

Thesis of Ph.D dissertation

**GENETIC STUDY AND STATISTICAL ANALYSIS OF SPECIFIC PRODUCTION
PARAMETERS OF THE HUNGARIAN LARGE WHITE, DUROC AND PIETRAIN
SOWS**

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2016

1. Introduction and goals of the doctoral dissertation

Animal husbandry as a production activity is one of the most ancient practices of humankind. Beside environmental factors, the character and standard of animal husbandry was determined by the level of social and economic development. It was influenced by nutritional demands, changes in the need for draft animals, technical and religious reasons as well as wars. It was affected by emerging and unsatisfied market demands in addition to scientific development.

Animal husbandry was brought on new grounds by population genetics, and the biotechnological and information technological discoveries of the last decade. The discovery of DNA in 1953 made it possible to create an application of genetic manipulation and genetic mapping. (SZABÓ, 2004).

Studies in animal genetics developed rapidly. Its goal is to utilize the biological traits and abilities of the animal in production to the highest possible extent. Consumer demands can be met by quantitative and qualitative production manufacturing and economical production practices. A possible way of increasing pork production is improving the indicators related to proliferation. The development of researches in animal biotechnology and genetic supplanted traditional selection processes. New methods emerged such as MAS (marker assisted selection), GAS (gene assisted selection) and GS (gene selection). These methods enabled us to apply new selection systems and the introduction of previously unused aspects into the selection criteria.

Based on markers, MAS identifies locuses that are closely related to the gene coding the desirable trait. The application of marker based selection is most advantageous in case of traits at which traditional selection is less effective.

The goals of my research were the following:

During the completion of this thesis we investigated factors that can be suitable to improve the profitability of large scale pig production on the pig farm of Hód-Mezőgazda cPlc.

1. In order to improve indicators connected to proliferation, I planned to identify the alleles of 7 previously identified, reproduction-related genes:

1. properdin (*BF*)
2. epidermal growth factor (*EGF*)
3. estrogen receptor (*ESR*)

4. follicle-stimulating hormone- subunit beta (*FSH β*)
5. Z member of H2A histon family (*H2AFZ*)
6. leptin (*LEP*)
7. prolactin receptor (*PRLR*)

My goal was to detect the gene the alleles of which effect the proliferation – related traits of sows during polimorphism study in the Hungarian large White, Duroc and Pietrain stocks. I wished to identify the most prolific genotype in the Hungarian Large White stock in addition to performing the genotypization of a few individuals of Duroc and Pietrain sows kept on the farms site in order to measure rhe genotype occurrence despite the low number of elements. My conclusion pertains only to the Hungarian Large White stock kept on the studied site.

The following tarits were analyzed which impacted the number of produced piglets by one sow:

- interval betwen litters (IBL)
- percentage litter (PL)
- number litters (NL)
- number born alive (NBA)
- number born dead (NBD)
- total number born (TNB)
- mean born alive (MBA)
- mean born dead (MBD)
- mean born total (MBT)
- number 21 days (M21D)
- growth rate (GR)

2. My aim was to perform the survival analysis between the genotypes in relation to economical production in order to improve profitability. My tasks was to determine the risk of culling and the time sows spent in production for the Hungarian Large White, Duroc and Pietrain breeds on the mentioned production farm. The determination of the survival expected life performance of the sows enables us to plan the most optimal sow rotation.

2. Material and method

2.1. Genetic studies

Sampling

We took blood samples from 117 pigs on the sites of Hód-Mezőgazda cPlc. from the blood samples used in the study 93 came from the Hungarian Large White sows, 9 from Duroc and 15 from Pietrain genotype sows.

The sampling was performed with disposable needles into 5 ml plastic blood tubes containing EDTA. We took 2.5-3.0 ml blood from each individual and stored the samples at – 20°C until further use.

Genomic DNA isolation

We isolated genomic DNA based on the method by ZSOLNAI & ORBÁN (1999) from the blood samples.

Used chemicals:

- Blood-cleaner buffer (10 ml 1M Tris /pH 7.5/ + 1ml 0.2M EDTA /pH 8/)
- Proteinase K stored at -20°C (15 mg Proteinase K powder enzyme + 1 ml steril distilled water) (Promega, Madison, USA)
- Lysis buffer (10mM Tris /pH 7.5/ + 50 mM KCl + 0.5% Tween-20)

We diluted the mix of chemicals with 1000 ml distilled water and sterilized it in autoclaves at 110 °C.

We added 0.5 ml blood-cleaner buffer into 1.5 ml Eppendorf tubes and washed 0.5 ml blood sample into it. We mixed the liquid in a vortex-mixer then we centrifugated it at the speed of 12 000 rpm. We removed the superfluous compound from the pellet. We repeated the process twice and added a mixture of 0.4 ml proteinase K enzyme and 10 ml lysis buffer. We dissolved the pellet from the bottom of the Eppendorf tube with a vortex-mixer. The samples were incubated at 56 °C for 61 minutes and then at 94 °C for 10 minutes. The genomic samples we gained were stored at –20 °C until further study.

PCR and RFLP in the research of proliferation-related genes

I successfully amplified the section of the genes enclosed by primers (SNP) through polymerase chain reactions (PCR)

Investigated genes:

- properdin (*BF*)
- epidermal growth factor (*EGF*)
- estrogen receptor (*ESR*)
- follicular stimulating hormone subunit beta (*FSH β*)
- *H2AFZ* gene of the Z member of the H2A histon family (H2A.Z)
- leptin (*LEP*)
- prolactin receptor (*PRLR*)

The following chemical concentrations were mingled in 15 μ l volume for the PCR reaction:

- distilled water
- 10x buffer (Promega, USA)
- 2 mM MgCl₂ (Promega, USA)
- 0,2 mM dNTP mix (dATP, dCTP, dGTP, dTTP) (Promega, USA)
- 0,1 μ M primer forward (1. táblázat) (SIGMA-ALDRICH, USA)
- 0,1 μ M primer reverse (1. táblázat) (SIGMA-ALDRICH, USA)
- 0,1 U goTaq DNS polimeraze (Promega, USA)
- 100 μ M genomic DNS

Table 1. Characteristics of the primer used during PCR analysis

Gene Features	Forward primer's sequence (5'-3')	Reverse primer's sequence (5'-3')	The length of the amplified product (bp)	Adhesion temperature (°C)	Restriction Enzyme	Size of allele		References
						A (bp)	B (bp)	
<i>BF</i>	ACTGCTATGACGG TTACACTCTCCG	TCCAAGAGCCACC TTCTTGG	390	60	SmaI	237 és 153 (SmaI-)	390 (SmaI+)	Jiang & Gibson (1998)
<i>EGF</i>	GAAACAATTCCCG TGTTCTCT	TCACTTCCACACC TGTAACATCT	875	54	-	1 527	652	Mendez et al. (1999)
<i>ESR</i>	CCTGTTTTTACAG TGACTTTTACAGA G	CACCTCGAGGGTC AGTCCAATTAG	120	55	PvuII	120	55 és 65	Short et al. (1997)
<i>FSHβ</i>	GTATACCAGGTCC TAAG	GTCTCGTACACCA GCTCCTT	1650	60	α TaqI	1650	675 és 975	Linville et al. (2001)
<i>H2AFZ</i>	GGTGGTTTCTGCTG TCTGG	TCATGGCTGGTTCG TCCTAG	333	57-60	Bsu15I	158 és 175	333	Zhang et al. (2009)
<i>LEP</i>	TGCAGTCTGTCTCC TCCAAA	CGATAAATTGGATC ACATTTCTG	152	60	Hinfl	84 és 68 (C allél)	152 (T allél)	Neuenschwander et al. (1996)
<i>PRLR</i>	CGGCCGAGAATC CTGCTGC	ACCCCACTTGTA ACCCATCATCC	170	62	AluI	127	35 és 92	Cajdócsi et al. (2008)

The PCR conditions were the following:

Steps	Temperature(°C)	Timespan	
initial denaturation	95	90 (sec)	} 35 cycles
denaturation	95	30 (sec)	
attachment of primers	Table 1.	30 (sec)	
elongation	72	15 (sec)	
closing section	72	5 (min)	

I checked the successful amplification of the PCR reaction with agarose gel electrophoresis then added 3 µl mixture of different restriction enzymes to the 10 µl (except for *EGF*, since I applied the AS-PCR method with them, which makes it unnecessary to apply restriction enzymes, but I acquired the genotype of the individual during the electrophoresis directly after the PCR reaction):

- 0.65 µl restriction enzyme (Fermentas, USA) (table1.)
- 1.3 µl buffer (Fermentas, USA)
- 1.05 µl distilled water

Digestion took place at 37 °C during 4 hours in a PCR appliance. The digested PCR products were scanned with 2% agarose gel.

Separation of nucleic acids with electrophoresis

Substances required for gel-electrophoresis:

- 1 X TAE buffer (Tris-Base, EDTA, distilled water, acetic acid)
- 0.5 mg/ml Gel red dye
- 2 % agarose(Qbiogene, USA)

I applied a gel made of agarose which contained 2% Gel red dye to detect and check amplified products. 4 g agarose powder was mixed and heated up with 200 ml 1 × TAE buffer until the powder was completely dissolved. I added gel red dye to the liquid gel. The liquid gel was poured into a tray equipped with a comb and it became solid. After the removal of the comb the samples were placed into the gel slots. The samples were put into the electrophoresis appliance and scanned for 20-60 minutes at 120V depending on their size. We checked the results under UV ray.

