2). They are negative, positive, close to zero or close to significance level, and significant for one extender ($p \leq 0.05$). This concerns the extender characterized by the shortest semen survival time and the lowest increase in the percentage of sperm with damaged chromatin.

Discussion

Sperm motility is commonly applied for evaluating boar semen quality and, according to many researchers, it affects fertility results in addition to the number of spermatozoa in a dose (TARDIF et al., 1999; DUBÉ et al., 2004; NEHRING and STAEHR, 2001).
The our results indicate that the presence or absence of a correlation between the chromatin abnormalities and semen survival time can be determined by the type of the environment in which semen is stored. In our earlier studies with different antioxidants added to boar semen extenders, we found a positive effect of magnesium fumarate on semen survival time parallel to a significant increase in the percentage of sperm with damaged chromatin (SZCZĘŚNIAK-FABIAŃCZYK at al., 2003).
Studies with humans also showed the lack (EVENSON et al., 1999; SPANO et al., 1999) or weak (BOCHENEK et al., 2004) relationship between the increased sensitivity of chromatin to denaturation and such parameters as motility, morphology or state of acrosomes.
Assuming the negative effect of sperm chromatin damage on fertility, a practical conclusion from our observations is that it is necessary to use the SCSA as an additional criterion of laboratory semen testing in addition to motility. The use of this method of evaluation can improve the selection of boars and ejaculates in terms of the extender used and semen storage time. This procedure may result in higher farrowing rates and greater litter sizes from the inseminated pigs.

References

ARAVINDAN, G.R.; BJORDAHL, J.; JOST, L.K.; EVENSON, D.P.:
Susceptibility of human sperm to in situ DNA denaturation is strongly correlated with DNA strand breaks identified by single-cell electrophoresis, Exp Cell Res **236**(1997), 231-237

BALLACHEY, B.E.; HOHENBOKEN, W.D.; EVENSON, D.P.:
Heterogenity of sperm nuclear chromatin structure and its relationship to bull fertility, Biol. Reprod. **36 (**1987), 915-925

BOCHENEK, M.; SMORĄG, Z.; PILCH, J.:
Sperm chromatin structure of bulls qualified for artificial insemination analysed by flow cytometry, Theriogenology **56** (2001), 557-567

BOCHENEK, M.; GOGOL, P.; JANECZKO, J.:
Sperm chromatin structure of man from infertile couples (Struktura chromatyny plemnikowej mężczyzn z niepłodnych par). In: Kuczyński, W. (editor) Biology of Reproduction and human fertility health (Biologia rozrodu oraz zdrowie reprodukcyjne człowieka), Białystok (2004), 103

DUBÉ, C.; BEAULIEU, M.; REYES-MORENO, C.; GUILLEMETTE, C.; BAILEY, J.L.:
Boar sperm storage capacity of BTS and Androhep Plus: viability, motility, capacitation, and tyrosine phosphorylation. Theriogenology **62** (2004), 874-886

EVENSON, D.P..
Flow cytometric analysis of male germ cell quality. In: Methods in cell Biology, Vol **33** (1990), San Diego, Academic Press, 401-410

EVENSON, D.P.; JOST, L.K.; MARSHALL, D.; ZINAMAN, M.J.; CLEGG, E.; PURVIS, K.; DE ANGELIS, P.; CLAUSSEN, O.P.:
Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod. **14** (1999), 1039-1049

GILLMORE, J.A.; LIU, J.; PETER, A.T.; CRITSER, J.K.:
Determination of plasma membrane characteristics of boar spermatozoa and their relevance to cryopreservation. Biol. Reprod. **58** (1998), 28-36

GOLAN, R.; SHOCHAT, L.; WEISSENBERG, R.; SOFFER, Y.; MARCUS, Z.; OSCHRY, Y.; LEWIN, L.M.:
Evaluation of chromatin condensation in human spermatozoa: a flow cytometric assay using acridine orange staining. Mol. Hum. Reprod. **3** (1997), 47-54

GRAHAM, J.K.; MOCÉ, E.:
Fertility evaluation of frozen/thawed semen. Theriogenology **64** (2005), 492-504

HRSTKOVA, P.; CEROVSKY, J.; ROZKOT, M.:
Boar spermatozoa survival rate in different selected commercial extenders for artificial insemination. Arch. Tierz., Dummerstorf **44** (2001), Special Issue, 134-137

KĄTSKA-KSIĄŻKIEWICZ, L.; BOCHENEK, M.; RYŃSKA, B.:
Effect of quality of sperm chromatin structure on in –vitro production of cattle embryos. Arch. Tierz., Dummerstorf **48** (2005), 32-39

NEHRING, H.; STAEHR, B.:
Efficient use of bulls and boars for AI. Arch. Tierz., Dummerstorf **44** (2001), Special Issue, 121-133

SPANO, M.; CORDELLI, E.; LETER, G.; LOMBARDO, F.; LENZI, A.; GANDINI, L.:
Nuclear chromatin variations in human spermatozoa undergoing swim-up and cryopreservation evaluated by the flow cytometric sperm chromatin structure assay. Mol. Hum. Reprod. **5** (1999), 29-37

SZCZĘŚNIAK-FABIAŃCZYK, B.; BOCHENEK, M.; SMORĄG, Z.; RYSZKA, F.:
Effect of antioxidants added to boar semen extender on the semen survival time and sperm chromatin structure. Reproductive Biology **3** (2003), 81-87

TARDIF, S.; LAFOREST, J.-P.; CORMIER, N.; BAILEY, J.L.:
The importance of porcine sperm parameters on fertility in vivo. Theriogenology **52** (1999), 447-459

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Arch. Tierz., Dummerstorf **50** (2007) Special Issue, 107-115

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Characteristics of genetic parameters and genetic gain in breeding herd of PL pigs over 25- year breeding work period

Abstract

Data from PL pigs over 1973-1999 and the line PL-23 in 1984-1999 maintained in the closed pig herd in Pukarzów were used to estimate genetic parameters and genetic gain for reproduction traits. Data sets were analyzed by using restricted maximum-likelihood programs. In the case of the PL breed, genetic progress was positive for body weight as defined on the day of assessment and daily gain was on average .50 kg/yr for (weight -W) and .39 g/yr for (adjusted daily gain -ADG). For backfat thickness and percentage meat content, the genetic reactions were negative and were between -.05 mm/yr and –.05 %/year respectively. The estimated genetic progress for the PL-23 line was: .39 kg/yr for body weight on the day of assessment, .08 g/yr for daily gain, -.03 mm for backfat thickness and .29 for the percentage content of meat /year.

Key Words: genetic trend, genetic parameters, fattening performance, slaughter performance, pig

Zusammenfassung

Titel der Arbeit: **Charakteristik der genetischen Parameter und des genetischen Fortschrittes für Mast- und Schlachtmerkmale einer Sauenherde der Polnischen Landrasse im Zeitraum von 25 Jahren**

Zur Bewertung genetischer Parameter und des Zuchtfortschrittes der geschlossenen Zuchtherde der Polnischen Landrasse in Pukarzów standen Daten aus den Jahren 1973-1999 und für die Linie PL-23 Daten aus den Jahren 1984-1999 zur Verfügung. Die Berechnungen erfolgten mittels der REML-Methode (restricted maximum-likelihood program). Bei der Polnischen Landrasse betrug der genetische Fortschritt ausgedrückt als linearer Regressionskoeffizient im erfassten Zeitraum für das Körpergewicht 0,5 kg und für die tägliche Zunahme 0,39 g. Für die Speckdicke wurde ein Rückgang von -0,05 mm und für den prozentualen Fleischanteil von -0,05 beobachtet. Der geschätzte jährliche Zuchtfortschritt in der Pl-23 Linie betrug für die vier genannten Merkmale 0,39 kg, 0,08 g, -0,03 mm, dagegen für den prozentualen Fleischgehalt 0,29.

Schlüsselwörter: Zuchtfortschritt, genetische Parameter, Mastleistungsmerkmale, Schlachtleistungsmerkmale, Schwein

The effects of the breeding work expressed as the genetic improvement are of special importance because they are stable and, on account of that, they come down to the following generations. The effects are particularly influenced by the accuracy of breeding value estimation. Therefore, one of the basic issues included in animal improvement appears to be evaluation of animal genetic value. The genetic value is determined on the basis of individual phenotypic value or phenotypic value of the blood-relationes. The assumed proceeding course depends mainly on the specific properties of the evaluated attributes, whose character facilitates these attributes direct determination on a given individual or on its blood-relations exclusively. Only very few characteristics may be evaluated on the grounds of animal utility. In most cases, a level of the analyzed parameters is estimated on the grounds of the blood-relatins utility ,i.e. offspring and all siblings [RÓŻYCKI, 1983].

Animal utility is a resultant of two groups of factors: genetic and environmental. A basis for the genetic improvement is the differentiation in what part the stated utility depends on an individual genotype (handed down to offspring by an animal)and in what part it is subject to the environmental factors. Observing variation of particular attributes of great economic consequence, it is evident that there are potentials for genetic improvement of most of these characteristics that determine the usability of this animal species for production.

High fecundity, intensive selection and short time intervals between generations facilitate the growth in pig performance value at a relatively short span of time. High productive effects obtainable in every herd lie entirely with, among others, a choice of an appropriate breeding work method, a hereditability level of a trait towards which a selection is conducted and genetic correlations, genetic variation of the selected individuals as well as compliance with the nutrition and maintenance requirements. For a number of years the breeders have focused on the evaluation methods and their implementation in the pig improvement. A conception of the organized breeding work on livestock in the Polish breeding centers, constituting the close herds, was elaborated in the early 1970s [DUNIEC and RÓŻYCKI, 1972; RÓŻYCKI, 1974, 1977]. However, a main drawback of this work proved to be a lack of verification of the set breeding aims as well as its efficiency evaluation from the genetic point of view. Traits hereditability and the estimated correlation coefficients including constant and random factors are specific for a given population living at the defined time. Hence, a reliable estimation may be of vital importance to efficient improvement of a breeding program for a given population. The present work aims at estimation of the genetic parameters and breeding progress in a close herd of PL breed pigs and the line PL-23 in Pukarzów.

Material and methods

The analytic material was made up by the data enclosed in the zootechnical records and stored in the farm archive. The data dealt with the fattening and slaughter performance of gilts and young boars born at the farm in the years 1973-1999. This elaboration covers the results of the PL breed in the fourteen generations at the 26 years span and the line 23 in the ten generations over 15 years. A fattening and slaughter value of gilts and young boars was estimated on the basis of 17429 boars and 3598 gilts of PL breed as well as 1431 young boars and 591 gilts of the PL-23 line. It included:

1. body weight of estimation day – W(kg)
2. standard daily gain – ADG(g)
3. mean standard backfat thickness – BF (mm)
4. height of loin"eye" – LH (mm) from 1993
5. level of meat content – LMC from 1995

For each characteristics, hereditability coefficients were estimated. The correlation coefficients between the mentioned performance features were computed. There was employed Eildert Groneveld computer program VCE 4.2.5. with REML method (Restricted Maximum Likelihood Procedure) that facilitates the relatively best approximation [GROENEVELD et al., 1992] and individual model. Characteristics estimation was carried out based on the following model:

$$Y_{ijklmn} = \mu + R_i + (RxS)_{ij} + P_k + \beta_1 (mc_{ijkl} - \overline{mc}) + \beta_2 (w_{ijkl} - \overline{w}) + a_m + e_{ijklmn}$$

Y_{ijklmn} –the phenotype value of the individual
μ - population mean
R_i- the fixed influence of the birth year
$(RxS)_{ij}$- the fixed influence of the year x season interaction
P_k- the fixed influence of gender
$\beta_1 (mc_{ijkl} - \overline{mc})$- body mass regression
$\beta_2(w_{ijkl}-\overline{w})$- age regression
a_m –random additive value of the individual
e_{ijklm}- random effect of the environment.

To determine the efficiency of the breeding work, genetic improvement estimates were applied, which was expressed as a rectlinear regression coefficient. The basis for the genetic trends determination in an investigated population comprised the records for a year of an individual birth depicting the changes of genetic quality in time. The computations were performed with computer program BLUPf90 by IGNACY MISZTAL (1994) using a multitrait individual model covering the same random factors and constants, like in the case of genetic parameters. Calculating the genetic parameters and a genetic trend, the standardization of backfat thickness was made at 110kg body weight.

Table 1
Hereditability coefficients on a diagonal table (h^2)and genetic correlation coefficients (r_G) for the PL breed

h^2	W	ADG	BF	LH	LMC
W	*0.284*	r_G – (-0.746)	r_G – (0.240)	r_G – (-0.351)	r_G – (-0.758)
ADG	r_G – (-0.746)	*0.277*	r_G – (-0.004)	r_G – (0.321)	r_G – (0.960)
BF	r_G – (0.240)	r_G – (-0.004)	*0.115*	r_G – (-0.219)	r_G – (-0.245)
LH	r_G – (-0.351)	r_G – (0.321)	r_G – (-0.219)	*0.117*	r_G – (0.834)
LMC	r_G – (-0.758)	r_G – (0.960)	r_G – (-0.245)	r_G – (0.834)	*0.589*

Table 2
Heritability coefficients on a diagonal table (h^2)and genetic correlation coefficients (r_G) for the line PL-23

h^2	W	ADG	BF	LH	LMC
W	*0.082*	r_G – (-0.178)	r_G – (0.145)	r_G – (-0.271)	r_G – (-0.264)
ADG	r_G – (-0.178)	*0.459*	r_G – (0.292)	r_G – (0.256)	r_G – (0.006)
BF	r_G – (0.145)	r_G – (0.292)	*0.152*	r_G – (-0.311)	r_G – (-0.870)
LH	r_G – (-0.271)	r_G – (0.256)	r_G – (-0.311)	*0.115*	r_G – (0.718)
LMC	r_G – (-0.264)	r_G – (0.006)	r_G – (-0.870)	r_G – (0.718)	*0.165*

Results

The hereditability coefficients of the examined gilts and young boars body weight are given in Table 1 and 2.Their value ranged from h^2=0,284 (PL breed) to h^2=0,082 (line PL-23). It was found that a daily gain belonged to the medium hereditable traits. There was also established low hereditability for backfat thickness for both breeds. The hereditability coefficients of loin "eye" height and % meat content were for PL breed and the line PL-23 h^2=0,117 and 0,589 and h^2=0,115 and 0,165, respectively. The values of the determined hereditability indices imply that selection towards daily gain

and % meat content is supposed to be the most effective. Relatively low and positive dependences were obtained between W and BF (r_G=0,240 – PL and r_G=0,145 – line PL-23). It was noted that along with daily gain growth, the loin ”eye” grows. The correlations between daily gain and % meat content estimated for the PL breed were high and reached r_G=0,960, while for the line PL-23 appeared very low and averaged r_G=0,006.

The trends of genetic changes of the fattening and slaughter characteristics in PL breed are presented in Figure 1 and 2.

Fig. 1: Genetic trends (PL)
Genetische Trends (Rasse PL)

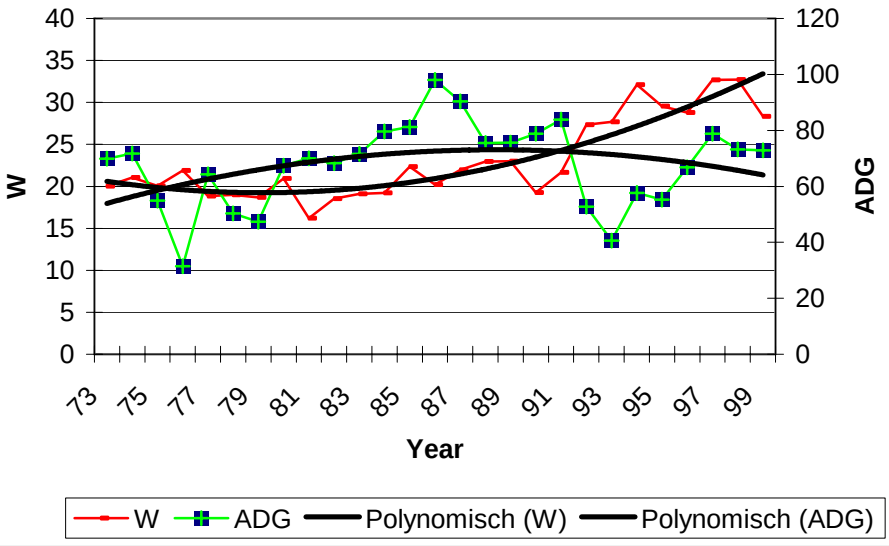
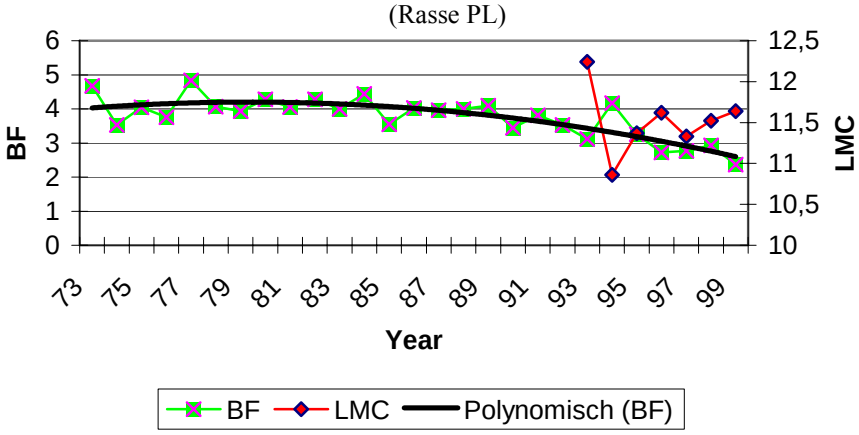


Fig. 2: Genetic trends for backfat thickness and lean meat content percentage (PL)
Genetische Trends für die Speckdicke und den Fleischgehalt (Rasse PL)



Body weight analyzed in the experimental years showed an upwards tendency. The highest values of the genetic trends as compared to daily gain were recorded in the years 1986, 1991 and 1997, whereas the lowest in 1976 (Figure 1). Analyzing percentage of meat content, it was stated that the highest genetic value was shown by the animals born in 1993 (Figure 2).

A genetic improvement was positive for the body weight determined on the estimation day and daily gains and averaged for PL breed (Tab. 3) – ,50kg for W and ,39g for ADG. The positive genetic trends were also established for backfat thickness (-,05 mm). Whereas for percentage meat content the genetic reactions proved negative (-,05%). The estimated breeding progress for the line PL-23 was as following: 39kg for W; ,08g for ADG. -,03 mm for BF and ,29 for LMC (Tab. 3).

Table 3
Genetic advances in the analysed pig population

Breed	Characteristics	Straight-line regression coefficient	
PL	W	0.504	
			P≤0,69
	ADG	0.391	
			P≤0,00
	BF	-0.049	
			P≤0,00
	LMC	-0.055	
			P≤0.00
Line PL-23	W	0.395	
			P≤0.00
	ADG	0.076	
			P≤0.00
	BF	-0.034	
			P≤0.00
	LMC	0.290	
			P≤0.00

A graphic picture of the trend value with regard to daily gains (line PL-23) over the analyzed ten generations indicates that the most valuable genetically individuals were born in 1985, 1990 and from 1994 to 1999. From 1995 the gains growth was recorded along with decreasing body weight (Figure 3). Analyzing the changes in backfat thickness, some positive tendencies in the genetic values of the animals were noted from 1995 to 1999 (Fig. 4).

Fig. 3: Genetic trends for weight and adjust daily gain (PL-23).
Genetische Trends für die Körpermasse und die standardisierten täglichen Zuwächse (Linie PL-23)

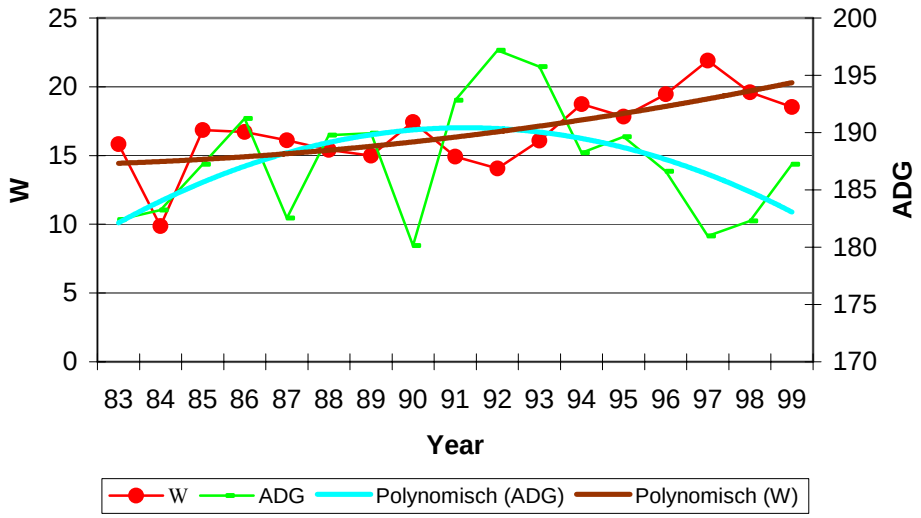
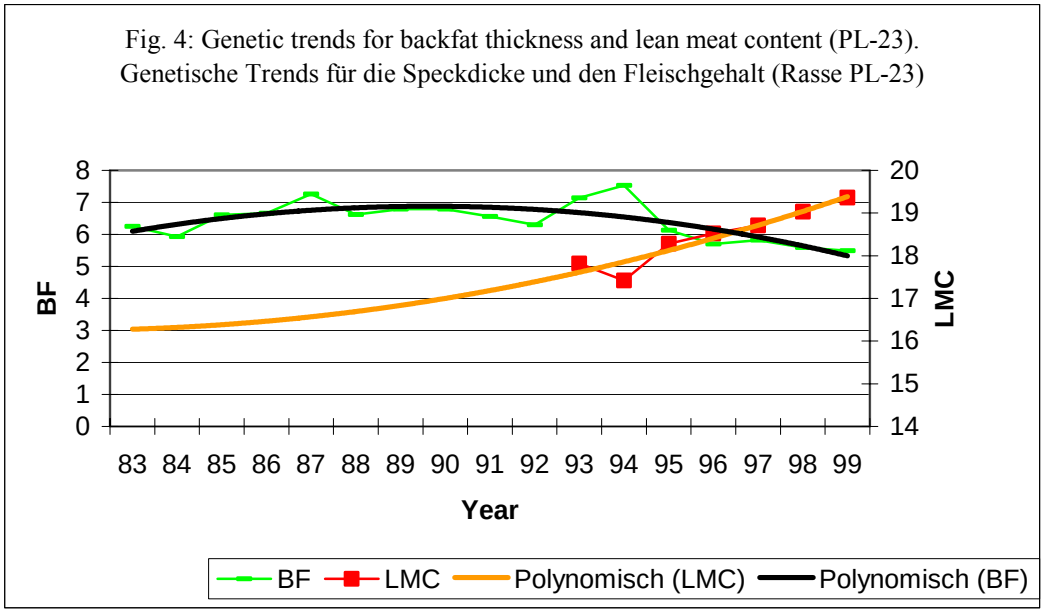


Fig. 4: Genetic trends for backfat thickness and lean meat content (PL-23).
Genetische Trends für die Speckdicke und den Fleischgehalt (Rasse PL-23)



A genetic progress estimated for the line PL-23 was slight, yet it was characterized by a positive trend of changes in all the considered traits (Tab. 3, Fig. 3 and 4).

Discussion

Hereditability being a measure of conformability between a genotype and phenotype within daily gains of the PL breed and the line PL-23 proved comparable with the results of SILVA et al. [1992]. For the Landrace and Large White breeds maintained in Brasil, the authors give the hereditability coefficients: h^2=0,40 and h^2=0,26, respectively. The convergent results are presented in the studies run by CHEN et al. [2002]. KAPŁON et al. [1991] estimated the hereditability of daily gains of PLW breed animals kept in the years 1978-1987 at the level h^2=-,27. Far lower values of the analyzed parameter for gilts Swedish Landrace evaluated in two herds were presented by KOSOVAC et al. [1997] (h^2=0,15 and h^2=0,11) as well as GUERRA et al. [1992] (h2=0,19). Similarly, MERKS in Holland [1988] assessed hereditability of gains of Dutch Landrace and Dutch Yorkshire at the following level: h^2=0,12 and h^2=0,18. In the present investigations, it was found that backfat thickness is a low hereditable trait, that is convergent with the results of GUERRA et al. [1992]. MERKS [1988] reports that hereditability of backfat tickness in Dutch Yorkshire and Dutch Landrace breeds is contained within 0,23-0,28. A slightly higher hereditability level of this trait is mentioned by KOSOVAC et al. [1997]. According to these authors, this parameter value ranged from 0,34 to 0,33, while the highest indices were recorded by BUCZYŃSKI [1988] (h^2=0,956 for young boars and h^2=0,516 for gilts). This author holds that hereditability in relation to body weight was 0,326 (young boars), 0,439 (gilts) for the gain h^2=0,394 and h^2= 0,840. Whereas, the investigations of FERRAZA and JOHNSON [1993] on the Landrace and Large White pigs showed the following hereditability indices of daily gains from 30 to 104 kg: 0,23-0,34 and 0,40-0,50 for backfat thickness. The differences in this parameter are attributed to the employment of different methods for the genetic parameters estimation as well as considering different populations.

The analysis of dependences between gain and backfat thickness was performed by numerous authors, among others SILVA et al. [1992], MERKS [1988], BIZELIS et al. [2000]. In the studies of the mentioned above scientists, the correlation coefficients ranged from 0,14 and 0,61 up to 0,78, subject to a breed and station. From the data presented in Table 1, it follows that in PL breed there occur some minor genetic connections between a gain and backfat thickness (r_G=-0,004). Correlation between ADG and BF in the case of the line PL-23 (Tab. 2) was r_G=0,292 and proved convergent with the results given by KAPŁON et al. [1990a] for PLW breed estimated over the years 1978-1987. A meat content was negatively correlated with backfat thickness (Tab. 1, 2). The obtained correlation coefficients between BF and LMC (r_G=-0,245) for PL breed and (r_G=-0,870) for the line PL-23 did not deviate from those evaluated for the gilts and young boars of Swedish Landrace by UREMOVIC et al. [1995]. They claim that dependences between backfat thickness and meat content percentage amounted to r_G=-0,42 to r_G=-0,79 (thickness of backfat between rib 3 and 4) 60 mm off the central dorsal line, r_G=-0,27 to r_G=-0,63 behind the last rib 65 mm from the central dorsal line.

The obtained values of correlation coefficients between BF and LH (r_G=-0,311) proved similar to those reported by SILVA et al. [1992]. For the Swedish Landrace, UREMOVIC et al. [1995] describe the following relationships between the mentioned traits: r_G=0,18-0,52 and r_G=0,14-0,45. Different correlation coefficients obtained by the authors may result from the interspecies differences, variation of productive environment where the researches were made and the different measurement methods. The highest positive correlation determined in the present investigations was recorded for meat quantity and loin “eye” height (Tab. 1, 2). The interdependences between the mentioned traits were estimated at the level of r_G=0,834 (PL) and r_G=0,718 (line PL-23). On these grounds it is affirmed that these traits constitute the proper indices characterizing the conformation, thus the selection including one of them will lead to meatiness growth. For the pig population of PLW breed, the genetic trends were estimated by KAPŁON et al. [1990b] for the years 1978-1987. They found the lower estimates of the breeding improvement for the fattening and slaughter traits as compared to the present investigations. The genetic trends, according to KAPŁON et al. [1990b] developed like that: 0,4+-0,4 for ADG and -,009 +-0,01 for BF.

In the present studies on backfat thickness, there were obtained the similar trends just like HUDSON and KENNEDY [1985] reported for Yorkshire, Landrace, Hampshire and Duroc breeds animals born between 1976 and 1983 in Canada (-,12: -,18: -,02: -,02 mm/year, respectively). According to CHEN et al. [2002] the genetic trends for this trait were -,39 mm/year. However, the researches conducted by CSATO et al. [1994] analyzing the breeding work over the years 1986-1996 on the Hungarian Landrace and Hungarian White did not exhibit any differences in backfat thickness. In France, the genetic trends were evaluated for LW and FL over the years 1969 – 1981. While, WEBB [1995] recorded for gains: 2,9g and 1g respectively and for backfat thickness -,26 mm and -,16 mm/year. The genetic trends computed by LUNDHEIM and ERIKSSON [1984] and ROEHE et al. [1993] for Landrace and Yorkshire developed as following: 6g and 4g for daily gain and ,40 and ,58 for % meat content.

In the light of the obtained results, the genetic improvement for the analyzed pig population was shown beneficial within daily gain and backfat thickness, whereas for the line PL-23 for % meat content as well. The positive values of genetic trends

confirm the proper direction of the breeding work, while the low values imply poor selection efficiency. Therefore, in order to attain better effects of performance parameters, the results determining the breeding animals value with BLUP method should be implemented in practice as this method serves better prediction.

References

BIZELIS, J.; KOMINAKIS, A.; ROGDAKIS, E.; GEORGADOPOULOU, F.:
Genetic parameters for production and reproductive traits in on a farm testeg Danish Large White and Landrace swine in Greece. Arch. Tierz., Dummerstorf **43** (2000), 287-298

BUCZYŃSKI, J.:
Kształtowanie się niektórych cech produkcyjnych u czterech pokoleń świń rasy polskiej białej zwisłouchej, hodowanych w stadzie zamkniętym. Rozprawy naukowe, Rozprawa hab. Rocz. AR w Poznaniu, Zeszyt 184, 1988.

CHEN, P.; BAAS, T.J.; MABRY, J.W.; DEKKERS, J.C.M.; KOEHLER, K.J.:
Genetic parameters and trends for lean growth rate its components in U.S. Yorkshire, Duroc, Hampshire and Landrace pigs. J. Anim. Sci., **80** (2002), 2062-2070

CSATO, L.; GROENEVELD, E.; FARKAS, J.; RADBOCZI, L.:
Genetische Trends in der Ungarischen Schweinezucht. Zanost i Praksa u Poljoprivredi i Prehrambenoj Tehnologiji, 24:1, 11-16; Paper presented at the 2nd International Symposium (Animal Scientific Days), Rovinj, Croatia, 21-31 September; 17 ref., 1994.

DUNIEC, H.; RÓŻYCKI, M.:
Wytyczne dotyczące organizacji centrów hodowlanych trzody chlewnej. Wyd. Min. Roln. 8608/72, 1972.

DUNIEC, H.; RÓŻYCKI, M.:
Możliwość zastosowania metody BLUP z wykorzystaniem modelu zwierzęcia do oceny świń. Biul. Inf. IZ., R XXXI, 5, 6, 1993, 5-13

FERRAZ, J.B.; JOHNSON, R.K.:
Animal model estimation of genetic parameters and response to selection for litter size and weight, growh and backfat in closed seedstock populations of large white and Landrace swine. J. Anim. Sci., **71** (1993), 850-858

GRONEVELD, E.; KOVAC, M.; WANG, T.:
Computing algoritm in a general purpose BLUP package for prediction and estimation. Arch. Tierz., Dummerstorf **35** (1992), 4-8

GUERRA, D.; DIEGUEZ, F.J.; SANTANA, I.; GERARDO, L.; TRUJILLO, C.:
Genetic and phenotypic parameters of a pig paternal synthetic breed. Cuban Journal of Agricultural Science, **26** (1992) 1, 11-15

HUDSON, G.F.S.; KENNEDY, B.W.:
Genetic trend of growth rate and backfat thickness of swine in Ontario. J. Anim. Sci.. **61** (1985) 1, 92-97

KAPŁON, M M.; ROTHSCHILD, M.F.; BERGER, P.J.; HEALEY, M.:
Wskaźniki genetyczne dla cech tucznych i reprodukcyjnych w zarodowej populacji świń rasy wbp. Materiały na LV Zjazd Naukowy PTZ, 20-21 września w Szczecinie, ART Olsztyn, 1990a, 3-4

KAPŁON, M.; ROTHSCHILD, M.F.; BERGER, P.J.; HEALEY, M.:
Trendy genetyczne i fenotypowe w zarodowej populacji świń rasy wbp. Materiały na LV Zjazd Nauk. PTZ, 20-21 września w Szczecinie, ART Olsztyn, 1990b, 4-5

KAPŁON, M.J.; ROTHSCHILD, M.F.; BERGER, P.J.; HEALEY, M.:
Population parameters estimates for performance and reproductive traits in polish large white nucleus herds. J. Anim. Sci., **69** (1991), 91-98

KOSOVAC, O.; PETROVIC, M.; SRECKOVIC-RAMSEY, D.; RADOJKOVIC, D.:
Genetska varijabilnost proizvodnih osobina nazimica i efekti selekcije. Genetic variation of performance traits in gilts and the effect of selection. Biotehnologija u Stocarstvu, 5-6, 1997, 311-318

LUNDEHEIM, N.; ERIKSSON, J.A.:
Estimating genetic change in the Swedish pig population by using mixed model methodology (BLUP). Acta Agriculturae Scandinavica. **34** (1984), 97-106

MERKS, J.W.M.:
Genotype × environment interactions in pig breeding programmes. III. Environmental effects and genetic parameters in on-farm testet. Livestock Production Science. **18** (1988) 2, 129-140

MISZTAL, I.:
MTC – Program REML do szacowania komponentów wariancji. Opis programu komputerowego, 1994.

ROEHE, R.; KRIETER, J.; KALM, E.:
The effect of number of sows and service time of boars on selection response and inbreeding using an animal model in a closed nucleus herd. journal of Animal Breeding and Genetics. **110** (1993) 2, 114-125

RÓŻYCKI, M.:
Organizacja pracy hodowlanej nad trzodą chlewną w stadzie zamkniętym. Wyd. IZ, Kraków, 1974.

RÓŻYCKI, M.:
Podstawy genetyczne pracy hodowlanej nad trzodą chlewną w stadzie zamkniętym. IZ, Wyd. własne – Rozprawy hab. Kraków, 1977.

RÓŻYCKI, M.:
Główne kierunki badań Instytutu Zootechniki w zakresie genetycznego doskonalenia zwierząt gospodarskich. Biul. Inf. IZ, Kraków, Rok XXI, NR 4-5-6, 1983, 4-11

SILVA, M. DE A.; CATALAN, G.; TORRES, R. DE A.; CARNEIRO, L.H.D.M.:
Estimativas de componentes geneticos de caracteristicas de importancia economica, em tres diferentes racas de suinos. Estimates of genetic components of economically important traits in three different pig breeds. Revista da Sociedade Brasileira de Zoot, 21, 5, 1992, 923-932

UREMOVIC, M.; UREMOVIC, Z.; SPERANDA, T.; SPERANDA, M.:
Correlations between ultrasonic measurements on live pigs and carcass measurements. Zbornik Biotehniske Fakultete Univerze v Ljubljani, Kmetijstvo. No. Supp 22, 1995, 149-154

WEBB, A.J.:
Future challenges in pig genetics. Animal Breeding Abstract **63** (1995) 10, 731-736

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Arch. Tierz., Dummerstorf **50** (2007) Special Issue, 116-124

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Estimates of genetic parameters and genetic gain for reproductive traits in the herd of Polish Landrace sows for the period of 25 years of the breeding work

Abstract

Evaluation of the 25-year breeding work on PL sows (4816) over 1973-1999 and the line PL-23 (925) in 1984-1999 maintained in the closed pig herd in Pukarzów. Data sets were analyzed by using restricted maximum-likelihood programs. The estimated heritability factors of breeding usability characteristics remained at a low level and were, in the case of the population of the PL race sows: $h^2 = 0.023$ for the number of live piglet births (NBA), $h^2 = 0.027$ for the number of piglets on the 21st day (NW21), $h^2 = 0.030$ for the litter weight (LW21). The heritability of analogous indicators for line PL-23 sows was: 0.061, 0.058 and 0.075 respectively. Average genetic gain for PL were -.05 pigs/yr, -.04 pigs/yr, -.48 kg/yr for NBA, NW21 and LW21 respectively. Genetic trends for reproductive performance PL-23 breed were .17 pigs/yr, .10 pigs/yr, .54 kg/yr for NBA, NW21 and LW21 respectively.

Key Words: genetic gain, genetic parameters, reproductive traits, pig

Zusammenfassung

Titel der Arbeit: **Bewertung der genetischen Parameter und des Zuchtfortschritts für Reproduktionsmerkmale in der Mutterschweinezuchtherde der Rasse Polish Landrace für einen Zeitraum von 25 Jahren**

Untersucht wurden die Reproduktionsmerkmale von 4816 Sauen in Pukarzów, einer geschlossenen Herde Polnischer Landrasse der Jahre 1973-1999 sowie im gleichen Betrieb 925 Sauen der Linie PL-23 aus den Jahren 1984-1999. Die Berechnungen erfolgten mittels der REML-Methode. Die geschätzten Heritabilitätskoeffizienten waren relativ niedrig und lagen bei der Polnischen Landrasse für die Anzahl lebend geborener Ferkel bei 0,023, für die Ferkelzahl am 21. Tag 0,027 und das 21 Tage Wurfgewicht bei 0,030. Die analogen Schätzwerte für die Linie PL-23 waren 0,061, 0,058 bzw. 0,075. Die linearen Regressionskoeffizienten für den genetischen Trend in den untersuchten Jahren lagen für diese drei Merkmale bei der Polnischen Landrasse bei -0,05, -0,044 bzw. -0,484. Die analogen Schätzwerte für die Linie PL-23 betrugen 0,166, 0,099 bzw. 0,544.

Schlüsselwörter: Zuchtfortschritt, genetische Parameter, Geburts- und Aufzuchtleistung, Schwein

In the late 1960s in Poland, in spite of the use of a strict selection, a lack of the genetic gain in the scope of breeding usefulness was ascertained. Besides that, in fattening and carcass characteristics, a regression was observed. For that reason, to allow the further improvement of the stock, with the beginning of the 1970s, work over the new organization of pig farming in Poland began, intended on consolidation of pure-breeds [DUNIEC and RÓŻYCKI, 1972; RÓŻYCKI, 1974, 1977]. Having these premises in mind, breeding centres were appointed in 1974 (closed herds) to produce specialized lines, intended in the future for interbreed crossing and interlinear crossing, and to prevent disease conveyance. The essential target, which inspired the idea to create large breeding units, was the differentiation of the population of pigs within national breeds and the limitation of the inflow of pigs' genes resulting from importation.

Bearing in mind the minimization of inbreeding, it was assumed that at least 8 groups of sows and 8 groups of boars should enter the breeding centre [RÓŻYCKI, 1977]. Breeding and selective work were held on the basis of a previously established breeding program. The next emphasized problem in the breeding and selective work at breeding centres was the useful characteristics' improvement, important from the economic point of view. In this aspect, special attention was paid to the improvement of the growth rate and better utilization of the fodder, the improvement of meat characteristics and reproductive usefulness. The program of genetic improvement of useful characteristics of the national pig herd was realized in Poland in 32 economic(breeding) centres, attaining different stages of program realization and diverse level of productivity coefficients [BUCZYŃSKI, 1988; SŁOMKOWSKI, 1985].

The basic imperfection was the lack of exact verification of established breeding targets, with reached performance. Although to work out the breeding programs was of great importance, most often there was not sufficient time or patience to check into which measure these plans became realized. The minute recognition of effectivness of the breeding work held in Poland in the scope of pigs [SŁOMKOWSKI, 1985; BUCZYŃSKI, 1988; KAPŁON, 1991; LECHOWSKA, 1998] determined the encouragement to enter upon research concerning this problem.

In this work an attempt has been made to present an assessment of the results of breeding work with a herd of breeding pigs in Pukarzow. Genetic parameters and genetic trends have been assessed, since knowing the values of these parameters for characteristics which should be improved when breeding in a specific population using sound assessments is indispensable for the efficient development of a breeding programme for future years.

Materials and methods

The material for the study consisted of data relating to 16,528 litters including 4,816 first litters (PL) and 2,417 litters which included 925 first litters (the PL-23 line), recorded in zootechnical documentation held at the Pig Breeding Farm in Pukarzow for the period 1973 to 1998. This performed work includes results concerning the PL breed in fourteen generations concerning a time period of 26 calendar years, and lines PL- 23 in ten generations for the cycle of 15 years.

Heritability and repeatability indicators were calculated for the following characteristics: fertility (the number of live piglet births - NBA), the number of piglets raised (NW21), litter weight (LW21).

Genetic correlations were defines between the following characteristics:

1. fertility and the number of piglets on the 21st day,
2. fertility and the litter weight on the 21st day,
3. the number of piglets raised and the litter weight on the 21st day.

The heritability and repeatability of the characteristics and genetic correlation was assessed applying Eildert Groeneveld's VCE 4.2.5 computer program using the REML (Restricted Maximum Likelihood Procedure) method. In estimating the parameters, an individual model was used because an animal model takes into account all genetic and environmental links so the calculations are not affected by selection errors, and hence they appear to give the most credible value estimates for breeding animals [ADAMEC and JOHNSON, 1997; GROENEVELD et al., 1992; CHEN et al., 2003;

HANENBERG et al., 2001; KAPŁON, 1991; KENNEDY et al., 1988; SOUTHWOOD and KENNEDY, 1991].

Breeding usability indicators for PL breed sows were calculated using the model:

$$\mathbf{Y_{ijklmno} = \mu + R_i + S_{ij} + RM_{ijk} + M_{ijkl} + p_m + a_n + c_m + e_{ijklmno}}$$

$Y_{ijklmno}$ – the phenotype value of the individual

μ - population mean

R_i - the fixed influence of the sow's birth year

S_{ij} - the fixed influence of the litter birth season

RM_{ijk} - the fixed influence of the litter birth year

M_{ijkl} - the fixed influence of the subsequent litter

p_m - the random influence of the maternal environment

a_n –the random additive genetic value of the individual

c_m - the random genetic influence of the mother

$e_{ijklmno}$ - the random influence of the environment.

In the case of the sows of the PL-23 line the calulations were carried out using the following model:

$$\mathbf{Y_{ijklmn} = \mu + R_i + S_{ij} + RM_{ijk} + M_{ijkl} + p_m + a_n + e_{ijklmno}}$$

The basis for determining genetic trends in population studies were solutions for the year of birth of the individual describing changes in genetic quality over time. Genetic progress was expressed as a straight-line regression coefficient. The calculations were carried out using Ignacy Misztala's BLUPf90 computer program utilising a multi-characteristic individual model, taking into account the same fixed and random factors as in the case of the genetic parameters.

Results

The estimated heritability factors of breeding usability characteristics remained at a low level and were, in the case of the population of the pbz race sows: $h^2 = 0.023$ for the number of live piglet births (NBA), $h^2 = 0.027$ for the number of piglets on the 21st day (NW21), $h^2 = 0.030$ for the litter mass (LW21) (Tab. 1). The heritability of analogous indicators for line pbz-23 sows was: 0.061, 0.058 and 0.075 respectively (Tab. 2). The estimated parameters for characteristics linked to reproduction showed an admittedly small, but significant influence of genetic principles on the fertility characteristics of the females.

Table 1
Heritability coefficients on a diagonal table (h^2)and genetic correlation coefficients (r_G) for the PL breed

h^2	NBA	NW21	LW21
NBA	*0.023*	r_G – (0.985)	r_G – (0.781)
NW21	r_G – (0.985)	*0.027*	r_G – (0.823)
LW21	r_G – (0.781)	r_G – (0.823)	*0.030*

Table 2
Heritability coefficients on a diagonal table (h^2)and genetic correlation coefficients (r_G) for the PL-23 line

h^2	NBA	NW21	LW21
NBA	*0.061*	r_G – (0.984)	r_G – (0.891)
NW21	r_G – (0.984)	*0.058*	r_G – (0.901)
LW21	r_G – (0.891)	r_G – (0.901)	*0.075*

The values of the genetic correlation coefficients between the breeding usability characteristics of the PL race sows analysed are presented in Table 1. From the data therein it can be concluded that the high dependencies between the characteristics means that it is permissible to take just one of them into account when selecting, e.g. the number of piglets reared to the 21st day of life.
The number of farrows born alive was significantly correlated (r_G = 0.985) with the number of farrows at day 21 of life (r_G = 0.781) and with the litter weight at day 21. The correlation between the number of farrows at day 21 of life and with the litter weight in this period achieved the level of r_G = 0.823. Genetic correlation coefficients were also high for the PL- 23 breed (Tab. 2). As ascertained in the author's own research, there is a high positive correlation between the sow breeding useful characteristics.
The next very important parameter in breeding and selective work is the repeatability of production characteristics (i.e. the ability to repeat the same values in subsequent litters). The results given in Table 3 show that the estimated repeatability of the useful breeding characteristics ranged from 0.116 to 0.236.

Table 3
Repeatability coefficients of the breeding usage characteristics r^2

Item	PL breed	Line PL-23
NBA	0.117	0.209
NW21	0.116	0.193
LW21	0.122	0.236

Table 4
Genetic advances in the analysed pig population

Breed	Characteristics	Straight-line regression coefficient	
PL	NBA	-0.005	
			p≤0.69
	NW21	-0.044	
			p≤0.00
	LW21	-0.484	
			p≤0.00
Line PL-23	NBA	0.166	
			p≤0.00
	NW21	0.099	
			p≤0.00
	LW21	0.544	
			p≤0.00

It was shown that estimated genetic trends for sows of the PL breed (Tab. 4, Fig. 1, 2) had been very minor and not always desirable. Negative directions of changes in number of born alive and reared farrows (Fig. 1) appeared in 1975-1983 and 1985-1992. Analysing the formation of the genetic trend for litter weight (Fig. 2), the rise of estimated breeding values in sows by 1983 was ascertained, whereas from 1985 the values were decreasing, and the renewed positive trend appeared from the year 1992. The results of the tendency valuation expressed with the standard deviation for the line PL-23 were shown in Fig. 3 and 4. The graphic image of the trend value evidences the positive direction of changes, both for the number of born and reared farrows, and for the litter weight.

