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Relationship of *PIT-1* Gene Polymorphism with Breeding Parameters and Body Weights of Cows and Calves

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ABSTRACT

The aim of this work was to determine the dependency between polymorphism in the PIT-1 and fertility rates, body weight of cows and calves, the course of parturition and viability of calves in Polish Black-and-White Holstein-Friesian cattle. The analysis included 1024 cows and 3663 calves of the Polish Black-and-White Holstein-Friesian breed (phf). The study included an analysis of one polymorphic place PIT-1 (c.1178G>A). The source data used to calculate the indicators of reproduction were collected from cows inseminated by semen of Black-and-White Holstein-Friesian bulls and giving birth to single calf. The following information was taken from each animal: age at first insemination, age at first calving, number of semen portions attributable to successful insemination, length of pregnancy and periods of: insemination service, postpartum resting period, interpregnancy period and intercalving period. The study also determined weight of the cow and weight of the calf at calving (including gender). Statistical calculations were performed using the statistical package SAS v.9.3. The following procedures were used: MEANS and GLM. In black and white Holstein-Fresian cattle there is genetic variety in analysed locus c.1178G>A gene *PIT-1*. Frequency of genotypes and alleles was respectively: AA=0.0469, AG=0.3154, GG=0.6377, A = 0.2046 and G = 0.7954. AA homozygotes had the highest age at first insemination and the lowest insemination index. Cows with this genotype after calving and calves (both gender) from AA homozygotes had the biggest weight after calving. In contrast GG homozygotes had the longest intercalving period. The results prove the existence of dependencies between genu *PIT-1* gene polymorphism in locus c.1178G>A, reproductive potential and body weight of cows and calves. More favorable results were observed in AA homozygotes. It is possible to use these dependencies in genetic selection of farm animals. In order to confirm the obtained results it would be advisable to conduct studies in a larger population of cows with consideration of other breeds.

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JP conceived and designed the study, executed the experimental work. IA statistically analyzed the data. ECP wrote the article.

Key words
Holstein-Friesian cattle, Polymorphism gene *PIT-1*, Indices of reproduction.

INTRODUCTION

PIT-1 is a transcription factor known also as POU1F1. It takes essential part in body development process as: activator of growth hormone gene (GH), receptor of hormone which is releasing growth hormone (GHRH-R), prolactin activator (PRL) and Thyroid-stimulating hormone (TSHβ). Khatib *et al*. (2009) demonstrated pathway of reproduction connected to POU1F1 and suggest, that single genes as well as all pathways can be used in selection programs focused on improve cattle fertility. According to Munir *et al*. (2017) all identified alleles of the gene PIT-1 will be useful for animal selection *i.e.* an ideal tool for marker assisted selection of animals for breeding. Lan et al. (2013) research on several dairy cattle population state relationships between polymorphism of the genes involved in reproduction pathway and cow and bull fertility. There are several groups that are proposing that cow mass impact on the milk value. Body weight of the mother during calving is also a factor that influences the occurrence of any difficulties during parturition. In contrast, the birth weight of calves is a parameter that significantly affects their survival and later development.

It is believed that single base modification in different locus *PIT-1* impact on gene function. There were a few amounts of researches about a dependency between *PIT-1* gene polymorphism and breeding parameters and body weight of cows and calves. From a practical point of view, it is important to conduct research on loci because of economic and production reasons.

The aim of this work was to determine dependency

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between polymorphism in the *PIT-1* and fertility rates, body weight of cows and calves in Polish Black-and-White Holstein-Friesian cattle.

MATERIALS AND METHODS

Animals

The analysis included 1024 cows and 3663 calves of the Polish Black-and-White Holstein-Friesian breed (PHF). The animals came from six farms located in the Wielkopolska region.

DNA isolation and PCR amplification

DNA was isolated from peripheral blood using the phenol-chloroform method (Sambrook *et al*., 1989). One polymorphic place *PIT-1* (c.1178G>A) located in sixth exon mutation type G→A was amplified using a TGradient thermocycler. The primers of Woollard *et al*. (1994) were used for the PCR reaction:

PIT-1E6F 5'- AAACCATCATCTCCCTTCTT - 3'

i PIT-1E6R 5'- AATGTACAATGTCCTTCTGAG - 3'.

The reaction mixture in a volume of 15 µl contained 100 ng of genomic DNA, 0.6 U of *Taq* polymerase, 10 pmol of each primer, 1,5 mM $MgCl_2$, 200 µM dNTP, 1.5µl of PCR buffer – $(NH_4)_2SO_4$ (10x) and 0.75 µl DMSO.

Preliminary denaturation for locus c.1178G>A were performed in temperature 95° C for 300 sec. After this process 30 cycles of denaturation (95°C/30 s) were performed, primer connection 51°C/30 s and synthesis (72°C/50 s), followed by final synthesis (72°C/300 s).

The amplification products were digested with *HinfI* restriction enzyme at 37°C, buffer R was used. Duration of the process was 3 h. Composition of the reaction mixture (11µl) for one sample was as follows: 5µl of the PCR product, 1µl of the restriction enzyme at a concentration of 10U/µl (Fermentas), 1µl of buffer to the enzyme (fermentas) and 4 µl of H_2O.