Statistical analysis of the study on proliferation-related genes

I studied the following productional data of sows:

- interval between litters (IBL)
- percentage litter (PL)
- number litters (NL)
- mean born alive (MBA)
- mean born dead (MBD)
- mean born total (MBT)
- number born alive (NBA)
- number born dead (NBD)
- total number born (TNB)
- number 21 days (M21D)
- growth rate (GR)

During the study of pig production data, we investigated the expected occurrence between the alleles of different genes and the genotype occurrence with Chi-square test (χ^2) in order to check the Hardy-Weinberg balance of the population. We analyzed the variables with two-way analysis of variance with SPSS 22.0 program. Fixed factors were the breed and the genotype that corresponds to the locus and the interaction between these. Due to the unequal number of elements we considered the estimated Marginal Means and applied the Bonferroni correction to study the multiple factor comparisons (LENTH 2016).

2.2. Survival analysis of sows

We performed the survival analysis of the Hungarian Large White, Duroc and Pietrain raised on the pig farm of the Hód-Mezőgazda cPlc. in Hódmezővásárhely. The study included 295 Hungarian Large White, 76 Duroc and 91 Pietrain sows.

We considered the following traits related to sows: age of sows at the time of their inclusion into breeding, their age at the time of culling, time spent in production, number of inseminations, number of parities, intervals between litters, number of piglets born alive and born dead, mean of piglets born alive, mean of piglets born dead, number of raised piglet litters, number of raised piglets, weight of raised litters, mean of the litter weight raised, parity percentage and raising rate.

We used the SPSS 22.0 software for statistical analysis. We applied single factor analysis of variance, Kaplan-Meier analysis and Cox proportional hazard model. The determination of the significance of risk rates differences was done through the Wald chi-square test.

3. Results

The polymorphism study of the seven candidate genes I investigated comprises the first part of the description of results in terms of the 11 traits that have a huge impact on economical pig production. First I compared the data of the Hungarian Large White, Duroc and Pietrain sows, then due to the low number of Duroc and Pietrain individuals I introduce the results of the Hungarian Large White in a separate chapter.

In the second half I specified the so-called survival ($[S(t)]$) and hazard ($[h(t)]$) curves projected on the days of culling of the specific pig genotypes by using single factor variance analysis and the most important non-parametric methods of survival analysis (Kaplan-Meier analysis and Cox proportional hazard model). We calculated risk rates based on which we evaluated the life performance and survival of genotypes raised on the same farm.

3.1 Result of the studies genes

3.1.1. Results of the Hungarian Large White, Duroc and Pietrain sows

Polymorphism of propedin (*BF*) and the correlation between traits

I identified two alleles of the propedin gene (*BF*) in pig stocks, allele A and B. The occurrence of allele A of the Hungarian Large White is 14% for allele B it is 86%. Those of Duroc genotype are 17% for allele A and 83% for allele B and those of the Pietrain are 10% and 90%.

I detected AB and BA genotypes in sows. In the stock of the Hungarian Large White and Duroc BB genotypes made up 67% with the rest comprising AB. 80% of Pietrain sows was homozygous and 20% heterozygous.

According to the results of the statistical analysis the number of piglets born dead was significantly lower in the Hungarian Large White stock than in the Duroc and Pietrain stock. The Hungarian Large White produced 3.34 ($P \leq 0.013$) fewer vital piglets than the Duroc and 4.72 fewer piglets ($P < 0.001$) than Pietrain. The outcome resulted in a significant difference in the mean of piglets born dead. The mean of the Hungarian Large White sow was exceeded on the average by 0.76 dead piglets for Duroc ($P \leq 0.001$) and 1.06 ones ($P < 0.001$) for Pietrain.

The Hungarian Large White pork pigs showed a significantly higher mean of piglets born alive (MBA) which is 2.03 more than the result of Pietrain ($P \leq 0.004$) and 1.91 more than the result of Duroc ones ($P \leq 0.014$).

The number at 21 days at the age of selection (M21D) for the Hungarian Large White proved to be statistically higher. It exceeded the M21D of Pietrain sows with 1.19 pieces ($P \leq 0.01$) and 1.59 pieces ($P \leq 0.001$)

Correlation between genotypes and breed

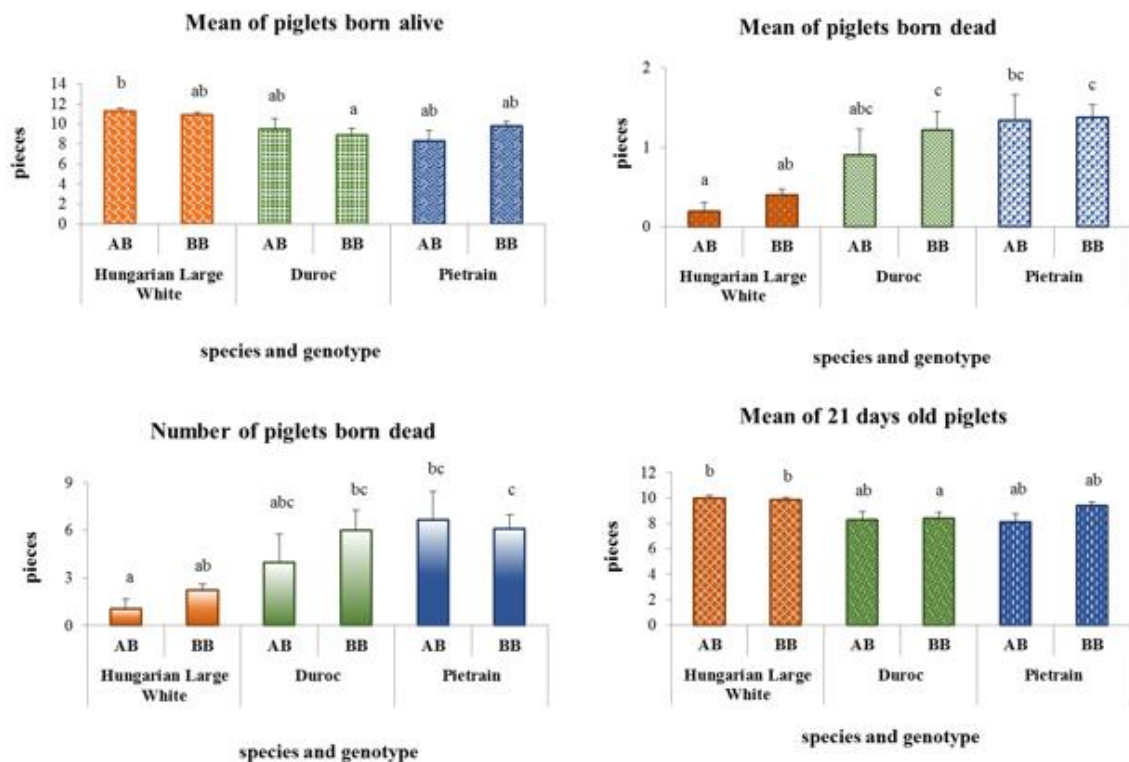


Figure 1.: Traits that indicate significant difference in the breed genotypes in case of the *BF* gene

The polymorphism of properdin gene resulted in a significant difference in the mean and number of the piglets born dead, the mean of piglets born alive and the number of piglets at the time of selection (figure 1.). In case of the Hungarian Large White and Duroc AB genotypes showed better results at NBD and MBD traits. The mean of piglets born alive from heterozygous Hungarian Large White sows exceeded the mean produced by Pietrain. The number of piglets at the time of selection - except for the Hungarian Large White - was higher in the BB genotype.

Polimorphism of epidermal growth factor (*EGF*) and the correlation between traits

Two alleles of *EGF* gene (A and B) were detectable in all the three breed. The allele occurrence in the Hungarian Large White was A (0.21), and B (0.79) in Duroc it was (0.11) and (0.89). It was the lowest in Pietrain sows with the occurrence of allele A making up 3% and allele B made up 97%.

All the three genotypes were present in the Hungarian Large White and in the Duroc stock but in Pietrain sows only AB and BB were present (figure 2.). In the Hungarian Large White the homozygous BB type represented the highest rate (66.5%) type AA made up only 6.5%. Heterozygous ones represented 27% while BB type individuals from Duroc stock represented 77.8% and heterozygotes showed 22.2%. 93% of Pietrain sows belonged to the BB genotype 6.7% belonged to the 6.7% heterozygous type.

The significant differences between breed manifested themselves in terms of interval between litters, mean and number of piglets born dead and the number of piglets at the age of selection.

The longest interval (IBL) between two parities in the population was detected in the Pietrain. Significant difference was detected between Pietrain and the Large White (80.56 days, $P < 0.001$) and between Pietrain and Duroc (89.89 days, $P < 0.001$). The statistical analysis showed that the number of piglets born dead was considerably lower in the Hungarian Large White than in Pietrain sows (-4.85 piglets, $P \leq 0.016$). This result led to a significant difference in the mean of piglets born dead (-1.51 piglets, $P < 0.001$). In case of Duroc sows the mean of piglets born dead was also higher than in Hungarian large sows (0.73 piglets, $P < 0.01$). Hungarian Large also showed a significantly higher number of piglets with 1.43 more pieces at 21 days of age (M21D) than Duroc sows.