Fig. 1: Genetic trends in PL
Genetische Trends (Rasse PL)

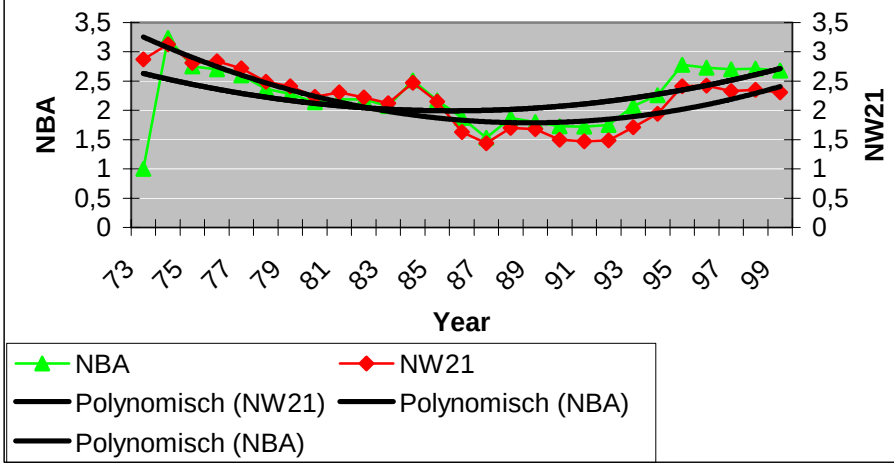


Fig. 2: Genetic trends for LW21 (PL)
Genetische Trends der Wurfmasse (PL)

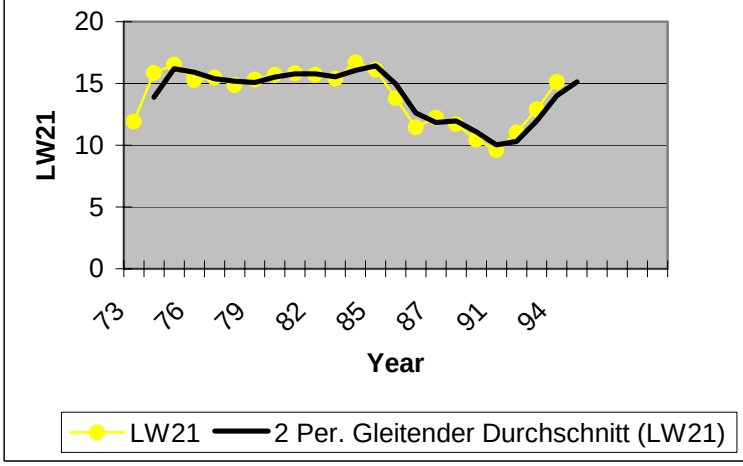


Fig. 3: Genetic trends (PL-23)
Genetische Trends der Linie PL-23

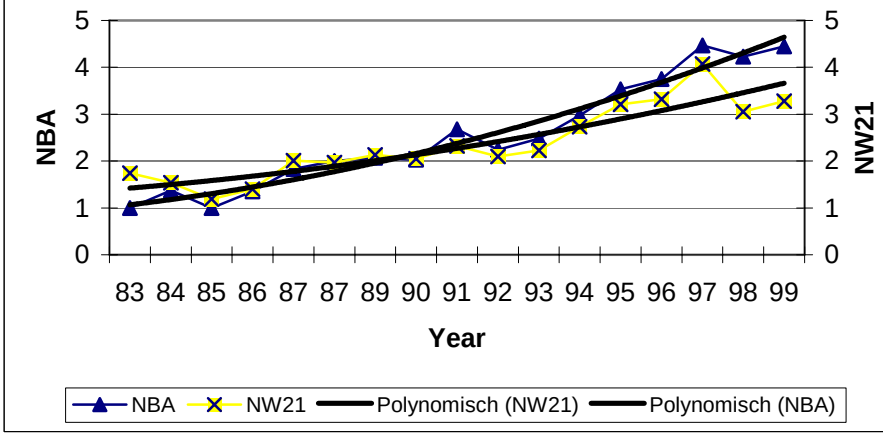
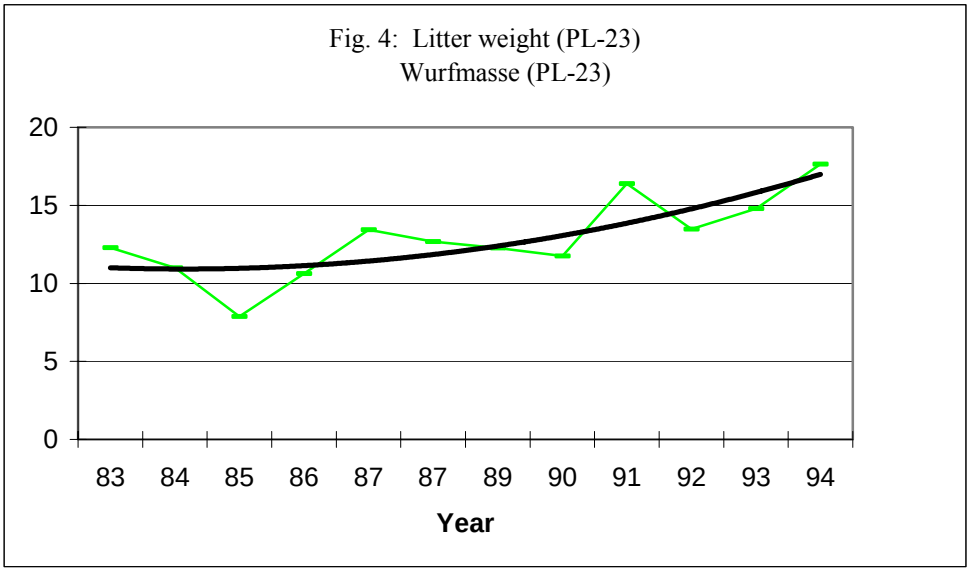


Fig. 4: Litter weight (PL-23)
Wurfmasse (PL-23)



Discussion

The planned target will not be achieved by the selection based on the animal phenotype, and often undesirable results may be achieved. Separation of genetic variability in the population, and by that a heritability coefficient, is extremely important, as this parameter determines the effectivity of the selection and the level of predicted genetic gain, with respect to the certain period of time and at the specific selective difference. The numerical values of breeding characteristics' heritability obtained in this work correspond with the results for the wbp breed reported by BIZELIS et al. [2000], KAPŁON et al. [1990a], HANENBERG et al. [2001] (for sows Dutch Landrace) and HAMMANN et al. [2004] (0.09 – 0.15 for the number of farrows born alive for the breed Pietrain and Teutons Landrace, respectively). Convergent results regarding the number of farrows born alive (h^2 =0.12) and the litter weight (h^2 =0.09) were obtained by ADAMEC and JOHNSON [1997] who investigated the sows of the breed Large White and Landrace from pedigree herds in Czech Republic. However, BUCZYŃSKI [1988] obtained the following heritability coefficients: h^2= 0.152 (for the number of farrows of day 1.), h^2 = 0.096 (for the number of farrows at day 21.) and h^2 = 0.154 (for litter weight). Higher coefficients of this parameter were obtained by LEWCZUK et al. [1991c]. According to them, the average heritability for the total number of farrows born alive, in the whole of the sow's productive life for the PL and PLW breeds, amounted to respectively 0.256 and 0.267, and farrow at day 21 numbered 0.238 and 0.255, and litter weight 0.403 and 0.410. Similar results are reported by LECHOWSKA [1998]. Low heritability coefficients indicate not only the susceptibility of the breeding characteristics on the activity of environmental factors (which actively control the efficiency of a sow organism), but also on the great constancy of the genetic information being descended from the parents. The reported values show that the genetic lability has a small share in the entire phenotypic liability, observed between sows. Thus, it can be inferred from the obtained h^2 values that phenotype-based selection not greatly increases the chance of the breeding characteristics' improvement. The selection based on the results of breeding value estimation should be done in the sow herd of the PL breed, by use of the most currently reliable BLUP method. In Denmark, for example, a combined procedure is

applied to estimate the breeding value in respect of the breeding use, emphasizing the h^2 and repeatability of the feature, using the selection focused on the optimum litter size [SORENSEN, 1991].

The analysis of breeding characteristics in the author's own research showed a high level of genetic connectedness, which evidences the possibility to limit the selective criteria to one feature only. Similar results concerning correlation between the characteristics useful for breeding in sows were achieved by KAPŁON et al. [1990a] for sows of the PLW breed (0.91 NBA-NW21, 0.68 NBA-LW21, 0.81 NW21-LW21). Slightly lower coefficients of the above parameter were received by LEWCZUK et al. [1992]. These authors showed the following correlations in sows of the pbz breed, investigated in the years 1970-1985: between the number of farrows born alive and the number of farrows at day 21 $r_G = 0.770$, between the number of farrows born alive and litter weight $r_G = 0.671$ and between the number of farrows born alive at day 21 and litter weight $r_G = 0.797$.

For sow breeding success the knowledge of the characteristics' repeatability in the following litters is crucial. The above parameter for breeding characteristics accepted values from 0.12 to 0.24. A slightly different value for the analysed feature in sows of pbz breed was achieved by LEWCZUK et al. [1991c] ($r_P = 0.169$ LU, $r_P = 0.169$ LO, $r_P = 0.183$ M21). Repeatability coefficients for the PLW breed [LEWCZUK et al., 1991c] totalled respectively: 0.157 NBA, $r_P = 0.136$ NW21, $r_P = 0.175$ LW21. Then, according to KUJAWIAK and RATAJSZCZAK [1992] it fell into the following ranges: for NBA $r_P = 0.196$-0.195, NW21 $r_P = 0.162$-0.165 and LW21 $r_P = 0.226$-0.199. However, according to BUCZYŃSKI [1988], the repeatability of the farrow number at birth amounted to $r_P = 0.148$, numbers of reared farrows at day 21 $r_P = 0.193$ and the litter weight $r_P = 0.242$. LECHOWSKA [1998] and ORZECHOWSKA [1998] asserted that the value of this parameter for breeding use reaches the level of 0.14 – 0.23. As a result of the low values of repeatability coefficients of the described characteristics, the accuracy of the estimation of the reproductive ability in sows increases along with the quantity of the following litters taken into account. For that reason, a late estimation of the multiparous sows increases the accuracy. However, it is responsible for the extension of the distance between generations, which simultaneously bears on the decrease of the genetic gain.

The primary target of the breeding work is the improvement of the genetic value of the pedigree herd. Changes in productivenesses, appearaing over long time periods, and caused by the differences of the average value of the genetic population, are qualified as genetic trends [ATIL, 2000; KUNAKA et al., 2001]. Understanding the value of genetic changes gives much of the most valuable information concerning, first of all, estimation of the efficiency of practical breeding methods. The genetic trends for each feature analysed in this work for the population of sows were estimated on the grounds of solutions for the following years of birth, received at the simultaneous estimation of constant and accidental influence, on the grounds of individual model equations, i.e. a BLUP method. It has to be stated that the genetic reactions for characteristics useful in breeding in the analysed population of sows were small and not always desirable. Genetic progress related to the PL breed for NBA amounted to on average –. 005, for NW21 –. 04 and for LW21 –. 48 (Tab. 3). The lack of efficiency in selection made for breeding characteristics, estimating sows of the PL breed from southeastern Poland, was also reported by LECHOWSKA [1998]. However, in the author's own research,

the regression of the number of farrows born alive, reared farrows and the litter weight in sows of the PL-23 line was proved as positive. Estimated values of the genetic progress of the above characteristics totalled respectively: . 17 (NBA), . 10 (NW21) and . 54 (LW21). The results concerning genetic trends shown in the presented work are difficult to compare because of the scarcity of reports in the national literature concerning these issues. The trends estimated on the grounds of the genetic animal value with the utilization of the REML method by KAPŁON et al. [1990b], for the PLW breed were as follows: . 01 ± . 01 for the number of farrows born alive and farrows at day 21, and . 45 ± . 06 for litter weight. In France, as a result of the breeding work from 1977-1998, positive changes in the genetic value for the survival rate of embryos and numbers of farrows born alive were reported [TRIBOUT et al., 2002]. As SOUTHWOOD and KENNEDY [1991] reported for the Yorkshire and Landrace breeds included in the breeding program from 1977-1987, the genetic trends were very small and negative, as indicated by results from -.012+/-.004 to .004+/-0.002 for farrows per year. Very small, though positive trends, were achieved in Brazil [FILHO et al., 2005]. In the United States CHEN et al. [2003] estimated the efficiency of breeding work in the herd of sows from the National Register of Pigs. The authors stated that genetic changes were positive and amounted to . 018 farrows/ year and . 114 kgs for the weight of farrows at day 21/year.

The obtained positive values of genetic trends prove the correctness of direction in the breeding work and proper selection of animals for mating. However, their low values prove a low selection efficiency, which probably results from including a large number of characteristics into the breeding program, and the use of selective criteria relying only on the animals' own phenotype. As a consequence, it can be predicted that the continuation of the work in herd with the utilization of presently available methods of genetic analysis - BLUP animal model, will permit a more precise estimation of productivity coefficients, and simultaneously the use of these values to direct the selection.

References

ADAMEC, V.; JOHNSON, R.K.:
Genetic analysis of rebreeding intervals, litter traits, and production traits in sows of the National Czech nucleus. Livest. Prod. Sci. **48** (1997), 13- 22

ATIL, H.:
Genetic relationships between days open and days dry with milk yield in a herd of Holstein Friesian cattle. Arch. Tierz., Dummerstorf **43** (2000), 573-582

BIZELIS, J.; KOMINAKIS, A.; ROGDAKIS, E.; GEORGADOPOULOU, F.:
Genetic parameters of production and reproductive traits in on a farm tested Danish Large White and Landrace swine in Greece. Arch. Tierz., Dummerstorf **43** (2000), 287-298

BUCZYŃSKI, J.:
Kształtowanie się niektórych cech produkcyjnych u czterech pokoleń świń rasy polskiej białej zwisłouchej, hodowanych w stadzie zamkniętym. Rozprawy naukowe, Rozprawa hab. Rocz. AR w Poznaniu, Zeszyt 184, 1988.

CHEN, P.; BAAS, T.J.; MABRY, J.W.; KOEHLER, K.J.; DEKKERS, J.C.M.:
Genetic parameters and trends for litter traits in U.S. Yorkshire, Duroc, Hampshire and Landrace pigs. J. Anim. Sci. **81** (2003), 46-53.

DUNIEC, H.; RÓŻYCKI, M.:
Wytyczne dotyczące organizacji centrów hodowlanych trzody chlewnej. Wyd. Min. Roln. 8608/72, 1972.

FILHO, R.DEA.T.; TORRES, R.DEA.; LOPES, P.S.; PEREIRA, C.S.; EUCLYDES, R.F.; DE ARAUJO, C.V.; SILVA, MDEA.:

Genetic trends in the performance and reproductive traits of pig. Genetic Molec. Biol. **28** (2005), 97-102

GRONEVELD, E.; KOVAC, M.; WANG, T.:
Computing algoritm in a general purpose BLUP package for prediction and estimation. Arch. Tierz., Dummerstorf **35** (1992), 4-8

HAMANN, H.; STEINHEUER, R.; DISTL, O.:
Estimation of genetic pareameters for litter size as a sow and boar trait in German herdbook Landrace and Pietrain swine. Livestock Prod. Sci. **85** (2004), 201-207

HANENBERG, E.; KNOL, E.; MERKS, J.:
Estimates of genetic parameters for reproduction traits at different parities in Dutch Landrace pigs. Livestock Prod. Sci. 69, 2001, 179-186.

KAPŁON, M.J.; ROTHSCHILD, M.F.; BERGER, P.J.; HEALEY, M.:
Population parameters estimates for performance and reproductive traits in polish large white nucleus herds. J. Anim. Sci., **69** (1991), 91-98.

KENNEDY, B.W.; SCHAEFFER, L.R.; SORENSEN, D.A.:
Genetic properties of animal models. J. Dairy Sci. **71** (Suppl.2), (1988), 17-26

KUJAWIAK, R.; RATAJSZCZAK, M.:
Odziedziczalność i powtarzalność cech użytkowości rozpłodowej świń rasy złotnickiej białej. Rocz. AR w Poznaniu, CCXXXIX, Zoot. **43** (1992), 91-100.

KUNAKA, J.W.; MAKUZA, S.M.; WOLLNY, C.B.A.; BANDA, J.W.:
Genetic trends for milk and protein in the Zimbabewan Holstein–Friesian population from 1973 to 1994. Arch. Tierz., Dummerstorf **44** (2001), 23-33

MISZTAL, I.:
MTC – Program REML do szacowania komponentów wariancji. Opis programu komputerowego. 1994.

LECHOWSKA, J.:
Próba oceny zmian cech reprodukcyjnych świń rasy pbz pod wpływem pracy hodowlanej w latach 1960-1990 w rejonie południowo-wschodniej Polski. Praca doktorska. Rzeszów, 1998.

LEWCZUK, A.; JANISZEWSKA, M.; GRUDNIEWSKA, B.; BOCHNO, R.:
Odziedziczalność i powtarzalność cech dzielności rozrodczej loch hodowanych na terenie działalności OSHZ w Olsztynie. Acta Acad. Agricult. Tech. Olst., Zoot, **34** (1991c), 23-32

ORZECHOWSKA, B.:
Możliwości poprawy użytkowości rozpłodowej świń metodami hodowlanymi. Mat. sem. nt. „Genetyczne i środowiskowe aspekty intensyfikacji produkcji trzody chlewnej". Balice, 29-30 październik, Kraków, 1998, 21-30

ORZECHOWSKA, B.; ŻAK, G.:
Wyniki oceny użytkowości rozpłodowej loch . Stan hodowli i wyniki oceny świń w roku 1994, Kraków, 1995.

RÓŻYCKI, M.:
Organizacja pracy hodowlanej nad trzodą chlewną w stadzie zamkniętym. Wyd. IZ, Kraków, 1974.

RÓŻYCKI, M.:
Podstawy genetyczne pracy hodowlanej nad trzodą chlewną w stadzie zamkniętym. Inst. Zoot., Wyd. własne – Rozprawy hab.. Kraków, 1977.

SŁOMKOWSKI, K.:
Ocena zrealizowanego postępu w zamkniętych populacjach świń. Praca dokt. Kraków, 1985.

SOUTHWOOD, O.I.; KENNEDY, B.W.:
Genetic and environmental trends for litter size in swine. J. Anim. Sci. **69** (1991), 3177-3182

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Arch. Tierz., Dummerstorf **50** (2007) Special Issue, 125-135

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Estimation of conditionality of pork sensory quality by using multivariate analysis

Abstract

The aim of the work was analysis of conditionality of the raw pork meat sensory quality defined in coherence with its technological quality and carcass meatiness with employment of multivariate statistic analysis.
The research was executed on material taken from 50 hogs originated from crossing of PLW x PL and Naïma sows with hybrids P76-PenArLan boars. All animals were slaughtered at 100 kg body weight. Content of meat was estimated by using of CGM apparatus. The samples were collected from *Longissimus lumborum* muscle and next pH in 45 minutes and 24 post mortem as well as drip loss were measured. Sensory quality of raw meat was evaluated in 48 h and 96 h after slaughter. Intensity and homogeneity of meat colour, marbling, drip loss and acceptability were also evaluated. The results were elaborated by using principal component and cluster analysis. The studied group of fatteners was characterized by high meatiness (56% of meat in carcass) and good meat quality (lack of faulty meat). Principal component analysis showed that approximately 64-71% of variability was explained by 3 or 4 main component related to marbling, acceptability, ultimate pH, slaughter weight. pH_1 and meatiness. The cluster analysis by applying the Ward method gave a possibility singling out three homogeneous groups. Received results indicated strong dependence of acceptability of meat from its marbling and relationship with drip loss and pH changes getting after slaughter in meat.

Key Words: pig, meat quality, sensory raw meat quality, PCA, cluster analysis

Zusammenfassung

Titel der Arbeit: **Prüfung von Bedingungen der sensorischen Schweinefleischqualität unter Nutzung mehrdimensionaler Analysen**
Das Ziel der Arbeit war die Prüfung der sensorischen Qualität von rohem Schweinefleisch im Zusammenhang mit seiner technologischen Qualität und der Fleischigkeit der Schweinehälften unter Nutzung mehrdimensionaler statistischer Analysen.
Untersucht wurden 50 Mastschweine aus der Kreuzung von PWL x PL und Naima Sauen mit P76-PenArlan Hybridebern. Die Schlachtung der Tiere erfolgte mit 100 kg. Nach der Schlachtung wurde der Fleischgehalt der Hälften als Muskeldurchmesser am *M. longissimus dorsi* und der Rückenspeckdicke mit dem CGM Apparates bestimmt. Die pH Messungen 45m und 24h p.m. sowie des Dripverlustes erfolgten am *M. longissimus lumborum*. Die sensorische Qualität wurde 48h und 96h p.m. durch Fleischfarbe, intramuskuläres Fett und Wasserbindung bestimmt. Die Tiere zeigten einen relativ hohen Fleischanteil von 56 % verbunden mit hoher Fleischqualität. Es werden die einzelnen Ergebnisse der Hauptkomponentenanalyse vorgestellt, analysiert und diskutiert. Die Ergebnisse zeigen, dass die Verbraucherakzeptanz maßgeblich von der Marmorierung sowie dem Dripverlust und der pH-Veränderung nach der Schlachtung bestimmt wird.

Schlüsselwörter: Schwein, Fleischanteil, Fleischqualität, sensorische Qualität, Hauptkomponentenanalyse, Klusteranalyse

Introduction

Pork meat is the most popular kind of meat in Europe and it comprises nearly 60% of total meat consumption. In Poland about 56% and in European countries about 20-

30% of this amount is sold as a row meat. Many researches carried out in recent 30 years showed that the increase of pigs meatiness causes a greater incidence of raw meat quality defects (SELLIER, 1998; ROSENVOLD and ANDERSEN, 2003). The breeds of high muscularity foreign pigs often have a gene adverse to meat quality (stress sensitivity $RYR1^T$ or acid meat RN^- gene) which is the reason of defects such as PSE, acid meat or boosted drip loss from muscle tissue after slaughtering (BERTRAM et al., 2000).

The sensory perception of meat depends from many factors such as the characteristics of the fresh matter employed, breed, weight, sex, diet and the biochemical changes that occur during further processing, slaughtering, maturation, heat treatment and cooking (RISVIK, 1994; FLORES et al., 1999).The eating pork quality evaluated as sensory perceptions during consumption consist of several attributes among the more important ones are tenderness, juiciness, flavour and absence of off-flavours (BRYHNI et al., 2003). In the case of raw meat bought by consumers for house consumption the significant traits are: the amount of visible fat and colour. Consumers tend to chose a light-pink meat without or with little fat without any marbling and also without any drip loss (POŁOM and BARYŁKO-PIKIELNA, 2004; RESURRECCTION, 2004). Acquaintance of like of consumer is important question for meat industry and capability to singling out in foothold about simple indices of proper meat of qualities.

The aim of the study was analysis of conditionality of the raw pork meat sensory quality defined in coherence with its technological quality and carcass meatiness with employment of multivariate statistic analysis.

Material and methods

In the study the group of 50 fatteners derived from crossing of PLW x PL and Naïma sows with hybrids P-76 PenArLan boars. The pigs were produced and reared in the same conditions and fatten by using a standard diet. The animals were slaughtered at 100 kg live weight. The percent of meat in carcass after slaughter (on the basis of measurement of muscle *Longissimus dorsi* thickness and back fat thickness) was estimated by CGM apparatus. For meat quality evaluation the samples were taken from the *Longissimus lumborum* muscle in 45 minutes and 24 h after slaughter. Value of pH was measured by CP-311 pH-meter after slaughter at 45 minutes and 24h. Drip loss was estimated at 48 h after slaughter according to the HONIKEL method (1987).

The 30 meat samples of different pH were taken for sensory analysis (remaining meat samples were repeated). Sensory quality of raw meat were estimated after 48 and 96 hours *post mortem*.

For sensory assessment the scaling method (PN-ISO 4121) was used. Assessor used a linear scale converted after collection into 0-10 conventional unit scale (c.u.). The quality of raw meat was estimated based on intensity and homogeneity of the colour, marbling (intramuscular lipids), drip and acceptability of appearance. The analyses were carried out using individual score sheets.

Analytical panel of 10 members (ISO 8586-2:1994) made each evaluation in duplicate – so that each mean result was based on 20 individual measurements.

Raw pork chops were placed on white, disposable polystyrene foam trays which are common used in the supermarket. Trays with meat were packed with polyethylene foil just as they used to be at the supermarket. Samples were analyzed at random order.

Sensory analysis were carried out in daylight room at room temperature. Conditions and the procedure of evaluation were established according to literature (MEILGAARD et al., 1999).
Research were carried in Division of Catering Technology and Food Hygiene, Faculty of Human Nutrition and Consumer Sciences, Warsaw Agricultural University – SGGW.
The results were analyzed by using principal component (PCA) and cluster analysis and also by analysis of variance with application of SPSS statistical program.

Results

The results showed that studied hogs were characterized by satisfactory carcass value and technological or sensory meat quality. In studied group was not said a faulty meat. It belongs to call attention to fact of greatest variability in traits as drip loss and marbling of meat (Table 1). Analysis of relations between traits described carcass and technological pork quality with sensory raw meat quality shown a significant relationship between pH_{24} and drip loss evaluated by sensory method and between marbling of meat and drip loss estimated by Honikel method (Table 2). These relationship showed that growth pH_{24} or marbling of meat would cause the decrease of drip loss from muscle tissue.

Table 1
Characteristics of technological and sensory raw meat quality for studied group of pigs

Traits	Mean	S. d.
Hot carcass weight (kg)	81,35	5,54
Meat in carcass (%)	56,61	2,21
Loin thickness (mm)	56,09	3,09
Backfatt thickness (mm)	13,84	3,49
pH_1	6,34	0,19
pH_{24}	5,50	0,08
Drip loss48 h (%)	6,95	2,46
Sensory quality of raw meat In 48 and 96 h post mortem (expressed on 0-10 conventional unit graphical scale):		
Homogenity of color 48 h	6,21	1,29
Intensity of color 48 h	5,67	1,34
Marbling 48 h	4,56	1,64
Drip loss 48 h	4,19	2,19
Acceptability 48 h	5,87	1,07
Homogenity of color 96 h	5,40	1,09
Intensity of color 96 h	6,02	1,04
Marbling 96 h	4,50	1,75
Drip loss 96 h	5,69	1,41
Acceptability 96 h	5,90	0,91

The analysis of variability by using of PCA method showed that total variance can be explained by 3 (64,41%) or 4 (71,63%) principal component depending on times of sensory raw meat evaluation (Table 3). In case of evaluation of the sensory quality of raw meat in 48 h after slaughter the first component (that explained 31,37% of variance) was coherent most strongly with color evaluation, marbling and acceptability, the second (explained 19,93% of variance) with pH_{24} and drip loss, and the third with hot carcass weight, pH_1 and drip loss (Table 3 and 4). However other results were received for estimation in 96 h after slaughter. The first three components were approximated in their participation in explaining total variability and with coherence with analyzed traits but the fourth component appeared with relation to the

percent of meat in carcass, color and pH_1 (Table 4). The traits which the most differentiated studied pigs were pH24, drip loss, marbling and acceptability (Figure 3 and 4).

Table 2
The relationship between traits described carcass and technological meat quality with sensory raw meat quality

	HCW	MC	LT	BT	pH1	pH24	DLHM
HC 48 h	0,11	0,09	-0,30	-0,21	-0,03	-0,30	0,13
IC 48 h	-0,05	0,29	-0,19	-0,36*	-0,17	-0,23	0,13
Mar 48 h	0,02	-0,17	0,01	0,18	0,19	0,17	-0,39*
DL 48 h	0,03	-0,08	0,04	0,10	-0,14	-0,52*	0,42*
Acc 48 h	-0,06	0,20	-0,16	-0,26	-0,04	-0,17	0,12
HC 96 h	0,04	0,24	-0,25	-0,34	-0,26	0,02	-0,07
IC 96 h	-0,07	0,02	-0,06	-0,05	-0,22	0,18	0,15
Mar 96 h	-0,02	-0,20	-0,09	0,17	0,23	0,14	-0,38*
DL 96 h	0,33	-0,04	0,19	0,11	-0,09	-0,38*	0,37*
Acc 96 h	0,14	0,11	0,14	-0,06	-0,19	-0,10	0,27

Explanation: HC – homogeneity of color, IC – Intensity of color, Mar – marbling, DL – drip loss evaluated by sensory method, Acc – acceptability, HCW – hot carcass weight, MC – meat in carcass, LT – loin thickness, BT – backfat thickness, DLHM – dripp loss evaluated by Honikel method, * - significant at $P < 0,05$

Table 3
Results of PCA analysis for relations between technological and sensory raw meat quality evaluated in 48 and 96 h after slaughter

Principal component	Evaluation in 48 h			Evaluation in 96 h		
	Eigen value	% of variance	cumulative	Eigen value	% of variance	cumulative
1	3,14	31,37	31,34	2,76	27,55	27,55
2	1,99	19,93	51,29	1,80	18,03	45,58
3	1,31	13,12	64,41	1,46	14,63	60,21
4	0,94	9,41	73,82	1,14	11,42	71,63
5-10	2,62	26,17	100,00	2,84	28,37	100,00

Table 4
The relations between measured traits characterized carcass, technological and sensory raw meat quality in studied group of pigs and important principal component (after rotation) in relations to time of evaluation after slaughter

Traits	Time of sensory evaluation						
	48 h			96 h			
	Principal component						
	1	2	3	1	2	3	4
Hot carcass weight (kg)			0,76			0,90	
Meat in carcass (%)	0,30	-0,39	-0,35				0,71
pH_1			0,70		0,41	0,45	-0,37
pH_{24}		-0,81			0,82		
Drip loss48 h (%)		0,68	-0,50	0,30	-0,80	-0,32	
Homogenity of color	0,71						0,79
Intensity of color	0,76			0,78			
Marbling	-0,87			-0,84			
Drip loss		0,80			-0,68	0,54	
Acceptability	0,94			0,88			

Analysis of cluster for search of hogs group with similar quality of carcass and meat was applied. This analysis showed a four groups of pigs which differed in technological and sensory quality of meat evaluated in 48 h post mortem (Table 5, Figure 1 and 3). Group noted as first was characterized by normal pH fall, average drip loss, intensity and homogeneity of color, low marbling and drip loss and also the higher acceptability. In next groups the differences were observed in pH_1, pH_{24}, drip

loss, marbling and in acceptability (Table 5). The higher drip loss was observed in group (2 and 3) with low pH_{24} and marbling. The lower acceptability of raw meat was noted in group (2, 3 and 4) characterized by higher marbling or drip loss (Table 5). Cluster analysis for carcass and meat quality traits with sensory evaluation in 96 h *post mortem* showed three groups with significant differences (Table 6, Figure 2 and 4). Similar dependences are observed as formerly in these groups. Differences in number of groups and changes in the group of membership may be explain by changes during maturation of meat (Table 6, Figure 1 and 2).

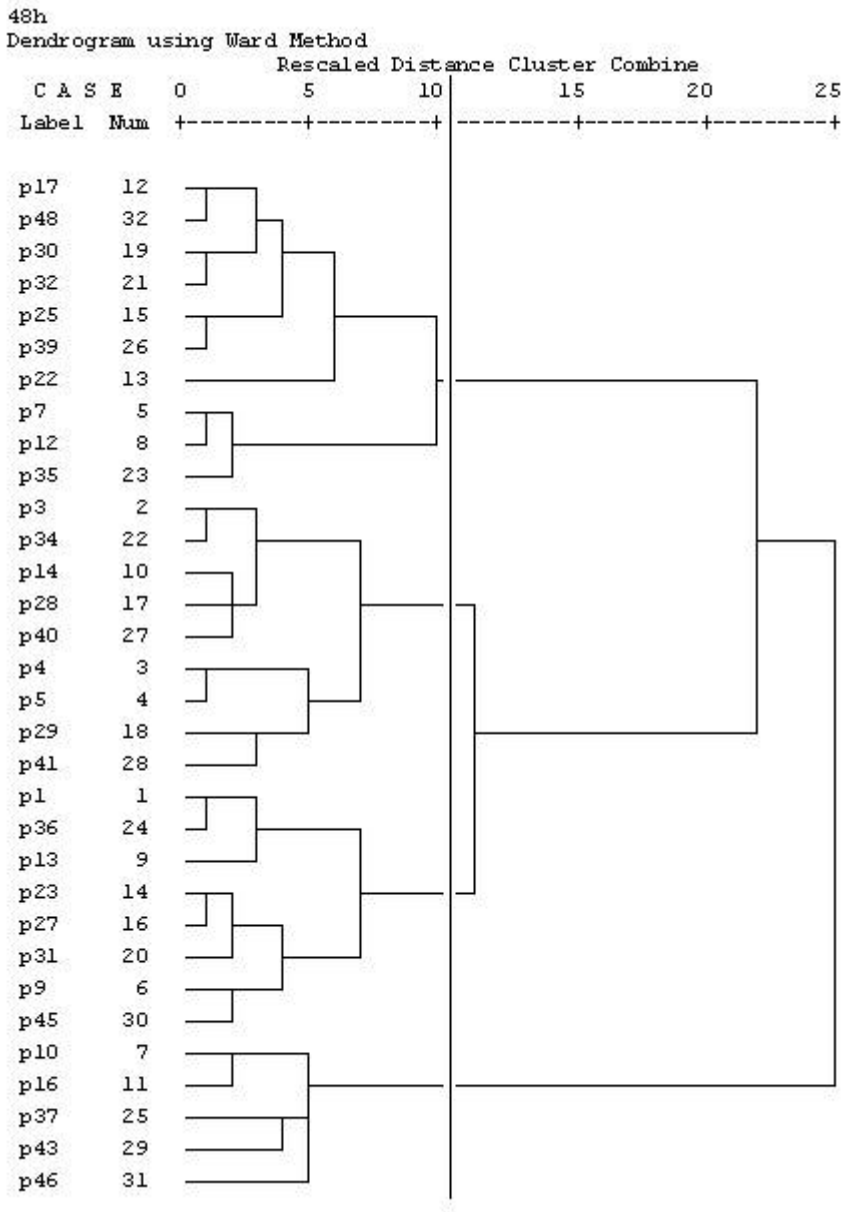


Fig. 1: Dendrogram showing course of cluster analysis with case of sensory analysis of meat in 48 h *post mortem* with differentiation of four group of pigs

Table 5
Characteristics of carcass value and meat quality of group of hogs differentiated by cluster analysis (sensory quality of raw meat was estimated in 48 h *post mortem*)

Traits	Number of cluster			
	1	2	3	4
Number of animals	10	9	8	5
Hot carcass weight (kg)	82,20±6,66	78,62±3,81	83,94±4,13	80,42±6,81
Meat in carcass (%)	57,48±1,92	57,01±1,65	55,50±2,36	55,96±3,02
pH_1	a 6,39±0,15	b 6,16±0,12	a 6,38±0,14	a 6,51±0,23
pH_{24}	a 5,54±0,07	b 5,42±0,03	b 5,47±0,09	a 5,55±0,08
Drip loss48 h (%)	a 6,04±1,09	b 9,65±1,95	a 6,67±0,91	a 4,37±1,83
Homogenity of color	a 6,79±0,81	a 6,33±1,17	a 6,58±0,75	b 4,24±1,33
Intensity of color	6,27±1,42	6,02±1,19	5,30±1,12	4,50±1,02
Marbling	a 3,43±1,19	ac 4,02±1,34	bc 5,21±1,06	b 6,79±1,18
Drip loss	a 2,34±1,10	b 5,50±2,15	b 5,93±1,24	a 2,75±1,13
Acceptability	a 6,83±0,91	ac 5,88±0,71	bc 5,47±0,70	b 4,57±0,66

Means with a row with different letters are significantly different $p<0.05$

Table 6
Characteristics of carcass value and meat quality of group of hogs differentiated by cluster analysis (sensory quality of raw meat was estimated in 96 h *post mortem*)

Traits	Number of cluster		
	3+4	1	2
Number of animals	14	7	11
Hot carcass weight (kg)	ab 81,28±5,93	a 86,00±4,40	b 78,48±3,70
Meat in carcass (%)	56,19±2,81	57,04±1,30	56,88±1,84
pH_1	a 6,46±0,16	a 6,38±0,16	b 6,17±0,11
pH_{24}	ab 5,51±0,09	a 5,55±0,08	b 5,43±0,04
Drip loss48 h (%)	a 5,41±1,65	a 6,45±1,85	b 9,23±2,00
Homogenity of color	5,02±0,97	5,74±0,95	5,67±1,25
Intensity of color	a 5,25±0,83	b 6,82±0,46	b 6,49±0,91
Marbling	a 5,70±1,53	b 3,53±1,62	b 3,60±1,15
Drip loss	5,29±1,37	5,79±1,87	6,12±1,11
Acceptability	a 5,25±0,65	b 6,66±0,67	b 6,23±0,77

Means with a row with different letters are significantly different $p<0.05$

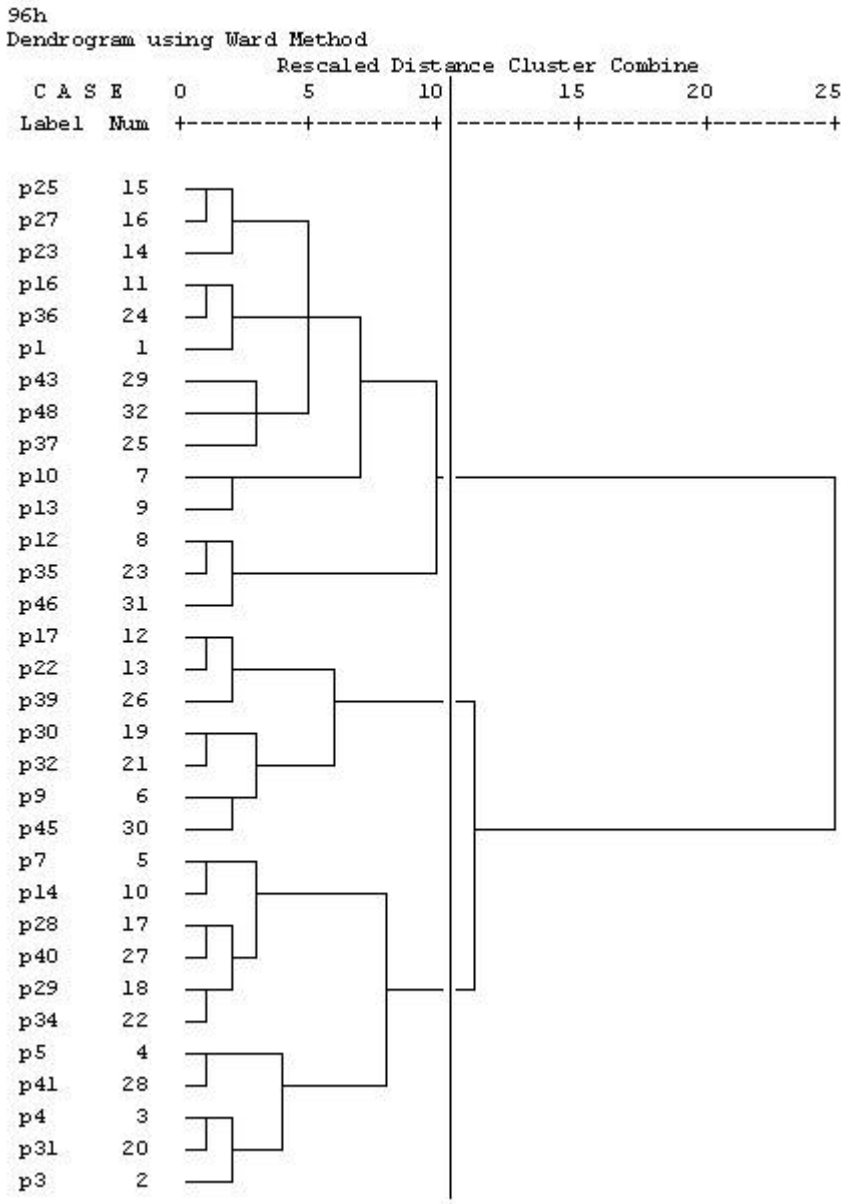
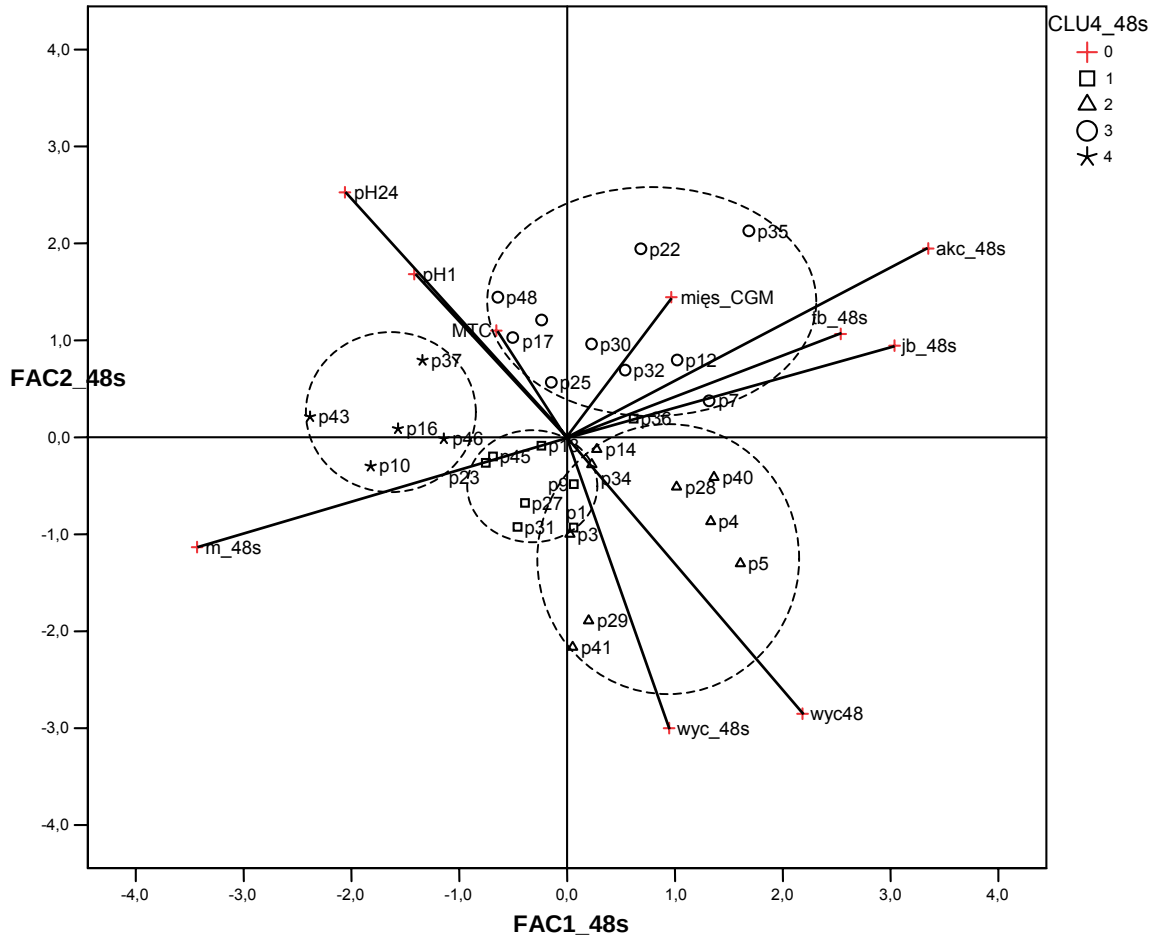


Fig. 2: Dendrogram showing course of cluster analysis with case of sensory analysis of meat in 96 h *post mortem* with differentiation of three group of pigs

Discussion

The results with reference to carcass and meat quality were similar to results obtained by GRZEŚKOWIAK and BORZUTA (2004) and also KOĆWIN-PODSIADŁA et al. (1997), KRZĘCIO et al. (2003). However in presented research was observed higher variability in drip loss than in KRZĘCIO et al. (2003).

