ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Zootechnica 22(4) 2023, 55–62

www.asp.zut.edu.pl pISSN 1644-0714 eISSN 2300-6145 DOI: 10.21005/asp.2023.22.4.06

ORIGINAL PAPER

Received: 23.12.2023
Accepted: 26.04.2024

RELATIONSHIP BEWTEEN THE *LEPTIN* GENE POLYMORPHISM AND THE PRODUCTIVITY AND HEALTH TRAITS IN HOLSTEIN-FRESIAN CATTLE

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ABSTRACT

The aim of this study was to analyse the relationship between the *leptin* gene and the productivity of Polish HF cattle as well as their functional traits (health). The study was conducted on a sample chosen from one of the West-Pomeranian farms. The lowest percentage of pathogenic disease (*mastitis, endometritis*) was recorded in the cattle with *CT* genotype for leptin, whilst cows with *TT* genotype had fewest metabolic diseases. The study found that the *TT* cattle were significantly more efficient ($P \leq 0.01$; $P \leq 0.05$) than other groups in value and energy corrected milk (VCM and ECM) as well as in milk fat and protein yields in their first lactation.

Key words: leptin, dairy cattle, productivity and health traits, diseases, Holstein-Fresian cattle

INTRODUCTION

It seems that the selection towards increase in milk field in dairy Holstein-Fresian Polish cattle could have had such consequences as decrease in the cattle's health [Fleischer et al. 2001, Kashiwada et al. 2009]. Higher milk yield are correlated with lowered fertility, higher susceptibility to certain disease and pathogenic infections (most often mastitis) [Ruane 1997, Lyons and Freeman 2010, Verschoor et al. 2011]. The scientists asked therefore a question about the relationship between the polymorphism of certain genes and cattle health as well as their productivity [Gascoyne et al. 2009, Gazda 2011, Sender et al. 2012]. It seems to be a very important issue, as it is obvious that sick or ill animals will have lower productivity than health cows, and that can pose economic consequences for the owners of the farms.

So far, in cattle, there have been several genes identified, which seem to influence traits such as fat and protein content, somatic cell count, dry mass of milk, duration of lactation, milking size, fertility and length of the intervals between calvings. One of the most important genes here is the leptin gene [Zhang et al. 1994, Bado et al. 1998]. Its product, leptin, also known as satiety hormone [Houseknecht et al. 1998, Buettner et al. 2008], is synthesised in fat tissue [Kotz et al. 1998, Jang et al. 2000, Oprządek et al. 2005] and is correlated with regulation of hunger [Ghilardi et al. 1996, Cunningham et al. 1999], energetic balance of the organism [Dubey et al. 2007], fertility [Liefers et al. 2002, Giblin et al. 2010] as well as the immunity mechanisms [Liang et al. 2006, Kamizono et al. 2009]. This is why the research on the polymorphisms of the leptin gene and the genotypes frequency are lately so intense. The leptin gene seems to be important not only for the cattle health but also its productivity, as these factors are closely correlated [Szyda et al. 2011].

Leptin, an adipocyte-derived cytokine (adipokine) has plethora of physiological roles in the body, particularly in the onset of puberty, viz. reproduction and immune function of an animal. Moreover, body fatness

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which is a crucial determinant of meat quality is governed by blood leptin level. Since its discovery in 1994, various research groups have explored its potential role in animal production, reproductive fitness and immune function which ultimately determine the profitability of livestock rearing [Mukherjee et al. 2023].

The aim of this research was to determine whether and to what extent there is a relationship between the *leptin* gene and productivity of Holstein-Fresian Polish cattle as well as their health traits.

MATERIAL AND METHODS

The research was conducted on a sample randomly chosen from 700 Holstein-Fresian cows in one of the dairy farms in West-Pomeranian area of Poland. In the farm the cows were divided into six technological groups, there were four loose-housing cowsheds and one building for sick and poor milking cows as well as those just before calving. The calves were kept in the cowshed with mothers for the first few days of life, then in 'igloo' houses and finally they were moved to a calfhouse. Feeding was conducted three times a day by means of Sano mixer wagon. There was a libitum feeding scheme in a Total Mixed Rotation system with regard to the technological group. The forage was based on lucerne and corn silage, haylage, pulps, as well as soy for more efficient cows. Full forage and various additives were also administered. Milking took place three times a day in a milking parlor with 32 stands. In the building there was also a waiting area as well as a selection device with an automatic balance.