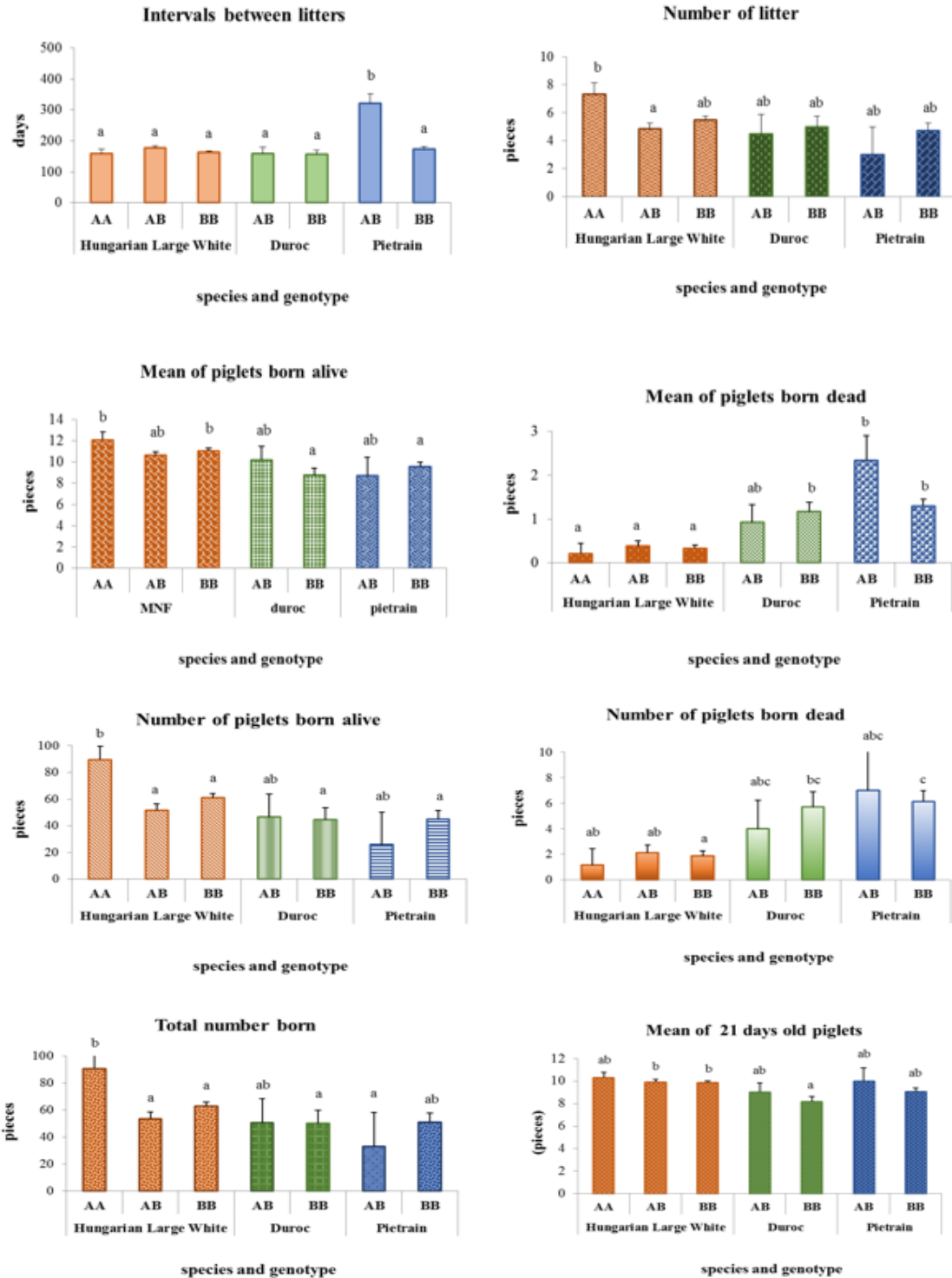


Figure 2.: Traits that indicate significant difference in the breed genotypes in case of the *EGF* gene

In case of the AB genotype individuals of the three breed sowed the shortest interval between litters. I detected the highest number of piglets born alive and the total number of piglets born in AA in the Large Hungarian Large White stocks, in AB in Duroc stocks and BB in Pietrain

stock. In the trait of parity rate the Hungarian Large White and Pietrain – like in case of the previous two traits – are showed the best results in AA and AB genotypes while in the Duroc stock in was homozygous B. The figures of selected pigs in the Hungarian Large White showed the dominance of the AA genotype while in case of the two other breeds the heterozygous individuals were overrepresented (figure 2.).

Overall we concluded that genotypes and differences between AA – BB and AA – AB alleles affected the number of piglets born. Homozygous AA individuals produce 39.44 more vital piglets ($P \leq 0.001$) than homozygous BB sows 48.19 more piglets than heteozygous AB genotypes ($P \leq 0.003$). The same result was traceable at the total number of the piglets born. Sows of the AA genotype produced a significantly higher number of piglets with 36.02 ($P \leq 0.005$) compared to BB genotypes and produced 44.98 more piglets ($P \leq 0.008$) than those of the AB genotype. It can have an impact on the trait of the *EGF* alleles, on the intervals between litters (IBL) and on litter number (NL). Data suggested that the longest interval between litters was with sows with AB allele, which is 54.32 days longer ($P \leq 0.001$) than that of BB genotypes. AB sows exceeded significantly the same trait of the AA genotype by 59.47 days ($P \leq 0.005$). The highest number of litters was shown by AA genotypes. The difference between the number of litters of the AA – BB genotypes was significant (3.22 piglets, $P \leq 0.018$) like the difference in AA – BB genotypes (2.27 piglets, $P \leq 0.031$). The highest number of piglets at the age of 21 days was in AA genotype sows and with AB type in Duroc and Pietrain breed for the lack of the AB genotype.

Polimorphism estrogen receptor gene (*ESR*) and the correlation between traits

I detected the A and B alleles of the *ESR* gene in the Hungarian Large White with an occurence (0.62) for A and (0.38) for B. The occurence of alleles in Pietrain sows was A (0.90) and B (0.10). I didn't find allele B in the individuals of Duroc.

I detected 2 genotypes (AA, BB) in the studied stock. I did not find BB genotype sows in neither of the breeds. The rate of homnozigous individuals was 24.74% and that of heterozygous ones was 75.26% in the Hungarian Large White stock. The Duroc stock comprised only of homozygous AA sows. Among the Piterain stock type AA was made up 80% ans AB genotype made up 20%.

The statistical analysis of *ESR* gene data showed that the mean of piglets born alive was higher with 2.06 piglets ($P \leq 0.004$) in case of the Hungarian Large White compared to the Duroc offsprings and they produced 1.59 more piglets ($P \leq 0.033$) compared to Pietrain. This

exceeded the results of Duroc offsprings with 22% and and the results of Pietrain offsprings with 16%. The Hungarian Large White sows produced 3.65 pieces fewer dead piglets than Duroc sows and 5.45 pieces fewer than Pietrain sows ($P \leq 0.003$ and $P < 0.001$). This tendency is present in the mean of piglets born dead because Duroc sows produced 0.82 more offsprings while Pietrain produced 1.26 more offsprings ($P < 0.001$). The mean of piglets at the age of 21 days was 1.50 pieces ($P \leq 0.001$) more than in Duroc breed and 0.5 more than Pietrain similarly to the mean of piglets born alive.