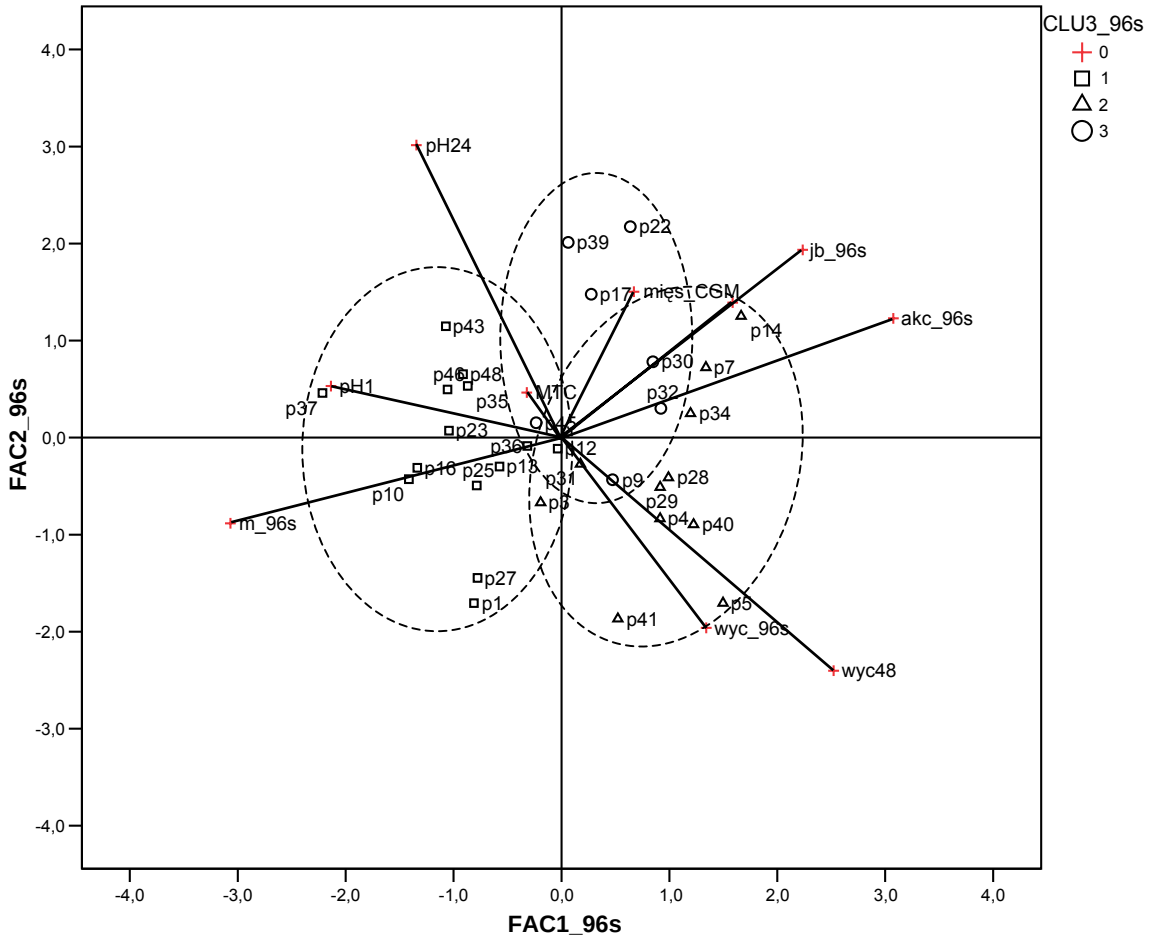
Despite small variability in range of carcass weight, meatiness and fatness highest variability ascertain in reference to marbling of meat. Written up variability in range of marbling meat is probably as effect of use in crossing boar's and sow's lines created with participation of Duroc and Meishan races with genetic predisposition to greatest level of intramuscular fat and marbling (JANSS et al. 1997, SELLIER 1998, MONIN et al. 1998, MICKLICH et al. 2002). The results of research of GRZEŚKOWIAK et al. (2003) in which was compared meat quality from polish large white breed with cross-



Explanation: tb_48s – homogeneity of color, jb_48s – Intensity of color, m_48sr – marbling, wyc_48s – drip loss evaluated by sensory method, akc_48s – acceptability, MTC – hot carcass weight, mięs_CGM – meat in carcass, wyc48– dripp loss evaluated by Honikel method,

Fig. 3: Results of cluster analysis showed on plot of the first two principal component (from PCA analysis) with presentation of measured variables and all studied hogs with case of sensory analysis of meat in 48 h *post mortem*

breed Naima x P76 showed a higher marbling these first pigs. Many authors showed that heritability of intramuscular fat (correlated with marbling) was around 50% and these traits playing a role in eating quality of pork (DE VRIES et al. 2000). As showed by JAWORSKA et al. (2006) meat from pigs with higher marbling was characterized after cooking as better sensory quality (texture). Positively effect of intramuscular fat on sensory quality of cooked meat was showed by many authors (FERNANDEZ et al. 1996, VAN LAACK et al. 2001; DASZKIEWICZ et al. 2005; POMMIER et al. 2004). The results of POŁOM and BARYŁKO-PIKIELNA (2004), FORTIN et al. (2005) and FERNANDEZ et al. (1996, 1999) showed, that more consumers prefered clearly pink color of meat with minimal fat thickness and without marbling and visible drip loss. RASMUSSEN et al. (1996) shown that low or medium marbling provide a high perceived value to the consumer. However it is known that consumers are not homogenous populations with equal preferences, often numerous groups of consumers which have different tastes can be found. That's the reason why a smaller group of consumers prefers meat which is dark and lean (POŁOM and BARYŁKO-PIKIELNA



Explanation: tb_96s – homogeneity of color, jb_96s – Intensity of color, m_96sr – marbling, wyc_96s – drip loss evaluated by sensory method, akc_96s – acceptability, MTC – hot carcass weight, mięs_CGM – meat in carcass, wyc48– dripp loss evaluated by Honikel method,

Fig. 4: Results of cluster analysis showed on plot of the first two principal component (from PCA analysis) with presentation of measured variables and all studied hogs with case of sensory analysis of meat in 96 h *post mortem*

2004). The described above relations were confirmed in PCA (Table 4) and in cluster analyses (Table 5 and 6). Similar results with reference to part of explained variance were obtained by JOSELL et al. (2003) and AASLYNG et al. (2003). The group of pigs obtained by employed cluster analysis differed in pH, drip loss, color, marbling and acceptability of meat. The group with higher marbling was characterized by lower acceptability. Meat with low marbling and low ultimate pH was characterized by higher drip loss. A significant effect of ultimate pH on drip loss and other meat quality traits was defined as "Hampshire effect" by many authors (MONIN and SELLIER 1985, WASSMUTH and GLODEK 1992, PRZYBYLSKI et al. 1998, CHAINERT et al. 2002). In presented results is also visible that higher drip loss except marbling and low pH can be related with lowest carcass weight. KRZĘCIO et al. (2003) also showed relations between these traits.

As already mentioned, analysis of relations between traits described carcass and technological pork quality with sensory raw meat quality shown a significant relationship between pH_{24}, drip loss and marbling of meat. These relationship showed

that growth pH_{24} or marbling of meat would cause the decrease of drip loss from muscle tissue.

PCA and analysis of cluster executed of Ward method has enabled singling out three different group of pigs and shown a big usefulness of method of multivariate analysis in research over conditionality of meat quality.

References

AASLYNG, M.D.; BEJERHOLM, C.; ERTBJERG, P.; BERTRAM, H.C.; ANDERSEN, H. J.:
Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. Food Quality and Preference. **14** (2003), 277-288

BERTRAM, H.C.; PETERSEN, J. S.; ANDERSEN, H.J.:
Relationship between RN genotype and drip loss meat from Danish pigs. Meat Science. **56** (2000), 49-55

BRYHNI, E.A.; BYRNE, D.V.; RODBOTTEN, M.:
Consumer and sensory investigations in relation to physical/ chemical aspects of cooked pork in Scandinavia. Meat Science, **65** (2003), 737-748

CHAINERT, W.; GLODEK, P.; BRANDT, H.; MÖLLERS, B.; HENNING, M.; KALLWEIT, E.; FISCHER, K.:
Systematische Gebrauchskreuzung als Möglichkeit der Erhaltung vom Aussterben bedrohter Landschweinerassen. Arch. Tierz., Dummerstorf, **45** (2002) 3, 35-43

DASZKIEWICZ, T.; BĄK, T.; DENABURSKI, J.:
Quality of pork with different intramuscular fat (IMF) content. Polish Journal of Food and Nutrition Sciences, 14/55, **1** (2005), 31-36

DE VRIES, A.G.; FAUCITANO, L; SOSNICKI, A.; PLASTOW, G.S.:
The use of gene technology for optimal development of pork meat quality. Food Chemistry. **69** (2000), 397-405

FERNANDEZ, X.; MONIN, G.; TALMANT, A.; MOUROT, J.; LEBRET, B.; BERNARD, P. :
Influence de la teneur en lipidem intramusculaires sur l`acceptabilite, par les comsommateurs, de la viande de prc et duj ambon cuit. Journess Rech. Porcine en France, **28** (1996), 163-170

FERNANDEZ, X.; MONIN, G.; TALMANT, A.; MOUROT, J.; LEBRET, B.:
Influence of intramuscular FAT content on the quality of pig meat-2. Consumer acceptability of m. longissimus lumborum. Meat Science **53** (1999) 1, 67-72.

FLORES, M.; ARMERO, E.; ARISTOY, M.-C.; TOLDRÁ, F.:
Sensory characteristics of cooked pork loin as affected by nucleotide content and post mortem meat quality. Meat Science. **51** (1999), 53-59

FORTIN, A.; ROBERTSON, W. M.; TONG, A.K.W.:
The eating quality of Canadian pork and its relationship with intramuscular fat. Meat Science. **69** (2005), 297-305

GRZEŚKOWIAK, E.; BORZUTA, K.:
Results of Meat Quality and Technological Stability Assessment of Fatteners Obtained from Mating Naima Sows with P-76 Boards. Polish Journal of Food and Nutrition Sciences, 13/54 (2004) 2, 199-202

GRZEŚKOWIAK, E.; BORUTA, K.; STRZELECKI, J.:
Wartość rzeźna oraz przydatność technologiczna mięsa tuczników uzyskanych z kojarzenia loch Naima z knurami P-76. Roczniki Instytutu Przemysłu Mięsnego i Tłuszczowego, XL, (2003), 13-23

HONIKEL, K.O.:
The water binding of meat. Fleischwirtschaft, **67** (1987) 9, 1098-1102

ISO 8586-2:
1994. Sensory analysis. General guidance for the selection, training and monitoring of assessors.

JANSS, L.L.G.; VAN ARENDONK, J.A.M.; BRASCAMP, E.W.:
Bayesian statistical analyses for presence of single genes affecting meat quality traits in a crossed pig population. Genetics. **145** (1997), 395-408

JAWORSKA, D.; PRZYBYLSKI, W.; KOŁOŻYN-KRAJEWSKA, D.; CZARNIECKA-SKUBINA, E.; WACHOWICZ, I.,; TRZĄSKOWSKA, M.; KAJAK, K.; LECH, A.; NIEMYJSKI, S.:
Evaluation of relations between traits described technological and sensory pork meat quality. Animal Sci. Pap. Rep. (2006), (in press)

JOSELL, A.; ENFALT, A-CH.; VON SETH, G.; LINDAHL, G.; HEDEBRO-VELANDER, I.; ANDERSSON, L.; LUNDSTROM, K.:
The influence of RN genotype, including the new V1991 allele, on the eating quality of pork loin. Meat Science **65** (2003), 1341-1351

KOĆWIN-PODSIADŁA, M.; KRZĘCIO, E.; PRZYBYLSKI, W.; KACZOREK, S.:
Carcass and slaughter values of crossbreed from pl-23 sows and Pietrain and P-76 boars. Current Problems on pig production. Konferencja Naukowa, Olsztyn, 7 V, (1997), 90

KRZĘCIO, E.; SIECZKOWSKA, H.; ZYBERT, A.; ANTOSIK, K.; PRZYBYLSKI, W.; KOCWIN-PODSIADŁA, M.:
Quality of raw material of two-breed fatteners originating from crossing of imported breeds. Annals of Animal Science, Supplement, **1** (2003), 65-69

MEILGAARD, M.; CIVILLE, G.V.; CARR, B.T.:
Sensory evaluation techniques (3rd ed.), Boca Raton, (1999), CRC Press.

MICKLICH, D.; MATTHES, H.D.; HARTUNG, M.; MÖHRING, H.:
Mast-, Schlachtleistung und Fleischqualität verschiedener Schweinerassen bei Stall- und Freilandhaltung (Kurzmitteilung). Arch. Tierz., Dummerstorf **45** (2002) 3, 247-253

MONIN, G.; SELLIER, P.:
Pork of low technological quality with a normal rate of muscle pH fall in the immediate post-mortem period: The case of the hampshire breed. Meat Science **13** (1985), 49-63.

MONIN, G. ; SELLIER, P.; BONNEAU, M.:
Trente ans d'évolution de la notion de qualité de la carcasse et de la viande de porc. Journeé de la Recherche Porcine en France **30** (1998), 13-27

POŁOM, A.; BARYŁKO-PIKIELNA, N.:
Analiza czynników decydujących o preferencjach polskich konsumentów mięsa wieprzowego. Żywność, Technologia, Jakość. 3, **40** (2004), 5-23

POMMIER, S.A.; MURRAY, A.; ROBERTSON, W.; AALHUS, J.; GIBSON, L.; DIESTRE, A.; SOSNICKI, A.; KLONT, R.:
Effect of genetics on meat quality and sensory properties of pork. 50 th International Congress of Meat Science and Technology, (2004), Helsinki, Finland.

PN-ISO 4121:
1988. Sensory analysis. Evaluation of food products using scaling metod.

PRZYBYLSKI, W.; KOĆWIN-PODSIADŁA, M.; KRZĘCIO, E.; KACZOREK, S.:
The frequency of RN^- gene in different group of pigs. Pol. J. Food Nutr. Sci. Vol. 7/48, No 4(S) (1998), 234-240

RASMUSSEN, K.B.; FERTIN, C.; ANDERSON, M.:
Preferences of German Pork Consumers – A Conjoint Analysis. "Meat for the Consumer" – 42nd ICOMST (1996), 274

RESURRECCION, A.V.A.:
Sensory aspects of consumer choices for meat and meat products. Meat Science, **66** (2004), 11-20

RISVIK, E.:
Sensory properties and preferences. Meat Science **36** (1994), 67-77

ROSENVOLD, K.; ANDERSEN, H.J.:
Factors significance for pork quality – a review. Meat Science **64** (2003), 219-237

SELLIER, P.:
Genetics of meat and carcass traits. in: The genetics of the pig. Rodshild M. F., Ruvinsky A. (eds). CAB Int. (1998), 463- 510

VAN LAACK, R.L.J. M.; STEVENS, S.G.; STALDER, K.J.:
The influence of ultimate pH and intramuscular fat content on pork tenderness and tenderization. Journal of Animal Science, **79** (2001) 2, 392-397

WASSMUTH, R.; GLODEK, P.:
Einfluβ des „Hampshirefaktors" und der Standzeit auf das glykolytische Potential und die Fleischbeschaffenheit bei Schweinen. Fleischwirtsch., **72** (1992) 9, 1299-1302

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Arch. Tierz., Dummerstorf **50** (2007) Special Issue, 136-143

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The Effect of HERB-mix® in Piglets' Diarrhea Prevention

Abstract

Prohibition of the use of feeder antibiotics, which has been put into execution makes the researchers look for alternative ways of diarrhea prevention. The aim of this study was to evaluate the HERB-mix® feed additive as a piglets' diarrhea prevention product, especially in the weaning period. The experiment was carried out on 12 litters divided into 2 groups according to the analogue rule: I – a control (standard feed with an addition of tylozyne and lincospectin), II – an experimental (standard feed with 0,5% addition of HERB-mix). Piglets were weighted on the 2nd, 14th, 28th (weaning) and 42nd day of life. On the 14th, 28th and 42nd day blood samples from chosen piglets were taken to estimate acid-base balance, acute phase proteins (haptoglobine, fibrynogene, serum amyloid A), total protein and its fraction in serum. The diarrhea accidents didn't occur in group II, and sporadically appeared in group I during lactation time. Within 2 weeks after weaning, a daily gain inhibition in group II was observed; diarrhea accidents were noticed too. Daily gains in group I did not decrease significantly. Diarrhea accidents were observed too, however on a lower scale compared to the experimental group. The acid-base parameters were more stable in the experimental group, which suggests a normalizing influence of evaluated feed additive. Estimated acute phase proteins were on a lower level in experimental group throughout the study. Last results of blood examination imply higher body homeostasis of piglets from group II in spite of a decrease in their daily gains.

Key Words: piglets, acute phase reaction, acid-base balance, herbs

Zusammenfassung

Titel der Arbeit: **Bewertung des HERB-mix® Zusatzes zur Durchfallvorbeugung bei Ferkeln**

Die Einführung des Verwendungsverbotes der Futterantibiotika bei der Schweinezucht zwingt zur Suche nach alternativen Methoden der Durchfallvorbeugung. Der Zweck der Untersuchungen war die Bewertung des Futterzusatzes HERB-mix der Firma OVER als eines durchfallvorbeugenden Mittels bei Ferkeln unter Produktionsbedingungen, besonders um die Absetzzeit. Die Untersuchungen wurden bei 12 Ferkelwürfen durchgeführt: Gruppe I - Kontrollgruppe (bekam das Standardgemisch in voller Ration um die Absetzzeit mit Zusatz von Tylosin und Lincospectin), Gruppe II - Versuchsgruppe (bekam dieselbe Mischung mit 0,5% Zusatz von HERB-mix). Die Ferkel wurden am 2.,14., 28. (Absetzung) und am 42. Lebenstag gewogen. Am 14., 28. und 42. Lebenstag wurde den ausgewählten Tieren Blut zur Bestimmung von : Säure-Basen–Balance, Akute Phase Proteine (Haptoglobin, Fibrinogen, Serum Amyloid A), Gesamteiweiß und dessen Fraktionen entnommen. Während der Aufzucht bei der Mutter wurden bei den Versuchsferkeln keine Durchfälle festgestellt, bei den Kontrollferkeln jedoch traten diese Erkrankungen auf. Nach dem Absetzen traten in der Versuchsgruppe Durchfälle seltener auf und dauerten auch nicht so lange wie in der Kontrollgruppe. Versuchsferkel wiesen mehr ausgeglichene Säure-Basen-Gleichgewichtsparameter und eine niedrigere Konzentration des Eiweißes in der akuten Phase auf, was auf den stabilisierenden Effekt dieses Zusatzes zurückzuführen ist.

Schlüsselwörter: Ferkel, Akut-Phase Reaktion, Säure-Basen–Balance, Kräuter

Introduction

Intestinal tract illnesses are one of the main reasons of a decrease in rearing efficiency of suckling and weaning piglets. It has been estimated that diarrhea in a suckling period occurs in 0-50 (100) % litters, especially in the first week of life and mortality

can range up to 60-70% of sick animals (HALL, 1989; SVENSMARK et al., 1989). This means that in spite of using feed antibiotics, diarrhea is one of the main causes of piglets' losses. The ban of antibiotic growth enhancers in pigs breeding, forced farmers to seek alternative diarrhea prevention products. The most popular additives used in feed industry are probiotics, zinc oxide, yeast, enzymes and herbs (CLOSE, 2000; TURNER et al., 2001; HILL et al., 2001; LIPIŃSKI and TYWOŃCZUK, 2004; MANZANILLA et al., 2004; PARTRIDGE and TUCKER, 2000).

The additives used are supposed, first of all, to support the development of "desired" bacterial flora through introduction of appropriate bacterial cultures (probiotics) into alimentary duct or providing conditions for optimal growth and development of non-pathogenic strains (probiotics, acidifiers, zinc oxide, yeast). These additives are to support digestion processes in still immature piglet's alimentary duct (acidifiers, yeast, enzymes). Among this wide range of additives herbs constitute a specific group. They feature multidirectional effect and influence of advantageous microflora in alimentary duct (antibacterial and antitoxic effect), as well as they can build enzymatic activity and show immunostimulatory effects (MANZANILLA et al., 2004; PARK et al., 2003).

The aim of this study was to evaluate in field conditions the HERB-mix® feed additive as a piglets' diarrhea prevention product, especially in the weaning period.

Material and Method

The experiment was carried out in field conditions in one farrowing room on 12 crossbreeds wbpxpbz piglets divided into 2 groups according to the analogue rule (sow age, litter size): I – a control and II- experimental. After weaning (28th day) all piglets were grouped in the nursing building in two pens, one for control group and the second for the experimental one. Observations of the piglets lasted until their leaving the nursery.

In the course of the experiment group I received the standard feed used on the farm, while group II was fed with the same feed with 0,5% addition of HERB-mix. This additive consists of the following herbs: *Myrtilli fructus, Quercus cortex, Rhizoma calami, Herba origani, Plantaginis ovatae semen, Cinnamomi cortex.*

The addition subjected to the assessment was mixed with another feed for piglets – the Starter and administred from the second week of life to the second week after weaning. In the control group during the same period, because of occurred sporadically diarrheas in all piglets, there was applied 2,5% addition of antibiotics: Tylosin and Lincospectin. The piglets were fed ad libitum from autofeeders. The composition of feed was shown in Table1.

Table 1
The composition of feed for piglets

Ingredients	Control Group I	Experimental Group II
Wheat	350 kg	
Barley	150 kg	
Maize	170 kg	
Ground soybean	80 kg	
Concentrate LNB 8347	250 kg	
Tylan	1 kg	-
Lincospectin	1,5 kg	-
Herb-mix	-	5 kg

In the course of the experiment the following parameters were assessed: clinical state, losses and the cases of treatment. To control daily gains piglets were weighted on the following days of life: - 2nd (classification into experimental groups, litter standardization), -14 th (beginning of experimental feed administration), -28th (weaning), -42th (the end of experiment). From selected clinically health piglets from both groups (6 from one group) there were collected blood samples for laboratory analysis on 14th, 28th, and 42nd day of life (basic morphology, acute phase protein/APP/: fibrinogen /Fb/, haptoglobin /Hp/, serum amyloid A /SAA/, total protein and its fraction in serum, acid-base balance /RZK/).
Fb was determined in whole blood with capillary method (MILLAR et al., 1971), the remaining proteins were determined in serum using: Hp-guayacol method (JONES and MOULD, 1984), SAA – according to the Tridelta phase™ range SAA test. Total protein in serum was determined by biurete method and its fractions using paper electrophoresis. RZK parameters were obtained from whole blood samples with the use of Rapidlab™ 348, Bayer.
The values obtained were analysed using oneway variance analysis and significant differences were evaluated by the t-Student test (Statistica software ver.7.1).

Results and discussion

Average results regarding the parameters assessed in the experiment were shown in the Figure and Tables 2-6.

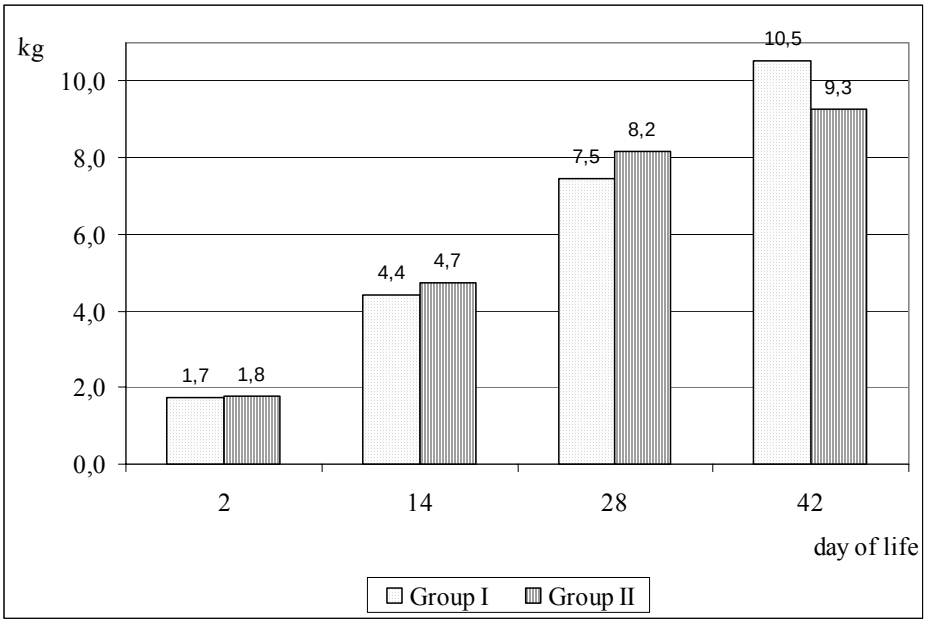

Figure: Body weight [kg]

Piglets' body weight in both groups was similar and even on the day of classification into groups and on the day the experiment began (Fig.). In further period, when the piglets were staying with sows, there were recorded significant differenciation between sucklers belonging to two groups. The piglets receiving HERB-mix showed high daily gains -240g/day, while the control ones -212 g/day. Moreover, in experimental group the values of standard deviation were lower, which points to higher equalization among piglets than in control group. Body weight on the weaning

day (experimental animals were heavier by 0,7 kg) expressed piglets' state of health and it should be assessed as a good one in the view of other reports found in the literature (KUHN et al., 2005; RZĄSA et al., 2003). Taking into account daily gains there was no influence of higher (about 0,5%) fiber content in feed foe the experimental group. During the whole suckling period in experimental piglets there were not diagnosed any cases of diarrhoea, while in control ones such cases occur. Observation of health state of the sucklers, as well as production results obtained prove considerable protective effect due to the preparation used in this period. It occurred even more effective in that period than this of antibiotics.

Table 2
Total serum protein and its fraction [g/l]

	Group I			Group II		
	14 day	28 day	42 day	14 day	28 day	42 day
Total protein	66,67	55,00	36,07	66,67	60,45	37,08
	5,16	3,16	10,07	4,65	3,73	4,65
Albumin	33,73	28,6	15,86	29,55	28,83	16,43
	2,43	3,91	5,76	3,96	1,74	2,02
α- globulin	13,33	12,26	7,86	14,14	13,46	8,86
	1,38	1,37	1,82	1,74	1,74	1,34
β- globulin	12,15	8,21	6,53	11,71	8,66	6,07
	1,75	1,06	2,34	1,65	0,85	0,89
γ- globulin	7,45	5,89	5,8	11,40	6,8	5,71
	0,71	0,79	1,71	5,3	1,14	1,29

Table 3
The level of hemoglobin [mmol·l^{-1}], erythrocytes [10^{12}·l^{-1} and leukocytes [10^{9}·l^{-1}]

Group	Hb 28	Hb 42	Ery28	Ery42	Leuk28	Leuk42
I	8,06	9,9	5,096	6,068	19,1	13,85
	0,4	0,7	0,7	1,06	0,27	0,23
II	7,81	9,96	5,298	7,348	18,26	13,85
	0,4	0,5	0,05	3,339	0,55	0,2

Table 4
Leukocyte image [%]

Group	granulocytes						agranulocytes			
	eozynophiles		neutrophiles							
			rods		segments		lymphocytes		monocytes	
	28 day	42 day	28 day	42 day	28 day	42 day	28 day	42 day	28 day	42 day
I	2,67	2,2	2,8	6,4	14,7	48,0	75,7	44,3	1,0	1,5
	2,1	0,4	2,7	1,8	9,2	10,6	2,5	9,9		0,7
II	1,8	1,75	4,25	5,5	35,8	50,83	58,4	42,0	1,5	1,5
	0,8	1,0	4,0	3,1	12,5	16,0	15,7	13,4	0,7	0,7

Table 5
Dynamics of acute phase proteins

Group	Fb [g/l]			Hp [g/l]			SAA mg/l		
	14 day	28 day	42 day	14 day	28 day	42 day	14 day	28 day	42 day
I	2,96	6,91	5,11	2,75	1,31	0,63	23,61	717,41	76,69
	1,13	1,01	0,69	1,9	1,0	0,7	17,7	388,2	79,7
II	3,75	6,43	4,53	1,33	0,9	0,04	12,96	697,97	10,73
	0,82	1,17	0,1	0,8	0,4	0,1	22,4	157,3	29,6

In the course of suckling period in both piglet groups there were recorded one loss case in each, which constitutes 1,67% piglets born alive in both groups. On the

weaning day 5 piglets from control group were left at adoptive sows becouse of low body weight and the same pattern followed only 1 piglet from experimental group.

Table 6
The parameters of acid-base balance

	Group I			Group II		
	14 day	28 day	42 day	14 day	28 day	42 day
pH	7,33	7,38	7,33	7,27	7,34	7,37
	0,03	0,09	0,04	0,1	0,07	0,1
pCO_2	52,76	48,78	61,99	54,08	56,33	50,72
	8,18	13,65	10,84	10,43	8,54	17,37
pO_2	21,3	48,1	31,83	18,27	33,63	40,02
	3,09	30,08	3,14	1,15	12,24	9,48
HCO_3	26,76	28,7	32,1	24,65	29,38	27,92
	3,7	2,35	4,29	3,32	1,73	4,1
BE	-0,22	3,07	4,24	-2,95	2,2	1,92
	3,01	2,15	3,38	3,98	2,2	2,69
Na		132,33	123,14		133,83	117,4
		3,98	3,85		2,69	6,11
Ca^{2+}		0,78	0,96		1,89	0,81
		0,25	0,14		0,22	0,22

After weaning, within two week time was recorded considerable inhibition of daily gains in experimental group and the cases of short-lasting diarrhea were also be found. In control group (which was administrated antibiotics in feed all the time), daily gains were only slightly lowered in relation to the period of staying at sows. In this group there also occurred single cases of diarrhea /loose stool, but less frequency and not as intensive as in experimental piglets. In spite of the fact that on the 42nd day of life the control piglets were heavier more than 1 kg and they were more diversified regarding this parameters compared to experimental ones. Inhibition or decreased of daily gains in this period is a common phenomenon, connected with enormous stress piglets face after weaning and moving to nursery building. In this time they are combined in more numerous groups and their basic diet becomes altered - they are not allowed to sows' milk (MANZANILLA et al., 2004). Generally, the mentioned stress is more visible in production results and state of health in the biggest individuals and this was the case of experimental group. On the basis of earlier own observation, it can be expected that in those individuals the phenomenon of growth compensation will occur and body weight, as well as the daily gains will become even in both groups providing good state of health is maintained. It seems that under the influence of circumweaning stress the piglets not protected by antibiotics are exposed to subclinical infections and thus to the need of defence against such infection, which can result in slower gains. This problem did not occur in control piglets receiving feed enriched with two antibiotics. On the day of moving weaners to fattening building average body weight of all assessed animals amounted 34 kg and there could be observed more advanced individual differentiation in control group.

It is interesting to consider alterations of total protein levels and its fractions. The decrease in total protein concentration, observed in analyzed period of piglets' growing was slower in experimental group than in control one. The decrease involved all protein fractions. Evident decrease in albumin concentration, which occurred after weaning can be connected with the necessity of adjusting to one source of feed – only solid. The stress described above certainly also caused, at the beginning, decreased feed intake and, therefore protein deficiency in a diet became a fact. Decreased

albumin level can be explained by altered diet, while diminished values of other fractions should be treated as advantegous phenomenon, speaking for general good state of health of the examined piglets. It is worth recording that until the 6th week of life in both groups there did not occur any increase in γ-globulin fraction, which can be interpreted as the lack of necessity of intensive defence against environmental treats, i.e. indirect proof of good zoohygienic conditions of the farm. However small difference between the groups can be noticed, namely in experimental group on 42nd day of piglets' life there was recorded further decrease in γ-globulin values, while in control group their level was nearly not changed since weaning, which probably resulted from two processes taking place at the same time: the decrease in colostrums immunoglobulin concentration in their serum and the increase in synthesis of own antibodies as a reply to stimulation by microorganisms effecting the piglets.

In control piglets average levels of determined acute phase proteins were higher than in experimental ones. Fb usually is not determined in pigs due to high content in blood. In the experiment presented comparing Fb dynamics with the remaining acute phase proteins proved that this protein can be used for interpretation of animals' reaction to the factors endangering their health. In control piglets Fb concentration in blood on 28th and 42nd day of life was distinctly higher than in experimental piglets and it exceeded upper limit of the norm. In experimental piglets only in circumweaning period the occurrence of Fb concentration was higher than the norm was recorded. These results can be treated as the consequence of higher threaten of infections states in control piglets (the presence of diarrhea in spite of administration of antibiotics) than in experimental animals.

Higher Hp levels in I group on 14th and 28th day confirm the hazard of infections (HALL et al., 1992) in this group (Tab. 5).

In our own investigations, similarly to HEEGARD et al. (1998) usefulness of SAA determination in monitoring state of health was also confirmed. HEEGARD (1998) observed only quantitative alterations of this protein in infected pigs. In our study it was recorded that in experimental pigs, within the whole observation was lower than in control pigs and on the day of weaning considerably moderate increase (tab.5) was recorded.

Lower levels of all APP in II group, determined on 42nd day of life indicate general good state of animals health. It seems that these result confirm the hypotesis of suggested growth compensation in later period, which could be proved when subsequent regrouping took place.

Determined morphological blood parameters in piglets did not exceed reference norms (EAGELI et al., 1998). In experimental piglets there was recorded slightly higher erythrocyte concentration. In all piglets it was stated moderate leukocytosis on the day of weaning, while white palettes image was typical for animals age. In control piglets on 28th day of life considerably strong lymphocitosis was recorded, which withdrew on 42nd day of life as the increase in granulocytes percentage and the shift of the image to the left (higher percentage of youthful forms) occurred. In experimental piglets the values of particular subpopulations were very strong.

The parameters of acid-base balance reflect animals' state of health, their nutrition, as well as maintenance (PATIENCE and CHAPLIN, 1997; HAMILTON et al., 2004). In subsequent terms of control there were not observed considerable disturbances (Tab. 6). The decrease in blood pH in control piglets, observed on 42nd day of their life can

prove higher feed intake (higher daily gain after weaning than experimental group) and therefore slight tendency to acidosis occurrence. On 28th day of life concentration of calcium ion in the blood of control piglets was very low, while in experimental piglets it was very high (SCHLUMBOHM and HARMEYER, 2004). In experimental piglets, on 42nd day of life, there did occur considerable decrease in calcium ion concentration, which can partly be treated as a result of insufficient feed due to stressful factors already described, lower feed consumption, as well as diarrhea occurring that time. Diarrhoea the piglets from II group suffered from found its reflexion in the decrease of Na level, as well as bicarbonate in comparison to the state on the day of weaning.

Summary

Piglets health and production results showed a protective effect of HERB-mix in suckle period. In fact, in this period the protective effect was even larger than this of antibiotics. Results of blood examination on the 42nd day of life imply higher body homeostasis of piglets from group II in spite of a decrease in their daily gains. This data suggests that a growth compensation phenomenon might be observed in later period, and body weight and daily gain would equalize in both groups.

References

CLOSE, W.H.:
Producing pigs without antibiotic growth promoters. Advances in Pork Production, vol. 11 (2000), 47-56

EAGELI A.K., FRAMSTAD T., MORBERG H.:
Clinical biochemistry, haematology and body weight in piglets. Acta Vet.Scand. **39** (1998), 381-393

HALL W.:
A review of colibacillosis in neonatal swine. Veterinary Medicine April (1989), 428-431

HALL W.F., EURELL T.E. HANSEN R.D., HERR L.G.:
Serum haptoglobin concentration in swine naturally or experimentally infected with Actinobacillus pleuropneumonae. JAVMA, **201** (1992), 1730-1733

HAMILTON D.N., ELLIS M., BERTOL T.M., MILLER K.D.:
Effects of handling intensity and live weight on blood acid-base status in finishing pigs. J. Anim. Sci, **82** (2004), 2405-2409

HEEGAARD P.M.H., KLAUSEN J., NIELSEN J.P., GONZALEZ-RAMON N., PINEIRO M., LAMPREAVE F., ALAVA M.A.:
The porcine acute phase response to infection with Actinobacillus pleuropneumoniae. Haptoglobin, C-Reactive Protein, Major Acute Phase Protein and Serum Amyloid A Protein are sensitive indicators of infection. Comp.Biochem.Physiol. vol 119B No2 (1998), 365-373

HILL G.M., MAHAN D.C., CARTER S.D., CROMWELL G.L., EWAN R.C., HARROLD R.L., LEWIS A.J., MILLER P.S., SHURSON G.C., VEUM T.L.:
Effect of pharmacological concentrations of zinc oxide with or without the inclusion of an antibacterial agent on nursery pig performance. J. Anim. Sci., **79** (2001), 934-941

JONES G.E., MOULD D.L.:
Adaptation of the guaiacol (peroxidase) test for haptoglobins to a microtitration plate system. Res. Vet. Sci. 37 (1984), 87-92

KUHN G., ENDER K., THOMANN R., TUCHSCHERER M., TUCHSCHERER A., STABENOW B., KRŰGER M., SCHRŐDL W.:
Einsatz von Echinacea-Extract bei tragenden und saűgenden Sauen. Arch. Tierz., Dummerstorf **48** (2005) 3, 270-282

LIPIŃSKI K., TYWOŃCZUK J.:
Oregano essential oils as an alternative to the use of antibiotics as growth promoters in weaner pig nutrition, Ann. Anim. Sci., Suppl., No 2 (2004), 155-159

MANZANILLA E.G., PEREZ J.F., MARTIN M., KAMEL C., BAUCELLS F., GASA J.:
Effect of plant extracts and formic acid on the intestinal equilibrium of early-weaned pigs. J. Anim. Sci. **82** (2004), 3210-3218

MILLAR H.R., SIMPSON J.G., STALKER A.L.:

An evaluation of the heat precipitation method for plasma fibrinogen estimation. J. Clin. Pathol. **24** (1971) 9, 827-830

PARK E-K., CHOO M-K., KIM E-J., HAN M.J., KIM D-H.:
Antiallergic activity of ginsenoside Rh2. Biol.Pharm.Bull. **26** (2003) 11, 1581-1584

PATIENCE J.F., CHAPLIN R.K.:
The relationship among dietary undetermined anion, acid-base balance, and nutrient metabolism in swine. J. Anim. Sci. **75** (1997), 2445-2452

PARTRIDGE G., TUCKER L.:
A healthy role for enzymes. Pig International **5** (2000), 28-31

RZĄSA A., POZNAŃSKI W., AKIŃCZA J., PROCAK A.:
The influence of primiparous sow litter standardization on their performance. Ann. Anim. Sci., Suppl., **2** (2003), 167-172

SCHLUMBOHM CH., HARMEYER J.:
Dietary additions of lactose, casein and soy protein exerted only moderate effects on calcium homeostasis in calcitriol deficient piglets. Journal of Steroid Biochemistry and Molecular Biology. **89-90** (2004), 605-609

SVENSMARK B., JORSAL S.E., NIELSEN K., WILLEBERG P.:
Epidemiological studies of piglet diarrhea in intensively managed Danish sow herds. I. Preweaning diarrhea. Acta vet. Scand. **30** (1989), 43-53

TURNER J.L., DRITZ P.S.S., MINTON J.E.:
Review: Alternatives to conventional antimicrobials in swine diets. The Professional Animal Scientist. **17** (2001), 217-226

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Arch. Tierz., Dummerstorf **50** (2007) Special Issue, 144-151

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Effect of linseed in pig diet on meat quality and fatty acid content

Abstract

The effect of different linseed content in pig diet on meat quality was studied on 40 fattening crossbreed gilts in the experiment. The experiment was carried out from 37 to 98 kg live weight in 4 groups (control group-L0, L1- 6,7 % of ground linseed, L2- 13,4 % of ground linseed and L3 -13,4% of linseed and 103 mg of α-tocopherol in the diet) of 10 fattening crossbreed gilts each. Linseed treatments did not affect (P>0,05) any monitored growth and carcass traits (average daily gain, lean meat percentage and intramuscular fat content). Feeding linseed to pigs significantly increased the content of linoleic acid (P<0,05), α-linolenic acid (P<0,001), arachidonic acid (P<0,05) and eicosapentaenoic (EPA) acid (P<0,05) content, but did not affect docosahexaenoic (DHA)acid content (P>0,05) in muscle tissue. The n-6/n-3 fatty acid ratio was decreased by the diet. The reduction was not statistically significant (P>0,05). The content of total n-6 and n-3 fatty acids was the lowest in control group. The oxidative stability of muscle lipids was higher in L3 group (α-tocopherol supplementation) compared with other groups.

Key Words: fatty acid, linseed, alpha-tocopherol, pig

Zusammenfassung

Titel der Arbeit: **Einfluss von Leinsamenzusätzen im Schweinemastfutter auf Schlachtmerkmale und Fettsäuremuster im Muskelgewebe**

An 40 weiblichen Kreuzungsmasttieren wurde der Einfluss von unterschiedlichen Leinsamenzusätzen im Mastfutter auf Schlachtmerkmale und das Fettsäuremuster im Muskelgewebe untersucht. Im Gewichtsabschnitt von 37 bis 98 kg wurden vier Gruppen mit je 10 Tieren geprüft (Kontrolle L0=ohne Leinsamen, L1 = 6,7%, L2 = 13,4% und L3 = 13,4% Leinsamen + 103 mg α-Tocopherol). Die Tiere wurden im gleichen Stall gehalten und erhielten Futter und Wasser ad libitum. Die Leinsamenzugabe hatte keinen signifikanten Einfluss auf Wachstums- und Schlachtmerkmale, jedoch auf das Fettsäuremuster. So erhöhte sich signifikant der Gehalt an Linolsäure, α-Linolensäure, Arachidonsäure und EPA (C20:5n-3). Kein Einfluss wurde auf den DHA (C22:6n-6) Gehalt im Muskelgewebe nachgewiesen (Die Proben wurden 24 h post mortem den Muskeln *longissimus dorsi* und *thoracis* entnommen). Der n-6/n-3 Quotient wurde durch die Zufütterung verengt, jedoch nicht signifikant. Der geringsten n-6 und n-3 Gehalte fanden sich in der Kontrollgruppe. Verglichen mit den anderen Gruppen war die Oxidationsstabilität in der L3-Gruppe am höchsten.

Schlüsselwörter: Fettsäuren, Leinsamen, α-Tocopherol, Schwein

Introduction

Fat is an carrier of essential fatty acids, fat soluble vitamins and it is an important component for eating quality. With breeding, feeding, keeping and biotechnology it is possible to change the fat content and composition (ENDER at al., 1997; BIEDERMANN et al., 2000). Dietary lipids have a principal effect on fatty acid profile in pork meat and adipose tissue. The fatty acid content and saturated and unsaturated fatty acids ratio determine physical and nutritional characteristics of fat. The n-6/n-3 fatty acids ratio is important due to its influence on human health. The mechanism by which the n-3 polyunsaturated fatty acids (PUFA) prevent fatal ventricular arrhythmias in animals and cultured heart cells, prevent sudden cardiac deaths and potential importance of these fatty acids in human nutrition is mentioned in

many studies (KANG and LEAF, 2000; PFEUFFER, 2001; DEMAISON and MOREAU, 2002; LEAF et al., 2003).
The level of food intake and composition of food regulates the rate of fatty tissue growth and the composition of lipids. There is a correlation between the amount of fatty tissue and fatty acid composition. The potential for dietary variation of lipid composition in monogastric animals is much greater then in ruminants (NUERNBERG et al., 1998). In pigs, dietary fatty acids are absorbed unchanged from the intestine and incorporated into tissue lipids. The PUFA linoleic and α-linolenic can not be synthesized and tissue concentrations respond rapidly to dietary changes. Saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) on the other hand are synthesized and their concentrations are less readily influenced by diet. Vitamin E, α-tocopherol, is the major lipid-soluble antioxidant in animal tissues which acts post-mortem to delay oxidative deterioration of the meat. Feeding animals with more unsaturated fatty acids to improve the P:S ratio or feeding n-3 PUFA as linseed or fish oil to lower n-6/n-3 ratio increases the susceptibility of the meat to oxidation. Concomitant increases in dietary vitamin E are therefore necessary to prevent flavour deterioration due to lipid oxidation (WOOD and ENSER, 1997; LAHUCKY et al, 2000; KRSKA et al., 2001; LAHUCKY, 2005). LAHUCKY et al. (2004) studied the impact of supplementation with magnesium oxide on the fatty acid composition, antioxidative capacity and meat quality parameters. Intramuscular fat content and concentration of linoleic acid, EPA and the total amount of n-3 fatty acids in *M.longissimus dorsi* was increased in supplemented pigs.
In pigs, the concentration of n-3 fatty acids should be enhanced, while that of n-6 fatty acids be lowered. This is achieved by increasing the share of n-3 rich oils (linseed, rapeseed, soy, fish) or linseed in the diet (ENDER, 1994; LEIBETSEDER, 1996; METGES, 2004).

Material and methods

The experiment was evaluated on 40 fattening crossbreed gilts (Czech Large White x Czech Landrace) x (Hampshire x Pietrain). The animals were divided into 4 groups, 10 gilts in each. The average initial live weight was 37 kg. The animals were fed by 4 types of feeding mixture- control group-L0, L1-6,7 % of ground linseed, L2- 13,4 % of ground linseed and L3 -13,4% of linseed and 103 mg of α -tocopherol/kg (Table 1). The average final live weight was 98 kg. The samples of *M. longissimus dorsi et thoracis* (200 g weight) for laboratory analysis of intramuscular fat content and fatty acid content were collected 24 hours after slaughtering, put in bags and frozen for further analyses, lean meat percentage were measured by FOM apparatus. Lipid fraction was isolated from the meat samples using method by FOLCH et al. (1957), preparation of methyl esters of fatty acids by CSN ISO 5509, methyl esters of fatty acids were analysed by gas chromatography (6890N Agilent Technologies) according CSN ISO 5508. Thiobarbituric acid-reacting substances (TBARS) test was used to assess lipid oxidation in muscle (method by PIETTE and RAYMOND, 1999) and the results were reported as mg of malonaldehyde/kg of muscle.
The statistical evaluation was performed using the computer program QCExpert and in R environment (R Development Core Team, 2005).Data were analysed using analysis of variance (ANOVA), logarithmic and radical transformation was applied for non-

normal data partition. Data were presented as the mean, standard deviation (SD) of each group and the significance levels.