2 µl of the loading buffer – gel loading solution I, 6x – were added to each sample after the period of digestion with restriction enzymes. Afterwards, the effects of digestion were examined by carrying out electrophoresis in a 3% agarose gel (BASICA GQT, Prona) in 1 x TBE buffer. Gene Ruler DNA Ladder Mix was used for the preparation of a mixture consisting of 2µl of the loading buffer, 1.5µl of DNA marker and 10.5µl of H_2O. The duration of electrophoresis and the applied voltage were as follows: 35 minutes and 140V. The digestion products were observed under UV light.

Identification of genotypes

The size of the amplified *PIT-1* gene for locus c.1178G>A fragment was 451bp. The following genotypes were identified: AA (-,-) – 451bp (lack of space recognizable by the restriction enzyme), AG (-,+) – fragments with lengths of 451bp, 244bp and 207bp as well as GG (+,+) – 207bp and 244bp.

Statistical analysis

A detailed analysis of the results of laboratory tests was carried out on the basis of performed calculations of true prevalence of genotypes and genes, and observed and observed and expected number of individuals for different polymorphic forms of given locus according to Hardy–Weinberg equilibrium. The accounts used test χ^2. The source data used to calculate the indicators of reproduction were collected from cows inseminated by semen of Black-and-White Holstein-Friesian bulls.

The following information was taken from each animal: age at first insemination, age at first calving, number of semen portions attributable to successful insemination, length of pregnancy and periods of insemination service, postpartum resting period, interpregnancy period and intercalving period. The study also determined weight of the cow and weight of the calf at calving (including gender). Body weight of calves was determined immediately after birth, and their mothers within 10 days of calving.

Statistical calculations were performed using the statistical package SAS (2003). The following procedures were used: MEANS and GLM. Significance of the effect of experimental factors on the studied indicators of reproduction and weight of the cow after calving and weight of the calf after parturition was estimated using a multivariate analysis of covariance according to the following linear model:

$$Y_{ijklmnop}=\mu+h_i+y_j+s_k+l_l+PIT\text{-}1E6_m+\beta_1 dl_n+\beta_2 my_o+e_{ijklmnop}$$

Where, $Y_{ijklmnop}$ is phenotypic value of the analysed trait, µ is average rate for population, h_i is permanent effect of a herd (i=1,…,6), y_j is permanent effect of calving year (j=1, …,17), s_k is permanent effect of calving season (k=1,…,4), l_l is permanent effect of calving group (l=1,…,10), PIT-$1E6_m$ is permanent effect of a genotype in locus c.1178G>A (m=1,2,3), β_1, β_2 is partial linear regression coefficients, dl_n is number of milking days in lactation, my_o is milk yield in lactation and $e_{ijklmnop}$ is random error.

The regression effects were not considered in the above model for the following traits: weight of the cow after calving and weight of the calf after parturition.

A detailed comparison of average rates was carried out using Duncan's multiple range test.

RESULTS

It has been shown that studied population of the Polish Black-and-White Holstein-Friesian cattle reach the

genetic equilibrium (Table I). It was found that frequency of genotypes in locus c.1178G>A is AA (-,-) = 0.0469, AG (-,+) = 0.3154, GG (+,+) = 0.6377, calculated frequency A = 0.2046 and G = 0.7954.

Table I.- Frequency of alleles in locus c.1178G>A gene *PIT-1* and actual and theoretical distribution of genotypes, as well as the observed and anticipated number of individuals in the examined population of black-white variety Polish Holstein-Friesian breed cows.

Specification	locus c.1178G>A			
	Genotype			∑
	AA	AG	GG	
Observed number of cows	48	323	653	1024
Theoretical of no. of cows	43	333	648	1024
AF of genotypes	0.0469	0.3154	0.6377	1.000
TF of genotypes	0.0419	0.3254	0.6327	1.000
Frequency of alleles	A = 0.2046		G = 0.7954	1.000
χ^2	0.6160	0.3169	0.0408	0.9737

**, $P \leq 0.01$; *, $P \leq 0.05$; AF, actual frequency; TF, theoratical frequency.

Table II contains the values of chosen indicators of reproduction and body weight of the mother and the calf (disaggregated by gender) after parturition with regard to genotypes in locus c.1178G>A. The performed statistical analysis showed a significant effect of genetic variants. The AA homozygotes were characterized by the highest age at first insemination (533 days) as well as the lowest value ($P \leq 0.05$) of insemination index (2.07) and the biggest weight of the cow (560.2kg) and calf both gender (♂ - 38.0 kg and ♀- 36.9 kg). Cows of genotype AA differed from other genotypes (at $P \leq 0.01$) weight of the cows after calving and calves after parturition has significant ($P \leq 0.01$) bigger weight than female calves from cows with genotype AG and GG. It has been shown that heifer with AA and AG genotype and male calves coming from homozygotes AA and heterozygotes AG differed from each other ($P \leq 0.05$) with the lowest age at first insemination and weight after calving. In contrast, the GG homozygotes differed from AG heterozygotes in terms of length of puerperium (92 days).