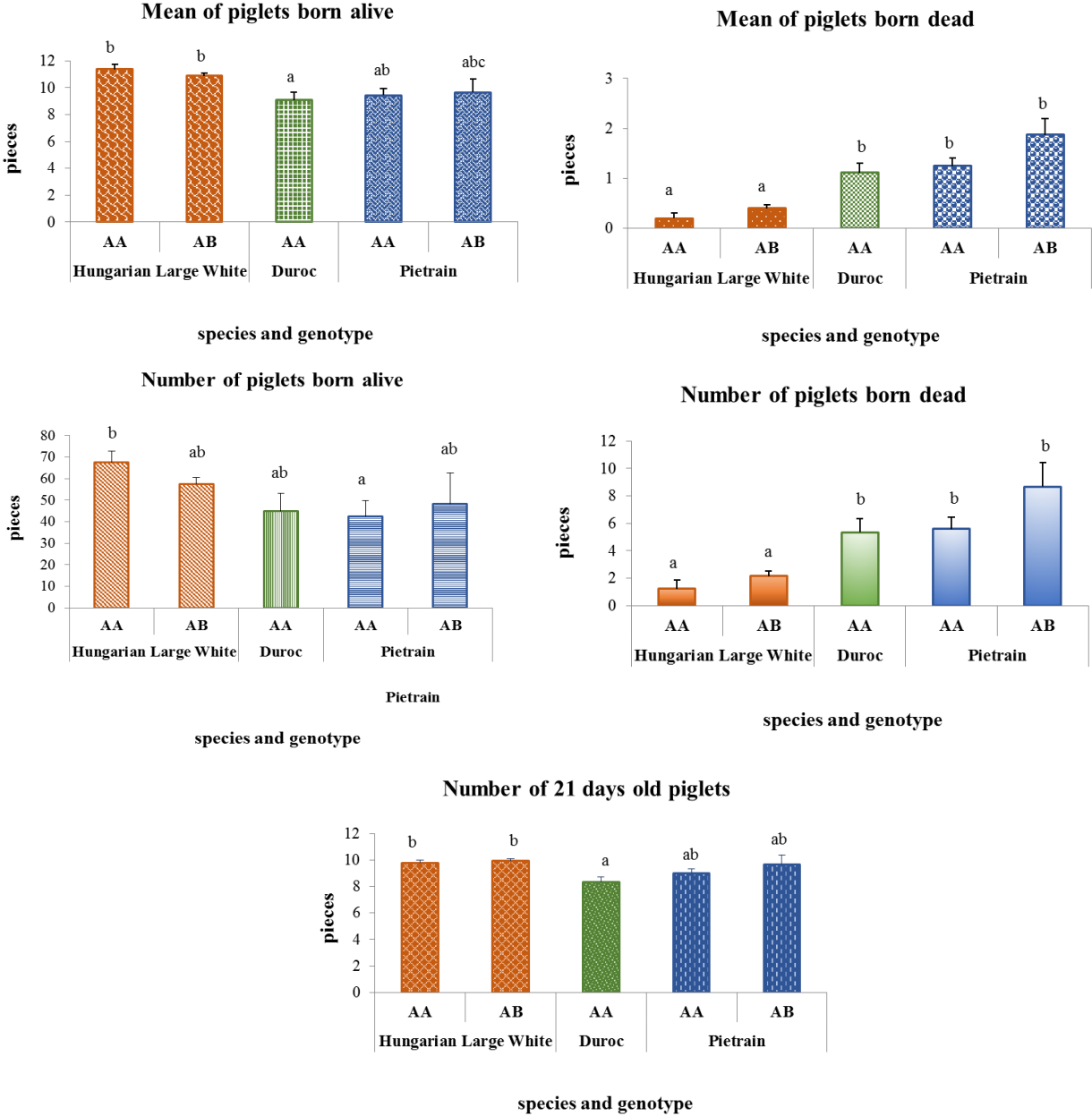


Figure 3.: Traits that indicate significant difference in the breed genotypes in case of the *ESR* gene

Sows of AA genotype gave birth to fewer stillborn piglets than sows of the AB genotype (figure 3.). The homozygous type A Hungarian Large White produced the highest mean of piglets born alive, while in the Pietrain stock heterozygous individuals showed the best performance. Heterozygous individuals of the Hungarian Large White and Pietrain breed produced the highest number of selected piglets.

Follicle-stimulating hormone- subunit beta (*FSHβ*) and the correlation between traits

We detected the A and B allele of the *FSHβ* gene with an occurrence rate of A (0.32) B (0.68). The rate of alleles was 20% and 80% in Pietrain sows. The Duroc group contained only allele B.

All genotypes were found (AA, AB, BB) in the Hungarian Large White breed with an occurrence of AA: 3.22%, AB: 57% and BB: 39.78%. There were only B homozygous individuals among the studies Duroc sows. The AA genotype was missing from the Pietrain group but 40% AB genotype was detectable besides 60% homozygous individuals.

During the analysis of the data of *FSHβ* we concluded that the N21D was higher with 1.22 piglets ($P \leq 0.026$) compared to Duroc and 0.48 piglets compared to Pietrain ($P \leq 0.647$). The number of piglets born dead was lower with 3.87 pieces ($P \leq 0.006$) compared to Duroc piglets and 4.89 pieces fewer than the result of Pietrain ($P < 0.001$). The number of piglets born dead of Duroc piglets exceeded with 0.85 pieces ($P \leq 0.001$) the mean of piglets born dead ($P \leq 0.001$) that same trait of the Hungarian Large White. Similarly to Pietrain sows Duroc individuals showed higher figures with 1.09 dead piglets ($P < 0.001$) compared to the proliferation of the Hungarian Large White.

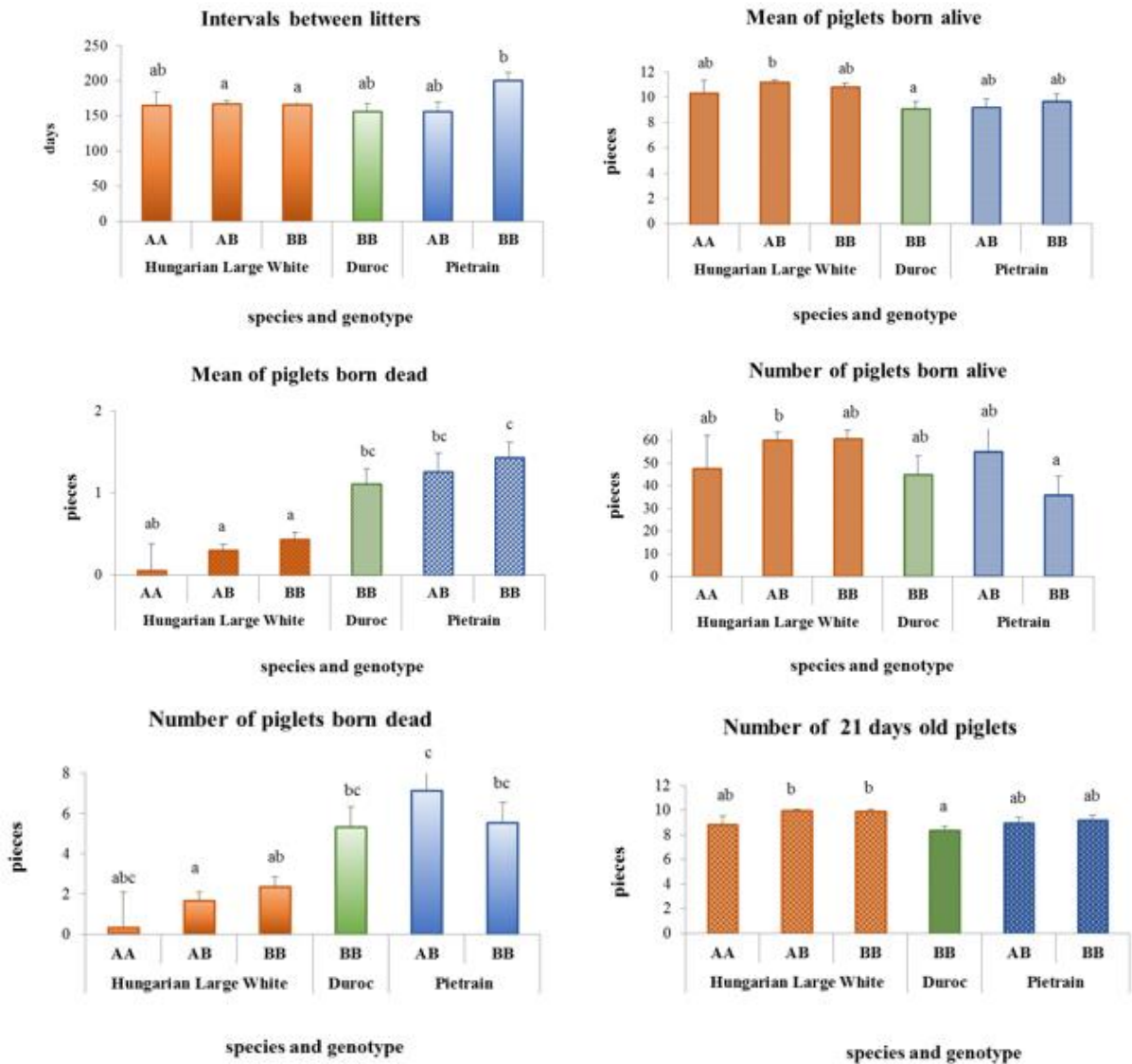


Figure 4.: Traits that indicate significant difference in the breed genotypes in case of the *FSH β* gene

The data of the piglets born dead showed that AA genotypes performed best (figure 4.). The analysis of the means of the data of the same trait revealed that AB sows performed better than BB individuals. The number of piglets born alive was the highest in the AB Hungarian Large White and in BB Pietrain breed. The ability of the breed to raise piglets had the same tendency described above.

Z member of H2A histon family (*H2AFZ*) and the correlation between traits

I detected two alleles of *H2AFZ*. I found an occurrence of A (0.85) and B (0.15) in the stock of the Hungarian Large White. The difference between their occurrence was found in the Duroc breed with A (0.44) and B (0.56) values.

The rate of AA genotype was 74.20%, for BB was 21.50% and the rate of heterozygotes was 4.30%. The occurrence of AA was at 22.2%, BB was at 33.3% and AB genotype was at 44.44% in the Hungarian Large. The Pietrain stock did not contain B homozygous individuals. The percentage of A homozygous individuals was 73.33% for heterozygous ones in was 26.67%.

By investigating the effect of breed we found that the mean and number of piglets born alive were lower in Duroc and Pietrain breed than in the Hungarian Large White. It produced more offsprings with 2.17 offsprings ($P \leq 0.009$), and 1.57 more ($P \leq 0.041$) than Pietrain. We found that in case of Duroc and Pietrain breed the number of piglets born dead was 3.97 pieces lower than ($P \leq 0.004$) at Duroc and Pietrain with 3.97 pieces ($P \leq 0.004$) and 4.97 pieces ($P < 0.001$). I detected a similar tendency comparing the means of the piglets born dead. The Hungarian Large White underperformed Duroc sows with 0.84 ($P \leq 0.001$) piglets born alive and Pietrain sows with 1.18 piglets.