Table 1
Components of feeding mixtures

Components	Diet L0	L1	L2	L3
Wheat meal (%)	45,3	35,3	26,9	26,9
Barely meal (%)	29,0	34,0	28,0	28,0
Soybean oil meal (%)	21,6	20,0	17,6	17,6
Linseed (%)	0	6,7	13,4	13,4
L-lysine (%)	0,3	0,3	0,3	0,3
L-threonin (%)	0,2	0,2	0,2	0,2
Methionin (%)	0,3	0,3	0,3	0,3
Sodium chloride (%)	0,4	0,4	0,4	0,4
Ground limestone (%)	1,5	1,5	1,5	1,5
ME (MJ/kg)	12,8	12,8	12,8	12,8
Crude protein (g/kg)	187,3	187,8	185,9	185,9
alpha-tocopherol (mg/kg)	31,3	31,4	31,4	103

Results

Linseed treatments did not affect (P>0,05) growth and carcass traits (Table 2). The average initial live body weight was 37 kg, final body weight was 98 kg. Average daily weight gain (ADG) was calculated from live body weight at the beginning and at the end of the experiment. The highest ADG (949,8 ± 94,3 g) was observed in L0 group but there was not significant difference and influence of the diet between groups (P >0,05). The average lean meat percentage value at the level 60,2% was investigated. The highest lean meat content was found in L2 group (60,9 ± 1,7 %) and the lowest was found in L3 group (59,2 ± 1,7 %). The differences were not significant (P>0,05). The highest IMF content was in L0 group (2,1 ± 0,4%) and the tendency of decreasing was observed but it was not significant (P>0,05).

Table 2
Carcass value traits in control and experimental groups

	Group L0 mean ± SE	L1 mean ± SE	L2 mean ± SE	L3 mean ± SE
Average daily weight gain (g)	949,8 ± 94,3	895,8 ± 57,2	893,3 ± 104,9	930,9 ± 75,9
Lean meat percentage (%)	60,6 ± 1,9	59,8 ± 2,6	60,9 ± 1,7	59,2 ± 1,7
Intramuscular fat content (%)	2,1 ± 0,4	1,8 ± 0,3	1,7 ± 0,3	1,9 ± 0,3

The fatty acid content was investigated in samples of *M. longissimus dorsi et thoracis* by gas chromatography. The composition of selected fatty acids is given in Table 3.
The n-6/n-3 ratio was calculated from total n-6 polyunsaturated fatty acids (PUFA) and total n-3 PUFA content. Feeding linseed to pigs significantly increased the relative content of α-linolenic acid. The increasing content of linoleic acid (from 7,53 ± 1,77 in L0 group to 11,57 ± 2,61 g/100g of total fatty acids in L3 group) was observed in the experiment (P<0,05). As well, α-linolenic acid content was increased (from 0,31 ± 0,05 in L0 group to 0,50 ± 0,09 g/100g of total fatty acids in L3 group; P<0,01) and arachidonic acid content was enhanced from 2,08 ± 0,67 in L0 group to 2,85 ± 0,76

g/100g of total fatty acids in L3 group (P>0,05). Alpha-linolenic acid is the precursor fatty acid for synthesis of EPA and DHA. The amount of these fatty acids was analysed as increasing but only in EPA was found significant (P<0,05). SFA content remained practically unchanged at the level about 39 g/100 g of total fatty acids. Monounsaturated fatty acid content was decreased from 49,17 ± 1,87 g/100 g in the control group to 44,28 ± 3,42 g/100 g of total fatty acids in L3 group, on the contrary polyunsaturated fatty acid content had rising tendency (P<0,05, resp. P<0,01). Both n-6 and n-3 fatty acid concentration was raised but it was more significant in n-3 fatty acids . The n-6/n-3 ratio was more favourable in L3 group (10,95 ± 0,55) than in the control group. However, the n-6/n-3 ratio decline was not significant (P>0,05) and intensive due to increase of both n-6 and n-3 total fatty acids amount. Correlation coefficients for intramuscular fat content, lean meat percentage and SFA, MUFA, PUFA and total unsaturated fatty acid content are in Table 4.

Table 3
Fatty acid content in *M.longissimus dorsi et thoracis* (g/100g of total fatty acids)

	Group			
	L0	L1	L2	L3
	mean ± SE	mean ± SE	mean ± SE	mean ± SE
Myristic C14:0	1,29±0,17	1,22±0,10	1,28±0,16	1,22±0,11
Palmitic C16:0	24,44±1,08	23,92±0,84	23,93±0,51	23,98±0,69
Stearic C18:0	12,78±0,52	12,44±0,63	12,58±0,58	12,62±0,67
Oleic C18:1 n-9	40,40 ± 1,53 ^{cd}	37,14 ± 2,46	37,20 ± 1,86^{a}	36,46 ± 2,77^{a}
Linoleic C18:2 n-6	7,53 ± 1,77 ^{cd}	10,70 ± 2,32	11,11 ± 1,82^{a}	11,57 ± 2,61^{a}
γ-linolenic C18:3n-6	0,10 ± 0,03	0,12 ± 0,04	0,12 ± 0,05	0,12 ± 0,03
α-linolenic C18:3 n-3	0,31 ± 0,05^{BCD}	0,49 ± 0,14^{A}	0,52 ± 0,06^{A}	0,50 ± 0,09^{A}
Eikosenoic C20:1 n-9	0,73 ± 0,08	0,68 ± 0,11	0,70 ± 0,11	0,67 ± 0,13
Arachidonic C20:4 n-6	2,08 ± 0,67	2,72 ± 0,71	2,37 ± 0,44	2,85 ± 0,76
EPA C20:5 n-3	0,12 ± 0,04^{d}	0,18 ± 0,04	0,16 ± 0,03	0,19 ± 0,05^{a}
DHA C22:6 n-3	0,08 ± 0,05	0,10 ± 0,04	0,09 ± 0,03	0,09 ± 0,04
Total SFA	39,23 ± 1,32	38,34 ± 1,45	38,70 ± 0,88	38,57 ± 1,17
Total MUFA	49,17 ± 1,87^{cD}	45,54 ± 2,85	45,27 ± 2,34^{a}	44,28 ± 3,42^{A}
Total PUFA	11,60 ± 2,88^{bD}	16,12 ± 3,42^{a}	16,03 ± 2,51	17,15 ± 3,94^{A}
Total n-6	10,65 ± 2,62^{d}	14,75 ± 3,18	14,69 ± 2,38	15,71 ± 3,61^{a}
Total n-3	0,95 ± 0,26^{bcD}	1,37 ± 0,29^{a}	1,34 ± 0,16^{a}	1,44 ± 0,34^{A}
n-6/n-3	11,38 ± 0,98	10,86 ± 1,35	10,97 ± 1,08	10,95 ± 0,55
MUFA/PUFA	4,51 ± 1,13^{BCD}	2,98 ± 0,75^{A}	2,92 ± 0,65^{A}	2,77 ± 0,85^{A}

^{a,b,c,d} significant differences at a significance level of 95%; ^{A,B,C,D} significant differences at a significance level of 99%

Table 4
Correlation coefficients for intramuscular fat content, lean meat percentage and SFA, MUFA, PUFA and total unsaturated fatty acid content

	SFA	MUFA	PUFA	Total unsaturated FA
Intramuscular fat content	0,198	0,377	-0,382	-0,198
Lean meat percentage	-0,247	-0,109	0,173	0,248

The oxidative stability was indicated as milligrams of malonaldehyde/kg of muscle. The addition of alpha-tocopherol enhanced the oxidative stability of muscle lipids. The lowest content of malonaldehyde (1,48 ± 0,26 mg/kg) was measured after 6 days in L3 group. Differences in malonaldehyde concentration was statistically significant at the1st (P<0,01) and 6th (P<0,05) day of storing. The malonaldehyde concentration is shown in the Figure.

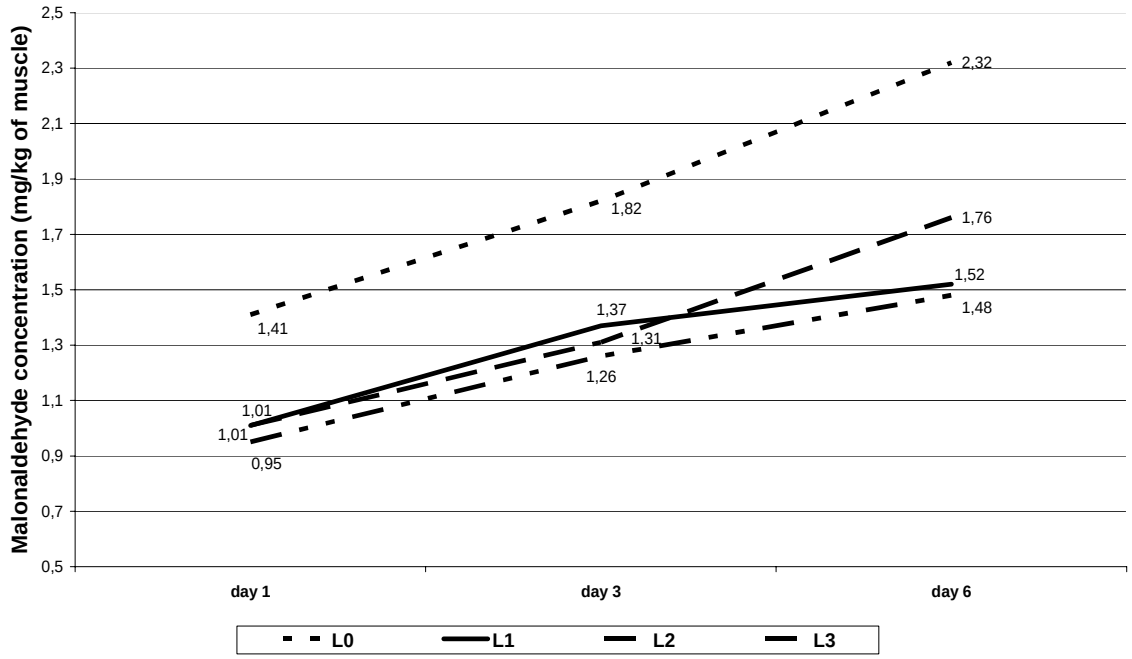

Figure: Effect of alpha tocopherol on oxidative stability of muscle lipids

Discussion

The carcass value traits, fatty acid content and meat quality was investigated in the experiment. No significant effect of linseed in pig diet on carcass value traits (average daily weight gain in the experiment, lean meat percentage and intramuscular fat content) was observed. The same result was published by WISEMAN et al. (2000). According to ROMANS et al.(1995) and KOUBA et al. (2003) flaxseed treatments did not affect (P>0,05) any monitored growth and carcass traits.

The effect of linseed oil and olive oil on lipid composition was mentioned in a study by NUERNBERG et al. (2005). Feeding linseed oil to pigs significantly increased the relative content of linolenic acid and increasing amount of this fatty acid was found out in the experiment to. The concentration of linoleic acid had increasing tendency.

The effect of linseed oil on the fatty acid composition was investigated by D´ARRIGO et al. (2002). The n-6/n-3 ratio was sharply reduced when pigs were fed diets enriched with linseed oil. The effect of n-3 fatty acid-enriched diets (in the form of linseed oil with sunflower oil or olive oil) and α-tocopheryl acetate supplementation on lipid oxidation (TBARS) was investigated in longissimus muscle by REY et al. (2001). Meat from pigs fed linseed oil-enriched diets had a higher proportion on n-3 fatty acid then meat from pigs in other dietary groups in neutral and polar lipids and 20% reduction in the n-6/n-3 ratio was observed. Total n-3 fatty acid content was enhanced

in the experiment. The difference between L3 group and control group was 0,49 g/100 g of total fatty acids (P<0,01). Also n-6/n-3 fatty acid ratio was reduced. However this decline was not statistically significant (P>0,05).
SHEARD et al. (2000), HOZ et al. (2003) studied influence of linseed-rich test diet on sensory quality, oxidative stability after conditioning and storage. There was no significant effect of diet on lipid oxidation and any significant effect of diet on colour changes of pork chops. The test diet resulted in higher α-linolenic acid levels, with major increases in total n-3 PUFA content. The n-6 PUFA content was reduced by the test diet. We observed this increase in α-linolenic acid content as well (P<0,001).
The oxidative stability was indicated as milligrams of malonaldehyde/kg of muscle. Similarly like in studies performed by LARICK et al. (1992), WAYLAN et al. (2002) and LAHUCKY et al. (2005) in which the importance of alpha tocopherol is mentioned, the effect of vitamin E on meat oxidative stability was documented in our experiment.
The contents of SFA and MUFA increase faster with increasing fatness then those content of PUFA resulting in decrease in the relative proportion of PUFA and consequently in the PUFA/SFA (P/S) ratio. For pork, the intramuscular fat level also affects the P/S ratio, but nutrition will have a larger impact. The fat level also influences the n-6/n-3 PUFA ratio, due to the difference of this ratio in polar and neutral lipids. However, these effects are much smaller then the effects that can be achieved by dietary means. After correction for fat level, breed or genotype differences in the MUFA/SFA ratio and in the longer chain C20 and C22 PUFA metabolism have been reported, reflecting the possible genetic differences in fatty acid metabolism (De SMET et al., 2004). The content of SFA is increased with increasing intramuscular fat content, it means the unsaturated fatty acid content is decreasing. The animals with higher meatness show lower SFA content and higher unsaturated fatty acids content in meat (ALTMANN et al., 1992). BIEDERMANN et al. (2000) investigated the carcass fat quality and fatty acid composition in the *M. longissimus* of castrated pigs and sows. There was no different in the fatty acid composition. The correlation coefficients between fatty acid composition and parameters of fattening performance and composition were in the low to medium range. The investigation corresponded with these publications. SFA and MUFA content was increasing with increasing intramuscular fat content (correlation coefficient 0,198 and 0,377), PUFA and total unsaturated fatty acid content was in negative correlation (-0,382 and -0,198) with fatness. On the contrary, there was reverse trend in correlation between lean meat percentage and fatty acid content.
It can be summarized that

- the carcass value traits were not influenced by adding linseed in pig diet
- the content of total saturated fatty acids were not affected, monounsaturated fatty acid content was decreased, polyunsaturated fatty acid content was increased
- the n-3 fatty acid content was increased but n-6/n-3 fatty acid ratio decrease was not statistically significant
- the increase of SFA and MUFA and decrease of PUFA and total unsaturated fatty acid content was found out with increasing intramuscular fat content
- and positive effect of alpha-tocopherol on meat oxidative stability was demonstrated

The fatty acid composition is important from the point of view of human health and it is reason to provide other experiments from this range to describe the problem in detail. There is possibility to change fatty acid profile in pork meat via animal diet, evaluate meat quality in term of beneficial fatty acid content and affect human health.

Acknowledgment

Supported by the Ministry of Agriculture of the Czech Republic (Project No. MZE 0002701403).

References

ALTMANN, M.; LENGERKEN, G. v.; PFEIFFER, H.:
Intramuskulärer Fettgehalt – Bedeutung, Ermittlung und Selektionsmaßnahmen in der Schweinezucht. Schweinezucht und Schweinemast, **40** (1992), 252-254

BIEDERMANN, G.; JATSCH, CH.; PESCHKE, W.; LINDNER, J.-P.; WITTMANN. W.:
Mast- und Schlachtleistungen sowie Fleisch- und Fettqualität von Pietrain Schweinen unterschiedlichen MHS-Genotyps und Geschlechts. Arch. Tierz., Dummerstorf **43** (2000), 165-178

Czech Standard CSN 57 0185.
Examination of meat, meat products and canned meat. Chemical and physical methods. Prague, 1985

Czech Standard CSN ISO 5509.
Animal and vegetable fats and oils. Preparation of methyl esters of fatty acids. Prague, 1994

Czech Standard CSN ISO 5508.
Animal and vegetables fats and oils. Analysis by gas chromatography of methyl esters of fatty acids. Prague, 1994

D´ARRIGO, M.; HOZ, L.; LOPEZ-BOTE, C. J.; CAMBERO, I.; PIN, C.; REY, A. I.; ORDONEZ, J. A.:
Effect of dietary linseed oil and α-tocopherol on selected properties of pig fat. Can. J. Anim. Sci. **82** (2002) 3, 339-346

DEMAISON, L.; MOREAU, D.:
Dietary n-3 polyunsaturated fatty acids and coronary heart disease-related mortality: a possible mechanism of action. Cell. Mol. Life Sci., **59** (2002), 463-477

DeSMET, S.; RAES, K. ; DEMEYER, D.:
Meat fatty acid composition as affected by fatness and genetic factors: a review. Anim. Res., **53** (2004), 81-98

ENDER, K.:
Zukünftige Anforderungen an die Fleischqualität. Arch. Tierz., Dummerstorf **37** (1994) Sonderheft, 137-142

ENDER, K.; NUERNBERG, K.; PAPSTEIN, H. J.:
Two parts of fat-A review. Arch. Tierz., Dummerstorf **40** (1997) 1, 35-45

FOLCH, J.; LEES, M.; SLOANE STANLEY, G.H.:
J. Biol. Chem. **226** (1957), 497

HOZ, L.; LOPEZ-BOTE, C. J.; CAMBERO, M. I.; D´ARRIGO, M.; PIN, C.; SANTOS, C.; ORDONEZ, J. A.:
Effect of dietary linseed oil and alpha-tocopherol on pork tenderloin (Psoas major) muscle. Meat Sci. **65** (2003) 3, 1039-1044

KANG, J. X.; LEAF, A.:
Prevention of fatal cardiac arrhythmias by polyunsaturated fatty acids. Am. J. Clin. Nutr., **71** (2000), 202-207

KOUBA, M.; ENSER, M.; WHITTINGTON, F. M.; NUTE, G. R.; WOOD, J. D:
Effect of a high-linolenic acid diet on lipogenic enzyme activities , fatty acid composition, and meat quality in the growing pigs. J. Anim. Sci., **81** (2003) 8, 1967-1979

KRSKA, P.; LAHUCKY, R.; KUECHENMEISTER, U.; NUERNBERG, K.; PALANSKA, O.; BAHELKA, I.; KUHN, G.; ENDER, K.:
Effects of dietary organic selenium and vitamin E supplementation on post mortem oxidative deterioration in muscles of pigs. Arch. Tierz., Dummerstorf **44** (2001), 193-201

LAHUCKY, R.; KRSKA, P.; KUECHENMEISTER, U.; NUERNBERG, K.; LIPTAJ, T.; NUERNBERG, G.; BAHELKA, I.; DEMO, P.; KUHN, G.; ENDER, K.:
Effect of vitamin E on changes in phosphorus compounds assessed by ^{31}P NMR spectroscopy and ATPase from postmortem muscle samples and meat quality of pigs. Arch. Tierz., Dummerstorf **43** (2000), 487-497

LAHUCKY, R.; NUERNBERG, K.; KUECHENMEISTER, U.; BAHELKA, I.; MOJTO J.; NUERNBERG, G.; ENDER, K.:
The effect of dietary magnesium oxide supplementation on fatty acid composition, antioxidative capacity and meat quality of heterozygous and normal malignant hyperthermia (MH) pigs. Arch. Tierz., Dummerstorf **47** (2004), 183-191

LAHUCKY, R.; KUECHENMEISTER, U.; BAHELKA, I.; NOVOTNA, K.; VASICKOVA, K.; ENDER, K.:
Effects of vitamin E by dietary supplementation and of calcium ascorbate by post mortem injection in muscle on the antioxidative status and on meat quality of pigs. Arch. Tierz., Dummerstorf **43** (2005), 592-600

LARICK, D.K.; TURNER, B.E.; SCHWEENHERR, W.D.; COFFEY, M.T.; PILKINGTON, D.H.:
Volatile compound content and fatty acid composition of pork as influenced by linoleic acid content of the diet. J. Anim. Sci., **70** (1992) 5, 1397-1403

LEAF, A.; XIAO, Y. F.; KANG, J. X.; BILLMANN, G. E.:
Prevention of sudden cardiac death by n-3 polyunsaturated fatty acids. Pharmacol. Therapeut., **98** (2003), 355-377

LEIBETSEDER, J.:
Beeinflussung der Zusammensetzung tierischer Fette durch die Fütterung. Arch. Tierz., Dummerstorf **39** (1996), 333-345

METGES, C. C.:
Importance of and opportunities for the production of nutrient enriched food of animal origin. Ernaehrungs-Umschau, **51** (2004), 484

NUERNBERG, K.; WEGNER, J.; ENDER, K.:
Factors influencing fat composition in muscle and adipose tissue of farm animals. Livest. Prod. Sci., **56** (1998), 145-156

NUERNBERG, K.; FISCHER, K.; NUERNBERG, G.; KUECHENMEISTER, U.; KLOSOWSKA, D.; ELIMINOWSKA-WENDA, G.; FIEDLER, I.; ENDER, K.:
Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. Meat Sci. **70** (2005) 1, 63-74

PFEUFFER, M.:
Physiologic effects of individual fatty acids in animal and human body, with particular attention to coronary heart disease risk modulation. Arch. Tierz., Dummerstorf **44** (2001) 1, 89-98

PIETTE, G.; RAYMOND, Y.:
Rancidity in processed meats-Comparative evaluation of methods. Fleischwirtsch. Int. 3 (1999), 36-39

R Development Core Team:
R:A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (2005)

REY, A.I.; KERRY, J.P.; LYNCH, P.B.; LOPEZ-BOTE, C.J.; BUCKLEY, D.J.; MORRISSEY, P.A.:
Effect of dietary oils and alpha-tocopheryl acetate supplementation on lipid (TBARS) and cholesterol oxidation in cooked pork. J. Anim. Sci. **79** (2001) 5, 1201-1208

ROMANS, J. R.; JOHNSON, R. C.; WULF, D. M.; LIBAL, G. W.; COSTELLO, W. J.:
Effects of ground flaxseed in swine diets on pig performance and on physical and sensory characteristics and omega-3 fatty acid content of pork: I. Dietary level of flaxseed. J. Anim. Sci., **73** (1995), 1982-1986

SHEARD, P.R.; ENSER, M.; WOOD, J. D.; NUTE, G. R.; GILL, B. P.; RICHARDSON, R. I.:
Shelf life and quality of pork products with raised n-3 PUFA. Meat Sci. **55** (2000), 213-221

WAYLAN, A. T.; QUINN, P. R.; UNRUH, J. A.:
Effect of modified tall oil and vitamin E on growth performance, carcass characteristics, and meat quality of growing-finishing pigs. J. Anim. Sci. **80** (2002), 1575-1585

WISEMAN, J.; REDSHAW, M. S.; JAGGER, S.; NUTE, G. R.; WOOD, J. D.:
Influence of types and dietary rate of inclusion of oil on meat quality of finishing pigs. Anim. Sci. **70** (2000), 307-315

WOOD, J. D.; ENSER, M.:
Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. Brit. J. Nutr., **78** (1997), Suppl. 1, 49-60

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Arch. Tierz., Dummerstorf **50** (2007) Special Issue, 152-160

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Investigations of the factors influencing damages of the spinal column and muscles during electrical stunning of swine

Abstract

The aim of the performed investigations was to study the effect of some live and technological factors such as: genotype, sex, body weight, class of meatiness, pre-slaughter rest as well as of the parameters of electrical stunning on the frequency of the vertebral fracture and the amount of bloody meat in the shoulder. Four experiments in two slaughterhouses on the total of 5556 fatteners from current market deliveries were carried out. On the basis of the *chi* square test, a significant influence of the genotype on the number of fractures of the spinal column was demonstrated, with Pietrain breed crossbreds and hybrids x revealing the highest proportion of carcasses with fractured spinal column (about 21% carcasses). The frequency of fractures was not found to be affected by either the class of meatiness, body weight and sex or the charge value of the electrical stunning within the range of the experimental charges from 9 to 15 coulombs. However, the number of the spinal column fractures was influenced significantly by the method of electrical stunning. Whereas the stunning by the current of the mains frequency of 50 Hz and the initial voltage of 250 V caused spinal column damages in each of the examined experimental groups, the phenomenon did not occur when the animals were stunned by the current of 2000 Hz frequency and the initial voltage of 250 V. In addition, about 20% less bloody meat was found in the shoulders of fatteners stunned using the current of increased frequency in comparison with the animals stunned with the current of the mains frequency. It is evident from the investigations that the method of electric stunning exerts a stronger influence on the number of the spinal column damages than live factors.

Key Words: pig, stunning, swine carcasses, spinal column fractures

Zusammenfassung

Titel der Arbeit: **Faktoren, die Knochenbrüche und Muskelbeschädigungen während der Elektrobetäubung bei Schweinen beeinflussen**

Es wurde der Einfluss bestimmter Lebens- und technologischer Faktoren, wie z.B.: Genotyp, Schlachtkörpergewicht, Klasse der Fleischfülle, Erholung der Tiere vor der Schlachtung und Parameter der Elektrobetäubung auf die Frequenz der Knochenbrüche (Wirbelbrüche) und den Anteil des blutigen Fleisches im Schulterbereich der Schweine untersucht. Dazu wurden vier Versuche in zwei Schlachthöfen an insgesamt 2143 Mastschweinen, die aus Marktlieferungen stammten, durchgeführt. Der Chi-Quadrat-Test beweist, dass der Genotyp der Tiere einen bedeutsamen Einfluss auf die Zahl der Knochenbrüche hatte, wobei Kreuzungen mit der Pietrain-Rasse und X-Hybriden den größten Teil der Schlachtkörperhälften mit Knochenbrüchen (ca. 21% Schlachtkörperhälften) bildeten. Auf die Höhe der Knochenbrüche hatten weder die Klasse der Fleischfülle noch die Größe der elektrischen Betäubungsladung im Bereich der untersuchten Parameter (von 9 bis 15 Coulomb) Einfluss. Den größten Einfluss auf die Zahl der Knochenbrüche hatte dagegen die Art der Elektrobetäubung. In jeder untersuchten Gruppe von Schweinen, die mit Strom mit einer Netzfrequenz von 50 Hz und der Anfangsspannung von 250 V betäubt wurden, gab es Knochenbrüche. Im Vergleich dazu gab es bei einer Betäubung mit Strom mit 2000 Hz Frequenz und einer Anfangsspannung von 250 V keinerlei Knochenbrüche. Außerdem wurde im Schulterbereich dieser Mastschweine, die mit dem Strom der größten Anfangsspannung betäubt wurden, weniger blutiges Fleisch festgestellt, als in dem Fall, wo die Tiere mit Strom von 50 Hz Netzfrequenz betäubt wurden. Diese Untersuchungen beweisen, dass die Art der Elektrobetäubung einen größeren Einfluss auf die Zahl der Knochenbrüche hat als die Lebensfaktoren.

Schlüsselwörter: Schwein, Betäubung, Schlachtkörper, Knochenbrüche

Introduction

For many years now, a steady progress in the improvement of technical solutions of both methods of fatteners' stunning, i.e. electrical and gas methods has been observed. In different countries, comparative experiments are being carried out in industrial conditions on large animal populations in which advantages and disadvantages of individual solutions bearing in mind such aspects as animal welfare, the impact of the applied method on meat technological and quality properties, work safety etc. are being assessed (KIEN, 1999; TROEGER, 1998, 1999; FAUCITANO et al., 1998; WENZLAWOWICZ, 1998).

KIEN (1997a) maintains that the stunning process may induce the following basic defects of the meat raw material: bone fracture (primarily of the spinal column), hemorrhages, muscle as well as pulmonary extravasations and meat quality defects of the PSE type. Hamorrhages as well as muscle and pulmonary extravasations are particularly common in the case of manual, low-voltage electric stunning (75 V, 0.3 A, 50 Hz, time - 15s), as confirmed by earlier experiments carried out at the Meat and Fat Research Institute (BORZUTA, 1971a, b). It was demonstrated then that, in the case of fatteners stunned in this way, hamorrhages occurred in 14.32% of hams, while surface extravasations in the muscles of the abdominal cavity and chest – in 8.17% carcasses and only about 40% lungs were free of extravasations. It was the above-mentioned factors as well as changes in regulations which imposed the application of stronger stunning current (working voltage at least 250 V at 50 Hz frequency) to deprive slaughter animals of their consciousness before slaughter (Directive 1999) that forced Polish slaughter-houses to abandon the application of the low-voltage method of stunning. At the present time, domestic slaughter-houses have begun introducing gas stunning but electric stunning continues to remain the dominant method of slaughter. When reporting the recent developments in investigations on swine stunning presented at an international conference in Billund, KIEN (1999) says that in response to the improved systems of gas stunning, new, three-electrode electric stunning systems were developed and the competition between the two systems continues unabated. Results of numerous quality investigations revealed that the systems of gas stunning utilizing high CO_2 concentrations as well as the automatic three-electrode systems can be treated as equal both with regard to their effectiveness and their impact on the meat quality (SILVEIRA, 1998; FAUCITANO et al., 1998; WENZLAWOWICZ et al., 1998, 1998a; VELARDE et al., 1998, 1999). In the case of both methods, bone fractures, extensive hamorrhages in muscles were eliminated and the frequency of the occurrence of the PSE meat in carcasses was reduced.

At present, the above-mentioned automatic stunning systems are used in very few slaughter-houses in Poland but there is no doubt that their numbers are increasing. Nevertheless, the dominant method, particularly in small and medium-sized slaughter-houses, is that which employs manual electrode tongs of various devices which can be divided into three groups: stunners using the initial voltage of approximately 250 V and 50 Hz frequency without any measurement of stunning parameters, stunners with a similar initial voltage but equipped in a system of an automatic on and off switch of the current and a system which allows measuring and signaling the electric charge during stunning and stunners with the 250 or 310 V initial voltage and frequency of 2000 Hz and a rectangular shape of the voltage wave. Both scientific reports (KIEN, 1997; ŁOBODA et al., 2004; TROEGER, 1999) as well as practical observations

indicate that especially the application of manually-operated tongs leads to frequent fractures of the spinal column bones. Attention is drawn to differences in the intensity of these fractures which indicate that the phenomenon may be affected by many pre-slaughter factors.

The aim of the performed investigations was to determine the effect of some live factors such as: genotype, body weight, sex, meatiness class, pre-slaughter rest and electric stunning parameters on the frequency of fractures of the spinal column and the amount of bloody meat in the shoulder.

Material and Methods

The investigations were carried out in the SOKOŁÓW S.A. processing plant, KOŁO branch and in the SKIBA slaughter-houses in Czersk. Fatteners from current deliveries were stunned employing the following domestic devices: EOT-1 with manually-operated tongs with the initial voltage of 250 V and frequency of 50 Hz using the electrical charge of 15 coulombs (method A), 12 coulombs (method B) or 9 coulombs (method C) and using an ENZ-2000 microprocessor power pack for animal electronarcosis with manually-operated tongs and the initial current voltage of 250 V and frequency of 2000 Hz (method D). The time of current application using methods A, B and C depended on the set charge and amounted to about 6, 8 or 10 seconds, respectively. The applied time was about 10 s when the stunning method with the increased current frequency was employed (method D). In the case of all the applied stunning methods, the time from the termination of current application to the moment of sticking ranged from 15 to 20 s. The following experiments were carried out:

- ***Experiment 1*** – impact of different genotypes on damages of the spinal column. The following 7 genotypes were investigated: hybrid X fatteners, crossbreds of polish large white x polish landrace (PLW x PL) sows with Pietrain boars, with Pietrain x Duroc boars, with Hampshire x Pietrain boars, with P-76 boars, hybrid Y fatteners and a group of fatteners of unidentified breeds. The total of 1253 carcasses was examined in group sizes given in Tab. 1. With the exception of the X hybrids, the remaining fatteners derived from the raw-material base of the slaughter-house in Koło and were delivered to the slaughter-house from the radius which did not exceed 100 km. The X hybrid fatteners were delivered from a farm situated about 180 km from the slaughter-house and were slaughtered after about 1 h or 8 hs of rest employing the B stunning method.
- ***Experiment 2*** – impact of the body weight and sex on damages of the spinal column. 168 hybrid X fatteners divided into the following weight groups were studied (Tab. 2): up to 95 kg (carcass weight up to 70 kg); from 95-105 kg (carcass weight from 70.1 to 80 kg) and above 105 kg. Following their transport for about 180 km and 1 h rest, the fatteners were slaughtered using method B.
- ***Experiment 3*** – impact of the A, B and C stunning methods on damages of the spinal column and the rate of after-slaughter changes in meat measured by the level of pH 45 minutes after slaughter (pH_1). The acidity was determined in the *longissimus lumborum* muscle at the height of the first lumbar vertebra using the Radiometer 80 Portable pH-meter equipped in a combined electrode. 168 hybrod X fatteners were examined in the first two groups (methods A and C) and 3413 fatteners of different breeds in group three (method D).

- ***Experiment 4*** – impact of the meatiness level of carcasses derived from different genotypes divided into classes according to the EUROP system (BORZUTA, 2000) on damages of the spinal column. The total of 501 fatteners were examined in group sizes shown in Tab. 3. Fatteners were stunned using method B.

Carcasses were assessed on the slaughter line recording visible fractures of the spinal column with hamorrhages in the muscles surrounding them. The proportion of carcasses with fractured vertebras was the basis for drawing conclusions about the effect of the examined live and slaughter factors on damages of the spinal column. In the case of Experiment 3, pH_1 changes as well as the proportion of bloody meat in the shoulder (estimated by weight during deboning) were also investigated.
The performed statistical calculations employed the *chi* square test to confirm the significance of differences between the proportions of damages in the spinal column in individual groups and the Student test to compare pH_1 differences between groups of fatteners stunned with different methods (RUSZCZYC, 1980).

Results and Discussion

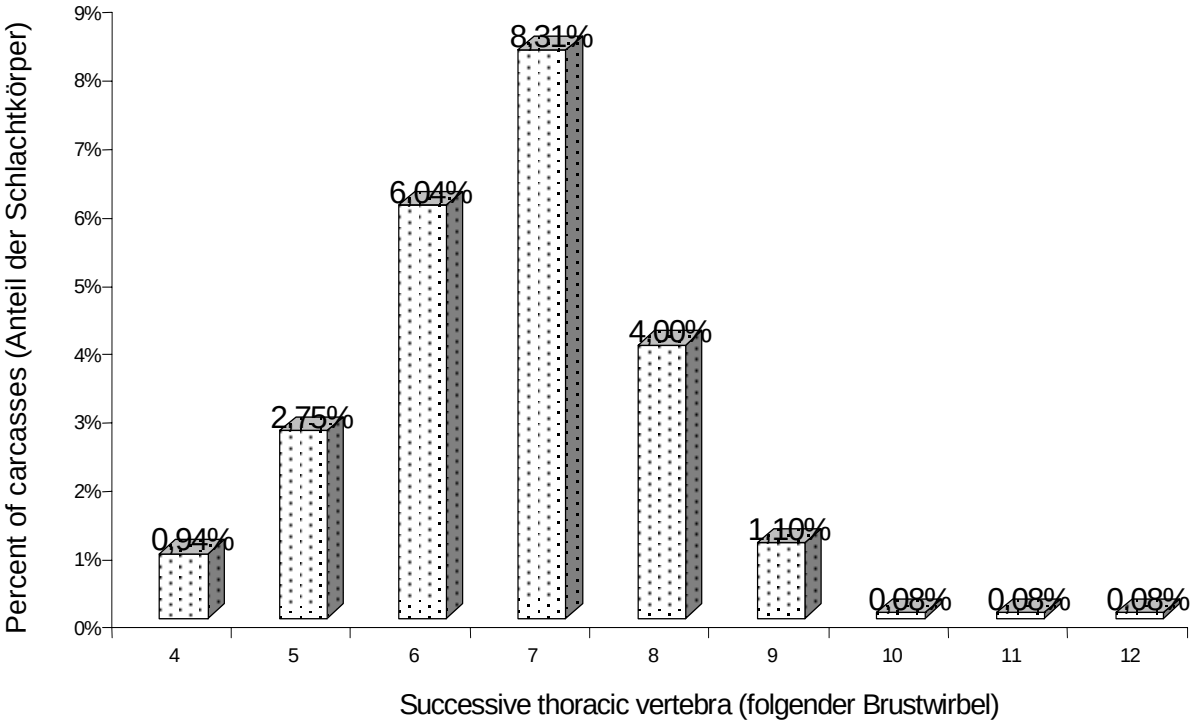
In the course of electrical stunning of pigs, the phenomenon of fracture of one or several vertebras was observed which required the removal of the damaged vertebras and the surrounding bloody meat as a veterinary confiscation causing definite losses. The amount of the removed meat together with the vertebra ranged from 0.5 to 1.0 kg. Observations carried out on 1275 carcasses obtained from fatteners which were stunned using method B showed that the 6^{th} and 7^{th} thoracic vertebra fractured most frequently (up to 8% of carcasses), 5^{th} and 8^{th} thoracic vertebra – less frequently (3 to 4%) and the 4^{th} and 9^{th} vertebra broke rarely (Fig.). None or only sporadic cracks were recorded in the remaining vertebras. However, irrespective of the location of the spinal column fractures, the most valuable part of the carcass was always damaged, i.e. the loin which cannot be sold. ŁOBODA and POSPIECH (2004) as well as VELARDE et al. (2001) maintain that the fractured bones are caused by exceptional mechanical strains during the contraction reaction caused by the applied electric current and possible fall of the animal. The above-mentioned authors also reported that vertebras situated in the loin and neck underwent fracture most frequently.
The performed investigations showed that there is a correlation between the proportion of damages of the spinal column and the breed origin of pigs (Tab. 1). Employing the *chi* square test, a statistically significant impact of the genotype on the amount of carcasses with fractured vertebras was demonstrated.

Table 1
Proportion of carcasses with fractured vertebra in fatteners of different breed origin

Breed	Number of carcasses with fractured vertebras	Number of carcasses without damages vertebras	% of carcasses with fractured vertebras
Hybrid X	72	257	21.88
(PLW x PL) x P	8	29	21.62
(PLW x PL) x (P x D)	9	199	4.33
Hybrid Y	3	37	7.50
(PLW x PL) x P-76	3	67	4.29
(PLW x PL) x (H x P)	0	35	0.00
Unidentified	42	492	7.87

$chi^2_{emp.} = 67.28$; $chi^2_{0.01} = 16.81$

Fig.: Proportion of pork carcasses with fractured thoracic vertebras in pigs stunned with method B (n= 1275)



The highest proportion of damages was found in crossbreds with the Pietrain breed and in hybrids X (more than 21% of carcasses). Three times fewer damages were observed in hybrids Y (about 7%) and more than 5 times fewer fractures occurred in hybrids after P-76 and P x D boars. No fractures were found in fatteners derived after the H x P father. The above observations may indicate a more delicate skeleton in hybrid X pigs and crossbreds with the Pietrain breed which are characterised by a very young age at slaughter and this may be one of the causes of more frequent damages of the vertebras. However, the above hypothesis is not confirmed by the results of investigations on the effect of the weight of fatteners on the number of vertebral damages (Tab. 2).

Table 2
Proportion of fractured vertebras in fatteners of different weights

Weight of fatteners in kg	Number of carcasses with damaged vertebras	Number of carcasses without damaged vertebras	% of carcasses with damaged vertebras
Up to 95	6	7	46.15
95 to 105	39	78	33.33
over 105	11	27	28.94

$chi^2_{emp.} = 1.29$; $chi^2_{0.05} = 5.99$

Despite the fact that the proportion of carcasses with fractured vertebras increases as the weight of fatteners decreases, nevertheless the difference turned out to be statistically non-significant. Similar observations were reported by ŁOBODA and POSPIECH (2004) who investigated a wider range of animal weights (weaners, fatteners, sows).

The performed investigations did not confirm the effect of the carcass meatiness on vertebral damages (Tab. 3).

Table 3
Proportion of carcasses with fractured vertebras in relation to the EUROP meatiness class

Carcass class	Number of carcasses with damaged vertebras	Number of carcasses without damaged vertebras	% of carcasses with damaged vertebras
E	62	165	27.31
U	46	116	28.40
R	25	62	28.74
O	7	18	28.00

$chi^2_{emp.} = 0.06$; $chi^2_{0.05} = 7.81$

The proportion of carcasses with fractured vertebras reached the level of about 28% in the five examined classes. Also ŁOBODA and POSPIECH (2004) failed to find significant differences in the percentage of carcasses with damaged vertebras between E, U, R, O and P classes; the determined level of these damages ranged from 10-12%. On the other hand, in the case of class S, the one with the best musculature with meat content over 60%, the same authors found three times more carcasses with fractured spinal columns and attributed this to the susceptibility of this group of animals to genetically preconditioned stress. This hypothesis was confirmed by investigations carried out by VELARDE et al. (2001) who reported more frequent fractures of the spinal column and blood extravasations in pigs susceptible to stress.
No effect of sex on the frequency of vertebral damage was observed as evident in data presented in Table 4.

Table 4
Proportion of carcasses with fractured vertebras in relation to the sex of fatteners stunned with method B

Gender	Number of carcasses with damaged vertebras	Number of carcasses without damaged vertebras	% of carcasses with damaged vertebras
Gilts	22	90	24.44
Hogs	10	39	25.64

$chi^2_{emp.} = 1.57$; $chi^2_{0.05} = 3.84$

These experiments were conducted on a uniform group of 162 pigs of the same breed (hybrid X) fattened on the same farm and delivered to the slaughter-house in one two-level truck. The proportion of fractures in gilts and hogs was similar and did not differ statistically significantly.
A similar material from the same farm was used in investigations on the impact of the pre-slaughter rest on the number of vertebral fractures. A batch of 299 hybrid X fatteners from Experiment 1 was divided into 2 groups – one of them was allowed to rest for 8 hours before slaughter and the other was slaughtered directly after the arrival at the slaughter-house. Results of this experiment are presented in Table 5.

Table 5
Proportion of carcasses with fractured vertebras in fatteners with and without pre-slaughter rest stunned with method B

Group of animals	Number of carcasses with damaged vertebras	Number of carcasses without damaged vertebras	% of carcasses with damaged vertebras
With rest	34	66	34.00
Without rest	32	167	16.08

$chi^2_{emp.} = 12.44$; $chi^2_{0.01} = 6.63$

The obtained results show that the application of the 8-hour pre-slaughter rest had a negative effect on the susceptibility of animals to fractures of the spinal column during stunning. The number of damaged vertebras in animals which were allowed to rest for 8 h before slaughter was two times higher than in animals which were slaughtered directly after the delivery. This phenomenon is difficult to explain. One of the possible explanations is that the 8 h spent in a new, hostile environment could have contributed to increased stress.
Table 6. presents the results of observations on the effect of the applied method of electrical stunning of experimental fatteners on the proportion of fractured vertebras.

Table 6
Proportion of carcasses with fractured vertebras in relation to the applied stunning method

Stunning method	Number of carcasses with damaged vertebras	Number of carcasses without damaged vertebras	% of carcasses with damaged vertebras
A (15 coulombs)	32	61	34.41
C (9 coulombs)	24	51	32.00

$chi^2_{emp.} = 0.11$; $chi^2_{0.05} = 3.84$

The stunning with the same EOT-1 device using different electrical charges (9 and 15 coulombs) during the process failed to exert a significant influence on the frequency of the occurrence of vertebral fractures. On the other hand, when the ENZ-2000 stunning device with the current frequency of 2000 Hz was employed, not a single carcass with fractured vertebras was found in 3413 examined carcasses. This shows a very significant impact of the applied electrical stunning device on the frequency of the spinal column damage. The employed high current frequency had a positive influence on the examined property as confirmed by other experiments (KIEN, 1999; BORZUTA, 1971). The above observations were used to design a new, three-electrode device in which, initially, current of increased frequency (400 – 800 Hz) is applied to the animal's head and, during the second stage, ordinary current of the mains frequency (50-60 Hz) is allowed to flow between the head and the heart. This method contributed to the improvement of meat quality (TROEGER, 1999).
The electrical charge of the stunning current at the applied range of 9 to 15 coulombs did not affect significantly the proportion of meat with quality defects (Tab. 7). Nevertheless, a trend can be observed towards increased number of carcasses with PSE meat in the group of fatteners stunned with the current used in method A (15 coulombs).

Table 7
Proportion of carcasses with defects in the *longissimus lumbarum* muscle in relation to the charge of the stunning current (n=168)

Meat quality group	Stunned with method A (15C)	Stunned with method C (9C)
PSE ($pH_1 \leq 5.80$)	5.4	2.6
Partially PSE ($pH_1 \leq 5.81 \div 6.00$)	14.2	12.0
Normal ($pH_1 > 6.00$)	80.4	85.4

chi^2emp.= 2.70; $chi^2$0.05 = 7.81

The tendency towards a greater proportion of PSE meat in pigs stunned by the stronger current charge appears to be corroborated by the mean pH_1 values determined in the *longissimus lumbarum* muscle which were as follows:
method A – 6.25±0.28
method C – 6.34±0.31

Differences between mean pH_1 values for the two stunning methods turned out to be statistically significant (t = 2.07) at P <0.04. A similar opinion was expressed by TROEGER (1999) who maintains that the application of the electrical charge ≤ 9C results in better meat quality.
The impact of stunning with the high frequency current turned out to be significant (t = 2,69^{xx}) from the point of view of the proportion of bloody meat in shoulders observed during bleeding as confirmed by the results presented in Table 8.