DISCUSSION

Zhao *et al*. (2004) studied mutations in the sixth exon (c.1178G>A) and received a similar frequency (0.44 and 0.45) of genotypes for: heterozygotes and homozygotes (+,+). By analysing the results of researches on other species made by other authors it was found out the dominance of frequency of G (+) gene than A(-). Woollard *et al*. (1994) obtain frequency of genes G and A at level 0.85 and 0.15 in Holstein cattle. According to Yan *et al*., (2006) Holstein cattle from China have frequency of A and G genes: 0.132 and 0.868. Zatoń-Dobrowolska *et al*. (2007) showed that frequency of G gene for polish red cattle was 0.863, Czech red cattle – 0.951 and German red cattle 0.921. Dybus *et al*. (2003) reported that Limousine cattle obtain frequency at level 0.2371. As well as

Table II.- Values of chosen factors of reproduction and also weight of the mother body and the calf after parturition in the cow studied population with regard to genotypes in locus c.1178G>A.

Traits	Significance effect	Genotype in locus c.1178G>A					
		AA		AG		GG	
		N	$\bar{x} \pm SD$	N	$\bar{x} \pm SD$	N	$\bar{x} \pm SD$
Age at first fertilization age (days)	*	48	533 ±61a	323	523 ±62a	653	528±72
Age at first calving (days)	NS	48	821±66	323	815±65	653	818±75
Postpartum resting period (days)	*	131	89±47	843	87 ±40a	1758	92±43 a
Insemination index	*	179	2.07±1.36ab	1166	2.14 ±1.48a	2411	2.14 ±153b
Period of insemination service (days)	NS	179	44±65	1166	50±72	2411	47±70
Interpregnancy period (days)	NS	130	145±85	836	149±88	1740	151±86
Pregnancy (days	NS	178	280±7	1158	279±7	2388	279±7
Dry period (days)	NS	130	65±21	836	63±23	1740	64±22
Intercalving period (days)	NS	130	397±48	396	398±48	1740	397±49
Weight of cow after calving (kg)	**	178	560.2 ±35.3AB	1158	554.2 ±30.0A	2388	553.7 ±29.6B
Calf weight at calving (kg) ♂	*	93	38.0 ±4.2a	586	37.3 ±4.0 a	1200	37.7±4.3
♀	**	83	36.9 ±3.9AB	554	35.9 ±4.3A	1147	36.0±4.2B

Effect influence: **, highly significant ($P \leq 0.01$); *, significant ($P \leq 0.05$); NS, non-significant ($P > 0.05$). Means denoted with identical letters (in rows) differ statistically: A and B, ($P \leq 0.01$); a and b, ($P \leq 0.05$).

di Stasio et al. (2002) conducted on Piemontese cattle obtain frequency of A allele at level 0.25. According to Zhang et al. (2009) for Qinchuan cattle frequency of A allele was 0.23. Selvaggi and Cataldo (2011) studied population of the Podolica cattle, the frequency of the A allele was 0.30.

The weight of a cow's body is an important factor that can affect its milk and reproductive production. Age of beginning reproductive use of heifer depends on the reproduction maturity that the animal obtains with appropriate development of body and body weight of about ¾ weight of adult cows. For Holstein Friesians, it is 14-16 months. Pytlewski and Antkowiak (2005) found that the age of first calving influences live production of milk, FCM milk, fat and protein per day of life. The most favourable results were obtained by cows that calved at the age of ≤ 26 months. It is widely recognized that cows should be inseminated approximately 6 weeks after calving. In the reproductive cycle, the length of the intercalving period, which directly affects the length of the production cycle, may be significantly different. The studies showed no differentiation between genetic groups due to the length of inter-pregnancy period. Pytlewski *et al*. (2006) showed that the length of that period included in the range from 121 to 160 days was the most favourable due to the length of use, milking and productivity of cows. Oprządek *et al*. (2006) found that cows with genetic variant AA in locus c.1178G>A had the lowest value of insemination index. However, subsequent studies of Edriss *et al*. (2009) conducted on Holstein cattle gave different results than own research. Authors showed that the biggest weight at calving had calves which mothers were GG homozygotes, AG heterozygotes had the longest intercalving period. Corrales-Álvarez *et al*. (2010) reported that absence of A allel in locus c.1178G>A is correlated with shorter intercalving period. Vargas *et al*. (2004) believe that Holstein-Fresian AA homozygotes in locus c.1178G>A have worse factor of reproduction.

The results prove the existence of dependencies between genu *PIT-1* gene polymorphism in locus c.1178G>A, reproductive potential and body weight of cows and calves. More favorable results were observed in AA homozygotes. It is possible to use these dependencies in genetic selection of farm animals. In order to confirm the obtained results it would be advisable to conduct studies in a larger population of cows with consideration of other breeds.

Statement of conflict of interest

Authors have declared no conflict of interest.

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