The data of piglets at the age of 21 days at selection proved that the Hungarian White Large has a better piglets raising ability and reproduction. The litters of the Hungarian Large White sows raised 1.24 more piglets ($P = 0.024$) than Duroc breed and 0.42 more piglets ($P \leq 0.917$) than Pietrain.

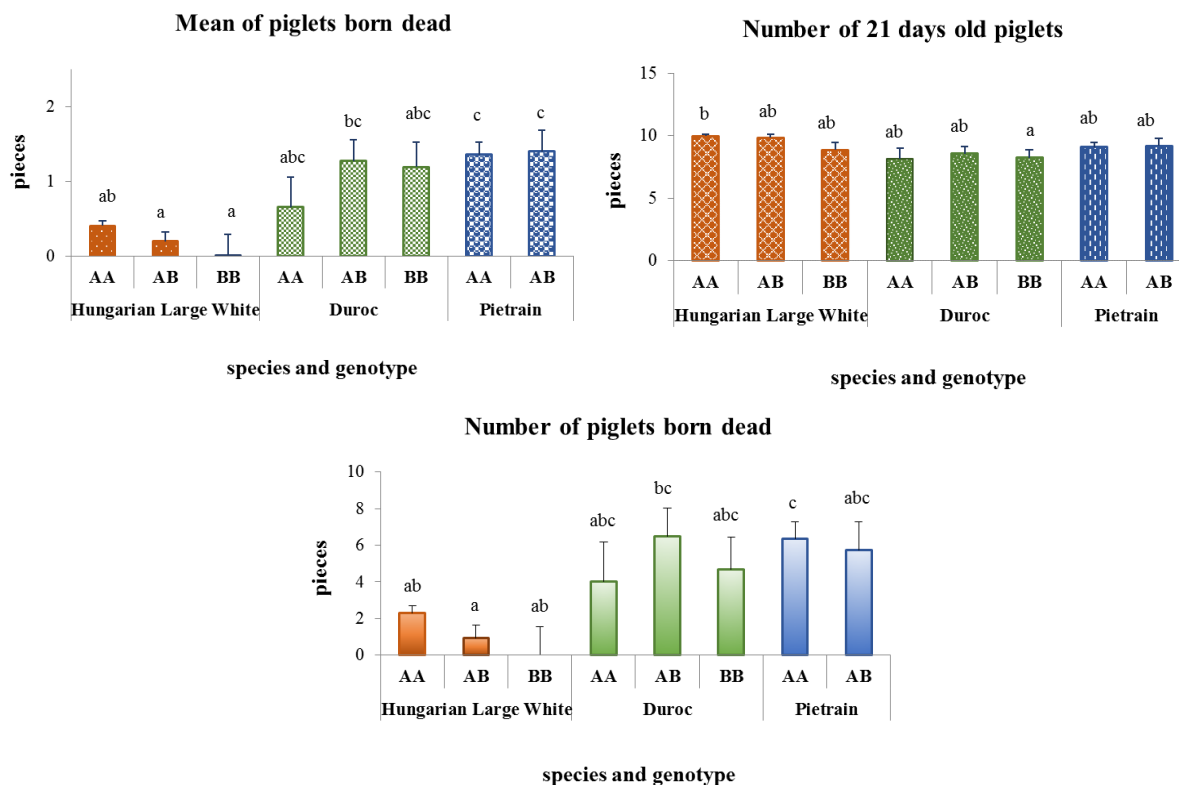


Figure 5.: Traits that indicate significant difference in the breed genotypes in case of the *H2AFZ* gene

The AA, AB and BB genotypes can be found in Hungarian Large White, Duroc and Pietrain breed and the Pietrain group lacked BB homozygous individuals (figure 5). BB Hungarian Large White sows and AB Duroc sows produced the lowest mean and number of piglets born dead.

In terms of the ability to raise piglets AA Large White sows and AB Duroc and Pietrain breed proved to be the most efficient.

Polimorphisms of leptin (*LEP*) and the correlation between traits

I recognized the T and C alleles during the analysis of leptin gene. The alleles were present in all three in similar proportions. In case of the Hungarian Large White the ratio was the following: T (0.87) and C (0.13) while that of the Duroc was T (0.87) and C (0.17) and Pietrian stock showed T (0.80) and C (0.20) ratio.

The rate of genotype TT was 77.41% in the studied Hunagrian Large White stock, CC homnozygous individuals represented 3.24% and heterozygous one made up 19.35%. Only genotypes TT and TC individuals were found in the Duroc stock the rate of homozygous

individuals was twice the as much as the rate of heterozygous ones. Similarly, the rate of genotype TT and TC was 60:40.

The analysis of the polymorphism of *LEP* showed that the Hungarian Large White breed gave birth to fewer dead piglets. The Duroc produced 3.09 fewer dead piglets ($P \leq 0.054$) and Pietrain produced 4.15 fewer dead ($P < 0.001$) offsprings. It showed a better result in the mean of the piglets born dead with 0.75 pieces ($P \leq 0.005$) than Duroc and 1.01 pieces more piglets ($P < 0.001$) than Pietrain. The Hungarian Large White produced 1.01 more piglets ($P < 0.001$) than Pietrain sows. The Hungarian Large White sows presented a higher number of piglets at the age of 21 days outperforming the result of Pietrain with 1.59 pieces ($P \leq 0.004$) and Duroc with 0.88 offsprings ($P \leq 0.087$).

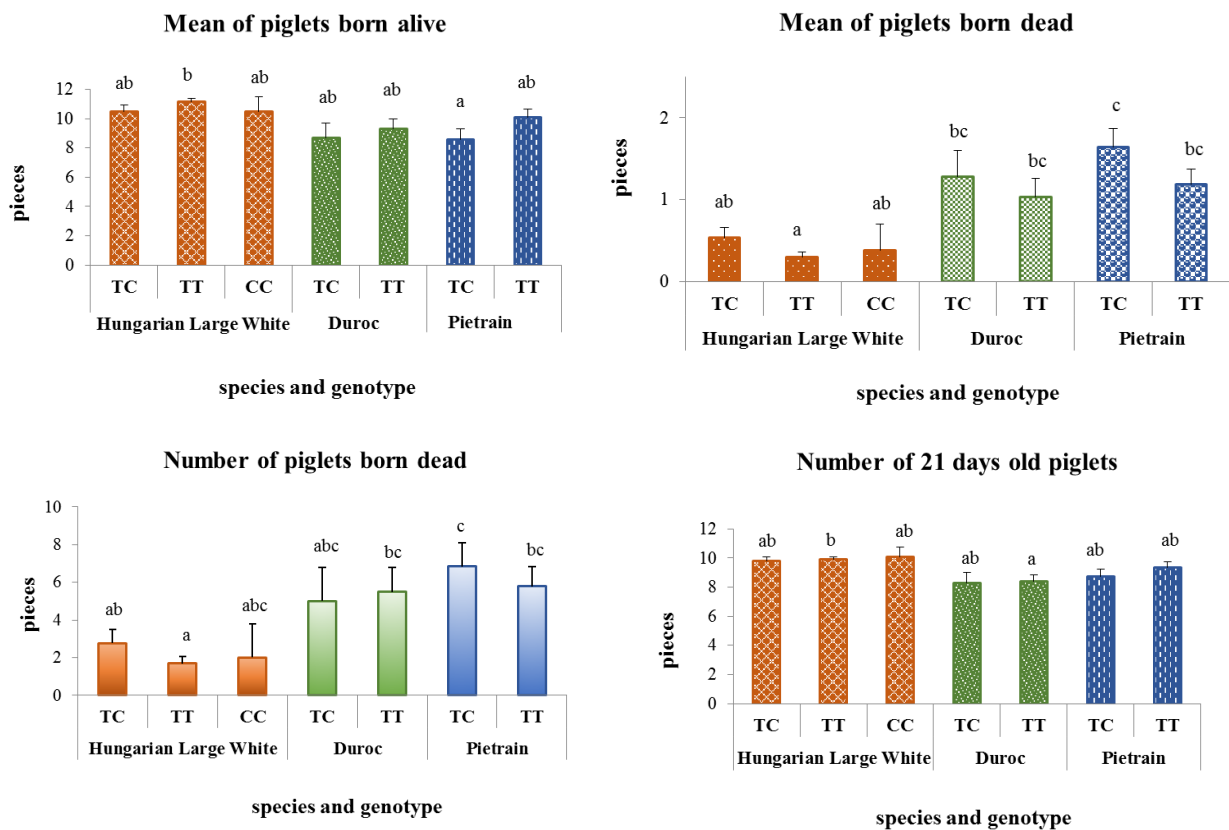


Figure 6.: Traits that indicate significant difference in the breed genotypes in case of the *LEP* gene

I measured TC, TT and CC genotype sows in the group of the Hungarian Large White. The frequency values of the genotypes are 19.35%, 77.41% and 3.24% in the above order. Type C homozygous individuals were missing from the Duroc and Pietrain stocks. In Duroc the genotype TT made up 60% and T homozygous individuals represented two-third among the Pietrain group. The Hungarian Large White produced a significantly lowest number of piglets born dead in the TT genotype and the mean of dead piglets indicated this too. CC sows gave

better result in the number of piglets at the age of selection. The number of piglets in the Duroc breed gave the edge to TC in the number of piglets born dead and to TT in the mean of piglets born dead. But the result was not significant. In terms of the piglets born dead from Pietrain sows, the T homozygous individuals performed best and were visible in the number of piglets at the age of selection (figure 6.).