Table 8
Proportion of bloody meat in shoulders in relation to the applied stunning method

Stunning method	Number of examined carcasses	Proportion of bloody meat in shoulder %
Stunned with method C	46	3.26±0.41
Stunned with method D	63	2.79±0.44
Difference		0.47
Test t		2.69^{xx}

Recapitulating the performed investigations, it should be said that the stunning of animals with the current of the ordinary mains frequency of 50÷60Hz results in frequent fractures of the spinal column, most often of the 6th, 7th and 8th thoracic vertebras. The frequency of the fractures depended on the phenotype of fatteners and the time of rest prior to slaughter. The highest number of spinal column damages was observed in crossbreds with the Pietrain breed and in hybrid X fatteners. Pigs which were allowed to rest 8 hours before slaughter developed more fractures of their spinal column than those which were slaughtered directly after delivery. The examined range of the current stunning charge of the mains frequency did not influence the proportion of carcasses with damaged vertebras. The stunning of experimental animals with the current of increased frequency eliminated fractures of vertebras and reduced the proportion of bloody meat in shoulders. It was also found that the application of higher current charges to stun animals (i.e. 15 C) at mains frequency reduced the pH of the meat 45 minutes after slaughter.
The remaining investigated live factors i.e. live weight, sex and meatiness class of carcasses did not exert any significant influence on the proportion of carcasses with damaged vertebras.

References

BORZUTA, K.:
Modern classication methods of pig carcasses. Med. Wet. **58** (2002) 1, 26-29
BORZUTA, K.:
Degree of blood draining and some meat quality characteristics of pigs stunned by high frequency current. Roczn. Inst. Przem. Mięs. VIII, 2, (1971), 17-24
BORZUTA, K.:
Techological usability of high frequency current for pigs stunning. Roczn. Inst. Przem. Mięs. VIII, 1, (1971), 27-35
FAUCITANO, L.; VELARDE, A.; GISPERT, M.; OLIVER, M.A.; MANTECA, X.; DIESTRE, A.:
Effects of electrical head – to – chest and carbon dioxide stunning on meat quality in slaughter pigs. Proc. 44th ICoMST, v.II, (1998), 1074-1075
KIEN, S.:
Review of pig stunning methods. Part I. Gosp. Mięsna XLIX, 8, (1997), 40-43
KIEN, S.:
Development of pre-slaughter pig stunning methods - a review. Roczniki Inst. Przem. Mięsn. i Tłuszcz., XXXVI, (1999), 5 – 19
ŁOBODA, P.; POSPIECH, E.:

Pigs stunning and its influence on backbone fractures of carcasses after slaughter. Roczn. Inst. Przem. Mięs. i Tł., XLI, (2004), 109-116

RUSZCZYC, Z.:
Metodology of zootechnic experiments. PWRiL Warszawa 1980

SILVEIRA, E.T.; SILVEIRA, N.F.A.; BERAQUET, N.J.:
The influence of stunning techniques on some quality aspects of pig meat. Proc. 44th ICoMST, v. II, (1998), 1072-1073

TROEGER, K.; NITSCH, P.:
(Technische Weiterentwicklungen bei der Elektrobetäubung von Schlachtschweinen). Development of electrical stunning technics in swine. Fleischwirtschaft **78** (1998) 11, 1134

TROEGER, K.:
Different electrical systems for stunning of pigs. Int. Workshop on Stum. Syst. for Pigs and Anim. Welfare Billung, (1999), Workshop Notes 8.

VELARDE, A.; FAUCITANO, L.; GISPERT, M.; OLIVER, M.A.; DIERTRE, A.:
A survey of the efficacy of electrical and carbon dioxide stunning on insensitivity in slaughter pigs. Proc. 44th ICoMST, v.II, (1998), 1076-1077

VELARDE, A.; GISPERT, M.; FAUCITANO, L.; ALONSO, P.; MANTECA, X.; DIESTRE, A.:
Effects of the stunning procedure and the halothane genotype on meat quality and incidence of haemorrhages in pigs. Meat Sci. **58** (2001) 3, 313-319

WENZLAWOWICZ, M.; SCHÜTTE, A.; HOLLEBEN, K.; ALTROCK, A.; BOSTELMANN, N.; ROEB, S.:
(Feld Untersuchung der MIDAS – Schweinebetäubungsanlage mit Inarco – System hinsichtlich Tierschutz und Schlachtkörperqualität) Study on MIDAS type device with Inarco system to stunning of pigs take into consideration animal welfare and quality of carcasses. Fleischwirtschaft **78** (1998) 10, 1096

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Arch. Tierz., Dummerstorf **50** (2007) Special Issue, 161-171

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Effect of husked and naked oat used in the diets supplemented with linseed oil on the growth performance of pigs, carcass and meat quality

Abstract

The aim of the experiment was to determine the effects of husked and naked oat grain used in the diets supplemented with oils, but not supplemented with vitamin E and selenium, on the meat quality and fattening results of pigs. 24 crossbred barrows were fed individually, from approx. 40 to 104 kg BW. The control diet (C) contained triticale and two experimental diets contained either 45% of husked oat (O) or 45% of naked oat (NO). The diets were formulated as prooxidative, i.e. they were supplemented with 3% of linseed oil and 1% of rapeseed oil during the grower period, as well as with 3% of linseed oil during the finisher period. All diets were isolipidic and isofibrous. Growth performance, carcass quality, as well as the pH, chemical composition, fatty acids, drip loss, color (L*, a*, b*), sensory properties, the content of vitamin E, vitamin A and TBARS in *longissimus dorsi* (LM*)* samples were determined. The experimental treatments had no effect on growth performance, meatiness, and contents of protein and fat in LM samples. Meat from pigs fed the diet with husked oat had a more desirable color (L*) than meat from pigs fed the control diet or the diet with naked oat. After 96 hours of storage at 4°C LM samples of pigs given the diet with naked oat had lower (P>0.05) drip loss (6.49%), as of pigs fed diets with husked oat (7.30%) or triticale (8.21%). Naked oat significantly increased the oxidative stability of meat, measured based on the formation of TBARS.

Key Words: pigs, oat, antioxidant properties, meat quality

Zusammenfassung

Titel der Arbeit: **Einfluss von Spelz- und Nackthafer bei mit Leinöl angereichertem Mischfutter auf Mast- und Schlachtleistungen sowie Schlachtkörperqualität und Fleischbeschaffenheit bei Mastschweinen**

Es wurde der Einfluss von Spelz- bzw. Nackthafer bei mit Lein-, Raps- bzw. Sojabohnenöl angereichertem Diätfutter ohne Vitamin E oder Selenzusatz auf die Mast- und Schlachtleistung sowie Fleischqualität untersucht. Die 25 Kreuzungsschweine wurden von 40 – 104 kg bis zur Schlachtung individuell gefüttert. Die Hauptkomponenten der drei Diätengruppen waren 1. Kontrolldiät mit Weizen und Roggen, 2. mit 45 % Spelzhafer, 3. mit 45 % Nackthafer, welche in den zwei Mastabschnitten bis bzw. nach 72 kg mit Lein-, Raps- bzw. Sojabohnenöl angereichert wurden. Erfasst wurden die Merkmale: Körpergewicht und Masttagszunahme, Futterverwertung, Schlachtkörperqualität, pH, Dripverlust, Fleischfarbe sowie sensorische Merkmale im *M. musculus longissimus dorsi*. Es konnte fast kein Einfluss der drei Diäten auf die genannten Merkmale festgestellt werden. Tiere der Gruppe 2 zeigten eine signifikant bessere Fleischfarbe, die der Gruppe 3 tendenziell geringere Dripverluste. In letztgenannter Gruppe konnte auch eine höhere Oxidationsstabilität des Fleisches nachgewiesen werden.

Schlüsselwörter: Schwein, Hafer, Mastleistung, antioxidative Merkmale, Fleischqualität

Introduction

A growing interest has been observed recently in the modification of the composition of diets for pigs, aimed at increasing the levels of polyunsaturated fatty acids (PUFA) as well as maintaining a more favorable balance between n-6 and n-3 PUFA. However, meat enriched with PUFA is more susceptible to oxidative deterioration. The products of lipid oxidation have a negative effect on the sensory, nutritional and

health properties of meat (JANITZ, 1985; WOOD et al., 2003). The oxidative stability of PUFA-enriched pork fat can be increased with the use of diets containing elevated levels of antioxidants, primarily α-tocopherol (HASTY et al., 2002; LAHUCKY et al., 2005). It seems that a similar role may be played by phytochemicals contained in feed, mainly in cereals. It is known that oat grains contain phytochemicals of various families that display antioxidant properties, such as tocopherols and tocotrienols, flavonoids (e.g. avenanthramides), phenolic acids in free and estrified form, and sterols (WHITE and ARMSTRONG, 1986; COLLINS, 1989; PETERSON, 2001; BRYNGELSSON et al., 2002). Avenanthramides occur in oat in a relatively high concentration and have high antioxidant activity. One of the forms of avenanthramides isolated from oat grain showed 60% of the activity exerted by α-tocopherol (DIMBERG et al., 1993). For the above reasons oat extracts are added to food products, to prevent lipid oxidation. It was found that methanolic extracts from oat added to soybean oil reduced the formation of peroxides during heating or storage (WHITE and ARMSTRONG, 1986; XING and WHITE, 1997, respectively). Oat can be also used as a source of antioxidants in diets for various animal species. A diet with husked oat enabled to reduce the concentrations of cholesterol oxidation products in broiler meat (LOPEZ-BOTE et al., 1998a) as well as the formation of thiobarbituric-reactive substances (TBARS) in rabbit meat (LOPEZ-BOTE et al., 1998b). Naked oat added to diets for cows significantly extended the oxidation induction period in milk fat (FAERON et al., 1998). Also CAVE and BURROWS (1993) observed reduced lipid oxidation in thigh meat of broilers fed naked oat diets. Literature on the subject provides no information on the results of such experiments on pigs.

Therefore, the aim of the present study was to determine the effects of husked and naked oat grain administred in the diets supplemented with linseed oil, but not supplemented vitamin E and selenium, on the meat quality and growth performance of pigs.

Material and Methods

Materials

Cereal grain (barley, triticale, husked oat and naked oat) was purchased from a local farmer. Husked oat grain was ground in a hammer mill, the grain of the other cereals was ground in roller mill with grooved rolls. Raw (unrefined) oils were purchased from a local oil plant. The levels of nutrients and antioxidants (α-tocopherol, total phenolic compounds (TPC) and the total antioxidant capacity (TAC) were measured in diets (Table 1).

Animals, diets and meat sample preparation

The experiment was performed on 24 crossbred barrows [(Large White×Polish Landrace)×Duroc] divided into three groups and fed individually from approx. 40 to 104±3.44 kg BW. Three grower (40-72 kg) and finisher (72-104 kg) diets were tested: the control diet (C) contained triticale and barley, and two experimental diets contained either 45% of husked oat (O) or 45% of naked oat (NO) as a substitution for triticale. The diets were formulated as prooxidative, i.e. they were supplemented with 3% of linseed oil and 1% of rapeseed oil during the grower period, as well as with 3% of linseed oil during the finisher period. The diets were not supplemented with vitamin

Table 1
Content of nutrients (% DM) and antioxidant properties of feeds

Feed	Nutrient				Antioxidant		
	dry matter	crude protein	ether extract	crude fibre	α-T[1] mg/kg	TPC[2] g/kg	TAC[3] mmol/kg
Barley	86.27	11.44	1.65	5.48	5.69	11.77	7.56
Triticale	86.54	11.30	1.69	3.10	4.42	6.70	3,56
Husked oat	87.33	11.60	5.70	11.60	2.09	5.81	3.54
Naked oat	89.64	13.34	8.51	4.89	3.46	5.26	3.93
Soybean meal	89.54	52.51	2.10	3.84	8.39	20.61	5.97
Wheat straw	92.06	5.26	0.76	45.27			
Linseed oil					154.3		
Rapeseed oil					156.0		
Soybean oil					192.0		

[1]α-T - α-tocopherol, [2]TPC – total phenolic compounds, [3]TAC – total antioxidant capacity in Trolox equivalents

Table 2
Ingredients, nutrients (% as-fed), antioxidants and fatty acid composition (% of total fatty acids) of diets

Item	Grower (40 -72 kg)			Finisher (72-104 kg)		
	C	O	NO	C	O	NO
Ingredients, %						
Barley	18.76	18.67	19.67	20.08	19.59	19.98
Triticale	39.0	6.0	-	48.0	13.0	9.0
Soybean meal	25.0	22.2	21.5	18.0	16.0	14.8
Husked oat (O)	-	45.0	-	-	45.0	-
Naked oat (NO)	-	-	45.0	-	-	45.0
Ground wheat straw	7.5	-	6.8	6.3	-	5.9
Linseed oil	3.0	3.0	3.0	3.0	3.0	3.0
Rapeseed oil	2.5	1.6	1.0	1.0	0.6	-
Soyabean oil	1.2	0.5	-	1.0	0.5	-
Limestone	1.0	1.0	1.0	0.8	0.8	0.8
Monoalcium phosphate	0.9	0.9	0.9	0.4	0.4	0.4
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Premix [1]	0.5	0.5	0.5	0.5	0.5	0.5
Lysine HCl (78%)	0.08	0.09	0.09	008	0.08	0.09
Methionine (98%)	0.06	0.04	0.04	0.04	0.03	0.03
Acidifier [2]	0.2	0.2	0.2	0.2	0.2	0.2
Analysed composition, %						
Crude protein	18.0	17.6	18.0	16.0	16.0	15.9
Ether extract	8.0	8.1	8.1	6.3	6.9	7.0
Crude fibre	6.1	6.4	6.6	5.8	6.3	6.2
NDF	19.8	19.7	18.6	18.4	20.3	19.0
SFA, % of total	13.5	14.3	15.4	13.4	14.6	14.5
MUFA, % of total	34.1	32.3	34.9	27.3	28.2	29.9
PUFA, % of total	52.4	53.4	49.7	59.3	57.3	55.6
Calculated composition, g/kg						
Lysine	9.84	9.79	9.79	8.15	8.19	8.16
Methionine	3.10	2.98	2.98	2.60	2.58	2.58
Ca	7.37	7.43	7.45	5.64	5.75	5.73
P total	5.46	5.76	5.49	4.38	4.62	4.37
ME, MJ/kg	13.74	13.42	13.54	13.54	13.24	13.47
α-tocopherol, mg/kg	15.7	12.2	10.7	12.9	10.	9.0
TPC [3], g/kg	1.77	1.99	1.87	1.66	1.87	1.72
TAC [4], mmol Trolox/kg	9.97	9.79	9.11	9.32	9.09	8.37

[1]The vitamin and mineral premix on limestone carrier, without vitamin E and Se, supplied per kg of diet: 9400 IU vitamine A; 1875 IU vitamine D_3; 1.26 mg vitamine K_3; 0.67 mg folic acid; 9.4 mg panthothenic acid; 18.8 mg niacinamid; 0.95 mg thiamine; 4.4 mg riboflavine; 1.0 mg pirydoxine; 18.8 μg vitamine B_{12}; 19.0 μg biotine; 170 mg cholinchloride; 45 mg Mn; 84.4 mg Zn; 84.4 mg Fe; 25 mg Cu; 0.57 mg Co; 0.84 mg I; 15 mg flavomycine; [2]Acidifier containing the mixture of organic acids (formic, phosphoric, acetic, lactic, citric, propionic) and their salts; [3]Total phenolic compounds; [4]Total antioxidant capacity

E and selenium, and were isolipidic and isofibrous. In addition, all diets contained barley, soybean meal, minerals, vitamins and amino acids. Rapeseed oil and soybean oil were added to achieve the crude fat level of diet NO, and ground wheat straw was added to achieve the fiber level of diet O (Table 2). The diets were offered wet (1:1 with water), in a restricted amount (from 1.8 to 3.0 kg/pig/day), and water was offered *ad libitum*. Daily gains and feed conversion ratios were calculated based on the live weight gains of pigs and feed intake. As the slaughter weight was achieved, the pigs were transported (25 km) to a slaughterhouse in two groups, each of 12 animals, at a week interval. The thickness of back fat and *longissimus dorsi* (LM) muscle were measured postmortem with an optical-needle choirometer (CGM 01-A, France), and carcass meatiness was calculated in accordance with the relevant standards applied at the slaughterhouse. Carcass yield was calculated based on live weight and hot carcass weight. The LM pH was determined 45 minutes and 24 hours after slaughter with a pH-meter (WPW-340 I, POL-EKO). On the day following slaughter, LM samples (weighing about 600 g) were taken from the area above the third and fourth lumbar vertebra. The samples were put into impermeable plastic bags and transported in a cooler to the laboratory. Four chops were sliced from each sample. The first chop (approx. 250 g) was used to determine the color, and then to evaluate the sensory properties of meat. Two chops, 2.5 cm in thickness, were used to determine drip loss. The fourth chop was trimmed of visible fat and connective tissue, and ground in a food grinder (RONIC, France). The levels of dry matter, nitrogen, fat, vitamin E, vitamin A and thiobarbituric acid-reactive substances (TBARS) were determined in ground fresh meat.

Chemical analysis

The content of dry matter, crude protein, ether extract and crude fiber in feed and diets were measured according to AOAC (1990). In the feeds tocopherol (α-T) was determined in accordance with the Polish Standard PN-EN ISO 6867:2002, and in meat samples - by the method described by RETTENMAIER and SCHÜEP (1992). TPC in feed was measured as described by SHAHIDI and NACZK (1995). TAC was determined by the method proposed by RE et al. (1999) and expressed as Trolox equivalent capacity. The fatty acid contents of diets and LM fat were determined by gas chromatography, following fat extraction from samples with petroleum ether and by methylation. A gas chromatograph (AT 6890 N) was equipped with a flame ionization detector and a capillary column (30 m × 0.32 mm). Helium (1.0 ml/min) was used as carrier gas. Injector, detector and column temperatures were 250, 225 and 180°C, respectively. The injector was used at a split : splitless ratio of 50:1. Fatty acid methyl esters were identified by a comparison with the standards run previously. The dry matter content of meat samples was expressed as a weight percentage after heating of ground tissue at 105ºC for 24 h. The fat content was measured as the total fat extracted from dried samples using the Soxtec 2043 extraction system (FOSS, Sweden). Protein content (N×6.25) was measured in fresh samples by the Kjeldahl method (2200 Kjeltec, FOOS). Meat lipid oxidation was determined by measuring the formation of TBARS, as described by SØRENSEN and JORGENSEN (1996).

Meat quality measurements

The surface color of LM was measured with a spectrophotometer (MiniScan XE Plus, Hunter Lab). Color measurements were taken in triplicate (in three different areas of the muscle) for each pork chop. Drip loss was determined on two chops using the suspension method as described by HONIKEL (1998). Measurements were made after 48 hour- and 96 hour-storage of the samples in a refrigerator at 4°C. The results were expressed as a percentage of initial weights. The sensory properties (aroma, tenderness, juiciness, taste) of meat were determined in accordance with the Polish Standards PN ISO 4121:1998. Meat samples subjected to evaluation were cooked in a 0.6% NaCl solution (meat : water ratio of 1:2) at 96°C, to reach a temperature of 75°C inside the sample. The evaluation was performed by five trained panelists, on a scale of 1 to 5 points.

Statistical analysis

The results were verified statistically by one-way ANOVA, and significant differences between the groups were determined by the Duncan′s multiple range test. The Tables contain the least-squares means and the standard errors of means (SEM). The differences were considered significant at $P \leq 0.05$.

Results

The feed used for formulation the experimental diets had a standard nutrient content (Table 1). As compared with husked oat, naked oat contained more total protein and crude fat, by 15% and 49% respectively, and 2.4-fold less crude fiber. However, the crude fiber content of naked oat was relatively high. Among cereal grain, the highest α-T concentration was recorded in barley, followed by triticale. Husked oat contained less α-T than triticale (over twofold) and naked oat. Oils, especially soybean oil, contained large amounts of α-T. Tocopherols belong to antioxidant compounds that protect plants against going rancid. Their concentration in cereal grain depends on genotype and environment, and may vary widely (PETERSON and QUERESHI, *1993*). The α-T content of feed recorded in our study was consistent with that reported by other authors (HIDIROGLOU et al., 1992). The level of TPC in husked and naked oat grain was comparable and slightly lower than in triticale and as much as twofold lower than in barley. The total antioxidant capacity was the highest in barley, and similar in triticale, husked oat and naked oat. ZIELIŃSKI and KOZŁOWSKA (2000) also observed higher antioxidant activity in barley than in oat.

The composition of diets as well as the levels of nutrients and antioxidants and the fatty acid profile are presented in Table 2. In order to evaluate the antioxidant properties of oat, the diets had a comparable nutritive value, including the concentrations of saturated, mono- and polyunsaturated fatty acids. The control diet had the highest calculated α-T content (15.7 and 12.9 mg/kg in the grower and finisher diet respectively). The α-T concentration in the diets with husked and naked oat grain was by 22% and 30% lower, respectively. It was caused primarily by a lower level of oils in diets with oat grain, especially that containing naked oat. The α-T content of grower diets, ranging from 10.7 to 15.7 mg/kg, was at a level recommended by NRC (1998*)* (11 mg/kg of feed for slaughter pigs). In finisher diets O and NO the α-T concentration was slightly below these standards. However, due to a high fat content

and the concentrations of polyunsaturated fatty acids exceeding 50%, the level of vitamin E in all diets could be insufficient to ensure the oxidative stability of meat. The concentration of TPC and TAC, calculated based on their levels in feedstuffs, was comparable in all diets.

Table 3
Daily gain and feed efficiency of pigs fed control (C), husked oat (O) or naked oat (NO) diets

Item	Diet			SEM	P-value
	C	O	NO		
Initial weight, kg	40.63	40.44	39.88	0.343	0.669
Final weight, kg	104.56	102.31	105.19	0.704	0.199
Average daily gain, g					
Grower	893	856	878	11.74	0.457
Finisher	924	847	919	16.28	0.089
Total	908	852	898	12.62	0.161
Feed efficiency, kg/kg					
Grower	2.40	2.51	2.43	0.034	0.441
Finisher	3.08	3.33	3.08	0.062	0.165
Total	2.73	2.90	2.75	0.046	0.249

The replacement of triticale with 45% of husked or naked oat grain in isolipidic and isofibrous diets had no significant effect on the growth performance of pigs (Table 3). A tendency towards lower daily gains and worse feed conversion in pigs fed the diet with husked oat (by 8% in comparison with the control group; P=0.089) was observed only over the finisher period. The nutritive value of all experimental diets was similar, regardless on the cereal component added, which may explain the small differences in the results of fattening. Non-significantly lower daily gains and feed conversion ratios in group O could result from a high lignin content in husked oat (BACH KNUDSEN, 1997), reducing nutrients digestibility. Good fattening results achieved in the group fed naked oat grain are consistent with the findings of other author (HARROLD et al., 1998).

Table 4
Carcass traits and composition of LM of pigs fed control (C), husked oat (O) or naked oat (NO) diets

Item	Diet			SEM	P-value
	C	O	NO		
Carcass traits					
Dressing percentage	78.1	78.7	78.9	0.228	0.307
backfat thickness, mm	13.4	14.3	12.4	0.537	0.378
LM thickness, mm	57.4	53.1	55.6	1.083	0.284
Carcass meat, %	57.3	55.6	57.4	0.488	0.231
Composition of LM					
Dry matter, %	26.49	26.11	26.65	0.212	0.585
N × 6.25, %	22.08	22.57	22.37	0.123	0.269
Ether extract, %	3.46	2.31	3.30	0.252	0.129
Fatty acid in LM, % of total					
SFA	39.8	39.7	40.6	0.504	0.746
MUFA	47.0	45.3	46.1	0.324	0.084
PUFA	13.1	14.9	13.2	0.577	0.384

There were no significant differences between the groups in carcass dressing percentage, back fat thickness and LM thickness (Table 4). Carcass meatiness was non-significantly lower (55.6%) in the group given husked oat diet, as compared with pigs fed the control diet or naked oat diet (57.3 and 57.4% respectively). LM samples

taken from the carcasses of pigs given the diet with husked oat contained statistically non-significantly less intramuscular fat than LM samples taken from the carcasses of animals fed the control diet and the naked oat diet (2.31% vs. 3.46 and 3.30, respectively; P>0.05). The intramuscular fat content affects the eating quality and technological suitability of meat. Pork should be delicate, with uniformly distributed thin fat fibers, responsible for the desirable juiciness, tenderness and palatability following thermal treatment. It was found that a fat content of 2.5 - 3.0% (FERNANDEZ et al., 1999) guarantees the optimum taste of meat. In our experiment LM meat fulfilled this condition.

The fatty acids profiles of LM fat were similar in all groups. The intramuscular fat contained high concentration of PUFA (from 13.1 to 14.9% of total fatty acids). The results demonstrated that fatty acids profiles of pork fat reflected those of the dietary fat. Similarly responses have been observed with pig fed diet supplemented with linseed (MATTHEWS et al., 2000).

Table 5
Fresh meat quality of LM of pigs fed control (C), husked oat (O) or naked oat (NO) diets

Item	Diet			SEM	P-value
	C	O	NO		
pH 45 min	6.52	6.62	6.53	0.049	0.691
pH 24 h	5.50	5.60	5.63	0.031	0.223
Drip loss 48 h, %	5,56	4.84	4.12	0.294	0.136
Drip loss 96 h, %	8.21	7.30	6.49	0.346	0.128
Colour					
L*	58.4[a]	55.9[b]	58.6[a]	0.412	0.005
a*	5.4[ab]	5.6[a]	4.5[b]	0.194	0.041
b*	13.9[a]	12.9[b]	13.0[b]	0.156	0.009
Sensory analysis[1]					
Aroma- intensity	4.8	5.0	4.8	0.054	0.276
Aroma- desirability	5.0	4.9	4.9	0.029	0.613
Tenderness	4.8	4.5	4.8	0.108	0.476
Juiciness	4.7	4.3	4.2	0.104	0.123
Taste- intensity	4.9	4.9	4.6	0.059	0,065
Taste- desirability	5.0	4.9	4.9	0.029	0.613
Vitamin E, μg/g	0.46	0.31	0.35	0.028	0.070
Vitamin A, μg/g	1.61	1.60	2.24	0.133	0.077
TBARS, mg/kg	0.613[a]	0.596[ab]	0.466[b]	0.024	0.050

[1]Scale from 1 to 5 points; [a,b] $P \leq 0.05$

The experimental diets had no influence on the values of pH_{45} and pH_{24h} in LM samples (Table 5). 24 hours postmortem pork in all groups was characterized by satisfactory pH levels (5.50 - 5.63). A tendency was observed towards lower drip loss in meat from pigs fed diets with oat, especially with naked oat. 48 and 96 hours postmortem drip loss in group NO was by 26% and 21% lower, respectively (P>0.05). Expressed per kg of meat, drip loss was by respectively 14.4 g/kg and by 17.2 g/kg lower after 48 and 96 hours of pork storage in a refrigerator. Drip loss is affected by a variety of factors, including integrity of cell membranes in muscles. Antioxidants (e.g. vitamin E) added to feed protect PUFA in the phospholipids of cell membranes against peroxidation (BUCKLEY et al., 1995). This may reduce drip loss from meat (BUCKLEY et al., 1995; LAHUCKY et al., 2000), but such an impact of vitamin E was not confirmed in other studies (HASTY et al., 2002). A similar antioxidative

function may be also performed by phenolic compounds contained in cereals, including oat. In the study of FORTIN et al. (2003) drip loss from LM samples of pigs fed wheat-barley diets was somewhat greater as compared with pigs given oat-based diets (6.71 vs. 6.1 - 6.2; $P>0.05$), despite the fact that all diets were supplemented with 100 mg/kg of vitamin E.

Meat from pigs fed a diet with husked oat grain was characterized by a significantly better color – it was darker than meat from pigs given the control diet or a diet with naked oat grain (55.8 vs. 58.4 and 58.6; $P<0.01$). The value of the attribute L* was high. Meat color is influenced by the levels of myoglobin and intramuscular fat. Meat with a higher fat content reflects more light and is characterized by a higher value of L* (WILFART et al., 2004), in comparison with meat with a low fat content (MURRAY et al., 2001). The meat of our pigs contained 2.3-4.4% of intramuscular fat. Diets containing husked oat or naked oat had a positive effect ($P<0.01$) on the color parameter b* - the meat from O and NO group was less yellow. The relatively high level of yellowness (b*) recorded in the experiment could be a consequence of high concentrations of PUFA in LM fat.

An evaluation of aroma, tenderness, juiciness and taste of meat showed that these sensory properties were not affected by husked oat or naked oat used in the diets (Table 5). Meat obtained from all barrows had a good quality. All quality attributes analyzed in the study received high scores (4.2 - 5 points). The effect of oat grain on the sensory quality of meat was not unequivocal, and was found to be significant only when the proportion of this cereal species in feed mixtures was high (FRIEND et al., 1988; POSTE et al., 1996).

The vitamin E content of LM meat from pigs of all groups was low, and varied from 0.31 to 0.46 μg/g. Meat from pigs fed the diet with naked oat contained larger amounts of vitamin A than meat from pigs of the other groups (2.24 vs. 1.60 μg/g; ($P=0.077$). The low concentration of vitamin E in meat was caused by its low content in the diets and by its high utilization by pigs, resulting from high quantity of PUFA consumption.

The oxidative stability of meat, analyzed based on the formation of TBARS, was the highest in the group that received naked oat grain (NO). The differences between this group and the control group fed the diet with triticale were statistically significant (0.466 vs. 0.613 mg/kg; $P<0.05$). The levels of TBARS, determined in meat samples collected 24 hours postmortem, were high. This could be a consequence of a low vitamin E content of meat as well as of the fact that pigs were fed diets enriched with large amounts of PUFA. A high and increasing concentration of TBARS in LM meat from pigs was observed by LOPEZ-BOTE et al. (2003) in consequence of an increase in the proportion of PUFA in meat. Similarly, to the results of our study, CAVE and BURROWS (1993) had also found less TBARS in the meat of broilers fed the diet with naked oat.

The highest oxidative stability of meat from barrows fed the diet with naked oat, containing the lowest amount of vitamin E and comparable to the other diets in terms of TPC and TAC levels, is difficult to explain. The results of the experiment suggest that the phenolic compounds (TPC) contained in naked oat grain could have great antioxidative potential, which enabled to save and accumulate larger amounts of vitamin A in meat. The results obtained by PÉREZ-JIMÉNEZ and SAURA-CALIXTO (2005) indicate that amount antioxidants released by cereal matrix into the

intestine may be higher than the one that can be expected from measurments in the usual aqueous-organic extracts.

The present results indicate that naked oat has higher influence on the meat quality than the husked oat. When used in the diet with high amount of fat (8% and 7% in grower and finisher diet, respectively) and deficit of vitamin E, it significantly increased oxidative stability of meat, based on formation of TBARS, as well as decreased (P>0.05) drip loss from meat of LM.

Conclusions

The results of the study show that diets containing 45% of oat grain had a positive effect on pork quality. The influence of naked oat was in this respect more beneficial than that of husked oat. The use of husked oat grain in the diets enabled to improve meat color (L*), whereas dietary inclusion of naked oat significantly increased the oxidative stability of pork, measured based on the formation of TBARS, as well as decreased (statistically non-significantly) the level of drip loss. Both oat species reduced the yellowness (b*) of meat

References

AOAC: Official Methods of Analysis. 15th ed. Assoc. Offic. Anal. Chem. (1990) Arlington. VA

BACH KNUDSEN, K.E.:
Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. **67** (1997), 319-338

BRYNGELSSON, S.; MANNERSTEDT-FOGELFORS,B.; KAMAL-ELDIN, A.; ANDERSSON, R.; DIMBERG, L.H.:
Lipids and antioxidants in groats and huls of Swedish oats (*Avena sativa* L.). J. Sci. Food Agric. **82** (2002), 606-614

CAVE, N.A.; BURROWS, V.D.:
Evaliation of naked oat (*Avena nuda*) in the broiler chicken diet. Can. J. Anim. Sci. **73** (1993), 393-399

BUCKLEY, D.J.; MORRISSEY, P.A.; GRAY, J.I.:
Influence of dietary vitamin E on the oxidative stability and quality of pig meat. J. Anim. Sci. **73** (1995), 3122-3130

COLLINS, F.W.;
Oat phenolics: avenanthramides, novel substitued N-cinnamoylanthranilate alkaloids from oat groats and hulls. J. Agric. Food Chem. **37** (1989), 60-66

DIMBERG, L.H.; THEANDER, O.; LINGNERT, H.:
Avenanthramides-a group of phenolic antioxidants inoats. Cereal Cem. **70** (1993) 6, 637-641

FEARON, A.M.; MAYNE, C.S.; CHARLTON, C.T.:
Effect of naked oats in the dairy cow's diet on the oxidative stability of the milk fat. J. Sci. Food Agric. **76** (1998), 546-552

FERNANDEZ, X.; MONIN, G.; TALMANT, A.; MOUROT, J.; LEBRET, B.:
Influence of intramuscular fat content on the quality of pig meat – 1. Composition of the lipid fraction and sensory characteristics of m. longissimus lumborum. Meat Sci. **53** (1999), 59-64

FORTIN, A.; ROBERTSON, W.M.; KIBITE, S.; LANDRY, S.J.;
Growth performance, carcass and pork quality of finisher pigs fed oat-based diets containing different levels of β-glucans. J. Anim. Sci. **81** (2003), 449-456

FRIEND, D.W.; FORTIN, A.; POSTE, L.M.; BUTLER, G.; KRAMER, J.K.G.; BURROWS, V.D.:
Feeding and metabolism trials, and asessement of carcass and meat quality for growing-finishing pigs fed naked oats (Avena nuda). Can. J. Anim. Sci. **68** (1988), 511-521

HARROLD, R.L.; MILLER, K.B.; ZIMPRICH, R.C.; CARTER, S.D.:
Barley-naked oat combination fo growing-finishing swine. J. Anim. Sci. **76**, suppl. 2 (1998), 56 (abstract)

HASTY, J.L.; van HEUGTEN, E.; SEE, M.T.; LARICK, D.K.:
Effect of vitamin E on improving fresh pork quality in Berkshire- and Hampshire-sired pigs. J. Anim. Sci. **80** (2002), 3230-3237

HIDIROGLOU, N.; CAVE, N. ATWAL, A.S.; FARNWORTH, E.R.; McDOWELL, L.R.:

Comparative vitamin E requirements and metabolism in livestock. Ann. Rech. Vet. **23** (1992), 337-359

HONIKEL, K.O.:
Reference methods for assessment of physical characteristics of meat. Meat Sci. **49** (1998) 4, 447-457

JANITZ, W.:
Interactions of meat fats and proteins with particular consideration of the effect of oxidized fats on the nutrition value of proteins. Rocz. A R Poznań. Rozprawy naukowe. (1985), No 147

LAHUCKY, R.; KRSKA, P.; KÜCHENMEISTER,U.; NÜRNBERG, K.; LIPTAJ, T.; NÜRNBERG, G.; BAHELKA, I.; DEMO, P.; KUHN, G.; ENDER, K.:
Effect of vitamin E on changes in phosphorus compounds assessed by 31 P NMR spectroscopy and ATPase from postmortem muscle samples and and meat quality of pigs. Arch. Tierz., Dummerstorf **43** (2000), 487-497

LAHUCKY, R; KUECHENMEISTER, U.; BEHELKA. I.; NOVOTNA, K.; VASICKOVA, K.; ENDER, K.:
Effects of vitamin E by dietary supplementation and of calcium ascorbate by post mortem injection in muscle on the antioxidative status and on meat quality of pigs. Arch. Tierz., Dummerstorf **48** (2005) 6, 592-600

LOPEZ-BOTE, C.J.; GRAY, E.A.; GOMAA, E.A.; FLIEGAL, C.J.:
Effect of dietary oat administration on lipid stability in broiler meat. Brit. Poultry Sci. **39** (1998a), 57-61

LOPEZ-BOTE, C.J.; SANZ, M.; REY, A.; CASTANO, A.; THOS, J.:
Lower lipid oxidation in the muscle of rabbits fed diets containing oats. Anim. Feed Sci. Technol. **70** (1998b), 1-9

LOPEZ-BOTE, C.J.; ISABEL, B.; DAZA, A.:
Effect of vitamin E supplementation and partial substitution of poly- witth mono-unsaturated fatty acids in pig diets on muscle, and microsome extract α-tocopherol concentration and lipid oxidation. Arch. Anim. Nutr. **57** (2003), 11-25

MATTHEWS, K.R.; HOMER, D.B.; THIES, F.; CALDER, P.C.:
Effect of whole linseed (*Linum usitatissimum*) in the diet of finishing pigs on growth performance and on the quality and fatty acid composition of various tissues. Brit. J. Nutr**. 83** (2000), 637-643

MURRAY, A.; ROBERTSON, W.; NATTRESS, F.; FORTIN,A.:
Effect of pre-slaughter overnight feed withdrawal on pig carcass an muscle quality. Can. J. Anim. Sci. **81** (2001), 89-97

NRC. Nutrient Requirements of Swine. 1998. 10th ed. Natl. Acad. Press, Washington, DC

PÉREZ-JIMÉNEZ, J.; SAURA-CALIXTO, F.;
Literature data may underestimate the actual antioxidant capacity of cereals. J. Agric. Food Chem. **53** (2005), 5036-5040

PETERSON, D.M.:
Oat antioxidants. J. Cereal. Sci. **33** (2001), 115-129

PETERSON,D.M.; QUERESHI, A.A.:
Genotype and Environment effects on tocols of barley and oats. Cereal Chem. **70** (1993), 2, 157-162

PN-ISO 4121:1998. Sensory analysis- Methodology – Evaluation of food products by methods using scales

PN-EN ISO 6867:2002. Animal feedingstuffs. Determination of vitamin E content

POSTE, L.M.; BUTLER, G.; CAVE, N.A.; BURROWS, V.D.:
Sensory analysis of meat from broiler chickens fed diets containing hulless oats (*Avena nuda*). Can. J. Anim. Sci. **76** (1996), 313-319

RE, R.; PELLEGRINI, N.; PROTEGGENTE, A.; PANNALA, A.; YANG, M.; RICE-EVANS, C.:
Antioxidant activity applaying an improved ABTS Radical cation decolorization assay. Free Radic. Biol. Med. **26** (1999), 1291-1237

RETTENMAIER, R.; SCHÜEP, W.:
Determination of vitamins A and E in liver tissue. Internat. Vit. Nutr. Res. **62** (1992), 312-317

SHAHIDI, F.; NACZK, M.:
Methods of analysis and quantification of phenolic compounds. In: Food Phenolic: Sources, Chemistry, Effects and Application; Shahidi,F.; Naczk, M.; Eds.; Lancaster/ Pensylvania: Technomic Publishing Company. (1995), 287-293

SØRENSEN, G.; JORGENSEN, S.S.:
A critical examination of the some experimental variables in the 2-thiobarbituric acid (TBA) test for lipid oxidation in meat products. Z. Lebensm. Unters. Forsch. **202** (1996),205-210

WHITE, P.J.; ARMSTRONG, L.S.:
Effect of selected oat sterols on the deterioration of heated soybean oil. J. Am. Oil Chem. Soc. **63** (1986) 4, 525-529

WILFART, A.; FERREIRA, J-M.; MOUNIER, A.; ROBIN, G.; MOUROT, J.:
Effet de différentes teneurs en acides gras n-3 sur les performances de croissance et la qualité nutritionnelle de la viande de porc. Journées Rech. Porc. **36** (2004), 195-202

WOOD, J.D.; RICHARDSON, R.I.; NUTE, G.R.; FISHER, A.V.; CAMPO, M.M.; KASAPIDOU, E.; SHEARD, P.R.; ENSER, M.:
Effects of fatty acids on meat quality:a review. Meat Sci. **66** (2003), 21-32
XING,Y.; WHITE, P.J.:
Identification and function of antioxidants from oat groats and hulls. J. Am. Oil Chem. Soc. **74** (1997) 3, 303-307
ZIELIŃSKI, H.; KOZŁOWSKA, H.;
Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. J. Agric. Food Chem. **48** (2000), 2008-2016

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Arch. Tierz., Dummerstorf **50** (2007) Special Issue, 172-180

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Effect of addition of feed antibiotic flavomycin or prebiotic BIO-MOS on production results of fatteners, blood biochemical parameters, morphometric indices of intestine and composition of microflora*

Abstract

The aim of the studies was to determine the effect of addition of feed antibiotic flavomycin (100 mg in 1 kg of 5% premix - group C) or prebiotic BIO-MOS (cell of *Saccharomyces cerevisiae* yeasts, *strain 1026,* 0.1% in the first growing period - group E) on production results, blood biochemical parameters, morphometric intestine properties and composition of intestinal microflora in fatteners. During slaughter of 32 crossbred pigs, blood samples were collected and the following biochemical indices were determined. Segments of small intestine were collected and morphometric analysis was carried out. *Post-mortem* quantitative and qualitative bacteriological tests and quantitative mycological tests on the determination of the contents of small and large intestine were carried out. The comparable results of fattening and slaughter evaluation of pigs in the groups were obtained. Significantly lower HDL ($P \leq 0.01$) and higher ALT ($P \leq 0.05$) content in blood serum of E fatteners was found as compared to the animals from C group. In group C, as compared to group E, significantly higher ($P \leq 0.01$) height of epithelial cells of crypts in duodenum and significantly lower one ($P \leq 0.01$) in jejunum was found; the results for ileum were comparable. Application of *Saccharomyces cerevisiae* yeasts feeding caused a favourable increase of the number of lactic acid bacteria in the contents of intestines and a decrease of the content of bacteria from *Enterobacteriaceae* family, including *Proteus vulgaris* and hypha fungi. The employment of BIO-MOS in feeding occurred to be favourable from the production and animal health point of view and seems to be a suitable alternative to feed antibiotics.

Key Words: pigs, feed additives, fattening, slaughter value, biochemical parameters of blood, morphometric indices and intestinal microflora

Zusammenfassung

Titel der Arbeit: **Einfluss des Antibiotikums Flavomycin sowie des Präbiotikpräparates BIO-MOS auf die Mast- und Schlachtleistungen, Blutparameter, morphometrische Darmeigenschaften und die Mikrodarmflora bei Mastschweinen**

Ziel der Arbeit war die Prüfung des Einflusses der Futtermittelzusatzstoffe Flavomycin (100 mg in 1 kg des 5%igen Prämixes – Kontrolle K) und des Präbiotikpräparates BIO-MOS (Hefezellen *Saccharomyces cervisiae,*Stamm 1026, 0,1% in der ersten Mastperiode – Versuchsgruppe D) auf die Mast- und Schlachtleistungen, biochemische Blutparameter, morphometrische Darmeigenschaften und die Mikrodarmflora bei Mastschweinen. Bei der Schlachtung der 32 Kreuzungsmastschweine wurden die Blutproben entnommen und die Blutparameter bestimmt. Für die morphometrischen Untersuchungen wurden Dünndarmausschnitte entnommen. *Post mortem* wurden bakteriologische Quantitäts- und Qualitätsuntersuchungen sowie mykologische Quantitätsuntersuchungen des Dünn- und Dickdarminhaltes durchgeführt. Bei den verglichenen Mast- und Schlachtmerkmalen ergaben sich zwischen Kontroll- und Versuchsgruppen keine signifikanten Unterschiede. Im Blutserum der Gruppe D fand sich, im Vergleich zur Gruppe K, wesentlich weniger HDL und mehr ALT. In Gruppe K fanden sich im Vergleich zur Gruppe D ein höherer Anteil von Kryptenepithelzellen im Zwölffingerdarm und ein kleinerer im Leerdarm. Die Ergebnisse im Hüftdarm waren ähnlich. Bei der Hefe-Versuchsgruppe vergrößerte sich der Anteil an Milchsäurebakterien und es verminderten sich die Bakterien der Familie *Enterobacteriaceae* einschließlich *Proteus vulgaris* und *Fusarium culmorum.* Der Einsatz von BIO-

* Supported by the State Committee for Scientific Research in Poland (Project no P06Z 026 25) and DS

MOS ergab eine positive Wirkung auf die Gesundheit und scheint eine richtige Alternative für Futterantibiotika zu sein.

Schlüsselwörter: Schwein, Futtermittelzusatzstoffe, Masteigenschaften, Schlachteigenschaften, biochemische Blutparameter, morphometrische Darmeigenschaften, Darmmikroflora

Introduction

The quantity and proportions of micro-organisms living in alimentary tract are relatively constant and typical of the particular periods of life of individual. They are subject to changes, depending on the consumed feeds, *inter alias*, on feed additives, in the state of health as well as during disease and stress situation (STAVRIC and KORNEGAY, 1995).

The subject of the studies is to determine the suitability and effectiveness of additives, replacing antibiotic growth promotors [AGP] (FLACHOWSKY and SCHULZ, 1997; HERICH et al., 1998; MIGUEL et al., 2004; MAXWELL et al., 2004). Natural additives stimulate development of favourable intestinal microflora (ZIMMERMANN et al., 2001; PICKARD et al., 2004, PETIGREW and MIGUEL, 2006). In the light of the so-far conducted experiments, the effectiveness of employing the prebiotics instead of AGP is, however, not univocal (HOUDIJK et al., 1999).

As affected by nutritional factors, the changes within the intestinal crypts take place and it increases or decreases proliferation activity of enterocytes. It causes the changes in secretory activity of bactericidal factors (VAN NEVEL et al., 2005).

The aim of the studies was to determine the effect of feed antibiotic additive or replacer - prebiotic on production results, blood biochemical parameters, morphometric indices and composition of intestinal microflora of fatteners.

Material and Methods

The observations covered 32 three-breed crossbred pigs (Polish Large White x Polish Landrace) x Duroc and (PLW x PL) x Belgian Landrace (1:1), gilts and barrows (1:1). Animals were allocated to control group (C) and experimental group (E) by the method of analogues.

The fatteners were managed and fed individually. In two-stage fattening, from the body weight of ca 21 kg up to 56 kg and from 56 kg up to 100 kg, the full-ration mixtures were administrated (Table 1) (ANONYMOUS, 1993). Mixtures for fatteners of C group contained 5% addition of premix with antibiotic – flavomycin (100 mg per 1 kg) and the experimental animals received premix without antibiotic. Group E received 0.1% addition of prebiotic mannan oligosaccharide BIO-MOS (during the first growing period) (obtained from cell walls of *Saccharomyces cerevisiae* yeasts, strain 1026).