Polimorphism estrogen receptor (*PRLR*) and the correlation between traits

I recognized the two alleles during the analysis of polimorphism of the *PRLR* gene. The occurrence of allele A was 63 % in the studied Hungarian Large White and allele B was 37%. In type Duroc the same allele occurred in a 17% and 83% ratio. I found the occurrence with the rate of A (0.59) and B (0.41)

We found three genotypes among the individuals (AA, AB and BB). Genotype AA showed 43%, BB 40 % in the Hungarian Large White and heterozygous individuals represented 17%. The Duroc genotype has no AA genotype while the other two genotypes were present in a two-third-one-third ratio in favour of homozygous individuals. We found genotypes AA and BB individuals in a ratio of 40% and 40% in Pietrain sows with 20% being homozygous B.

Statistical analysis showed that the interval between litters was 35.52 days shorter ($P < 0.001$) compared to Pietrain. It was 9.13 less than the figures of the Duroc sows. The mean of the piglets born alive was 2.02 higher ($P \leq 0.01$) for the Hungarian Large White compared to the Duroc breed. It took over Pietrain with 1.55 piglets ($P \leq 0.014$). The mean of selected piglets at the age of 21 days was less for Duroc and Pietrain than for the Hungarian Large White with 1.56 piglets ($P \leq 0.002$), and 0.65 piglets ($P \leq 0.187$). The number of piglets born dead was higher with 3.17 pieces ($P \leq 0.024$) for Duroc and 4.22 pieces Pietrain ($P < 0.001$) compared to the Hungarian Large White. The number of piglets born dead showed the same rate as above: sows of the Hungarian Large White produced more vital individuals with a rate of 0.72 pieces ($P \leq 0.003$) and 1.07 pieces ($P < 0.001$) than Duroc and Pietrain.

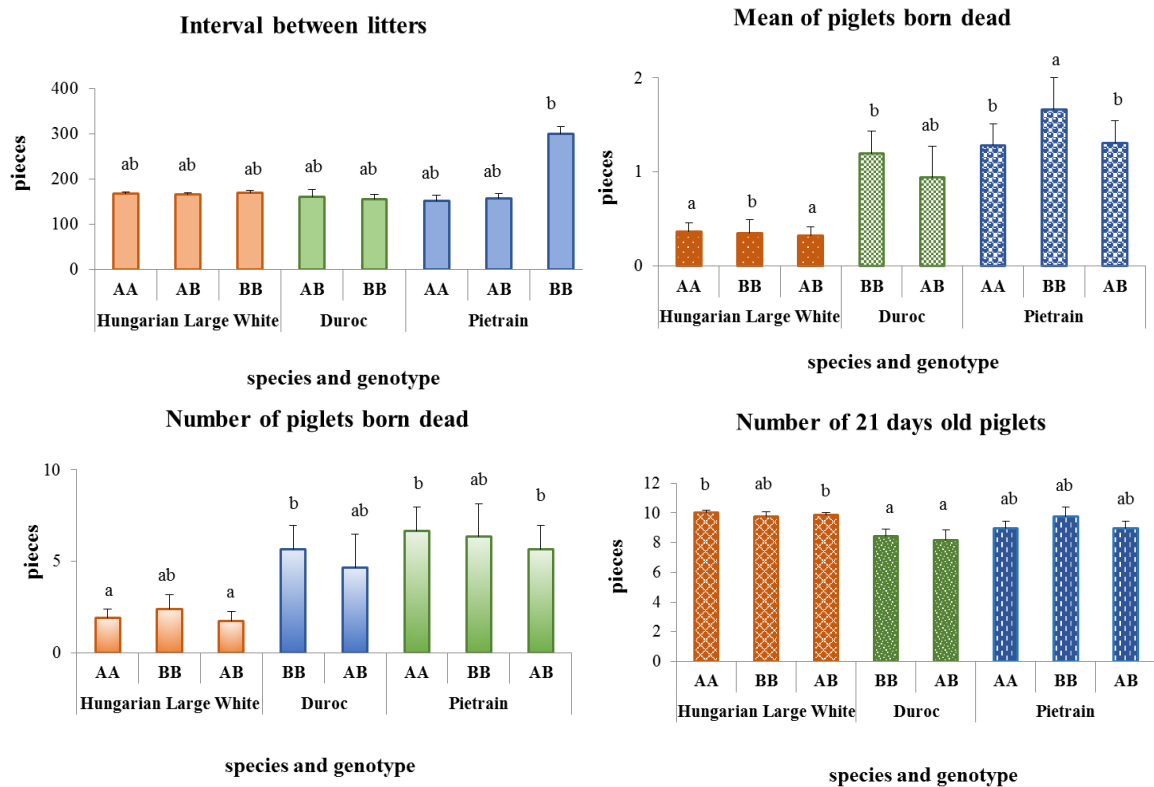


Figure 7.: Traits that indicate significant difference in the breed genotypes in case of the *PRLR* gene

The interval between parities in case of pigs carrying AB and AA alleles of the *PRLR* gene was considerably shorter than those with BB alleles (-46.98 days, $P < 0.001$, -48.12 days, $P < 0.001$). The mean of piglets born dead was higher in the parities of BB sows, and the best results were exhibited by AB individuals (figure 7.). In the ability of raising piglets Duroc and Pietrain AB sows stood out as well as AB sows from the Hungarian Large White stock.

3.1.2. Results of the study on the Hungarian Large White pork genotype

In this subchapter I focus only on the description of the relation between gene traits indicating significant difference.

The relation between the polymorphisms of properdin gene (*BF*) and the interval between litters

Two alleles, A and B of the properdin gene (*BF*) were identified in the Hungarian Large White stock. The occurrence of allele A was 14% while for allele B it was 86% in case of the

Hungarian Large White sows. AB and BB genotypes were detected in sows. 67% of the Hungarian Large White stock belonged to genotype BB and 33% to genotype AB.

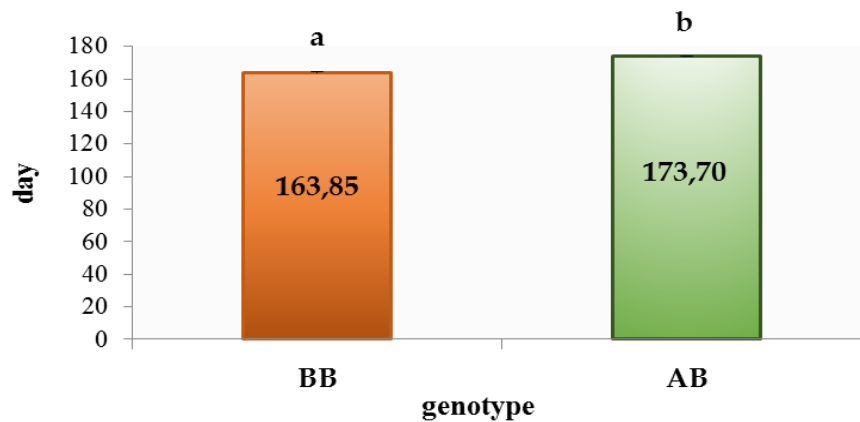


Figure 8.: The interval between litters in the genotypes of the Hungarian Large White in case of *BF* gene

The corrected mean of the interval between litters was significantly lower in case of genotype BB in the population compared to genotype AB. The difference is 9.84 days (figure 8.). For properdin (*BF*) no statistically detectable difference was found for the other studied traits.

The polymorphism of the epidermal growth factor (*EGF*) and the relation of the interval between litters, number of litters, number of piglets born alive and the total number of piglets born.

Two alleles (A and B) of the *EGF* were detectable. The allele occurrence was 21% for allele A and 79% for allele B in the Hungarian large White population. Here BB type homozygous individuals showed the highest percentage (66.5%) occurrence, type AA one showed only 6.5%. Heterozygous individuals represented 27%.

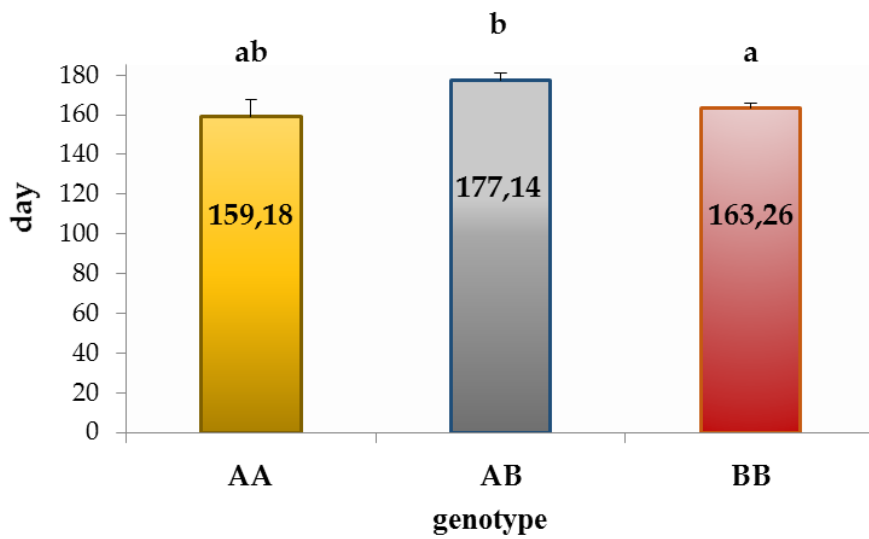


Figure 9. : The interval between litters in genotypes of the Hungarian Large White in case of *EGF* gene

The longest interval between litters was that of genotype AB in the studied stock. There was a significant difference between AB and BB genotype individuals (13.87 days difference) while the corrected mean of genotype AA (due to a higher deviation) does not differ from the other two genotypes (figure 9.).