Chemical analysis of raw materials and mixtures was conducted by AOAC (1990) method with an application of Tecator apparatus.

During slaughter, blood samples were collected. Serum biochemical indicators were determined: total protein (TP), albumins (ALB), glucose (GLU), urea nitrogen (BUN), alkaline phosphatase (ALP), triglycerides (TG), cholesterol (CH), lipoprotein fractions HDL, LDL, VLDL, CH/HDL ratio, aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

After completion of fattening and slaughter, the abbreviated carcass dissection was carried out (RÓŻYCKI, 1996).

Table 1
Composition (%) and nutritive value of mixtures

Item	Group			
	control		experimental	
	period			
	I	II	I	II
Ground barley	53.15	58.74	53.05	58.74
Ground wheat	25	25	25	25
Soybean meal	11.5	6	11.5	6
Meat bone meal	5	5	5	5
Premix with antibiotic[1]	5	5	-	-
Premix without antibiotic[2]	-	-	5	5
BIO-MOS*	-	-	0.1	-
L-Lysine	0.3	0.26	0.3	0.26
DL-Methionine	0.05	-	0.05	-
Metabolizable energy[x] (MJ)	12.3	12.2	12.3	12.2
Crude protein (g)	165	143	166	149

Composition of 1 kg of premix Lidermix T 5%: [1]vitamins: A 210000 IU; D_3 40000 IU; E 2000 IU; $B_1$30 IU; B_2 80 IU; B_3 400 mcg; B_6 45 mg; B_{12} 500 mcg; K 32.5 mg; H 500 mcg; choline 1500 mg; folic acid 9.0 mg; Synthetic amino acids: methionine 7.50 g; lysine 36.0 g; threonine 5000 mg; mineral components: Mn 1500 mg; Zn 2250 mg; Co 8.0 mg; Se 6.0 mg; Cu 500 mg; Fe 1800 mg; J 20 mg; Mg (total) 1.0 g, P (total) 27.5 g; Na (total) 25.0 g, Ca (total) 122.0g; Other components: antioxidant 300 mg; Betafin S_1 3750 mg, Flavomycin 100 mg; Ca panthotenate 200 mg; Pigor 757 – 4000 mg; brans – 4969000 mg.
[2] as above, without Flavomycin, * BIO-MOS – contains cellular walls of *Saccharomyces cerevisiae* yeasts, strain 1026; [x] – energy content – value from table on from POLISH SWINE NUTRITION REQUIREMENTS (1993)

The specimens of duodenum, ileum and jejunum were *post-mortem* collected on at random basis from 16 selected animals (8 per each group). The specimens were rinsed in 0.9% NaCl and preserved in 10% buffered formalin solution (24 hours). The collected specimens were treated by alcohol, xylanes and paraffin, then embedded in the paraffin wax blocks and cut into serial sections of 4 μm thickness. They were stained with hematoxylineosin (H-E) method. The slides were examined, using Olympus microscope (under 400 x magnification). At morphometric examination included the determination of number of fission cells per 1 crypt and height of epithelial cells in crypt. The results were shown as an average from 10 measured cells.
For *in vitro* microbiological tests approximately 1 g samples were collected under sterile conditions from the individual parts of gastrointestinal tract, that is, the duodenum, jejunum, ileum and large intestine. After dilution, removed material was used for plating, using poured plates and surface spread. Single colonies from mixture of various microorganisms were obtained using a streak plate method.
Morphological observations of microorganisms included macroscopic examination of the colonies, that is, size, shape, surface, consistency, border type and colour, and microscopic observations after Gram staining of cells. Examination and observations of microorganisms were made on Mueller-Hinton medium (for total bacterial count), Sabourand medium (for fungi), blood agar medium (for total bacterial count), on McConkey medium (for *Enterobacteriaceae*), on Martin medium (total number of yeasts and microscopic hypha fungi), on Eijkman medium (for acid-producing bacteria), and Rogosa, Mitchell, Wiesman media (for total of acidifying bacteria) (DUSZKIEWICZ-REINHARD et al., 1999).
To count the colonies-forming units (CFU), a counter of Stuart Scientific company was used. In qualitative analyses, the reducing surface inoculation (the scratch method) was applied. A quality evaluation was conducted on the ground of capability to grow, observation of macroscopic colonies and microscopic cells (preparations stained by the method acc. to Gram as well as and life performance preparations).

Identity of the species was corroborated using Bergey's Manual of Determinative Bacteriology (BUCHANAN and GIBBONS, 1974).
Results were statistically analysed by one-factor variance analysis with an application of the smallest square method (SPSS, 2000).

Results and Discussion

In the studies on intake and conversion of feed, growth rate and chosen slaughter performance traits, none statistically significant differences were found between the groups of fatteners, receiving the different feed additives (Table 2). Feed intake in fattening period was higher in group E, as compared to group C, and feed conversion was worse. Experimental fatteners, as compared to the control ones, grew slower by 2.42% (E).

Table 2
Production results

Specification	Group		S_e	Significance level	
	control	experimental		P	SPSS
Initial body weight, kg	21.6	21.2	0.504	0.573	ns
Body weight after finishing I period, kg	56.5	56.7	0.515	0.766	ns
Slaughter body weight, kg	100.7	100.6	0.587	0.940	ns
Length of fattening period: fattening period I, days	48.6	48.9	1.502	0.884	ns
fattening period II, days	53.3	55.1	1.764	0.489	ns
Length of fattening period, days	101.9	104.0	2.456	0.557	ns
Age of slaughter, days	171.6	174.5	2.561	0.537	ns
Feed intake: fattening period I, kg	81.8	81.0	3.026	0.853	ns
fattening period II, kg	133.9	139.3	4.089	0.352	ns
during fattening from 21 to 100 kg, kg	215.7	220.3	5.224	0.533	ns
Feed conversion per 1 kg of BW gain:					
fattening period I, kg/kg	2.35	2.28	0.072	0.506	ns
fattening period II, kg/kg	3.04	3.17	0.087	0.283	ns
during fattening period from 21 to 100 kg, kg/kg	2.73	2.77	0.065	0.662	ns
Daily gains:					
fattening period I, g	723	734	17.98	0.670	ns
fattening period II, g	841	805	23.59	0.290	ns
during fattening period from 21 to 100 kg, g	783	768	18.00	0.581	ns
Weight of half right cold carcass, kg	38.2	38.3	0.280	0.839	ns
Dressing percentage, %	75.9	76.1	0.370	0.700	ns
Mean thickness of backfat from 5 measurements, cm	2.26	2.28	0.094	0.847	ns
Loin "eye" area, cm^2	49.2	52.5	1.563	0.147	ns
Weight of loin without backfat and skin, kg	4.8	5.1	0.087	0.040	*
Weight of backfat and skin of loin, kg	1.5	1.5	0.061	0.540	ns
Weight of ham without backfat and skin, kg	7.4	7.5	0.121	0.628	ns
Weight of backfat and skin of ham, kg	1.6	1.5	0.059	0.321	ns
Meatiness of basic cuts, %	58.9	60.7	0.694	0.078	ns

* $P \leq 0.05$

The group piglets supplemented with BIO-MOS had higher body weights on d 84 ($P \leq 0.05$) and a tendency toward better feed utilization on d 29-84, compared with the control (MATRAS et al., 2006). On the other hand, HOUDIJK et al. (1999) employed the addition of indigestible oligosaccharides and observed the periodical decrease of feed consumption and of daily gains, having no effect on mean results obtained in the experiments. After using the mixture with prebiotic, for example mannanooligosaccharide, YOUZELA et al. (2002) did not observe the improvement of

body gain and feed conversion of growing pigs, but DAVIS et al. (2001), LeMIEUX et al. (2003) and BOBČEK et al. (2004) observed somewhat better results. According to ZIMMERMANN et al. (2001), oligosaccharides contribute to increase of absorptive surface of intestines; they bind the receptors of intestinal epithelium and occupy them or link with the lectins on a surface of pathogenic bacteria, preventing the adhesion of pathogens to epithelium.

The biochemical indicators in blood of C and E fatteners were found to be in the reference limits (FRIENDSHIP and HENRY, 1996). There were no differences between the groups in respect to majority of mentioned parameters, except HDL, beeing significantly lower in serum of experimental pigs ($P \leq 0.01$) (Table 3). Significantly higher ALT ($P \leq 0.05$) content in blood serum of experimental fatteners was found as compared to animals from control group. The effects BIO-MOS on biochemical parameters of blood were studied (CÖMERT, 2003; MATRAS et al., 2006). Differences regarding the average total cholesterol, triglyceride and HDL values in blood serum were statistically insignificant (CÖMERT, 2003). A considerable increase of glucose concentration in the blood serum was observed after the application of BIO-MOS in the diet of rats (ALESINA et al. 1993). None treatment differences in total blood protein and blood urea nitrogen have been observed when mannan oligosaccharides were administered to calves (HEINRICHS et al., 2003). The own results and other authors (MATRAS et al., 2006) do not indicate the unfavourable effect of BIO-MOS on indices of energy-protein changes in organism of growing pigs.

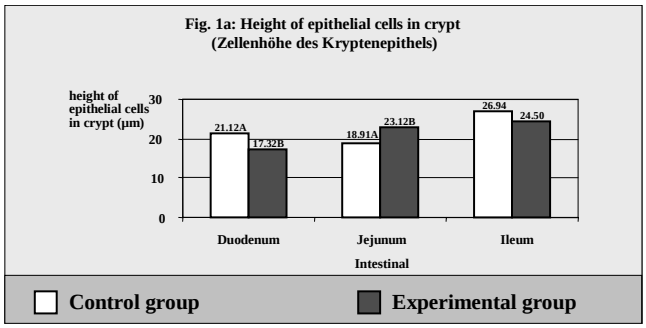
Table 3
Biochemical parameters in blood

Item	Group		S_e	Significance level	
	control	experimental		P	SPSS
Albumin (ALB), g l^{-1}	37.75	35.13	1.204	0.145	NS
Glucose (GLU), mmol l^{-1}	5.37	4.49	0.301	0.059	NS
Urea nitrogen (BUN), mmol l^{-1}	5.13	4.33	0.377	0.154	NS
Alkaline phosphatase (ALP), UL^{-1}	149.13	142.87	12.985	0.739	NS
Total protein (TP), mmol l^{-1}	65.75	62.00	1.668	0.134	NS
Triglycerides (TG), mmol l^{-1}	0.42	0.41	0.036	0.802	NS
Total cholesterol (CH), mmol l^{-1}	2.26	2.08	0.092	0.175	NS
HDL, mmol l^{-1}	0.90	0.77	0.028	0.005	**
LDL, mmol l^{-1}	1.19	1.14	0.087	0.716	NS
VLDL, mmol l^{-1}	0.17	0.16	0.015	0.653	NS
CHL/HDL Index	2.53	2.71	0.124	0.303	NS
Aspartate aminotransferase AST, UL^{-1}	47.87	48.97	6.882	0.911	NS
Alanine aminotransferase ALT, UL^{-1}	30.14	49.03	5.299	0.018	*
Index Ritisa	1.91	1.17	0.518	0.055	NS

** $P \leq 0.01$; * $P \leq 0.05$

The selected morphometric indices of intestine were analysed (Fig. 1a and 1b). The changes in ileal crypts might decrease the proliferation activity of enterocytes (ABBAS et al., 1989; EKELUND and EKBLAD, 1999). When feed additives were used, the increase of intestinal villi was observed *post mortem* (THOREUX et al., 1998) as well as the higher number of crypts in mucosa (McCULLOGH et al., 1998) and proper changes in alimentary tract (ROTKIEWICZ et al., 2000) were found.

Table 4 presents a quantitative compilation of the microorganisms from the individual sections of the digestive tract. Number of bacteria from *Enterobacteriaceae* family and lactic acid bacteria (LAB) in 1 g of dry matter of the intestinal content of fatteners in



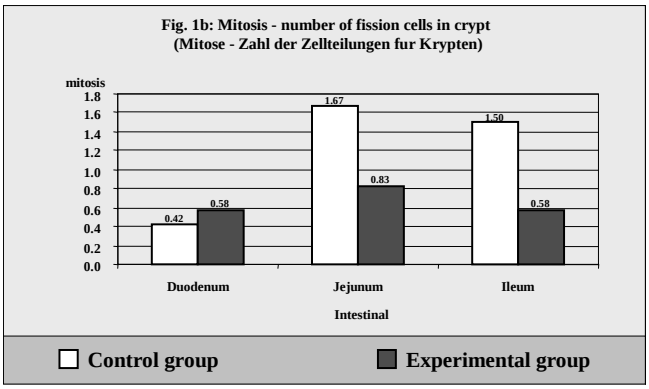


Table 4
Number of microbial cells in individual segments of the digestive tract, per 1 g dry weight of intestine content

Group feeding	Segment of digestive tract	Number of the chosen groups of micro-organisms, g^{-1} DM^{-1}						
		Type of medium used						
		Mueller-Hinton	McConkey	Eijkman	Rogosa, Mitchell, Wiesman	Martin	Nutrient agar + blood	Wilson -Blair
Control	duodenum	10^{10}	1.15×10^{8}	2.72×10^{7}	3.11×10^{7}	3.40×10^{7}	1.46×10^{8}	+
	jejunum	10^{9}	1.14×10^{8}	4.60×10^{7}	1.46×10^{8}	2.30×10^{6}	1.46×10^{8}	+
	ileum	10^{9}	8.70×10^{7}	2.57×10^{7}	4.95×10^{7}	4.33×10^{7}	7.10×10^{7}	+
	large intestine	10^{8}	1.63×10^{8}	2.43×10^{8}	3.17×10^{7}	1.15×10^{7}	1.19×10^{7}	+
Experi-mental	duodenum	10^{10}	5.65×10^{7}	1.50×10^{10}	1.66×10^{7}	2.58×10^{5}	-	+
	jejunum	10^{10}	3.23×10^{7}	1.50×10^{10}	1.82×10^{8}	3.56×10^{5}	-	-
	ileum	10^{10}	1.58×10^{6}	4.75×10^{10}	1.58×10^{7}	8.01×10^{4}	8.01×10^{2}	-
	large intestine	10^{10}	2.86×10^{6}	2.58×10^{9}	8.60×10^{7}	8.60×10^{4}	-	+

group C was equal to $10^{7} – 10^{8}$. In contents of large intestine, the ratio of micro-organisms from two mentioned above groups was also similar. In the contents of the intestine of fatteners from group E, number of bacteria from *Enterobacteriaceae* family amounted to ca 10^{7} whereas number of acidifying bacteria was higher than in group C ($10^{9} – 10^{10}$ in 1 g of dry matter). The decrease of number of bacteria from *Enterobacteriaceae* family in group E in relation to group C may be considered as phenomenon exclusively favourable. From among the types of *Escherichia*, the species *Escherichia coli* belongs to the mentioned above family. The most of its representatives reveal the opportunistic pathogenicity. They may cause infections of alimentary tract; the presence of genes, located in plasmid and determining generation of haemolysins in haemolytic *E. coli* strains affects a quick spreading out of infection (BUCHANAN and GIBBONS, 1974). Prebiotics decide on the composition of the intestinal microflora of pigs and affect favourably the host's organism (SCHREZENMEIR and De VRESE, 2001; ZIMMERMANN et al., 2001; MAXWELL et al., 2004).

In group E, as compared to group C, bacteria from LAB group were 1000-times more numerous. They had probably an influence on the 10 -100th fold decrease of number of bacteria from *Enterobacteriaceae* family in relation to group C. The application of probiotic (strain of *Pediococcus acidilactici* bacteria MA18/5M) caused the increase of acidifying bacteria number and decreased number of bacteria from *Enterobacteriaceae* family (REKIEL et al., 2005b). The potentially probiotic strains were much more numerous in the case of the group fatteners which received feed containing greater fibre content (REKIEL et al., 2005a). The antagonistic metabolites, produced by autochthonous lactic acid bacteria include, among others, bacteriocins. They are active in relation to Gram-negative bacteria, e.g. *Clostridium.* By generation of pores in cytoplasmic membrane of sensitive bacteria and outflow of electrolytes and low-molecular metabolites, they cause the death of a cell (KOT et al., 2000). *Clostridium perfringens* produces toxins which affect the secretion activity of intestine, increase a difference between potential in ions' transport, damage the cells or tissues and change the activity of smooth muscles in the intestine (NIEMIAŁTOWSKI and TOKA, 1996). The presence of *Clostridium perfringens* in the contents of fatteners' intestine was found more frequently in group C than in group E. The limitation of their number decreased their participation in competition for nutrients and was favourable for better productivity of the experimental animals.

Number of fungi and *Proteus vulgaris* bacteria in group E as compared to group C, was lower (Table 4). An action of mannan oligosaccharide consisting in "effect of raft" and "death by starvation" of bacterial cell has an inhibitory influence on pathogenic bacteria *E. coli, Salmonella typhimurium, Clostridium botulinum* and a stimulating effect on beneficial bacteria: *Bifidobacterium longum, Lactobacillus casei, L. acidophilus* and *L. delbrueckii* (SCHREZENMEIR and De VRESE, 2001). The studies, conducted by HANCOCK et al. (2003) on the presence of *Escherichia coli* in faeces of piglets who received 0.2% addition of BIO-MOS to diet, did not demonstrated any significant differences between the experimental group and the control negative one. Significant differences were found for pH of faeces. None significant differences were found in the analysis of faeces of the piglets, obtaining antibiotic (carbadox) or mannan oligosaccharide what has confirmed the suitability of BIO-MOS as AGP replacer.

A high similarity of the composition of intestinal microflora in fatteners from two examined groups was observed. Microorganisms, isolated from animals fed with antibiotic growth promoters - AGP (C) or BIO-MOS (E) included: *Escherichia, Pseudomonas, Streptococcus, Enterococcus, Lactobacillus, Lactococcus, Staphylococcus, Proteus, Clostridium, yeasts* and *microscopic hypha fungi* (*Hyphomycetes*). The absence of pathogenic ß-haemolytic strains: *Escherichia coli* and *Salmonella sp* in alimentary tract of the examined animals of the two feeding groups was stated; it was an evidence of their good health state.

The obtained results confirm generally the possibility to employ biostimulators instead of feed antibiotic what is favourable from production viewpoint and seems to be a suitable alternative to antibiotic.

References

ALESINA, M.I.; SUKACHOVA, O.O.; ZILBERMAN, S.T.; KONOVALENKO, O.O.; NESTERENKO, G.O.; BESKROVNYI, O.M.:
State of the endocrine system in rats of various ages under immobilization stress and influence of adaptogenic biomos. Fiziol Zh., **39** (1993), 78-83

ANONYMOUS:
Polish Swine Nutrition Requirements. [Normy żywienia świń] (Pl) IFiŻZ PAN. Omnitech-Press, Warszawa 1993

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS:
Official Methods for Analysis of the Associated of Official Analytical Chemists. 15th ed., Chapter 32, Washington, DC, 1990

ABBAS, B.; HAYES, T. L.; WILSON, D. J.; CARR, K. E.:
Internal structure of the intestinal villus: morphological and morphometric observations at different levels of the mouse villus. J. Anat., **162** (1989), 263-273.

BOBČEK, B.; MRAZOVA, J.; BOBČEK, R.:
The influence of mannan oligosaccharides „BIO-MOS" on the production and quality parameters in pig fattening. J. Centr. Europ. Agricult., **5** (2004), 69-70

BUCHANAN, R. E.; GIBBONS, N. E.:
(Ed.) Bergey's Manual of Determinative Bacteriology. 8thed., Williams & Wilkins Co., Baltimore 1974

CÖMERT, N.:
The effects of avilamycin, BIO-MOS, cylactin and Yussa Schidigera extract added to the corn-soybean meal based diets on fattening performance, slaughter results and some blood and intestinal parameters of male broiler turkey. [Misir-soya esasli etlik erkek hindi yem karmalarina katilan avilamycin, Bio-Mos, cylactin, Yucca Schidigera ekstraktinin besi performansi, kesim sonuclari ile bazi kan ve bağirsak parametreleri űzerine etkileri] (T) Ph. D. Thesis. (2004), 1-57

DAVIS, M.E.; MAXWELL, C.V.; DE RODAS, B.Z.; BROWN, D.C.; JOHNSON, Z.B.; DVORAK, R.A.:
Efficiency of mannan oligosaccharide (Bio-Mos®) addition with two levels of copper sulfate in the diets of growing-finishing pigs. J. Anim. Sci., **79** (2001), 472

DUSZKIEWICZ-REINHARD, W.; GRZYBOWSKI, R.; SOBCZAK, E.:
Theory and Exercises in General and Technical Microbiology [Teoria i ćwiczenia z mikrobiologii ogólnej i technicznej] (Pl). SGGW, Warsaw 1999

EKELUND, K.M.; EKBLAD, E.:
Structural, neuronal, and functional adaptive changes in atrophic rat ileum. Gut **45** (1999), 236-245

FLACHOWSKY, G.; SCHULZ, E.:
Futterergänzungen und ihre Bedeutung für Leistung und Ökologie. Arch. Tierz., Dummerstorf **40** (1997), 101-107

FRIENDSHIP, R.M.; HENRY S.C.:
Cardiovascular system, hematology and clinical chemistry. In: Diseases of swine, Eds. Leman, A.D.; Straw, B.E.; Mengeling, W.L.; D'Allaire, S.; Taylor, D.J.; Iowa State Univ. Press, USA, (1996), 3-11

HANCOCK, J.D.; JONES, C.L.; STARKEY, C.W.:
Mannan oligosaccharides in diets for nursery pigs. Swine day. Report of Progress 920 (2003), 128-131

HEINRICHS, A.J.; JONES, C.M.; HEINRICHS, B.S.:
Effects of mannan oligosaccharide or antibiotics in neonatal diets on health and growth of dairy calves. J. Dairy Sci., **86** (2003), 4064-4094

HERICH, R.; BOMBA, A.; NEMCOVÁ, R.; GANCARČIKOVÁ, S.:
The influence of application of probiotic on the immune system of 2-3 months old calves. Arch. Tierz., Dummerstorf **41** (1998), 565-572

HOUDIJK, J.G.M.; BOSCH, MW.; TAMMINGA, S.; VERSTEGEN, M.W.A.; BERENPAS, E.B.; KNOOP, H.:
Apparent ileal and total- tract nutrient digestion by pigs as affected by dietary non-digestible oligosaccharides. J. Anim. Sci., **77** (1999), 148–158

KOT, B.; JAKUBCZAK, A.; BUKOWSKI, K.:
The antagonistic activity of lactic acid bacteria against selected bacteria [Antagonistyczne działanie bakterii fermentacji mlekowej w stosunku do wybranych drobnoustrojów] (Pl) Medycyna Wet., **56** (2000), 53-57

LeMIEUX, F.M.; SOUTHERN, L.L.; BIDNER, T.D.:
Effect of mannan oligosaccharides on growth performance on weanling pigs. J. Anim. Sci., **81** (2003), 2482-2487

MATRAS, J.; CZECH, A.; KLEBANIUK, R.; ROSZKOWSKI, T.; GRELA, E.R.:
Responses to dietary fructooligosaccharide (couch grass) and mannan oligosaccharide (Bio-Mos®) in piglets diets. Nutritional Biotechnology in the Feed and Food Industries. Proceedings of Alltech's 22nd Annual Symposium. April 23-26, 2006. Lexington, KY, USA. 2006, (suppl. 1), 52

MAXWELL, F.J.; DUNCAN, S.H.; HOLD, G.; STEWART, C.S.:
Isolation, growth on prebiotics and probiotic potential of novel bifidobacteria from pigs. Anaerobe **10** (2004), 33-39

McCULLOGH, J. S.; RATCLIFFE, B.; MANDIR, N.; CARR, K. E.; GOODLAD, R. A.:
Dietary fibre and intestinal microflora: effect on intestinal morphometry and crypt branching. Gut **42** (1998), 799-806

MIGUEL, J.C.; RODRIGUES-ZAS, S.L.; PETTIGREW, J.E.:
Efficacy of a mannan oligosaccharide (Bio-Mos®) for improving nursery pig performance. J. Swine Health Prod. **12** (2004), 296-307

NIEMIAŁTOWSKI, M.; TOKA, F.N.:
The bacteria and their toxins – from microscope to molecular biology. Infections and invasive diseases of intestinal tract human and animals [Bakterie jelitowe i ich toksyny – od mikroskopu do biologii molekularnej. w: Choroby zakaźne i inwazyjne przewodu pokarmowego ludzi i zwierząt] (Pl). Mat. Conf. Kazimierz Dolny, 24-26.10.1996

PETIGREW, J.E.; MIGUEL, J.C.:
Impact of Bio-Mos® on microbial of the pig digestive tract. Nutritional Biotechnology in the Feed and Food Industries. Proceedings of Alltech's 22nd Annual Symposium. Edited by TP Lyons, Jacques, K.A., Hower, J.M. (2006), 115-120

PICKARD, K.M.; BREMNER, A.R.; GORDON, J.N.; MacDONALD, T.T.:
Microbial-gut interactions in health and disease. Immune responses. Best Prac. Res. Clin. Gastroenterol. **18** (2004), 271-285

REKIEL, A.; GAJEWSKA, J.; TOPOL, K.; SAWOSZ, E.:
Effect of intensity of feeding on the intestinal microflora of pigs. Pol. J. Microb., **54** (2005a), 331-334

REKIEL, A.; GAJEWSKA, J., WIĘCEK, J.; MISZCZYK, A.:
Effect of addition of feed antibiotic or probiotic on performance and composition of intestinal microflora of pigs. Electr. J. Pol. Agric. Univ., Anim. Husb., **8** (2005b), #32

ROTKIEWICZ, T.; WIŚNIEWSKI, M.; BOMBA, A.; DEPTA, A.; ROTKIEWICZ, Z.:
The effect of *Lactobacillus casei* inoculation on morphology of digestive tract in gnotobiotic piglets [Wpływ kolonizacji *Lactobacillus casei* na morfologię przewodu pokarmowego prosiąt gnotobiotycznych] (Pl) Przegl. Hod., Zesz. Nauk. PTZ, Chów i Hodowla Trzody Chlewnej. **48** (2000), 131-139

RÓŻYCKI, M.:
Procedures of pig evaluation at pig performance testing stations. State of breeding and evaluation results of swine [Zasady postępowania przy ocenie świń w Stacjach Kontroli Użytkowości Rzeźnej Trzody Chlewnej. Stan hodowli i wyniki oceny świń] (Pl) IZ XIV (1996), 69-82

SCHREZENMEIR, J.; De VRESE, M.:
Probiotics, prebiotics, and synbiotics – approaching a definition. Am. Society Clin. Nutr., **73** (2001), 361-364

SPSS,:
SPSS 10.0 for Windows user's guide, 2000 by SPSS Ins. USA.

STAVRIC, S.; KORNEGAY, E.T.:
Microbial probiotics for pigs and poultry. In: Biotechnology in Animal Feeds and Animal Feeding. [In:] Wallace R.J., Chesson A.: VCH Verlagsgesellschaft mbH, Wienheim, RFN, (1995), 206 – 231

THOREUX, K.; BALAS, D.; BOULEY, C.; SENEGAL–BALAS, F.:
Diet supplemented with yoghurt or milk fermented by Lactobacillus casei DN-114 001 stimulated growth and brush-border enzyme activities in mouse small intestine. Digestion **59** (1998), 349-359

VAN NEVEL, C.J.; DECUYPERE J.A., DIERICK N.A., MOLL K.:
Incorporation of galactomannans in the diet of newly weaned piglets: effect on bacteriological and some morphological characteristics of the small intestine. Arch. Anim. Nutr., **59** (2005), 123-138

ZIMMERMANN, B.; BAUER, E.; MOSENTHIN, R.:
Pro- and prebiotics in pig nutrition – potential modulators of gut health? J. Anim. Feed Sci., **10** (2001), 47 - 56

YOUZELA, C.; TEVES, P.; SILVA, M.; REGO, O.; BORBA, A.;
The effect of mannano oligosaccharide (Bio-Mos) on growth performances and carcass characteristics in pigs. 53rd Ann. Meet. EAAP, Cairo, (2002), Poster N4.49

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Arch. Tierz., Dummerstorf **50** (2007) Special Issue, 181-185

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Retinol binding protein 4 gene and reproductive traits in pigs

Abstract

The aim of the study was to use the DNA mutations in the *RBP4* gene to determine associations between the different genotypes and litter size in Polish Large White sows. Reproductive traits investigated were: number of piglets born alive (NBA) and number of piglets weaned (NW). The polymorphism in *RBP4* gene was detected using the PCR-RFLP method, with specific primers and the restriction enzymes *Msp*I. Two different alleles of *RBP4* gene were identified: allele A (0.65) and B (0.35) and three genotypes: AA (0.46), AB (0.37) and BB (0.17). The relationship between the *RBP4* genotypes NBA and NW were analyzed. The analysis showed in first parity sows statistically significant ($P \leq 0.05$) differences between sows carrying different *RBP4* genotypes. In later parities sows with the BB genotype still had the largest litter size compared to AA and AB sows, but the difference was statistically not significant.

Key Words: *RBP4* gene, pigs, polymorphism, reproductive traits

Zusammenfassung

Titel der Arbeit: **Das *RBP4*-Gen und Wurfleistungsmerkmale beim Schwein**

Ziel der Arbeit war die Untersuchung von DNA Mutationen im *RBP 4*-Gen und deren Zusammenhänge mit Wurfleistungsmerkmalen wie der Anzahl lebend geborener Ferkel (NBA) und Anzahl abgesetzter Ferkel (NW) bei Sauen der Großen Polnischen Rasse. Der Polymorphismus des *RPB4*-Gens wurde bei Anwendung der PCR-RFLP- Methode unter Heranziehung spezifischer Starter und dses *Msp*I-Restriktionsenzyms bestimmt. Es wurden die zwei Allele des *RPB4*-Gens A-Allel (0,65) und B-Allel (0,35) sowie die drei Genotypen AA (0,46) AB (0,37) und BB (0,17 identifiziert. Die Zusammenhänge zwischen den einzelnen Genotypen des *RPB4*-Gens und den Wurfleistungsmerkmalen konnten analysiert und diese bei den Erstlingswürfen der Sauen signifikant nachgewiesen werden. Diese Zusammenhänge fanden sich tendenziell auch bei späteren Würfen aber sie waren nicht signifikant.

Schlüsselwörter: Schwein, *RBP4*-Gen, Polymorphismus, Wurfleistungsmerkmale

The advancement in research on swine genome enabled identification of polymorphic loci of individual genes that control the level of reproductive traits, which are know to have influence on reproductive performance in sows (WANG et al., 2006; TERMAN, 2005; LINVILLE et al., 2001; DROGEMULLER et al., 1999; ROTHSCHILD et al., 1996) and boars (TERMAN et al., 2006; MAĆKOWSKI et al., 2004; SCHLINGMANN et al., 2002; KMIEĆ et al., 2001).

Molecular techniques can now be used to increase rate of response to selection. It has been proposed that "candidate gene" analyses be used to identify individual genes responsible for traits of economic importance (ROTHSCHILD and SOLLER, 1997).

One of these genes can be retinol binding protein 4 gene which was localized in chromosome 14 in pigs. HARNEY et al., (1993) have shown that there is an increasing *RBP4* gene expression in gravid porcine endometrium from d 10 to 12. Their results support an important role for this vitamin A transport protein in uterine and conceptus

physiology during the establishment of pregnancy. Therefore, *RBP4* was investigated as a candidate gene for litter size owing to its role at the time of high embryonic mortality rate.

A *Sac*I polymorphism was detected in pig genomic DNA by hybridization of Southern blots with a pig retinol-binding protein 4 (RBP4) probe (MESSER et al., 1996). This polymorphism was significantly associated with litter size in pigs (DROEGEMULLER et al., 2001; ROTHSCHILD et al., 1996, 2000).

The purpose of this study was conducted to examine the *RBP4* gene polymorphism and its effects on reproductive traits in Polish Large White sows. Reproductive traits investigated were: number of piglets born alive (NBA) and number of piglets weaned (NW).

Materials and Methods

The experimental populations included in total 101 Polish Large White sows. The animals were bred and raised at a farm in Western Pomerania (Poland). Rearing and feeding conditions were equalized for all animals. Genomic DNA was extracted from blood sample using Master Pure kit of Epicentre Technologies.

Genotypes of the *RBP4* gene were determined by the PCR-RFLP method, but only for the sows that had farrowed successfully. The *RBP4* gene fragment was amplified from genomic template using the PCR with designed primers in exons 2 and 4 of sequences reported by ROTHSCHILD et al., (2000). The region of the *RBP4* gene containing the polymorphic *Msp*I site was amplified using primers: forward – GAGCAAGATGGAATGGGTT and reverse – CTCGGTGTCTGTAAAGGTG in a 15 µl PCR containing using 90 ng porcine genomic DNA, 200 µM of each dNTP, 1.5 mM $MgCl_2$ and 0.6 units of *Taq* DNA polymerase (MBI Fermentas) in a standard 1x PCR buffer. Primers were used at a concentration of 10 pmol with a thermal cycling regime of 93^0C for 3 minutes followed by 40 cycles of 93^0 from 30 second, 56^0C for 45 second, 72^0C for 45 second and ending with a final step of 72^0C for 5 minutes. Digestion of PCR product (550 bp) was performed with 3 I.U. of appropriate restriction endonuclease *Msp*I at 37ºC overnight. PCR product was then examined by electrophoresis on a 2% agarose gel stained with ethidium bromide. After that the gels were analyzed in UV rays. Performance traits data were collected from farm documentation and they contained: number piglets born alive (NBA) and numbers of piglets weaned (NW). The relations between *RBP4* genotypes and studied reproductive traits were analyzed with one-way analysis of variance and the significance of differences was verified using Duncan test with computer program Statistica'99.

The following model was used:

$$y_{ijklm} = \mu + G_i + Y_j + M_k + P_l + S_m + e_{ijklm}$$

where:

y_{ijklm} – observed value,
μ – overall mean,
G_i – effect of i-th genotype (i = AA, AB, BB),
Y_j – effect of j-th year of farrowing (j = 1, 2, 3,…, 6),
M_k – effect of k-th month (k = 1, 2, 3,…, 12),

P_l – effect of l-th parity (l = 1, 2, 3, ≥4),
S_m – effect of m-th sire,
e_{ijklm} – random residual.

Results

Two different *RBP4* alleles were identified in the sows herd under study: allele A and allele B that controlled three genotypes, namely AA, AB and BB. The lengths of restriction fragments detected during the experiment are given in Table 1. The allele and genotype frequency were distributions in Hardy-Weinberg equilibrium.

Table1
Endonuclease and allele sizes of *RBP4* gene

Candidate Gene	PCR product size (bp)	Endonuclease	Allele size (bp)	Source
RBP4	550	*Msp*I	A – 190, 154, 136, 70 B – 190, 136, 125, 70, 29	ROTHSCHILD et al. (2000)

In the analyzed sows herd the allele A occurred with the frequency 0.65, whereas the allele B – with the frequency 0.35. The AA genotype occurred with the frequency 0.46, AB with frequency 0.37 and BB with 0.17 – Table 2.

Table 2
The frequency of *RBP4* genotypes and alleles of Polish Large White sows

Breed		Genotype			Allele	
		AA	AB	BB	A	B
Large White	number	47	37	17	0.65	0.35
	frequency	0.46	0.37	0.17		

Table 3
Effects of *RBP4* genotypes on reproductive traits of sows

RBP4 genotype	Parity	n	NBA[1]	NW[1]
AA	I	43	$9.07^{a} \pm 2.27$	$8.12^{A} \pm 2.24$
AB		37	$9.05^{a} \pm 2.43$	$7.92^{A} \pm 2.38$
BB		17	$9{,}41^{b} \pm 2.42$	$9.00^{B} \pm 1.84$
AA	II	37	$8.73^{a} \pm 2{,}61$	$7.59^{a} \pm 2{,}01$
AB		29	9.17 ± 2.83	8.07 ± 2.68
BB		9	$10.67^{b} \pm 3.00$	$9.78^{b} \pm 2.63$
AA	III	27	8.98 ± 3.10	8.07 ± 2.37
AB		22	9.68 ± 2.85	8.59 ± 2.65
BB		5	10.00 ± 2.45	9.40 ± 2.30
AA	≥ IV	76	9.35 ± 2.70	7.84 ± 2.10
AB		111	9.68 ± 2.64	8.23 ± 2.55
BB		13	10.38 ± 2.10	8.69 ± 1.75

Small letters (a, b) denoted significance difference (P≤0.05); capital letters (A, B) denoted significance difference (P≤0.01),
[1] NBA – number of piglets born alive; NW – number of piglets weaned,
n – number of sows within parities.

The relationship between the *RBP4* genotypes and litter size were analyzed – Table 3. The analysis of the number born piglets alive (NBA), showed in first and second parity sows statistically significant (P≤0.05) differences between sows carrying BB

genotypes compared to AA and AB genotypes. The sows with BB genotypes had also larger number of piglets weaned (NW) than sows carrying AA and AB genotypes and this differences were statistically significant (P≤0.01) in first parity and (P≤0.05) in second parity – Table 3.
In later parities sows with the BB genotype still had the largest litter size compared to AA and AB sows, but the difference was statistically not significant.
Analysis of the interaction PARITY x *RBP4* showed small and non-significant differences.

Discussion

A similar frequency of allele A (0.62) was observed in German Landrace x Duroc crossbreed sows (DROEGEMÜLLER et al. 2001; ROTHSCHILD et al., 2000). A higher frequency of allele A (0.67) was observed by DROEGEMÜLLER et al. (2001), who studied the breed German Landrace. A lower frequency of allele A compared to the present study was revealed in Landrace (0.59), Large White (0.55) and Landrace x Large White crossbreed (0.42) pigs (ROTHSCHILD et al., 2000; WANG et al., 2006; LINVILLE et al., 2001).
In the current study, many candidate gene markers for litter size was found including alleles at the *ESR* gene locus, the *PRLR* locus, and the *CYP21* locus (ROTHSCHILD et al., 1996, 2000; SHORT et al., 1997; VINCENT et al., 1998; TERMAN, 2005; ZIEMAK and GRZESIAK, 2006). ROTHSCHILD et al. (2000), using data of nearly 2,800 litters of 1,300 *RBP4* genotyped sows of six commercial lines, reported a significant additive effect associated with RBP4 of +0.15 NBA. ROTHSCHILD et al. (2000) stated that it is difficult to determine whether it is linked to one or more genes having this effect. WANG et al. (2006), showed that sows with BB genotype of *RBP4* locus had more piglets per litter than sows with AA and AB genotypes. These results are comparable to the results reported in this study.
The analysis of the total number born (TNB) and number born alive (NBA) reported by DROEGEMÜLLER et al. 2001, showed no significant effect *RBP4* locus on litter size in pigs.

Conclusion

The preliminary study of *RBP4* gene showed that the sows with the BB genotype produced more piglet than sows with the AA and AB genotypes. This result was confirmed statistically (P≤0.05) in first and second parity. The present study showed that the *RBP4* gene is strongly associated with litter size in sows. The analysis of the number piglets born alive (NBA), numbers piglets weaned (NW) in later parities showed small and statistically not significant differences between sows carrying different *RBP4* genotypes. The obtained results suggest a possibility of utilisation of the polymorphism in breeding that aims at an improvement of some reproductive performance traits of sows.

References

DROEGEMÜLLER, C.; HAMANN, H.; DISTL, O.:
Candidate gene markers for litter size in different German pig lines. J. Anim. Sci. **79** (2000), 2565-2570
DROEGEMÜLLER, C.; HAMANN, H.; THIEVEN, U.; KRIETER, J.; DISTL, O.; HARLIZIUS, B.:

Influence of the genome region surrounding the estrogen receptor (ESR) gene on litter size in a German Landrace population. Arch. Tierz., Dummerstorf **42** (1999) Special Issue, 175-177

HARNEY, J.P.; OTT, T.L.; GEISERT, R.D.; BAZER, F.W.:
Retinol-binding protein gene expression in cyclic and pregnant endometrium of pigs, sheep, and cattle. Biol. Reprod. **49** (1993) 5, 1066-1073

KMIEĆ, M.; DYBUS, A.; TERMAN, A.:
Prolactin receptor gene polymorphism and its association with litter size in Polish Landrace. Arch. Tierz., Dummerstorf **44** (2001) 5, 547-551

LINVILLE, R.C.; POMP, D.; JOHNSON, R.K.; ROTHSCHILD, M.F.:
Candidate gene analysis for loci affecting litter size and ovulation rate in swine. J. Anim. Sci. **79** (2001), 60-67

MAĆKOWSKI, M.; ŚWITOŃSKI, M.; MAĆKOWSKA, J.; PERZ, W.
Polymorphism of the GPX-5 gene and characteristics of boar semen. Arch. Tierz., Dummerstorf **47** (2004) 2, 165-171

MESSER, L.; WANG, L.; YELICH, J.; POMP, D.; GEISER, R.; ROTHSCHILD, M.F.:
Linkage mapping of the retinol-binding protein 4 (RBP4) gene to porcine Chromosome 14. Mamm. Gen. (1996) 7, 396

ROTHSCHILD, M.F.; MASSER, L.A.; DAY, A.; WALES, R.; SHORT, T.; SOUTHWOOD, O.; PLASTOW, G.:
Investigation of the retinol-binding protein 4 (RBP4) gene as a candidate gene for increased litter size in pigs. Mamm. Gen. **11** (2000) 1, 75-77

ROTHSCHILD, M. F.; SOLLER M.:
Candidate gene analysis to detect genes controlling traits of economic importance in domestic livestock. Probe (1997) 8, 13–22

ROTHSCHILD, M.; JACOBSON, C.; VASKE, D.; TUGGLE, C.H.; WANG, L.; SHORT, T.; ECKARDT, G.; SASAKI, S.; VINCENT, A.; MCLAREN, M.; SOUTHWOOD, O.; VAN DER STEEN, H.; MILEHAM, A.; PLASTOW, G.:
The estrogen receptor locus is associated with major gene influencing litter size in pigs. Proc. Natl. Acad. Sci. USA **93** (1996), 201-205

SCHLINGMANN, C.; DIETL, G.; RÄDER, I.:
Association von Polymorphismen im Promotbereich des porcinen HSP 70.2-Gens bei Ebern mit der Wurfgrősse. Arch. Tierz., Dummerstorf **45** (2002) 2, 171-180

SHORT, T.H.; ROTHSCHILD, M.F.; SOUTHWOOD, O.I.; MCLAREN, D.G.; DE VRIES, A.; VAN DER STEEN, H.; ECKARDT, G.R.; TUGGLE, C.K.; HELM, J.; VASKE, D.A.; MILEHAM, A.J.; PLASTOW, G.S.:
Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines, J. Anim. Sci. **75** (1997) 12, 3138-3142

TERMAN, A.; KMIEĆ, M.; POLASIK, D.:
Estrogen receptor gene (ESR) and semen characteristics of boars. Arch. Tierz., Dummerstorf **49** (2006) 1, 71-76

TERMAN, A.:
Effect of the polymorphism of prolactin receptor (PRLR) and leptin (LEP) genes on litter size in Polish pigs. J. Anim. Breed. Genet. **122** (2005) 6, 400-404

VINCENT, A.L.; EVANS, G.; SHORT, T.H.; SOUTHWOOD, O.I.; PLASTOW, G.S.; TUGGLE, C.K.; ROTHSCHILD, M.F.:
The prolactin receptor gene in associated with increased letter size in pigs. Proceedings 6th World Cong. Genet. App. Liv. Prod. **27** (1998), 15-18

WANG, X.; WANG, A.G.; FU, J.; LIN, H.:
Effects of ESR1, FSHB and RBP4 genes on litter size in Large White and Landrace Herd. Arch. Tierz., Dummerstorf **49** (2006) 1, 64-70

ZIEMAK, J.; GRZESIAK, W.:
Associations between polymorphism of the steroid 21-hydroxylase gene (*CYP21*) and litter size of Polish Large White × Polish Landrace sows. Arch. Tierz., Dummerstorf **49** (2006) 2, 158-164

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Differences in muscle fibre composition and tenderness of *m. longissimus lumborum* between heterozygous and homozygous negative Polish Landrace pigs for the *RYR1**

Abstract

The objective of this study was to investigate the effect of *RYR1* genotype on muscle fibre traits and meat tenderness of *m. longissimus lumborum* in fatteners. The study was carried out on 16 heterozygous Nn and 24 RYR1-free NN Polish Landrace gilts. Muscle samples were taken to categorize muscle fibre types (I, IIA and IIB) according to their mATP-ase activity and to determine the following technological parameters of meat: hardness and Warner-Bratzler shear force. Muscle fibre percentage, diameter and phenotypic correlation between muscle fibre traits and meat tenderness were estimated. The results obtained indicated that *RYR1* genotype had no effect on muscle fibre proportion, meat hardness and shear force. On the other hand, significant differences between groups were found in the size of muscle fibres. The *m. longissimus lumborum* of the Nn fatteners had significantly larger diameter of all examined muscle fibre types than muscle from group NN. The phenotypic correlations between histological and meat quality traits were generally low. A positive correlation was found between hardness and shear force and percentage of type IIB fibres, in contrast to a negative correlation between hardness and shear force and percentage of type I fibres. On the other hand, size of type I fibres, unlike that of type IIB fibres, positively correlates with meat quality traits. In addition, correlations between fibre type percentages were negative, in contrast to positive correlations between fibre type diameters.

Key Words: pigs, muscle microstructure, *RYR1,* tenderness, *m. longissimus lumborum*

Zusammenfassung

Titel der Arbeit: **Einfluss des *RYR1* Genpolymorphismus auf Muskelfasereigenschaften und Zartheit im M. *longissimus lumborum* bei hetero- und homozygot negativen Polnischen Landrasseschweinen**

Vorliegende Arbeit untersucht den Einfluss von Genpolymorphismus im Lokus *RYR1* auf Muskelfasereigenschaften und Zartheit im M. *longissimus lumborum* bei Schweinen. Einbezogen waren 16 heterozygote (Nn) und 24 *RYR1* freie Homozygote (NN) Jungsauen der Polnischen Landrasse. Anhand der Myosin-ATP-ase Aktivität wurden drei Muskelfasertypen (I, IIA und IIB) sowie deren Dicke (Durchmesser) und ihr prozentualer Anteil bestimmt. Weiterhin wurden zwei technologische Merkmale, nämlich der Härtewert und die Schnittstärke, untersucht. Der *RYR1* Genotyp hatte keinen signifikanten Einfluss auf den Fasertypenanteil, den Härtewert und die Schnittstärke. Signifikant wurde durch das Gen dagegen die Muskelfaserdicke beeinflusst. Im Vergleich zu den NN Tieren wiesen die Nn Tiere signifikant dickere Muskelfasern bei allen Typen auf. Die phänotypischen Korrelationskoeffizienten zwischen den histologischen Merkmalen und den Qualitätseigenschaften des Schweinefleisches waren sehr niedrig. Der Härtewert und die Schnittstärke zeigten positive Beziehungen zum Muskelfaseranteil des Typs IIB, dagegen negative zum Typ I. Andererseits ergaben sich bei der Anzahl der Typ I Fasern im Gegensatz zu den IIB Typen positive Korrelationen zu den Fleischqualitätsmerkmalen. Insgesamt waren die Beziehungen zwischen den Anteilen der Muskelfasertypen negativ, jedoch im Gegensatz hierzu die Beziehungen zwischen der Dicke einzelner Fasertypen positiv.

Schlüsselwörter: Schwein, Muskelfaser, *RYR1*, Zartheit, *m. longissimus lumborum*

Introduction

Meat quality describes the attractiveness of meat to consumers. The three major attributes of meat, which affect consumer satisfaction, are tenderness, juiciness and

* This study was funded by the Polish Ministry of Science and Information Society Technologies, grant no. PBZ-KBN-113/P06/2005

flavour. Tenderness is probably the most significant in determining the overall acceptability of meat. Evidence from the literature (KARLSSON et al., 1993; MIGDAŁ et al., 2004) indicates that muscle characteristics and particularly fibre type percentages may be an important source of variation in eating quality. Muscle fibre composition is influenced by environmental factors (GENTRY et al., 2004), nutrition (KARLSSON et al., 1993; MIGDAŁ et al., 2004), exercise (PETERSEN et al., 1998), genetics (BROCKS et al., 1998; WOJTYSIAK and MIGDAŁ, 2006) and their possible interactions. The *m. longissimus* is the most frequently used indicator muscle in meat quality studies in pigs. Fibres of mammalian skeletal muscle have been classified on the basis of various physiological, morphological and biochemical parameters and attempts have been made to make functional correlations. PETER et al. (1972) correlated aerobic-oxidative and glycolytic capacities of muscle fibres to physiological measurements and myosin ATPase activity to give rise to the classification system, slow-twitch oxidative (SO), fast-twitch-oxidative-glycolytic (FOG), and fast-twitch-glycolytic (FG). Using histochemical methods these fibre types are designated as types I, IIA and IIB by BROOKE and KAISER (1970), types βR (red), αR and αW (white) by ASHMORE et al. (1972), respectively. Muscle metabolism is the summation of the activities of the individual muscle fibres, which comprise the muscle.

For many years pig breeding has been very successful in selection for improved lean meat production and reduced fat level. Selection for improved performance of pigs in the fattening period can have a negative influence on meat quality. In the last decades a large number of indicators and tests for stress susceptibility have been developed. One of the major genes affecting meat quality traits is the halothane sensitivity gene (*HAL*), known since 1991 as mutated ryanodine receptor gene (*RYR1*) (FUJII et al., 1991). Three genes, *i.e. RYR1*, *RYR2*, and *RYR3*, have been identified coding for a skeletal muscle, cardiac muscle, and brain isoform, respectively. The skeletal muscle isoform (*RYR1*) is one of the key proteins involved in excitation-contraction (E-C) coupling in skeletal muscle, where it functions as a Ca^{2+} release channel in the sarcoplasmic (SR) membrane. This can lead to rapid glycogenolysis immediately postmortem while the carcass is cooling, resulting in myosin denaturation, loss of water-holding capacity, and inferior meat quality (WARNER et al., 1997). There is no doubt that a very close causal relation exists between the mutation of the *RYR1* and the porcine stress syndrome as well as the poor meat quality. Moreover, ryanodine receptor *RYR1* has gained special interest because it was shown to be involved in malignant hyperthermia. In stress susceptible pigs, a higher incidence of PSE (pale, soft, exudative) meat was found (O'BRIEN, 1987). Meat from mutant genotypes is often tougher than that from normal animals, which could in part be due to reduced postmortem proteolysis (MURRAY et al., 1989; LUNDSTROM et al., 1995). Several researches have revealed the effect of stress susceptibility on meat quality and muscle fibre traits (ESSEN-GUSTAVSSON and FJELKNER-MODIG, 1985; FIEDLER et al., 1993, 1999), whereas others reported no differences in muscle fibre characteristics (ACKERMAN and SALOMON, 1991).

Therefore, the objective of this study was to examine a relation between genotypes at the *RYR1* loci and muscle fibre traits and meat tenderness of *m. longissimus lumborum* in Polish Landrace fatteners.

Material and methods

Animals

The study was carried out on 40 Polish Landrace gilts. Animals were fed *ad libitum* and slaughtered at 105 kg body weight. Pigs were transported 20 km to a commercial slaughterhouse and lairaged overnight before slaughter. Feed was withdrawn 12 h before slaughter but water was freely available in lairage. Fatteners were stunned with CO_2 and processed according to the normal slaughterhouse procedures (exsanguinated, scalded, dehaired and eviscerated).

Genotyping of *RYR1* polymorphisms

DNA was isolated from the whole blood drawn into sterile tubes containing EDTA. Isolation was carried out using a MasterPure™ Genomic DNA Purification Kit (Epicentre Technologies, USA), according to manufacturer's instructions. Polymorphisms at the porcine loci *RYR1* were identified using the PCR-RLFP method described by KAMIŃSKI et al. (2002). On this basis the pigs were assigned to the two groups: 16 heterozygous (Nn) and 24 RYR(1)-free (NN).

Histochemical muscle fibre analysis

For histochemical analysis muscle samples were taken from the right carcass-side from the *m. longissimus lumborum* (LL) 20-min postmortem at the level of the 5th lumbar vertebra and deep within the muscle. Samples were immediately cut into 1×1×1-cm pieces (parallel to the muscle fibres) and frozen in isopentane that was cooled using liquid nitrogen and stored at -80ºC until histochemical analyses were performed. Samples were mounted on a cryostat chuck with a few drops of tissue-freezing medium (Tissue-Tek; Sakura Finetek Europe, Zoeterwoude, The Netherlands). Serial tissue sections (10-µm thick) obtained from cryostat (Slee MEV, Germany) sectioning at -20ºC were stained for myosin ATP-ase after both acid (pH 4.6) and alkaline (pH 10.3) preincubation at room temperature. The slow-twitch-oxidative type I fibres stained dark in the sections preincubated under acidic conditions whilst the fast-twitch-glycolytic type IIB fibres and fast-twitch-oxidative and glycolytic type IIA fibres appeared light under microscopy. Preincubation under alkaline conditions has the inverse effect, namely type I fibres appeared light but type IIB fibres dark and type IIA fibres intermediate (BROOKE and KAISER, 1970; GUTH and SAMAHA, 1970). The Multi Scan v. 14.02 computer image analysis software was used to evaluate percentage and muscle fibre diameter. A minimum of 300 fibres was counted in each section. Additionally, phenotypic correlation between muscle fibres traits and meat tenderness was estimated.

Shear force and hardness measurement

Meat samples for analysis were taken from right half-carcasses after 24 h cooling at 4°C. For analysis of Warner-Bratzler shear force and hardness chops were roasted at 180°C to reach an internal temperature of 78°C and then cooled to room temperature. Next ten cores, 14 mm in diameter, were taken from each chop parallel to the muscle fibre orientation. Shear force was measured using a Texture Analyser TA-XT2 (Stable-Micro Systems, UK) with Warner-Bratzler unit and with a triangular blade. The cores for hardness determination were doubly compressed by a cylinder (SMS P/25, base diameter 50 mm) to 70% of their height, at the rate of 2 mm/s and with a 3-

second break between the storage of compression. Hardness was analysed using a Texture Analyser TA-XT2 (Stable-Micro Systems, UK).

Statistical analysis

Differences between the examined groups in histochemical characteristics of the muscle fibres were tested using analysis of variance by Statgraphics 5.0 (STSC Inc., Rockville, MD) and tested for differences by the Tukey test. Body weight at slaughter was included as a covariant. Differences were considered significant at $P \leq 0.05$.

Results and Discussion

In the present study the effect of genotype at *RYR1* loci was analysed in relation to meat tenderness and muscle fibre traits in LL muscle. The microstructural characteristics of fibre types and traits of tenderness from LL muscle in both genotype groups are presented in Table 1.

Table 1
Percentage, diameter of muscle fibre type (I, IIA and IIB), and hardness and Warner-Bratzler shear force of *m. longissimus lumborum* from two examined genotype groups of fatteners

traits	group NN $\bar{x}$	group Nn $\bar{x}$	SE	Sign. level
Percentages of muscle fibres [%]				
IIB	72.48	75.57	1.64	Ns
IIA	11.21	10.16	0.68	Ns
I	16.31	14.27	0.56	Ns
Diameter of muscle fibre [µm]				
IIB	76.96	84.67	1.24	*
IIA	57.12	64.21	1.19	*
I	52.92	61.03	1.02	*
Shear force [kg/cm^2]	4.54	5.24	0.67	Ns
Hardness [N]	130.42	132.05	7.26	Ns

* significant at $P \leq 0.05$
Ns – not significant

There was no significant effect of *RYR1* on percentages of all examined muscle fibre types. However, in the heterozygous Nn fatteners a tendency towards an increased number of type IIB fibres, and a declining number of type I fibres compared to homozygous NN fatteners were observed. A similar tendency was observed in earlier studies. FIEDLER et al. (1999) showed that pigs which were homozygous for the malignant hyperthermia mutation nn had significantly more fast-twitch-glycolytic fibres than pigs heterozygous Nn or without the mutation NN, between which no significant differences were observed. Moreover, the present study showed a significant difference between the examined group of fatteners in the size of muscle fibres. The LL muscle of the Nn fatteners had significantly larger diameter of all examined muscle fibre types compared to the muscle from group NN. The fact that *RYR1* genotype had an effect on fibre size confirms the earlier results of FIEDLER et al. (1993, 1999) and KŁOSOWSKA et al. (2004). KŁOSOWSKA et al. (2005) also observed an effect of the *RYR1* genotype on muscle microstructure characteristics, but only on the diameter of fast-twitch-glycolytic fibres. FIEDLER et al. (1999) suggested that the differences in fibre diameter indicate that the muscle growth in LL muscle in pigs carrying the mutant Nn allele is more marked than in the NN pigs. On the other

hand, ACKERMANN and SALOMON (1991) found no major differences in muscle fibre characteristics between normal and malignant hyperthermia susceptible pigs.
GENTRY et al. (2004) suggest that the presence or lack of differences in fibre type composition observed in literature may be affected by a lack of uniformity in the classification of muscle fibre types, especially type II, and additionally, by different histochemical methods used for the identification of muscle fibre types. It is also possible that some of the controversial data found in literature result not only from different methods of muscle fibre classification but from the fact that the physiological differentiation of muscle fibres is in a dynamic balance, which may change during growth or in response to changes in the work pattern of a muscle (SWATLAND, 1994). Variation in the fibre composition may be connected with transformation of fibre type I and IIA, being dependent on oxygenic metabolism, into type IIB fibres dependent on glycolytic metabolism. Moreover, it is also possible that variation in the fibre composition and size is affected by various examined parts of *longissimus* muscle (MORITA et al., 2000; WOJTYSIAK et al., 2004).
The present study also showed that shear force and hardness were similar for the two-genotype groups of pigs (Tab. 1). However, a tendency towards lower values of shear force and hardness was observed in NN pigs compared with Nn pigs. The lack of effect of the genotype on shear force values between NN pigs and Nn pigs agrees with the observation of LEACH et al. (1996), DE SMET et al. (1996) and MILLER et al. (2000). On the other hand, higher shear force values and lower tenderness panel scores for stress-susceptible pigs were mentioned by MURRAY et al. (1989), BOLES et al. (1991) and HAMILTON et al. (2000). CASTEELS et al. (1995) also concluded that selection against the halothane gene would positively influence sensory meat quality traits. The effect of genotype on meat quality has been described by other authors. MONIN et al. (1999) reported that for most traits of meat quality, the heterozygous were intermediate between the two homozygous, but they were closer to NN than to nn pigs. The presence (or lack) of differences in meat tenderness probably resulted not only from differences in muscle fibre composition but also are connected with numerous factors (such as collagen content/cross-linking and sarcomere length), which contribute to the tenderness of the LL muscle (WHEELER et al., 2000).

Table 2
Phenotypic correlations between hardness, shear force and *longissimus lumborum* muscle fibre characteristics

	Percentage (%)		Diameter Ø			Shear force	Hardness
	IIA	I	IIB	IIA	I		
% IIB	-0.88*	-0.80*	ns	ns	ns	0.01*	0.05*
% IIA		ns	ns	ns	ns	ns	ns
% I			ns	ns	ns	-0.08*	-0.06*
Ø IIB				0.82**	0.77**	-0.01*	-0.04*
Ø IIA					0.86**	ns	ns
Ø I						0.06*	0.09*

* significant at $P \leq 0.05$
** significant at $P \leq 0.01$

Phenotypic correlations between muscle fibre characteristics and meat quality traits are presented in Table 2. The phenotypic correlation between fibre type percentages was significantly negative, whereas correlations between fibre type size were all positive and highly significant. These results confirm the earlier results of LARZUL et al. (1997) and WOJTYSIAK et al. (2004). On the other hand, the correlations between

fibre percentages and diameter were not significant. In contrast, LARZUL et al. (1997) showed low correlations between fibre percentages and fibre size. The phenotypic correlations between muscle fibre traits and hardness and shear force were generally low. Diameters of type I fibres, unlike that of type IIB fibres, positively correlate with hardness and shear force, similarly to the results by CAMERON et al. (1998). Moreover, the present study showed a positive phenotypic correlation between hardness, shear force and percentage of type IIB fibres, in contrast to a negative correlation between hardness, shear force and percentage of type I fibres. This is in accordance with the data of CAMERON et al. (1998). On the other hand, these results still remain open to criticism. In literature, one may find diverse opinions. SAZILI et al. (2005) suggests that exactly how muscle fibre composition and meat tenderness are correlated is unclear and a number of inconsistent reports exist. KARLSSON et al. (1993) in pigs and O'HALLORAN et al. (1997) in cattle have shown that the percentages of type IIB fibre is negatively correlated with toughness. On the other hand, RENAND et al. (2001), in a study on bulls, found a positive correlation between toughness and the proportion of type I fibres. MALTIN et al. (1998), however, supported a positive relationship between the percentage of type I fibres and sensory tenderness and a negative relationship between percentage of type IIB fibres and tenderness. The positive relationships between type I fibres frequency and tenderness is perhaps not surprising. There is evidence that m calpain, a proteolytic enzyme thought to be crucially involved in postmortem proteolysis, is preferentially localized in type I fibres. This is consistent with the high rates of protein synthesis in type I fibres (GARLICK et al., 1989). Hence, an association between type I fibres frequency and tenderness might be predicted (MALTIN et al., 1998).

Conclusion

The genotype of *RYR1* had no effect on muscle fibre percentage and meat hardness and shear force, but influenced the diameter of muscle fibre. The LL muscle of the Nn pigs had significantly larger diameter of all examined muscle fibre types than muscle from NN pigs. Phenotypic correlations between fibre type percentages were negative, in contrast to positive correlations between fibre type diameters. In addition, correlations between fibre type percentages were negative, in contrast to positive correlations between fibre type diameters. On the other hand, correlations between histological and meat quality traits were generally low.

References

ACKERMANN, M.; SALOMON, F.V.:
Histochemische und morphometrische Befunde am m. longissimus des Schweines bei maligner Hyperthermie. Anat. Hist. Embr. **20** (1991), 280-289

ASHMORE, C.R.; TOMPKINS, G.; DOERR L.:
Postnatal development of muscle fibre types in domestic animals. J. Anim. Sci. **34** (1972), 1, 37-41

BOLES, J.A.; PARRISH, F.C.; SKAGGS, C.L.; CHRISTIAN, L.L.:
Effect of porcine somatotropin, stress susceptibility, and final end point of cooking on the sensory, physical, and chemical properties of pork loin chops. J. Anim. Sci. **69** (1991), 2865

BROOKE, M.H.; KAISER, K.:
Muscle fibre type: how many and what kind? Arch. Neurol. **23** (1970), 369-370

BROCKS, L.; HULSEGE, B.; MERKUS, G.:
Histochemical characteristics in relation to meat quality properties in the Longissimus lumborum of fast and lean growing lines of Large White pigs. Meat Sci. **50** (1998), 4, 411-420

CAMERON, N.D.; OKSBJERG, N.; HENCKEL, P.; NUTE, G.R.; BROWN, S.N.; WOOD, J.D.:

Relationships between muscle fibre traits with meat and eating quality in pigs, In: Proceedings of BSAS Annual Meeting, March 1998 Scarborough (1998), 123

CASTEELS, M.; VAN OECKEL, M.J.; BOSCHAERTS, L.; SPINCEMAILLE, G.; BOUCQUÉ, C.V.:
The relationship between carcass, meat and eating quality of three pig genotypes. Meat Sci. **40** (1995), 253

DE SMET, S.M.; PAUWELS, H.; DE BIE, S.; DEMEYER, D.I.; CALLEWIER, J.; EECKHOUT, W.:
Effect of halothane genotype, breed, feed withdrawal, and lairage on pork quality of Belgian slaughter pigs. J. Anim. Sci. **74** (1996), 1854-1863

ESSEN-GUSTAVSSON, B.; FJELKNER-MODIG, S.:
Skeletal muscle characteristics in different breeds of pigs in relation to sensory properties of meat. Meat Sci. **13** (1985), 33-47

FIEDLER, I.; ENDER, K.; WICKE, M.; LENGERKEN, G. v.:
Zusammenhänge zwischen der Mikrostruktur des Muskelgewebes bei Schweinen der Landrasse und ihrer Stressempfindlichkeit (Halothanreaktion). Arch. Tierz., Dummerstorf **36** (1993), 525-538

FIEDLER, I; ENDER, K.; WICKE, M.; MAAK, S.; LENGERKEN, G. v.; MEYER, W.:
Structural and functional characteristics of muscle fibres in pigs with different malignant hyperthermia susceptibility (MHS) and different meat quality. Meat Sci. **53** (1999), 9-15

FUJII, J.; OTSU, K.; ZORZATO, F.; DE LEON, S.; KHANNA, V.K.; WEILER, J.E.; O'BRIEN, P.J.; MAC LENNAN, D.H.:
Identification of a mutation in porcine ryanodine receptor associated with Malignant Hyperthemia. Science **253** (1991), 448-451

GARLICK, P.J.; MALTIN, C.A.; BAILLIE, A.G.; DELDAY, M.I.; GRUBB, D.A.:
Fibre-type composition of nine rat muscles. II. Relationship to protein turnover. Am. J. Physiol. **257** (1989), 828-832

GENTRY, J.G.; McGLONE, J.J.; MILLER, M.F.; BLANTON J.R.:
Environmental effects on pig performance, meat quality, and muscle characteristics. J. Anim. Sci. **82** (2004), 209-217

GUTH, L.; SAMAHA, F.:
Procedure for the histochemical demonstration of actomyosin ATPase. Exp. Neurol. **28** (1970), 365-367

HAMILTON, D.N.; ELLIS, M.; MILLER, D.K.; McKEITH, F.K.; PARRET, D.F.:
The effect of the Halothane and Rendement Napole genes on carcass and meat quality characteristics of pigs. J. Anim. Sci. **78** (2000), 2862-2867

KAMIŃSKI, S.; RUŚĆ, A., WOJTASIK, K.:
Simultaneous identification of ryanodine receptor 1 (*RYR1*) and estrogen receptor (*ESR*) genotypes with the multiplex PCR-RFLP method in Polish Large White and Polish Landrace pigs. J. Appl. Genet. **43** (2002), 3, 331-335

KARLSSON, A.; ENNFALT, A.; ESSEN-GUSTAVSSON, B.; LUNDSTROM, K.; RYDHERM, L.; STERN, S.:
Muscle histochemical and biochemical properties in relation to meat quality during selection for increased lean tissue growth rate in pigs. J. Anim. Sci. **71** (1993), 930-938

KŁOSOWSKA, D.; KURYŁ, J.; ELIMINOWSKA-WENDA, G.; KAPELAŃSKI, W.; WALASIK, K.; PIERZCHAŁA, M.; CIEŚLAK, D.; BOGUCKA J.:
A relationship between the PCR-RFLP polymorphism in porcine *MYOG*, *MYOD1* and *MYF5* genes and microstructural characteristics of *m. longissimus lumborum* in Pietrain x (Polish Large White x Polish Landrace) crosses. Czech J. Anim. Sci. **49** (2004), 99-107

KŁOSOWSKA, D.; KURYŁ, J.; ELIMINOWSKA-WENDA, G.; KAPELAŃSKI, W.; WALASIK, K.; PIERZCHAŁA, M.; CIEŚLAK, D.; BOGUCKA J.:
An association between genotypes at the porcine loci *MSTN (GDF8)* and *CAST* and microstructural characteristics of *m. longissimus lumborum*: a preliminary study. Arch. Tierz., Dummerstorf **48** (2005) 1, 50-59

LARZUL, C.; LEFAUCHEUR, L.; ECOLAN, P.; GOGUE, J.; TALMANT, A.; SELLIER, P.; LE ROY, P.; MONIN, G.:
Phenotypic and genetic parameters for longissimus muscle fibre characteristics in relations to growth, carcass, and meat quality traits in Large White pigs. J. Anim. Sci. **75** (1997), 3126-3137

LEACH, L.M.; ELLIS, M.; SUTTON, D.S.; McKEITH, F.K.; WILSON, E.R.:
The growth performance, carcass characteristics, and meat quality of halothane carrier and negative pigs. J. Anim. Sci. **74** (1996), 934-943

LUNDSTROM, K.; KARLSSON, A.; HAKANSSON, J.; HANSSON, I.; JOHANSSON, M.; ANDERSSON, L.; ANDERSSON, K.:
Production, carcass and meat quality traits of F2-crosses between European Wild Pigs and domestic pigs including halothane gene carriers. Anim. Sci. **61** (1995), 325-331

MALTIN, C.A.; SINCLAIR, K.D.; WARRIS, P.D.; GRANT, C.M.; PORTER, A.D.; DELDAY, M.I.:

The effect of age at slaughter, genotype and finishing system on the biochemical properties, muscle fibre type characteristics and eating quality of bull beef from suckled calves. Anim. Sci. **66** (1998), 341-348

MIGDAŁ, W.; PAŚCIAK, P.; WOJTYSIAK, D.; BAROWICZ, T.; PIESZKA, M.; PIETRAS, M.:
Effect of CLA addition in feed for fatteners for meat quality, eating quality and dietetic value of *m. longissimus dorsi.* Meat Sci. **66** (2004), 863-870

MILLER, K.D.; ELLIS, M.; McKEITH, F.K.; WILSON, E.R.:
Influence of sire line and halothane genotype on growth performance, carcass characteristics, and meat quality in pigs. Can. J. Anim. Sci. **80** (2000), 319-327

MONIN, G.; LARZUL, C.; LE ROY, P.; CULIOLI, J.; MOUROT, J.; ROUSSET-ARKIM, S.; TALMANT, A.; TOURAILLE, C.; SELLIER, P.:
Effects of the halothane genotype and slaughter weight on texture of pork. J. Anim. Sci. **74** (1999), 408-415

MORITA, S.; IWAMOTO, H.; FUKUMITSU, Y.; GOTOH, T.; NISHIMURA, S.; ONO, Y.:
Heterogeneous composition of histochemical fibre types in the different parts of m. longissimus thoracis from Mishima (Japanese native) steers. Meat Sci. **54** (2000), 59-63

MURRAY, A.C.; JONES, S.D.M.; SATHER, A.P.:
The effect of preslaughter feed restriction and genotype for stress susceptibility on pork lean quality and composition. Can. J. Anim. Sci. **69** (1989), 83

O'BRIEN, P.:
Etiopathogenetic defect of malignant hyperthermia: Hypersensitive calcium-release channel of skeletal muscle sarcoplasmic reticulum. Vet. Res. Commun. **11** (1987), 527-559

O'HALLORAN, G.R.; TROY, D.J.; BUCKLEY, D.J.; REVILLE, W.J.:
The role of endogenous proteases in the tenderisation of fast glycolysing muscle. Meat Sci. **47** (1997), 187-210

PETER, J.B.; BARNARD, R.L.; EDGERTON, V.R.; GILLESPIE, C.A.; STEMPEL, K.E.:
Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. Biochem. **11** (1972), 2627-2633

PETERSEN, J.S.; HENCKEL, P.; OKSBJERG, N.; SØRENSEN, M.T.:
Adaptations in muscle fibre characteristics induced by physical activity in pigs. Anim. Sci. **66** (1998), 733-740

RENAND, G.; PICARD, B.; TOURAILLE, C.; BERGE, P.; LEPETIT, J.:
Relationships between muscle characteristics and meat quality traits of young Charolais bulls. Meat Sci. **59** (2001), 49-60

SAZILI, A.Q.; PARR, T.; SENSKY, P.L.; JONES, S.W.; BARDSLEY, R.G.; BUTTERY, P.J.:
The relationship between slow and fast myosin heavy chain content, calpastatin and meat tendernss in different ovine skaletal muscles. Meat Sci. **69** (2005), 17-25

SWATLAND, H.J.:
The conversion of muscle to meat. In: Structure and Development of Meat Animals and Poultry. H. Swatland, ed. Technomic Publishing Co., Lancaster, U.K. (1994), 495

WARNER, R.D.; KAUFFMAN, R.G.; GREASER, M.L.:
Muscle protein changes post mortem in relation to pork quality traits. Meat Sci. **45** (1997), 339-352

WEELER, T.L.; SHACKELFORD, S.D.; KOOHMARAIE, M.:
Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. J. Anim. Sci. **78** (2000), 958-965

WOJTYSIAK, D.; MIGDAŁ, W.:
Morphological characteristics of muscle fibres of m. Longissimus lumborum in crossbreed fatteners with different proportion of Hampshire breed. Polish Journal of Natural Sciences (2006), in press.

WOJTYSIAK, D.; PAŚCIAK, P.; MIGDAŁ, W.; POŁTOWICZ, K.:
Histochemical profile of two parts of *longissimus dorsi* muscle in relation to sex of fatteners. Anim. Sci. Pap. Rep. **22** (2004), 4, 587-593

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Arch. Tierz., Dummerstorf **50** (2007) Special Issue, 194-199

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RLFP/*Hinf*I polymorphism within intron 1 of glutathione-peroxidase-5 (*GPX5*) gene in AI boars

Abstract

GPX5 – one of the components of the enzymatic complex of factors protecting the organism against harmful peroxides – is a seleno-independent isoenzyme of glutathione peroxidase activity, specific to epididymis in male mammals. In pigs, *GPX5* gene locus is located on chromosome 7. *GPX5*/*Hinf*I restriction polymorphism within intron 1 was defined by PCR-RFLP method in 247 AI boars. Allele $GPX5^{1B}$ was found to occur with a frequency of 0.1964, whereas allele $GPX5^{2B}$ occurred with a frequency of 0.8036. In the studied herd of boars, $GPX5^{1B}GPX5^{1B}$ and $GPX5^{1B}GPX5^{2B}$ genotype frequencies were 0.0405 and 0.3117, respectively, whereas $GPX5^{2B}GPX5^{2B}$ genotype frequency was 0.6478. The analysis of associations between *GPX5* genotypes and the examined semen characteristics showed statistically significant associations ($P \leq 0.05$) and the results suggest the possibility of using this gene polymorphism to improve some reproductive traits in AI boars.

Key Words: glutathione-peroxidase-5 gene, *GPX5*, boars, reproduction traits

Zusammenfassung

Titel der Arbeit: **RFLP/Hinfl-Polymorphismus innerhalb des 1. Intron des Glutathion-Peroxydase-5 (*GPX5*) Gens bei Besamungsebern**

GPX5 ist eine der Komponenten des Enzymkomplexes von Faktoren, die den Organismus vor schädlichen Superoxyden schützen. Es ist ein selenunabhängiges, Isoenzym der Glutamin-Peroxydase Aktivität und für die Nebenhoden von Säugetieren spezifisch. Beim Schwein findet sich der Lokus des GPX5 Gens im 7. Chromosom. Der *GPX5* Restriktions Polymorphismus des 1. Introns wurde mit der PCR-RFLP-Methode bei 247 Besamungsebern mehrerer Rassen bestimmt. Es konnten die zwei Allele $GPX5^{1B}$ und $GPX5^{2B}$ mit den Frequenzen von 0,1964 bzw. 0,8036 identifiziert werden. In der untersuchten Eberpopulation traten die Genotypen mit folgenden Frequenzen auf: $GPX5^{1B}$ $GPX5^{1B}$ – 0.0405, $GPX5^{1B}$ $GPX5^{2B}$ – 0,3117 und $GPX5^{2B}$ $GPX5^{2B}$ – 0,678. Die Analyse ergab statistisch signifikante Abhängigkeiten zwischen den GPX5 Genotypen und den Spermamerkmalen und damit die Möglichkeit diesen Genpolymorphismus für die Beurteilung einiger Spermamerkmale von Besamungsebern zu nutzen.

Schlüsselwörter: Glutathion-Peroxydase-5-Gen, *GPX5,* Eber, Spermamerkmale

Introduction

Pork production profitability depends largely on the possibility to obtain numerous offspring from each sow in successive litters. Studies carried out by many research centres suggest that certain genes can be used as markers of reproductive traits in pigs, which may have practical application in early selection aimed at increasing the effectiveness of sow insemination and the number of piglets per litter. Both these parameters depend on, among others, the number of spermatozoa in a boar's ejaculate which are capable of interacting with egg cells. The percentage content of live, fully-developed spermatozoa per ejaculate is one of the criteria for a boar's reproductive utility.

In the reproductive systems of male mammals, there are many factors which have a negative effect on spermatozoa maturation, vitality and motility. The factors include

active oxidants – ROS (reactive oxygen species) and free radicals, which increase cell-membrane permeability and cause sperm immotility, which in turn leads to a decreased fertilization capability. Epididymis cells contain an enzymatic complex consisting of catalase, glutathione peroxidase (*GPX*) / glutathione reductase and other proteins capable of deactivating oxidizers (HALL et al., 1998). *GPX5*, one of the components of the complex, is a seleno-independent isoenzyme of glutathione peroxidase activity, specific to epididymis. It was proved (OKAMURA et al., 1997) that in the boar epididymis, *GPX5* protein binds to the spermatozoon acrosome and disappears during the acrosomal reaction. Moreover, *GPX5* protein was found to delay this reaction considerably when induced *in vitro*. It was suggested that *GPX5* gene product plays a role in preventing spermatozoa from premature acrosomal reaction and therefore maintains the fertilization capability of spermatozoa collected in the epididymis. *GPX5* may also be of importance for the correct process of sperm cell maturation (SCHWAAB et al., 1998).

GPX5 gene locus is located on chromosome 7, along with the genes of the major histocompatibility complex (SLA) (BERTANI et al., 1999). Within the partial *GPX5* gene sequence (2,849 base pairs) that is known, there occurs a mutation in the gene intron recognized by *Hinf*I restriction enzyme. Basing on this, two *GPX5* gene alleles can be identified: 1B and 2B (BERTANI et al., 1999). As the *GPX5* gene mutation in humans is known to cause a considerable decrease in fertility (HALL et al., 1998), it can be presumed that *GPX5/Hinf*I polymorphism in boars is likely to be linked to their lowered reproductive capability (decreased sperm quality and vitality, lowered egg-cell fertilization capability).

The aim of this study was to identify *GPX5/Hinf*I polymorphism in AI boars kept in Animal Breeding and Artificial Insemination Centres in Poland as well as to find possible associations between the identified *GPX5* genotypes and selected reproductive traits in boars.

Materials and methods

The study included 247 AI boars (Polish Landrace – 60, Polish Large White – 41, Pietrain – 15, Hampshire – 3, Duroc – 4, Duroc x Pietrain – 53, Hampshire x Pietrain – 34, Duroc x Hampshire – 8, Polish Synthetic Line – 10, PIC – 19) bred in Animal Breeding and Artificial Insemination Centres in Poland (Table 1.). All the boars were kept in the same conditions and used exclusively for reproductive purposes. The data on 9,456 ejaculates used in this study was collected from the 247 boars in the years 1997 – 2002 and concerned the following semen characteristics: ejaculate volume, sperm concentration, percentage of live sperm, live sperm count per ejaculate and number of insemination doses. The ejaculates were collected from boars aged 221 – 585 days to eliminate the effect of age on the characteristics under study. The semen collection period was divided into two seasons: the spring-summer season (1 April to 30 September) and the autumn-winter season (1 October to 30 March). The season as well as the year, breed and sire effect were taken into account while determining variability.

The DNA used in this study was isolated from all the peripheral blood samples collected into vacuum test tubes containing K_3EDTA as an anticoagulant. The isolation

was performed using the Master Pure^{TM} Genomic DNA Purification Kit from Epicentre Technologies.

Glutathione-peroxidase-5 genotypes were determined by PCR-RFLP method. The mutation located within introne 1 of the *GPX5* gene was detected by using specific starter sequences (forward primer: 5' – TTC ATG TAG AAC TTA TTT CTG – 3' and reverse primer: 5' – TGG TGC CTG TCA CGT CTT – 3') and an appropriate thermal profile (94°C/3 min., 40 x (94°C/40 sec., 52°C/45 sec., 72°C/2.5 min.) + 72°C/5 min.) for the PCR reaction. The amplified fragment was digested with 5 units of restriction enzyme *Hinf*I at 37°C for 4-5 hours. The obtained DNA restriction fragments were separated by electrophoresis on 2% agarose gels containing ethidium bromide in buffer 1xTBE. Afterwards, the gels were visualized and analysed under UV light and recorded using the Vilber Lourmat system.

Statistical analysis of the associations of concern was carried out with the SAS package (General Linear Model Procedure – the SAS System) according to the following model:

$$Y_{ijklm} = \mu + M_i + O_j + R_k + Z_l + Y_m + S_n + (ZSR)_{lmn} + W_S + G/H_t + e_{ijklmnstw}$$

where: Y_{ijklm} – observation; μ - mean for the herd; M_i – effect of *i*th dam; O_j – effect of *j*th sire; R_k – fixed effect of *k*th boar breed (k = 1, 2, ... , 7); Z_l – effect of *l*th insemination centre (l = 1, 2); Y_m – effect of *m*th year (m = 1, 2, ... , 6); S_n – effect of *n*th season (n = 1, 2); $(ZSR)_{lmn}$ – interaction effect of appropriate model effects; W_s – effect of *s*th boar's age; G/H_t – effect of *t*th genotype *GPX5/Hinf*I (t = 1, 2 and 3); $e_{ijklmnstw}$ – error.

The results are presented in tables, including mean and standard deviations, and the number of ejaculates under study. The significance of differences, verified with Duncan test, is also included.

Results and Discussion

The PCR product was digested with endonuclease *Hinf*I and the restriction fragments obtained thereby were subsequently separated on 2% agarose gels and thus different band patterns were obtained, which enabled to identify two alleles, *GPX5*^{1B} and *GPX5*^{2B}, which determined the occurrence of three genotypes: *GPX5*^{1B}*GPX5*^{1B}, *GPX5*^{1B}*GPX5*^{2B} and *GPX5*^{2B}*GPX5*^{2B}. The frequencies of the identified genotypes and *GPX5/Hinf*I alleles in the herd of AI boars under study are presented in Table 1. The frequency of allele *GPX5*^{1B} in the herd was 0.1964, whereas the frequency of allele *GPX5*^{2B} was 0.8036. A similar allele frequency in another herd of AI boars had been reported previously by MACKOWSKI et al. (2002): 0.28 for allele *GPX5*^{1B} and 0.72 for allele *GPX5*^{2B}. As can be seen in Table 1, the frequency of particular *GPX5/Hinf*I alleles varies from breed to breed. Allele *GPX5*^{1B} frequency ranged from 0.3235 in Hampshire x Pietrain crossbred boars to 0.125 in Duroc boars. MACKOWSKI et al. (2002) had reported a considerably higher frequency of allele *GPX5*^{1B} in Duroc and Hampshire AI boars (0.50 and 0.63, respectively), and the same frequency in Duroc x Hampshire crossbred boars (0.25). The analysis of glutathione-peroxidase-5 genotypes frequency in the studied herd of boars showed that the most frequent genotype was *GPX5*^{2B}*GPX5*^{2B} with a frequency of 0.6478. *GPX5*^{1B}*GPX5*^{2B} genotype frequency was 0.3117, whereas *GPX5*^{1B}*GPX5*^{1B} genotype frequency was 0.0405. MACKOWSKI et al. (2002) had reported a considerably lower frequency of *GPX5*^{2B}*GPX5*^{2B} genotype (0.51) and a higher frequency of *GPX5*^{1B}*GPX5*^{2B} genotype (0.46). Particularly worth

noting is the low frequency of *GPX5*^{1B}*GPX5*^{1B} genotype, which was found only in Polish Large White boars (0.0731) and Hampshire x Pietrain crossbred boars (0.2059). The heterozygous *GPX5*^{1B}*GPX5*^{2B} genotype was most frequent in Duroc x Pietrain crossbred boars (0.7358), and least frequent in Hampshire x Pietrain crossbred boars (0.2353). The frequencies of heterozygous genotypes determined by MACKOWSKI et al. (2002) had been different: this genotype had been found to be most frequent in Duroc (1.00) and Polish Large White (0.756) boars, and least frequent in Pietrain boars (0.16). The homozygous *GPX5*^{2B}*GPX5*^{2B} genotype was most frequent in Duroc boars (0.75) and least frequent in Duroc x Pietrain crossbred boars (0.2642).

Table 1
The frequency of *GPX5*/*Hinf*I genotypes and alleles of boars under study

Breed	Number of boars in breed groups	Genotyp *GPX5*			*GPX5* allele	
		GPX5^{1B}*GPX5*^{1B}	*GPX5*^{1B}*GPX5*^{2B}	*GPX5*^{2B}*GPX5*^{2B}	*GPX5*^{1B}	*GPX5*^{2B}
Polish Landrace	60	-	0.3167	0.6833	0.1583	0.8417
Duroc x Pietrain	53	-	0.7358	0.2642	0.1321	0.8679
Polish Large White	41	0.0731	0.3171	0.6098	0.2317	0.7683
Hampshire x Pietrain	34	0.2059	0.2353	0.5588	0.3235	0.6765
PIC	19	-	0.3684	0.6316	0.1842	0.8158
Pietrain	15	-	0.4000	0.6000	0.2000	0.8000
Polish Synthetic Line	10	-	0.4000	0.6000	0.2000	0.8000
Duroc x Hampshire	8	-	0.5000	0.5000	0.2500	0.7500
Duroc	4	-	0.2500	0.7500	0.1250	0.8750
Hampshire	3	-	0.3333	0.6667	0.1667	0.8333
Total	247	0.0405	0.3117	0.6478	0.1964	0.8036

No statistically significant differences were found in the studied herd of AI boars between the observed distributions and the expected ones estimated theoretically for *GPX5/Hinf*I genotypic groups according to the Hardy-Weinberg law.

Associations were analysed between *GPX5/Hinf*I polymorphism and the following semen characteristics: ejaculate volume, sperm concentration, percentage of live sperm per ejaculate and number of insemination doses per ejaculate. The mean ejaculate volume in the herd of boars under study was 219.2 cm^3 and it was lower than the mean ejaculate volume recorded by MACKOWSKI et al. (2002). Ejaculates of the highest volume were obtained from *GPX5*^{1B}*GPX5*^{2B} boars (225.2 cm^3), ejaculates of statistically significantly lower volume were obtained from *GPX5*^{2B}*GPX5*^{2B} boars, whereas ejaculates of the lowest volume came from *GPX5*^{1B}*GPX5*^{1B} boars. All the differences in ejaculate volumes between boars of different *GPX5/Hinf*I genotypes were confirmed statistically (Table 2). The mean sperm concentration in the studied ejaculates was 598.4 x 10^6/cm^3. The sperm concentration recorded in ejaculates of *GPX5*^{2B}*GPX5*^{2B} boars (604.5 x 10^6/cm^3) was higher than both the mean for the herd

under study and the mean for boars of the other *GPX5/Hinf*I genotypes (*GPX5*^{1B}*GPX5*^{2B} – 587.6 x 10^6/cm^3 and *GPX5*^{1B}*GPX5*^{1B} – 601.2 x 10^6/cm^3). The differences were confirmed statistically (Table 2.). A slightly lower sperm concentration in ejaculates had been reported by MACKOWSKI et al. (2002) in *GPX5/Hinf*I genotypic groups (445 x 10^6 for *GPX5*^{1B}*GPX5*^{2B} boars and 411 x 10^6 for *GPX5*^{2B}*GPX5*^{2B} boars), and the differences had been confirmed statistically. On the other hand, in their analysis of associations between *GPX5/Hinf*I polymorphism and Hampshire x Pietrain boar semen characteristics, KMIEĆ et al. (2002) had found the highest sperm concentration in the ejaculates of *GPX5*^{1B}*GPX5*^{2B} boars (634.1 x 10^6), and the lowest in the ejaculates of *GPX5*^{2B}*GPX5*^{2B} boars (604.7 x 10^6). The differences between the boars of different *GPX5/Hinf*I genotypes had also been confirmed statistically with $P \leq 0.05$.

Table 2
Values of studied semen traits in reference to *GPX5*/*Hinf*I genotype

Character		*GPX5*/*Hinf*I genotype			Total	
		GPX5^{1B}*GPX5*^{1B}	*GPX5*^{1B}*GPX5*^{2B}	*GPX5*^{2B}*GPX5*^{2B}		
Number of ejaculate		288	3363	5805	9456	
Ejaculate volume [cm^3]	Mean SD	204.0^{BC} 64.8	225.2 ^{AB} 84.9	216,6^{AC} 74.2	219.2	78.1
Sperm concentration [mln/cm^3]	Mean SD	601.2^{B} 123.5	587.6^{AB} 121.8	604.5^{A} 125.0	598.4	124.1
Sperm alive percentage	Mean SD	70.8^{BC} 2.8	72.9^{AB} 5.2	72.2^{AC} 4.5	72.4	4.7
Live sperm count in ejaculate [mld]	Mean SD	83.6^{AB} 28.2	93.5^{A} 31.7	90.8^{B} 30.3	91.5	32.1
Number of insemination doses	Mean SD	23.3^{AB} 7.5	24.4^{B} 9.4	24.1^{A} 8.1	24.2	8.6

Means in rows marked with the same letter differ significantly at $P \leq 0.05$.

The analysis of associations between *GPX5/Hinf*I genotypes and the mean percentage of live sperm per ejaculate shows that the mean percentage in the semen of *GPX5*^{1B}*GPX5*^{2B} boars was significantly higher than in the semen of *GPX5*^{1B}*GPX5*^{1B} boars and significantly higher than in the semen of *GPX5*^{2B}*GPX5*^{2B} boars. The differences were confirmed statistically (Table 2.). Similarly, the highest live sperm count per ejaculate was recorded in the semen of heterozygous *GPX5/Hinf*I boars. That count was significantly higher than the one in the ejaculates of *GPX5*^{1B}*GPX5*^{1B} boars. These significant differences prove the associations previously found but not statistically confirmed by KMIEĆ et al. (2002) in their study on Hampshire x Pietrain crossbred boars.

The lowest number of insemination doses was obtained from the ejaculates of *GPX5*^{1B}*GPX5*^{1B} boars, whereas the highest – from the ejaculates of boars with heterozygous *GPX5/Hinf*I genotype, and the difference was confirmed statistically (Table 2.). A statistically significantly higher number of insemination doses was also obtained from the ejaculates of *GPX5*^{2B}*GPX5*^{2B} boars than from the ejaculates of *GPX5*^{1B}*GPX5*^{1B} boars. A slightly lower number of insemination doses (23.4) obtained from the ejaculates of Hampshire x Pietrain crossbred boars had been reported previously in the study by KMIEĆ et al. (2002). The lowest number of insemination doses obtained in that study had been recorded in heterozygous *GPX5*/*Hinf*I boars. However, the differences had not been confirmed statistically.