The highest number of piglets born, 7.33 pieces was shown by genotype AA among the Hungarian Large White sows, followed by BB (5.48) and AB (4.85) pieces.

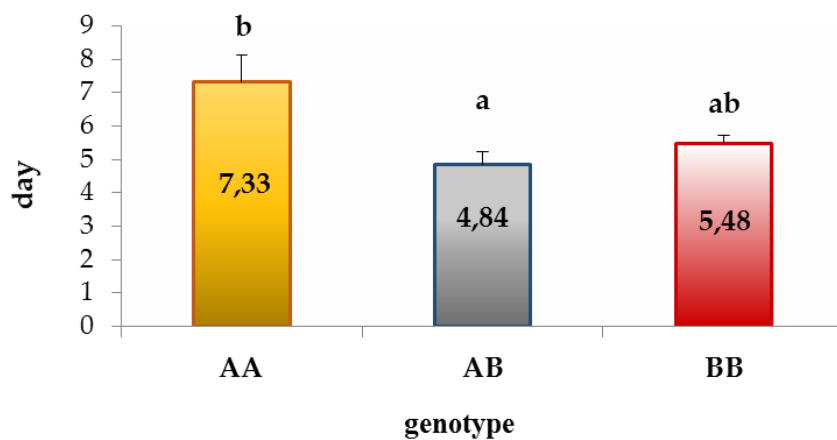


Figure 10: The number of parities in genotypes of the Hungarian Large White in case of *EGF* gene

AA and AB genotype individuals indicated a significant difference (2.49 parities difference) while the corrected mean of genotype BB does not differ from the other two genotypes (figure 10.).

During the analysis of the value measuring trait the number of piglets born alive I observed that genotype AA sows produced the highest number of piglets (89.50 pieces) followed by BB sows (60.84 pieces) finally AB individuals with 51.44 pieces.

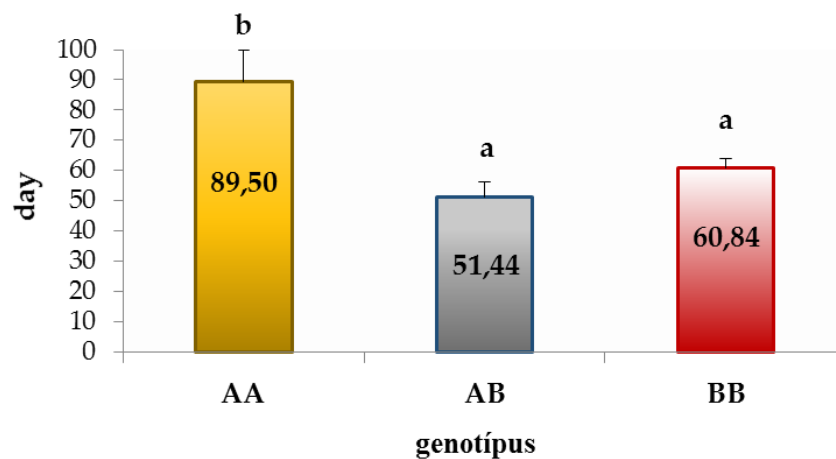


Figure 11. : The number of piglets born alive in genotypes of the Hungarian Large White in case of *EGF* gene.

Significant difference was shown between AA and AB (38.06) pieces in addition to AA and BB genotype animals (27.95 days difference). At the same time the corrected mean of AB and BB genotypes were not different.

The total number of piglets born alive was the highest with genotype AA individuals with 91.07 pieces followed by BB (62.72 pieces) and AB genotype with 53.56 pieces (figure 11.).

Studies by HOROGH et al. (2005) concluded that genotype BB sows – instead of AA genotype sows as my study proved – outperformed AB and AA genotype sows at the first and subsequent parities in the number of piglets born alive (NBA), the total number of piglets born (TNB) and the corrected number of selected piglets (CNW).

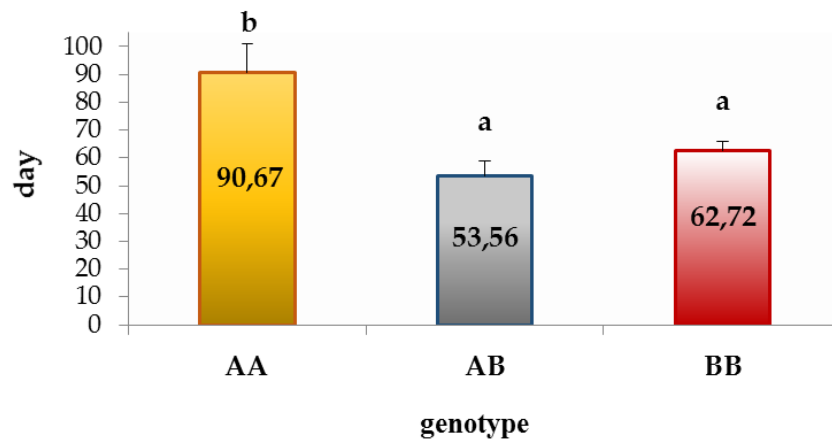


Figure 12. : The total number of piglets born in genotypes of the Hungarian Large White in case of *EGF* gene.

During the analysis of value measuring trait of the total number of piglets born there was a significant difference between AA and AB genotype individuals (37.11 piglets) and between AA and BB genotype animals (27.95 days difference). The corrected mean of AB and BB genotypes did not differ from each other (figure 12.)

3.2. Results of the survival analysis

Since data about sows were collected from three different breeds raised on the same farm I performed the comparison analysis of them. We emphasised the lifespan of sows and life performance and addressed the question of whether there is a significant difference between the sows in terms of production. First I investigated the different production parameters of the culled sows belonging to the three breeds.

Table 2. Description of the production parameters of the three breeds and the indication of significant differences

Factors examined	Hungarian Large White		Duroc		Pietrain	
	N°	mean±standard error *	N°	mean±standard error *	N°	mean±standard error *
Age of sows at the time of their inclusion into breeding (day)	291	233.4 ± 2.62a	73	214.68 ± 5.15b	83	230.04± 4.67a
Their age at the time of culling (day)	295	1080.4 ± 29.41a	76	832.20 ± 53.02b	91	906.06± 51.68b
Time spent in production (day)	291	845 ± 29.31a	73	613.99 ± 53.98b	83	650.22± 54.32b
Number of mating (piece)	295	5.6 ± 0.20a	76	3.96 ± 0.35b	91	4.67± 0.35ab
Number of parities (piece)	254	4.7 ± 0.18a	57	4.19 ± 0.35a	68	4.43± 0.35a
Intervals between litters (day)	254	117.4 ± 3.85a	57	100.23 ± 7.09a	68	102.79± 6.45a
Number of piglets born alive (piece)	254	52.6 ± 2.04a	57	36.95 ± 3.13b	68	38.33± 3.33b
Number of piglets born dead (piece)	203	5.8 ± 0.34a	45	5.71 ± 0.62a	59	7.10± 0.84a
Mean of piglets born alive (piece)	254	10.9 ± 0.09a	57	8.71 ± 0.21b	68	8.29± 0.21b
Mean of piglets born dead (piece)	254	0.9 ± 0.05b	57	0.98 ± 0.11b	68	1.38± 0.16a
Number of litters raised (piece)	252	4.7 ± 0.17a	54	4.09 ± 0.34a	67	4.34± 0.33a
Number of 21 days old piglets (piece)	254	48.5 ± 1.87a	57	30.96 ± 2.74b	68	35.75± 2.95b
Weight of 21 days old piglets (kg)	252	308 ± 11.80a	54	198.31 ± 17.78b	67	236.57± 19.42b
Mean of 21 days old piglets (piece)	254	10.3 ± 0.09a	57	7.62 ± 0.28b	68	8.05± 0.20b
Mean weight of raised litter (kg)	254	64.8 ± 0.62a	57	44.41 ± 1.88c	68	51.94± 1.37b
Percentage litter (%)	254	75.3 ± 1.36b	57	87.98 ± 1.93a	68	72.62± 2.50b
Raise percentage (%)	254	93.0 ± 0.81a	57	81.22 ± 3.20b	68	96.99± 2.55a

* the distinct letters mean significant difference at level $P \leq 0.05$ based on the Tukey test

After performing the single variance analysis it can be concluded that production indicators of the different breeds vary from one another in many cases based on table 2. The lowest age of sows at the time of their inclusion into breeding was exhibited by Duroc sows while the data at the two other breeds were significantly higher. In contrast the highest age of culling was characteristic of the Hungarian Large White while the sows of the other two breeds were culled considerably earlier. The time spent in production was characterised by the same tendency. The number of inseminations was the highest with the Hungarian Large White, only the performance of Duroc breed was significantly lower. The Tukey test of the variance analysis indicated no significant difference between the number of parities, the number of days

between two parities and the number of piglets born dead. The three breed showed the same rates in terms of the number of raised litters.

At the same time considering the whole of the production the number of piglets born alive from the Hungarian Large White outperformed the values found at the two other breed. A similar tendency was observed in the mean of the piglets born alive, number of raised litters, weight of raised litters, and the weight of litter of raised piglets. I found an opposite result investigating the mean of dead piglets since the figures of the piglets born dead from Pietrain sows highly exceeded the mean of the other two breed. The order of the breeds after investigating the mean of the weight of raised letters is the following: Hungarian Large White, Pietrain and Duroc. After analyzing the parity percentage we found an order that is different from the one above because Duroc was in the first place followed by Hungarian Large White and Pietrain. The last indicator we studied was the raising percentage where the sows of the Hungarian Large White and Pietrain showed no statistically significant difference but the performance of Duroc sows did not match up to performance of these two.

After this I created the survival and hazard curves of the different genotype sows. (figure 8. and 9.). The survival curves represent the probability for sows remaining in production in case of different breeds. By contrast the risk curves show the chance oif culling. The graphics show that the curves about individuals from the Hungarian Large White are detached from the curves of the other two breeds. This means that the time the Hungarian Large White spent in production was longer than that of the sows of the other two breed kept under the same conditions.

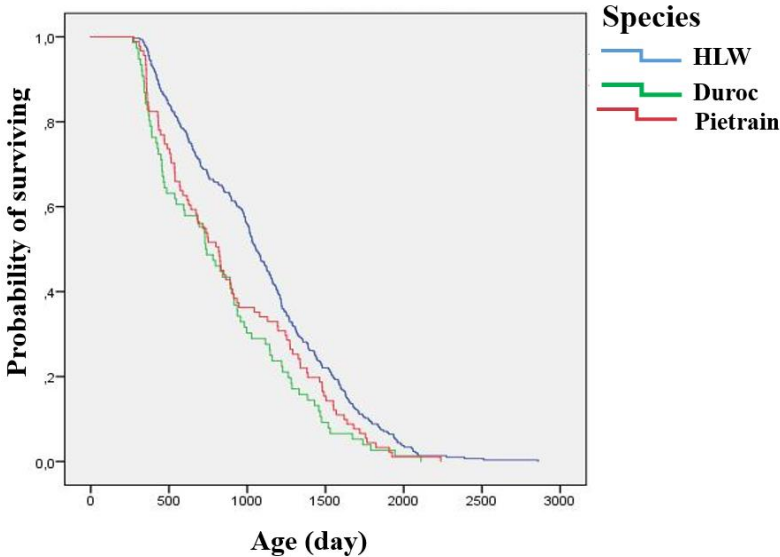


Figure 8.: Survival curves of sows per breed
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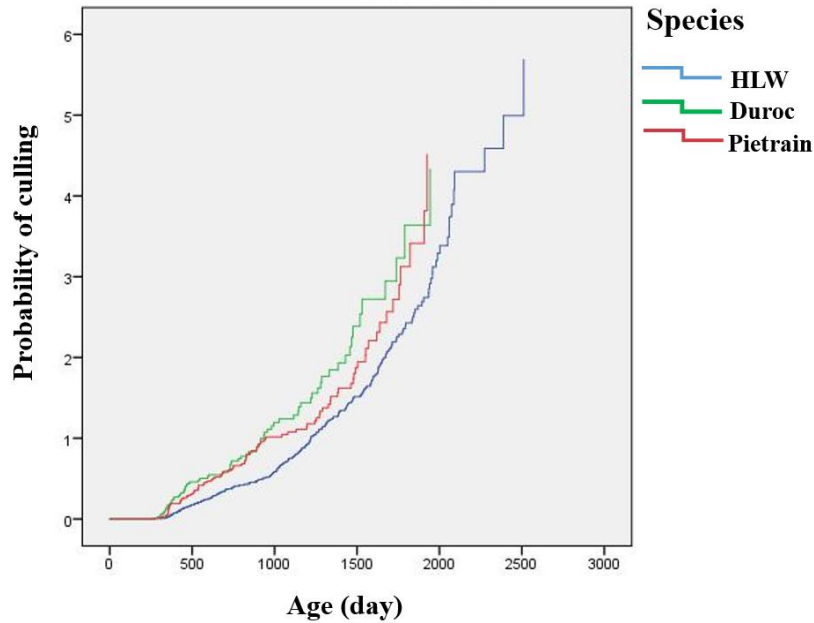


Figure 9.: Hazard curves of sows per breed
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The median of age of the three breed differed at the time of culling. In case of the Hungarian Large White the age at the time of culling was 1056 ($\pm 33,52$) days, Duroc and Pietrain breed this value was lower (735 ($\pm 73,56$) and 818 ($\pm 71,98$) day. This means that half of the Hungarian Large White individuals had lived for at least 1050 days before they were culled while individuals from the other two breed left production before the age of 710 days. The results from above are illustrated by figure 8. and 9. as they show the survival and hazard curves of the culling categories.

The same conclusion can be made based on the hazard curves. Based on the results of the log-rank test of survival analysis we can determine that the test showed a significant difference between the three breed ($\chi^2=16.981$; $P<0.001$), which means that the survival rates of the certain breed varied remarkably.

The risk rate of all three breed showed significant difference ($P<0.01$) compared to the risk rate of the Hungarian Large White. Since the risk rate of all three breeds were above 1 it means that the occurrence of culling was higher in case of any breed than for HLW. The risk rate of Duroc was 1.6 times ($P<0.001$) higher and Pietrain was 1.36 times ($P<0.01$) more likely to fall out of production than the Hungarian Large White.

Regarding the results above we have to emphasize that they were valid only in terms of the stock on the site we studied. At the same time we can conclude that in accordance with other

studies (DIJKHUIZEN et al., 1989; LUCIA et al., 2000; BALOGH et al., 2006; ENGBLOM et al., 2008), in case of the stocks difference in genetic makeup can be responsible for having a different production risks. For this reason in order to make production more efficient the breed needs to receive care in order for them to provide for the gilts and have more professional gilt raising (USUI és KOKETSU, 2015). By this their leave from production can be prevented.

4. New scientific results

In my thesis I can make the following statements that are considered novel in terms of the studies in this subject-matter based on my primary and secondary research:

1. During the polymorphism study of the 7 genes performed in the Hungarian Large White stock on the studied site I proved in terms of the different genotypes that the most significant impact on the reproduction-related trait was made by the *EGF* gene. In the studied breeds the interval between litters (IBL), the number of litters (NL), the number of piglets born alive (NBA), and the total number of piglets born (TNB) traits were influenced by the epidermal growth factor gene, *EGF* while the interval between litters (IBL) was influenced by properdin gene (*BF*).
2. Using survival analysis methods I demonstrated that the risk of being culled for sows from different breeds I investigated varied considerably. The risk of being culled for the Duroc breed proved to be 1.6 times higher ($P < 0.001$) than the risk for the Hungarian Large White while Pietrain sows are 1.36 times more likely to fall out of production ($P < 0.01$).

5. Applicability of the results

1. The epidermal growth factor gene (*EGF*) proved to be the most informative since its alleles indicated significant difference in four of the studied reproduction-related genes while properdin (*BF*) induced significant differences in one gene.

In the given site the selection of *EGF* for AA genotype Hungarian Large White sows can lead to the improvement of proliferation indicators.

2. It was proven that the culling risk of Duroc breed is 1.6 times higher than that of the Hungarian Large White among the stocks of the Hungarian Large White, Duroc and Pietrain kept on the pig farm of the Hód-Mezőgazda cPlc. Pietrain sows were 1.36 times more likely to drop from production in the given time frame. Based on the figures the planning of sow rotations create a continuous, safe and economical sow supply by which the efficiency of pig farming can be increased.

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7. List of publications



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Candidate: Ágnes Baginé Hunyadi
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MTMT ID: 10055715

List of publications related to the dissertation

Hungarian scientific articles in Hungarian journals (2)

1. **Baginé Hunyadi, Á.**, Kusza, S., Balogh, P.: Magyar nagyfehér, duroc és pietrain kocák túlélés elemzése.
Agrártud. közl. 69, 31-36, 2016. ISSN: 1587-1282.
2. **Baginé Hunyadi, Á.**, Balogh, P., Kusza, S.: Szaporaságra ható gének (LEP, PRLP, ESR BF, EGF, FSH-[Béta], H2A.Z) polimorfizmus vizsgálatának rövid áttekintése sertésben.
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3. **Baginé Hunyadi, Á.**, Kusza, S., Balogh, P.: Examination of the interval between litters (IBL) of different genotype HLW sows using survival analysis.
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Acta Biochim. Pol. 63 (2), 359-364, 2016. ISSN: 0001-527X.
DOI: http://dx.doi.org/10.18388/abp.2015_1188
IF: 1.187 (2015)



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9. **Baginé Hunyadi, Á.**, Jankóné Forgács, J.: A kendermagos magyar tyúk tojóival előállított genotípusok vágási teljesítménye és húsminőségi vizsgálata.
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A hús. 3-4, 97-102, 2008. ISSN: 1215-0665.

Total IF of journals (all publications): 1,187

Total IF of journals (publications related to the dissertation): 1,187

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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