Conclusions

The study carried out on a herd of AI boars demonstrated the existence of *GPX5/Hinf*I polymorphism. Two alleles were identified: $GPX5^{1B}$ with a frequency of 0.1964 and $GPX5^{2B}$ with a frequency of 0.8036. The alleles were found to determine the occurrence of three genotypes: $GPX5^{1B}GPX5^{1B}$ with a frequency of 0.0405, $GPX5^{1B}GPX5^{2B}$ with a frequency of 0.3117 and $GPX5^{2B}GPX5^{2B}$ with a frequency of 0.6487. The analysis of *GPX5/Hinf*I genotypic groups in the herd of AI boars under study did not show any disruption of genetic equilibrium between the observed distributions and the expected ones estimated theoretically according to the Hardy-Weinberg law.

The analysis of associations between *GPX5/Hinf*I genotypes and the examined quantitative and qualitative characteristics of semen suggest the possibility of using the existing polymorphism to improve reproductive traits in AI boars as heterozygous $GPX5^{1B}GPX5^{2B}$ boars were found to produce ejaculates of the greatest volume, the highest percentage of live sperm and the highest live sperm count per ejaculate, and from which the greatest number of insemination doses was obtained.

References

BERTANI, G.R.; MARKLUND, S.; HU, Z.L.; ROTHSCHILD, M.F.:
Mapping of the Glutathione-peroxidase-5 (GPX5) gene to pig chromosome 7. J. Anim. Sci. **77** (1999), 2855-2856

HALL, L.; WILLIAMS, K; PERRY, A.; FRAYNE, J.; JURY, J.A.:
The majority of human glutathione peroxidase type 5 (GPX5) transcripts are incorrectly spliced: implication for the role of GPX5 in the male reproductive tract. Biochem. J. **333** (1998), 5-9

KMIEĆ, M.; DYBUS, A.; KONIK, A.:
Polymorphism of Glutatione Peroxidase 5 (*GPX5*) gene and its effect on some semen characters in boars. J. Anim. Sci. Suppl. 2 (2002), 123-126

MACKOWSKI, M.; ŚWITOŃSKI, M.; MACKOWSKA, J.:
Polymorphism of the PGX-5 gene and characteristics of boar semen. Arch. Tierz., Dummerstorf **47** (2004) 2, 165-171

OKAMURA, N,; IWAKI, Y.; HIRAMOTO, S.; TAMBA, M.; BANNAI, S.; SUGITA, Y.; SYNTIN, P.; DACHEUX, F.; DACHEUX, J.L.:
Molecular cloning and characterization of the epididymis-specyfic glutathione peroxi dase-like protein secreted in the porcine epidymal fluid. Biochim. Biophys. Acta 1997 Jul 19; 1336 (1), 99-109

SCHWAAB, V.; LAREYRE, J.J.; VERNET, P.; PONS, E.; FAURE, J.; DUFAURE, J.P.; DREVET, J.R.:
Characterization, regulation of the expression and putative roles of two glutathione peroxidase proteins found in the mouse epididymis. J. Reprod. Fertil. Suppl. **53** (1998), 157-162

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Arch. Tierz., Dummerstorf **50** (2007) Special Issue, 200-204

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Restriction polymorphism *FABGL/Bbv*I in herd of sows derived from crossing Polish Large White x Polish Landrace breeds

Abstract

The *FABGL* gene encodes a nicotinamide adenine dinucleotide (NAD)-dependent 17β-hydroxysteroid dehydrogenase (17b-HSD) which is responsible mainly for immune response. It can also regulate the concentration of biologically active estrogens and androgens. It is appear as oxidative enzyme that inactivates estradiol, testosterone, and dihydrotestosterone as well as reductive enzyme by synthesis of estradiol from estrone. Investigations showed that *FABGL* gene is expressed within the ovaries and testes. Different variants of this gene may be associated with reproduction traits in pigs. The aim of this study was to determine polymorphism in the promoter region of *FABGL* gene as well as examine associations between particular genotypes and reproduction traits in Polish Large White x Polish Landrace sows. Polymorphism in *FABGL/Bbv*I was determined by applying *PCR-RFLP* and following frequency of alleles were obtained: *A* - 0.45, *B* - 0.55. Statistical analysis showed associations ($P \leq 0.05$, $P \leq 0.01$) between particular genotypes and some reproduction traits in investigated herd of sows.

Key Words: *FABGL* gene, sows, polymorphism, reproduction traits

Zusammenfassung

Titel der Arbeit: **Restriktionspolymorphismus des *FABGL/Bbv*I Gens bei Kreuzungssauen aus Große Polnische Weiße x Polnische Landrasse**

Das *FABGL*-Gen verschlüsselt die NAD-abhängige 17ß-Hydroxysteroid Dehydrogenase (17b-HSD), welche hauptsächlich für die Immunantwort verantwortlich ist. Da sie ein Oxydationsenzym ist, wird auch mit deren Hilfe die Konzentration des biologisch aktiven Östrogens und Androgens reguliert. Untersuchungen haben ergeben, dass das *FABGL*-Gen einer Expression in den Eierstöcken und Hoden unterliegt. Verschiedene Varianten dieses Gens könnten also mit dem Niveau von Wurfleistungsmerkmalen beim Schwein verbunden sein. Das Ziel vorliegender Arbeit war die Bestimmung des Polymorphismus im promotorischen Teil des *FABGL*-Gens sowie die Untersuchung von Zusammenhängen zwischen den einzelnen Genotypen und den Wurfleistungsmerkmalen bei Kreuzungssauen der Rassen Große Polnische Weiße x Polnische Landrasse. Bei Anwendung der *PCR-RFLP*-Technik konnte der *FABGL7Bbv*I-Polymorphismus bestimmt und Zusammenhänge zwischen den Genotypen und einzelnen Wurfleistungsmerkmalen festgestellt werden.

Schlüsselwörter: *FABGL*-Gen, Sau, Polymorphismus, Wurfleistungsmerkmale

Introduction

Steroid hormones act through specific receptors effect activation of gene transcription. The biological activity of these hormones is regulated at the pre-receptor level (MINDNICH et al., 2004). Several enzymes are committed in this process. Nicotinamide adenine dinucleotide (NAD)-dependent 17β-hydroxysteroid dehydrogenases (17β-HSD) are key enzymes acting in the last step of formation of androgens and estrogens. Eleven 17β-HSDs coded by different not homologous genes have been discovered, which vary in tissue distribution, catalytic preferences, substrate specificity, subcellular localization and mechanism of regulation. These enzymes are mainly involved in conversion at position 17 of sex steroids. They can also metabolize

different substrates including alcohols, bile acids, fatty acids and retinols (ADAMSKI and JAKOB, 2001).
17β-hydroxysteroid dehydrogenase VIII (17β-HSD8) protein efficiently catalyses the oxidation of estradiol, testosterone and dihydrotestosterone leading to their inactivation. It also appear reductive activity by synthesis of estradiol from estrone (FOMITCHEVA et al., 1996). Gene which codes this enzyme is known as *FABGL* gene as well as *KE6* or *RING2* gene. In swine it was assigned to chromosome 7. within the class II region of pig major histocompatibility complex. It is called *SLA* and located on the long arm in 7q1.1 (CHARDON et al., 1999). *FABGL* gene consist of nine exons with 86% and 82% homology to human and mouse mRNA sequences respectively (JACOBS et al., 2002). FOMITCHEVA et al. (1996) have demonstrated that *FABGL* gene is expressed within the ovaries and testes maintaining local level of sex steroids. Polymorphisms in this gene may be associated with the fertility and lipid metabolism in swine.
The aim of this study was to determine polymorphism in the promoter region of *FABGL* gene discovered by JACOBS et al., (2002) as well as examine associations between particular genotypes and reproduction traits in Polish Large White x Polish Landrace sows.

Materials and methods

Investigation were carried out on herd 305 sows derived from crossing Polish Large White x Polish Landrace breeds. Conditions of rearing and feeding were equalized for all animals. DNA was extracted from whole blood by using standard kit (MasterPure™ DNA Purification Kit for Blood, Epicentre). Primers for PCR reaction were designed according to JACOBS et al., (2002). To estimate optimal annealing temperature gradient thermocycler was used (TGradient, Biometra). PCR was performed for each sample in total volume 15µl contains: 1xPCR buffer (C), 2mM $MgCl_2$, 0.2mM dNTP mix, 15pmol of each primer, 0.75U Taq (Eurx), about 80ng of DNA, PCR grade water up to 15µl. Following thermal profile was applied: initial denaturation at 94°C for 5min, 35 cycles: 94°C/45s, 69°C/50s, 72°C/40s and the final extension at 72°C for 5min. 5µl of PCR product was checked on 1% agarose gel staining with ethidium bromide. After positive estimation it was digested by using 1U of *Bse*XI (*Bbv*I) restriction enzyme in 65°C overnight. Restriction fragments were separated on 2% agarose gels. To visualization and record gels Vilber Lourmat system was used.
The analysis of associations between *FABGL/Bbv*I genotypes and total number born (TNB), number of weaned (NW) and number falls (NF) were performed applying following models according to parity order:
first parity (I)
$Y_{ijk} = \mu + a_i + c_j + d_k + b(av) + e_{ijk}$
second and remaining (>I)
$Y_{ijk} = \mu + a_i + c_j + d_k + e_l + b(av) + e_{ijk}$
where:
Y_{ijk} - analyzed trait
μ - the overall mean
a_i - constant effect of *FABGL/Bbv*I genotype (i = 1, 2, 3)
c_j - fixed effect of sire (j = 1, ...38)

d_k - fixed effect of year-season (k = 1,…14)
e_l - fixed effect of parity order (l = 2, …14)
b(av) - the regression of age at first farrowing/value of trait
e_{ijk} - the random error

Results

Two alleles of *FABGL* gene were identified in investigated herd of sows: *A* and *B*. Presence of three genotypes: *AA*, *AB*, *BB* were confirmed. The following lengths of restriction fragments were observed: *AA* - 273bp, *AB* - 273, 150, 123bp, *BB* - 150, 273bp. Table 1 presents obtained frequency of alleles and genotypes of *FABGL/Bbv*I. The analysis of associations between polymorphism at *FABGL/Bbv*I locus and reproduction traits is showed in Table 2.

Table 1
Frequency of the *FABGL/Bbv*I alleles and genotypes

N	*FABGL/Bbv*I genotypes	Frequency	*FABGL/Bbv*I alleles	Frequency
59	*AA*	0.19	*A*	0.45
158	*AB*	0.52		
88	*BB*	0.29	*B*	0.55

Table 2
Means and standard deviations of investigated traits in relation to *FABGL/Bbv*I genotypes

*FABGL/Bbv*I genotypes	Litter	n	TNB[1]	NW[2]	NF[3]
AA	I	55	8.96±2.03	7.76±1.92	1.091±0.369
AB		137	8.93±2.21	7.73±1.83	1,022±0.426
BB		75	8.68±2.15	7.72±2.06	0,027±0.162
Total		267	8.87±2.15	7.73±1.91	0.713±0.319
AA	>I	233	10.32±2.54^{A}	8.80±2.16^{aB}	0.537±0.770
AB		660	10.00±2.43	8.45±2.06^{a}	0.441±0.705
BB		435	9.77±2.58^{A}	8.39±2.22^{B}	0.375±0.820
Total		1328	9.98±2.51	8.49±2.13	0.451±0.765

Means in lines with the same letters differ significantly, small letters - P≤0.05, capitals - P≤0.01
[1]TNB - total number born, [2]NW - number of weaned, [3]NF - number of falls

Discussion

There are many investigations concerning polymorphisms and their relationships with performance traits in domestic animals. In pigs mainly reproduction, carcass and meat quality traits are taking under consideration. Recent studies have proved that polymorphisms in some genes may be associated with reproduction traits in pigs. In sows have examined following genes: *ESR1*, *FSHB* (WANG et al., 2006, HUMPOLIČEK et al., 2006), *RBP4* (WANG et al., 2006), *CYP21* (ZIEMAK and GRZESIAK, 2006), *GPX*, *FUT1*, *ESR2* (BUSKE et al., 2006) *PRLR*, *LEP* (TERMAN 2006) *sFBP* (VALLET et al., 2005), *EPOR* (VALLET et al., 2005), however in boars: *ESR1*, *ESR2* (TERMAN et al., 2006), *GNRHR*, *PRL*, *PRLR*, *FSHB*, *LHB*, *FST*, *INHA*, *INHBA*, *INHBB* (LIN et al., 2006) *ACTN1*, *ACTN4*, *ACTG2* (WIMMERS et al., 2005). In our study we investigated *FABGL* gene on account of role in reproduction which

plays the product of this gene. We have found statistical significant associations between particular genotypes and total number born and number of weaned in second and remaining parities. Higher value for TNB characterized sows (P≤0.01) with genotype *AA* (10.32) in relation to genotype *BB* (9.77). Considering NW, higher value for this traits achieved sows with genotype *AA* (8.80) than sows with genotype *BB* (8.39) (P≤0.01) and *AB* (8.45) (P≤0.05). Differences in number of falls for *FABGL/Bbv*I genotypes were also observed but they were not statistical significant. There are no data in the literature concerning association study of *FABGL* gene. Only JACOBS et al. (2002) has given frequency of alleles in different breeds of pigs. In our study following frequency of alleles were obtained: *A* - 0.45, *B* - 0.55. Similar frequency were observed in two breeds: Czech Meat Pig *A* - 0.47, *B* - 0.53 and Landrace *A* - 0.42, *B* - 0.58. Higher frequency of allele *A* were found in Pietrain - 0.57 and Large White - 0.54. Allele *B* was not found in pigs belong to Meishan breed.
Results of our study indicates that genotype *AA* is favourable for TNB and NW. It may be suggested that *FABGL* gene is 'candidate gene' for reproduction traits but investigation should be verified on bigger population as well as on different breeds.

References

ADAMSKI, J.; JAKOB, F.J.:
A guide to 17β-hydroxysteroid dehydrogenases. Mol. Cell Endocrin. **171** (2001), 1-4.

BUSKE, B.; STERNSTEIN, I.; REIßMANN, M.; REINECKE, P.:
Analysis of association of GPX5, FUT1 and ESR2 genotypes with litter size in a commercial pig cross population. Arch. Tierz., Dummerstorf **49** (2006) 3, 259-268

CHARDON, P.; ROGEL-GAILLARD, C.; PEELMAN, L.; YERLE, M.; VELTEN, F.W.; RENARD, C.; VAIMAN, M.:
Physical organization of the pig major histocompatibility complex class II region Immunogenetics **50** (1999), 344–348

FOMITCHEVA, J.; BAKER, M.E.; ANDERSON, E.; LEE. G.Y.; AZIZ, N.:
Characterization of Ke 6, a New 17 β -hydroxysteroid dehydrogenase, and its expression in gonadal tissues. J. Biol. Chem. **273** (1998) 22664–22671

HUMPOLÍČEK, P.; URBAN, T.; HORÁK, P.:
Influence of *ESR1* and *FSHB* genes on litter size in Czech Large White sows. Arch. Tierz., Dummerstorf **49** (2006) 2, 152-157

JACOBS, K.; MATTHEEUWS, M.; VAN POUCKE, M.; VAN ZEVEREN, A.; PEELMAN, L. J.:
Characterization of the porcine *FABGL* gene. Anim. Genet. **33** (2002), 220–223

LIN, C.L.; PONSUKSILI, S.; THOLEN, E.; JENNEN, D.G.; SCHELLANDER, K.; WIMMERS, K.:
Candidate gene markers for sperm quality and fertility of boar. Anim. Reprod. Sci. **92** (2006) 349-363

MINDNICH, R.; MÖLLER, G.; ADAMSKI, J.:
The role of 17 beta-hydroxysteroid dehydrogenases. Mol. Cell Endocrin. **218** (2004), 7–20.

TERMAN, A.:
Effect of the polymorphism of prolactin receptor (PRLR) and leptin (LEP) genes on litter size in Polish pigs. J Anim Breed Genet. **122** (2005), 400-404

TERMAN, A.; KMIEĆ, M.; POLASIK, D.:
Estrogen receptor gene (ESR) and semen characteristics of boars. Arch. Tierz. Dummerstorf **49** (2006) 1, 71-76

WANG, X.; WANG, A.G.; FU, J.; LIN, H.:
Effects of ESR1, FSHB and RBP4 genes on litter size in a Large White and a Landrace Herd. Arch. Tierz., Dummerstorf **49** (2006) 1, 64-70

WIMMERS, K.; LIN, C. L.; THOLEN, E.; JENNEN, D. G. J.; SCHELLANDER, K.; PONSUKSILI, S.:
Polymorphisms in candidate genes as markers for sperm quality and boar fertility. Anim. Genet. **36** (2005), 152-155

VALLET, J.L.; FREKING, B.A.; LEYMASTER, K.A.; CHRISTENSON, R. K.:
Allelic variation in the secreted folate binding protein gene is associated with uterine capacity in swine. J. Anim. Sci. **83** (2005), 1860–1867

VALLET, J.L.; FREKING, B.A.; LEYMASTER, K.A.; CHRISTENSON, R. K.:

Allelic variation in the *erythropoietin receptor* gene is associated with uterine capacity and litter size in swine. Anim. Genet. **36** (2005), 97-103

ZIEMAK, J.; GRZESIAK, W.:

Associations between polymorphism of the steroid 21-hydroxylase gene (*CYP21*) and litter size of Polish Large White × Polish Landrace sows. Arch. Tierz., Dummerstorf **49** (2006) 2, 158-164

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Arch. Tierz., Dummerstorf **50** (2007) Special Issue, 205-213

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Association of growth hormone (GH) gene polymorphism with carcass and meat quality traits in PIC hybrid pigs

Abstract

The presented study was aimed at determining the association of growth hormone (*GH*) gene polymorphism with the traits that characterise carcass and meat quality and meat basic chemical composition in porkers with genotype CC with respect to *RYR1 locus*. The study was carried out on 126 PIC porkers originating after Camborough 22 sows and PIC 337 boars. The polymorphism of *GH* gene was identified respectively with restrictive enzymes *Msp*I, *Hae*II, *Cfo*I and *Apa*I. The allele frequencies were as follows: *GH*/*Msp*I A = 0.78 and B = 0.22; *GH*/*Hae*II A = 0.86 and B = 0.14; *GH*/*Cfo*I C1 = 0.09 and C2 = 0.03 and C3 = 0.18 and C4 = 0.70; *GH*/*Apa*I A1 = 0.28 and A2 = 0.72. The analysis of association of *GH* genotypes identified with four restrictive enzymes did not show any relationship between the *GH* gene polymorphism and the carcass slaughter traits determined with meatiness measuring apparatus CGM and the meat quality and meat basic chemical composition in PIC hybrid porkers. It may be stated, basing on the analysis performed, that *GH* gene is weakly related to the variability of analysed traits in PIC porkers.

Key Words: pig, GH genotype, meatiness, meat quality

Zusammenfassung

Titel der Arbeit: **Zusammenhänge zwischen dem Polymorphismus des Wachstumshormongens (GH) und Qualitätsmerkmalen des Schlachtkörpers bei PIC Hybridschweinen**

Ziel der Arbeit war die Untersuchung von Zusammenhängen zwischen dem Polymorphismus des Wachstumshormongens (GH) und Qualitätsmerkmalen des Schlachtkörpers bei Mastschweinen des Genotyps CC hinsichtlich des RYR1 Lokus. Einbezogen waren 126 Mastschweine aus Paarungen von Camborough-22 Sauen und PIC-337 Ebern. Der Polymorphismus des GH-Gens wurde mittels der Restriktionsenzyme *Msp*I, *Hae*II, *Cfo*I und *Apa*I identifiziert. Die Allelfrequenzen betrugen: GH/*Msp*I A = 0,78 und B = 0,22; GH/*Hae*II A = 0,86 und B = 0,14; GH/*Cfo*I C1 = 0,09, C2 = 0,03, C3 = 0,18 und C4 = 0,70; GH/*Apa*I A1 = 0,28 und A2 = 0,72. Die mit Hilfe von vier Restriktionsenzymen identifizierten GH Genotypen ergaben keine Zusammenhänge zwischen diesen und den untersuchten Qualitätsmerkmalen des Schlachtkörpers.

Schlüsselwörter: Schwein, GH Genotyp, Fleischanteil, Fleischqualität

Introduction

Meat quantity and quality are affected by a whole number of genetic, feeding and environmental factors and interactions occurring between them (DE VRIES et al., 1998; CAMERON, 1990; KUSEC et al., 2005). Genetic effects play a key role in shaping pig carcass composition and quality, though the quality of meat is less affected by genetic factors than its quantity. It is assumed that genetic factors determine meat quality in 10 to 30%, whereas the other part falls on environmental factors (DE VRIES et al., 1994). Studies show that animals belonging to various breeds but characterised by the same genotype with respect to *RYR1* locus demonstrate significant differences not only in carcass muscling, but also in meat quality traits. The occurrence of meat with PSE traits among animals with genotype *CC* at *RYR1* locus, or also of meat with

normal parameters in animals with genotype *TT* in *RYR1* locus, may come out of the effect of other genes on carcass and meat quality traits, modifying the *RYR1* gene effect (KOĆWIN-PODSIADŁA and KURYŁ, 2003).
The knowledge of genome and the compilation of genetic maps are indispensable for isolating and characterising the genes concerned. Quantitative trait loci (QTL) studies and candidate gene analysis have resulted in identification of important chromosomal regions and genes associated with economically important traits in pigs (WIMMERS et al., 2005).
A gene that arouses large interest is growth hormone (*GH*) gene, which is connected with the fact that growth hormone is directly involved in increasing intensity of the course of some metabolic conversions, in particular of protein synthesis (LE ROITH et al., 2001; TE PAS et al. 2003). Studies point at more rapid growth rate and thinner backfat in pigs with higher *GH* concentration in blood (ALTHEN and GERRITS, 1976; CHENG et al. 2000). Also ETHERTON (2000) reports that porcine somatotropin (PST) increases muscular tissue increment and decreases fatty tissue deposition during the pig growth. On the other hand, the results of studies on association of different *GH* gene variants with carcass quality traits are not unambiguous. KNORR et al. (1997) demonstrated significant association of *GH* genotypes with eight carcass fatness traits in Meishan x Pietrain pigs, whereas did not confirm this fact in the hybrids of wild boar x Pietrain pigs. WANG et al. (2003) showed an association of *GH*/*Apa*I gene polymorphism with carcass meat content in Yorkshire pigs, however not confirming this in Nanchang White pigs. Also the studies of LARSEN et al. (1995) on the hybrids of wild boar x Pietrain pigs and of URBAN et al. (2002) on Duroc porkers did not prove an association of *GH* gene polymorphism with carcass quality traits. On the other hand, PIERZCHAŁA et al. (1999; 2004), KŘENKOVÁ et al. (1999), KURYŁ et al. (2003), FRANCO et al. (2005) point to significant association of different *GH* gene variants with carcass fatness and meatiness in different pig breeds and lines.
The presented study was aimed at determining the association of growth hormone (*GH*) gene polymorphism with the traits that characterise carcass and meat quality and meat basic chemical composition in porkers with CC genotype with respect to *RYR1 locus*.

Material and methods

The study was carried out on 126 PIC hybrid porkers, which came from a farm running the pig production in Western Pomeranian province (Poland). Examination covered the offspring after PIC 337 boars and Camborough 22 sows, which was kept under the same environmental conditions and fed with a balanced feed-mix ad libitum. All porkers destined for the experiment were conveyed from a farm to the "Agryf" Meat Plant in Szczecin (Poland) by one means of transport in the evening and slaughtered in the morning on the next day after 4 hours long transportation from a distance of 250 km.
During slaughtering the animals, after stunning them with CO_2, blood samples were collected to EDTA-coated tubes for DNA analysis in order to identify both alleles and genotypes with respect to *RYR1* and *GH* loci. Furthermore, the sex of porkers was established on the slaughter line, among which there were 68 gilts and 58 barrows. Thereafter, carcass slaughter value was measured on the left-hand side half-carcass

with a needle-optical probe equipped CGM apparatus (Sydel, France) (Borzuta, 2004) and hot carcass weight of the examined porkers was determined. Mean hot carcass weight of porkers under examination was 78.98 ± 0.39 (mean value and standard error). After 24 hours long carcass cooling, meat samples were collected from *longissimus lumborum* muscle (LL) in 1-4 lumbar vertebrae section of the right-hand side half-carcass, in which meat pH_{24} value and drip loss volume from the muscular tissue determined after 48 hours from the slaughter were recorded according to HONIKEL (1987). Approximately 48 hours after slaughter, on minced muscular tissue, pH measurement was taken in aqueous solution, colour traits were determined, i.e. L* - lightness, a* - red colour, and b* - yellow colour by means of HunterLab apparatus using D65 light source and 10° observer (CIE, 1978), as well as meat water holding capacity with the method of Grau and Hamm in Pohja & Niinivaar's modification (1957), thermal drip according to Walczak [1959], water-soluble protein content with Kotik's method (1974) and basic meat chemical composition, i.e. total protein, fat, ash and dry matter (AOAC, 1990), were estimated.

Genomic DNA was extracted from blood using Master Pure kit of Epicentre Technologies (Madison, WI, USA). Genotypes of *RYR1* and *GH* were determined by PCR-RFLP method. *RYR*/*Hin6*I genotypes were identified using sequence of primers according to FUJI et al. (1991). All of the pigs were *CC/RYR1* genotype.

The *GH* fragments were amplified from a genomic template using the PCR with primer sequences reported by KIRKPATRICK et al. (1992), LARSEN and NIELSEN (1993). Information on primer sequences, restriction enzymes and allele sizes are given in Table 1. PCR reactions were performed in a total volume of 20 µl using 50 ng porcine genomic DNA, 0.5 µM of each primer, 100 µM of each dNTP, 3 mM $MgCl_2$ and 1 U of *Taq* DNA polymerase in standard PCR buffer. Thermal conditions were as follows: 94^0C for 5 min followed by 35 cycles of 45 s at 94^0C, 45 s at 57^0C (*GH*/*Msp*I and *GH*/*Hae*II) and 55 s at 94^0C, 50 s at 53^0C (*GH*/*Cfo*I and *GH*/*Apa*I) and last extension for 5 min at 72^0C. Digestion of the PCR product was performed with 5 IU of the appropriate restriction endonuclease (Table 1) at 37^0C overnight. The restriction fragments of DNA were separated by electrophoresis in 2% (*GH*/*Msp*I and *GH*/*Hae*II) and 2,5% (*GH*/*Cfo*I and *GH*/*Apa*I) agarose gel stained with ethidium bromide. The results were visualized using UV rays.

Table 1
Primer sequences, endonuclease and allele sizes of *GH* gene

Primer sequence (5'-3')	PCR product size (bp)	Endonuclease	Allele size (bp)	source
GCCAAGTTTTAAA TGTCCCTG	506	*Msp*I	A – 222, 147, 137 B – 284, 222	Kirkpatrick et al. (1992)
DTGTCCCTCCGGG ATGTAG	506	*Hae*II	A – 506 B – 333, 173	
TTATCCATTAGCAC ATGCCTGCCAG	605	*Cfo*I	C1 – 605 C2 – 497, 108 C3 – 448, 157 C4 – 448, 108, 49	Larsen and Nielsen (1993)
CTGGGGAGCTTAC AAACTCCTT	605	*Apa*I	A1 – 449, 101, 55 A2 – 316, 133, 101, 55	

Genetic equilibrium of analysed population was evaluated on the basis of chi-square test. A statistical analysis was performed to compare carcass and meat quality and basic chemical coposition traits between pigs of different genotypes, using the least squares method of the GLM procedure (Statistica 7.1) according to the following model:

$$Y_{ijk} = \mu + G_i + sex_j + \beta\,(x_{ijk} - \bar{x}) + e_{ijk}$$

where:
Y_{ijk} - trait measured;
μ - the overall mean;
G_i - the effect of a particular genotype at the *GH* locus (i = AA, AB, BB of *GH*/*Msp*I and *GH*/*Hae*II; C1C4, C2C4, C3C4, C4C4 of *GH*/*Cfo*I; A1A2, A2A2 of *GH*/*Apa*I);
sex_j - the effect sex (j = 1, 2);
β - linear regression coefficient for hot carcass weight;
x_{ijk} - hot carcass weight of *ijk*-th individual included as covariable;
$\bar{x}$ - mean for hot carcass weight;
e_{ijk} - the random error.

Results and discussion

The observed frequency of genotypes and alleles for the growth hormone (*GH*) gene identified with endonucleases *Msp*I, *Hae*II, *Cfo*I and *Apa*I PIC hybrid porkers is presented in Tables 2 and 3. The chi-square analysis showed that genotype frequencies for *GH*/*Msp*I, *GH*/*Cfo*I and *GH*/*Apa*I loci ($p<0.01$) did not present the Hardy-Weinberg equilibrium. Higher frequency was stated of allele A at *GH*/*Msp*I and *GH*/*Hae*II loci, which is also confirmed by the results of allele estimation in the porkers after Large White boars, as well as after Large White x Pietrain (KŘENKOVÁ et al., 1999) and Duroc ones (URBAN et al., 2002). ERNST et al. (2003) found in wild boar a higher frequency of allele A (0.61) for *GH*/*Hae*II polymorphism and a higher frequency of allele B (0.61) in case of *GH*/*Msp*I genotype.

Table 2
Frequency of GH alleles in PIC pigs

GH/*Msp*I		*GH*/*Hae*II		*GH*/*Cfo*I				*GH*/*Apa*I	
A	B	A	B	C1	C2	C3	C4	A1	A2
0.7817	0.2183	0.8571	0.1429	0.0873	0.0278	0.1825	0.7024	0.2817	0.7183
78.17%	21.83%	85.71%	14.29%	8.73%	2.78%	18.25%	70.24%	28.17%	71.83%

Table 3
Frequency of GH genotypes in PIC pigs

	GH genotype													
	*GH/Msp*I			*GH/Hae*II			*GH/Cfo*I					*GH/Apa*I		
	AA	AB	BB	AA	AB	BB	C1C3	C1C4	C2C4	C3C4	C4C4	A1A1	A1A2	A2A2
n	86	25	15	97	22	7	2	20	7	44	53	3	65	58
%	68.3	19.8	11.9	77.0	17.5	5.6	1.6	15.9	5.6	34.9	42.1	2.4	51.6	46.0
χ^2	11.43**			3.37			10.03**					9.50**		

In the present study, from among three genotypes at locus *GH*/*Msp*I and *GH*/*Hae*II, the lowest frequency was stated in genotype BB, while the highest one for genotype AA. KURYŁ et al. (2003), who examined Pietrain, Polish Landrace, Złotnicka Spotted, Torhyb line and PIC line pigs, found that the frequency of particular variants of *GH* gene differed and depended on pig breed or line. In that study, genotype AA frequency in PIC hybrid porkers was lower than that of genotype BB at locus *GH*/*Msp*I, as well as higher frequency was stated of genotype AB at locus *GH*/*Hae*II.
In the examined PIC hybrid porkers, the highest frequency was found of allele C4 at locus *GH*/*Cfo*I, whereas the higher one for allele A2 at locus GH/*Apa*I. From among GH/*Cfo*I genotypes, the highest frequency was stated for genotype C4C4, while the lowest for genotype C1C3. Moreover, similar frequency was stated of genotypes A1A2 and A2A2 and the lowest of genotype A1A1 at locus *GH*/*Apa*I. Due to low frequency of genotypes C1C3 *GH*/*Cfo*I and A1A1 *GH*/*Apa*I in the PIC porkers, they were not included in statistical analysis with GLM procedure.

Table 4
The LSQ means and their standard errors (SE) and relationship between growth hormone (GH) genotypes for analysed traits in PIC pigs

Traits	LSQ mean	SE	*GH* genotype			
			*Msp*I	*Hae*II	*Cfo*I	*Apa*I
meatiness, %	56.90	0.33	n.s.	n.s.	n.s.	n.s.
thickness muscle, mm	55.39	0.69	n.s.	n.s.	n.s.	n.s.
fat thickness, mm	13.02	0.31	n.s.	n.s.	n.s.	n.s.
Total protein, %	22.31	0.05	n.s.	n.s.	n.s.	n.s.
Fat, %	2.07	0.05	n.s.	n.s.	n.s.	n.s.
Ash, %	1.16	0.00	n.s.	n.s.	n.s.	n.s.
Dry matter, %	25.57	0.06	n.s.	n.s.	n.s.	n.s.
pH_{24}	5.80	0.01	n.s.	n.s.	n.s.	n.s.
pH_{48}	5.70	0.01	n.s.	n.s.	n.s.	n.s.
L*	55.81	0.19	n.s.	n.s.	n.s.	n.s.
a*	15.70	1.09	n.s.	n.s.	n.s.	n.s.
b*	6.88	0.07	n.s.	n.s.	n.s.	n.s.
Drip loss, %	6.08	0.17	n.s.	n.s.	n.s.	n.s.
WHC, % of free water	17.94	0.33	n.s.	n.s.	n.s.	n.s.
WHC, % of bound water	75.90	0.44	n.s.	n.s.	n.s.	n.s.
Thermal drip, %	25.43	0.18	n.s.	n.s.	n.s.	n.s.
Water-soluble protein, %	10.04	0.11	n.s.	n.s.	n.s.	n.s.

F-test: n.s. = not significant

In the examined PIC hybrid porkers, free of stress-susceptibility gene ($RYR1^T$), no effect of the growth hormone (*GH*) gene polymorphism, identified with endonucleases *Msp*I, *Hae*II, *Cfo*I and *Apa*I, was found on carcass slaughter value, i.e. on the percent content of meat in carcass and the thickness of backfat and *longissimus dorsi* (LD) muscle determined with a meatiness measuring apparatus CGM (Table 4). KURYŁ et

al. (2003) found differences in the level of some carcass traits depending on the *GH* genotype, each relationship occurring in one line or breed not being confirmed in another. These authors stated in PIC hybrid porkers a relation between the *GH* gene polymorphism and the slaughter value of ham, whereas did not confirm it for meat content and carcass fatness, as it was found in our study. They found that AA genotypes at locus *GH*/*Hae*II and BB genotypes at locus *GH*/*Msp*I proved to be the least advantageous for ham weight and ham meat when compared with other genotypes with respect to these loci. On the other hand, genotype BB at *GH*/*Msp*I was associated with the increased carcass length. Also PUTNOVÁ et al. (2001) showed that the length of carcass is related to the *GH* gene polymorphism identified with enzyme *Dde*I. In the study of PIERZCHAŁA et al. (2004), an association was demonstrated of genotype BB at locus *GH*/*Msp*I and genotype AA at locus *GH*/*Hae*II with higher ham weight and ham meat weight and higher carcass ham content in the porkers after Polish Large White, Duroc and Pietrain boars and Polish Large White x Polish Landrace sows. In their earlier study however, KŘENKOVÁ et al. (1999) did not find an association between the *GH*/*Hae*II gene polymorphism and carcass muscling and fattening traits in hybrid porkers, while showing unfavourable effect of AA *Msp*I-*Hae*II haplotype on these traits. On the other hand, PIERZCHAŁA et al. (1999) stated unfavourable effect of genotype AA *GH*/*Hae*II only, while AA *Msp*I-*Hae*II haplotype influenced positively carcass quality traits in hybrid porkers.

It is well-known that carcass slaughter value as well as meat quality in pigs is related to stress-susceptibility gene RYR1. KŘENKOVÁ et al. (1999) did not find an interaction between *GH* genotypes identified with enzymes *Hae*II and *Msp*I and RYR1 genotypes in carcass quality traits. It was showed in the study of FRANCO et al. (2005) on Landrace pigs free of stress-susceptibility gene ($RYR1^T$) that the polymorphism of *GH* gene identified with enzyme *Dde*I is associated with the thickness of backfat. However, in the study of URBAN et al. (2002) no association was showed of the *GH*/*Msp*I gene polymorphism with carcass meatiness and fatness in Duroc porkers with genotype CC/RYR1.

The quality of meat depends on many genetic and extragenetic factors. Therefore, studies on the effect of specific genes on meat quality of porkers should be carried out under most standardised environmental conditions (DE VRIES et al., 1998). In the present study, all porkers were treated the same way, in particular during the pre-slaughter handling and the slaughtering, as well as carcasses directly after slaughter, as it is well-known that these procedures affect meat qualitative traits sometimes even more than genetic factors.

In the examined PIC hybrid porkers no association was found between the polymorphism of growth hormone (*GH*) gene identified with enzymes *Msp*I, *Hae*II, *Cfo*I and *Apa*I and the traits that characterise meat quality and its basic chemical composition. Also CASAS-CARILLO et al. (1997) did not show an association of the polymorphism of *GH*/*Dde*I and *GH*/*Hae*II genes with carcass quality traits and meat quality determined on the grounds of pH measured in *longissimus dorsi* and *semimembranosus* muscles in pigs originating after Yorkshire, Landrace, Hampshire pigs and their crossbreds. According to these authors, the polymorphism of *GH* gene is weakly related to the variability of growth and carcass quality traits. Also KNORR et al. (1997) did not find an association of *GH* gene variants with meat quality traits in the hybrids of wild boar x Pietrain pigs and of wild boar x Meishan pigs.

Conclusions

In the PIC hybrid porkers allele frequencies were as follows: *GH*/*Msp*I A = 0.78 and B = 0.22; *GH*/*Hae*II A = 0.86 and B = 0.14; *GH*/*Cfo*I C1 = 0.09 and C2 = 0.03 and C3 = 0.18 and C4 = 0.70; *GH*/*Apa*I A1 = 0.28 and A2 = 0.72. Basing on the performed study, no association was found between the polymorphism of growth hormone (*GH*) gene identified with enzymes *Msp*I, *Hae*II, *Cfo*I and *Apa*I and the traits that characterise carcass and meat quality and meat basic chemical composition in the PIC porkers with genotype CC at locus *RYR1* originating after Camborough 22 sows and PIC 337 boars. It may be stated, basing on the analysis performed, that *GH* gene is weakly related to the variability of analysed traits in PIC porkers.

References

ALTHEN, T. G.; GERRITS R.J.:
Pituitary and serum growth hormone levels in Duroc and Yorkshire swine genetically selected for high and low backfat. J. Anim. Sci. **42** (1976), 1490-1497.

AOAC.:
Association of Official Analytical Chemists - Official Methods of Analysis (1990) 15 th Ed., Washington DC.

BORZUTA, K.:
The evaluation of pig carcass quality – a review. Prace i Mater. Zootech. **15** (2004) Special Issue, 77-84

CAMERON, N. D.:
Genetic and phenotypic parameters for carcass traits, meat and eating quality in pigs. Livestock Prod. Sci. (1990) 26, 119-135.

CASAS-CARRILO, E.; PRILL-ADAMS, A.; PRICE, S. G.; CLUTTER, A. C.; KIRKPATRICK, B. W.:
Relationship of growth hormone and insulin-like growth factor-1 genotypes with growth and carcass traits in swine. Anim. Genet. **28** (1997), 88-93

CHENG, W. T. K.; LEE, C. H.; HUNG, C. M.; CHANG, T. J.; CHEN, C. M.:
Growth hormone gene polymorphisms and growth performance traits in Duroc, Landrace and Tao-Yuan pigs. Theriogenology **54** (2000), 1225-1237

COMMISION INTERNATIONAL DE I'ECLAIRAGE (CIE):
Supplement No. 2 to CIE publication No. 15. Colorimetry. Bureau Central de la CIE, Paris 1978.

DE VRIES, A. G.; SOSNICKI, A.; GARNIER, J. P.; PLASTOW, G. S.:
The role of major genes and DNA technology in selection for meat quality in pigs. Meat Sci. **49** (1998) Suppl. 1, 245-255

DE VRIES, A. G.; VAN der WAL, P. G.; LONG, T.; EIKELENBOOM, G.; MERKS, J. W. M.:
Genetic parameters of pork quality and production traits in Yorkshire populations. Livestock Prod. Sci. **40** (1994), 277-289

ERNST, M.; KUCIEL, J.; URBAN, T.;
Analysis of genetic variation of eight candidate genes in two wild boar subspecies. Czech J. Anim. Sci. **48** (2003) 12, 533-539.

ETHERTON, T. D.:
The biology of somatotropin in adipose tissue growth and nutrient partitioning. J. Nutr. **130** (2000), 2623-2625.

FRANCO, M. M.; ANTUNES, R. C.; SILVA, H. D.; GOULART, L. R.:
Association of PIT1, GH and GHRH polymorphisms with performance and carcass traits in Landrace pigs. J. Appl. Genet. **46** (2005) 2, 195-200

FUJI, J.; OTSU, K.; ZORZATO, F.; DE LEON, S.; KHANNA, V. K.; WEILER, J. K.; O'BRIEN, P. J.; MacLENAN D. H.:
Identyfication of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science **253** (1991), 448-451

HONIKEL, K. O.:
pH and water binding of meat. Fleischwirtschaft **67** (1987), 1098-1102

KIRKPATRICK, B. W.:
Identyfication of a cinserved microsatellite site in the porcine and bovine insulin-like growth factor-I gene 5' flank. Anim. Genet. **23** (1992), 543-548

KNORR, C.; MOSER, G.; MÜLLER E.; GELDERMANN, H.:
Associations of GH gene variants with performance traits in F_2 generations of European wild boar, Piétrain and Meishan pigs. Anim. Genet. **28** (1997), 124-128.

KOĆWIN-PODSIADŁA, M.; KURYŁ, J.:
The effect of interaction between genotypes at loci CAST, RYR1 and RN on pig carcass quality and pork traits - a review. Anim. Sci. Pap. Rep. **21** (2003), Suppl. 1, 61-75

KOTIK, T.:
Protein content in water extracts of meat as an index of its quality. Roczniki Instytutu Przemysłu Mięsnego **12** (1974), 47-52 [in Polish, English abstract]

KŘENKOVÁ, L.; KUCIEL, J.; URBAN, T.:
Association of the *RYR1, GH, LEP* and *Tf* genes with carcass and meat quality traits in pigs. Czech J. Anim. Sci. **44** (1999), 481-486.

KURYŁ, J.; KAPELAŃSKI, W.; PIERZCHAŁA, M.; BOCIAN, M.; GRAJEWSKA, S.:
A relationship between genotypes at the *GH* and *LEP* loci and carcass meat and fat deposition in pigs. Anim. Sci. Pap. Rep. **21** (2003) 1, 15-26.

KUSEC, G.; BAULAIN, U.; HENNING, M.; KÖHLER, P.; KALLWEIT, E.:
Fattening, carcass and meat quality traits of hybrid pigs as influenced by MHS genotype and feeding systems. Arch. Tierz., Dummerstorf **48** (2005) 1, 40-49

LARSEN, N. J.; NIELSEN, V. H.:
ApaI and CfoI polymorphisms in the porcine growth hormone gene. Anim. Genet. **24** (1993), 71

LE ROITH, D.; SCAVO, L.; BUTLER, A.:
What is the role of circulating IGF-I? Trends in Endocrinology & Metabolism **12** (2001), 48-52

NIELSEN, V. H.; LARSEN, N. J.; AGERGAARD, N.:
Association of DNA-polymorphism in the growth-hormone gene with basal-plasma growth-hormone concentration and production traits in pigs. J. Anim. Breed. Genet. **112** (1995), 205-212

PIERZCHAŁA, M.; BLICHARSKI, T.; KURYŁ, J.:
Growth rate and carcass quality in relation to *GH*/*MspI* and *GH*/*Hae*II PCR-RFLP polymorphism in pigs. Anim. Sci. Pap. Rep. **22** (2004) 1, 57-64

PIERZCHAŁA, M.; KORWIN-KOSSAKOWSKA, A.; ZWIERZCHOWSKI, L.; ŁUKASZEWICZ, M.; ZIĘBA, G.; KURYŁ, J.:
*Hae*II and *Msp*I polymorphism of growth hormone gene in pigs and its association with production traits. Czech. J. Anim. Sci. **44** (1999), 441-445.

POHJA, M. S.; NIINIVAARA, F. P.:
Die Bestimmung der Wasserbindung des Fleisches mittels der Konstantdrückmethode. Fleischwirtschaft **9** (1957), 193-195

PUTNOVÁ, L.; KŘENKOVÁ, L.; VRTKOVÁ, I.; DVOŘÁK, J.; PIETRUSZKA, A.; CZARNECKI, R.:
Association od the DdeI growth hormone gene polymorphism with some performance traits in Polish Large White and Czech Large White x Polish Large White pigs. J. Appl. Genet. 42 (2001) 3, 317-324

Te PAS, M. F. W.; GERRITSEN, C. L. M.; VISSCHER, A. H.; DE GREEF, K. H.:
Relationship between performance traits and the expression of growth hormone, insulin-like growth factor-I, and insulin in pigs selected for growth or leanness. J. Anim. Breed. Genet. **120** (2003), 346-357

URBAN, T.; KUCIEL J.; MIKOLÁŠOVÁ, R.:
Polymorphism of genes encoding for ryanodine receptor, growth hormone, leptin and MYC protooncogene protein and meat production in Duroc pigs. Czech J. Anim. Sci. **47** (2002) 10, 411-417

WALCZAK, Z.:
A laboratory method for the determination of jelly content in canned meat. Roczniki Nauk Rolniczych **74-B-4** (1959), 619 [In Polish]

WANG, W.; HUANG, L.; GAO, J.; DING, J.; CHEN, K.; REN, J.; LUO, M.:
Polymorphism of growth hormone gene in 12 pig breeds and its relationship with pig growth and carcass traits. Asian-Australasian J. Anim. Sci. **16** (2003) 2, 161-164

WIMMERS, K.; MURANI, E.; SCHELLANDER, K.; PONSUKSILI, S.:
Combining QTL- and expression-analysis: identyfication of functional positional candidate genes for meat quality and carcass traits. Arch. Tierz., Dummerstorf **48** (2005) Special Issue, 23-31

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