DNA isolation was carried out from randomly selected 100 Polish Holstein-Fresian cows according to the protocol included with the DNA isolation kit, MasterPure™ (Epicentre Technologies). The PCR in T-Biometra Personal and Biometra Gradient thermocyclers was conducted using the starter sequences according to Haegemann et al. [2000]:

- LEP forward: 5′-GGGAAGGGCAGAAAGATAG-3′
- LEP reverse: 5′-TGGCAGACGTTGAGGATC-3′

The analysis concerned an SNP in the third exon of the *Lep* gene (U50365.1 GenBank). The following thermal profile was used: initial denaturation of the DNA templates 94°C (5 min), then 31 cycles: DNA denaturation 94°C (30 s), primers annealing 55°C (30 s), DNA synthesis 72°C (40 s) and then final synthesis 72°C (5 min). The product of amplification containing the *leptin* gene were digested with 5 units of restriction enzyme *HphI* for at least 3 hours at 37°C. The fragments were then separated through gel electrophoresis in 2% agarose gel with added ethidium bromide and in once-concentrated TBE buffer for 50 min. The results were subsequently archived (DOC-PRINT, Vilber Lourmat).

The data about productivity and health traits of the cattle were obtained from the farm documentation. The source data included: milk, fat and protein yield (kg) as well as milk, fat and protein percentage in 305-days lactations with distinction for first, second and third lactation. The milk yields were compared using the formulae for VCM (Value Corrected Milk) [Arbel et al. 2001] and ECM (Energy Corrected Milk) [Cichocki et al. 2007]:

$$\text{VCM} = 0.05 \times \text{kg milk} + 8.66 \times \text{kg fat} + \\ + 25.08 \times \text{kg protein}$$

$$\text{ECM} = 0.327 \times \text{milk (kg)} + 12.95 \times \text{fat (kg)} + \\ + 7.2 \times \text{protein (kg)}$$

The collected source data were statistically processed by calculating basic statistical measures. In order to compare the mean values of the groups, statistical analysis was performed according to the ANOVA test using the following linear model:

$$Yij = \mu + a_i + b_j + e_{ij}$$

where:

μ – general mean
a_i – effect of i-th genotype
b_j – effect of j-th lactation
e_{ij} – random error of observation.

Chi^2 test was used to determine the relationship between gene polymorphisms, causes of cull cows and their diseases. Microsoft Excel and Statistica 7.1 PL were used to evaluate the collected data.

RESULTS AND DISCUSSION

The genotyping procedure (PCR-RFLP) used the restriction enzyme *HphI*, which allowed differentiation of the studied polymorphic site. The DNA restriction fragments were separated through the means of electrophoresis, in the presence of a mass standard (pUC19/*MspI*), which allowed the determination of three genotypes: *CC*, 331 bp, *TT*, 311 and 20 bp, and *CT*, 331, 311 and 20 bp.

The most common genotype was the heterozygote *CT* (0.47), then homozygote *CC* (0.44). The least numerous were the homozygotes *TT* (0.09). The frequencies are shown in the Fig. 1. The frequencies of allele C and T were 0.675 and 0.325, respectively.

The frequencies of *leptin* genotypes obtained in this study differ slightly from those reported by Kulig [Kulig 2005, Kulig et al. 2010], who studied cattle breeds Jersey and Polish Holstein-Fresian, where *CC* genotype was the most common. However, the *TT* genotype was also the rarest. Similarly, Giblin et al. [2010], who investigated HF cattle, found that the *TT* genotype was non-existent, heterozygotes were noted very rarely and the

majority of cows had the *CC* genotype. Also Yazdani et al. [2009, 2010] obtained similar results in Iranian Holstein cattle, with the absence of *TT* genotype and the majority of cows having the *CC* genotype, as well as Corva et al. [2009] who investigated Angus, Brahman and Nelore cattle. It is worth mentioning that these investigations were conducted on much larget samples than the one in the current investigation (apart from the Iranian Holstein cows). Similar frequencies to those obtained in this study were published by Komisarek [2010] in PHF bulls, Machulskaya et al. [2017] in Holstein cows, and Kaygisiz et al. [2011] in Anatolian Black and Red and Brown Swiss cows, in which they found an even higher preference for heterozygotes over other genotypes than in their own study.

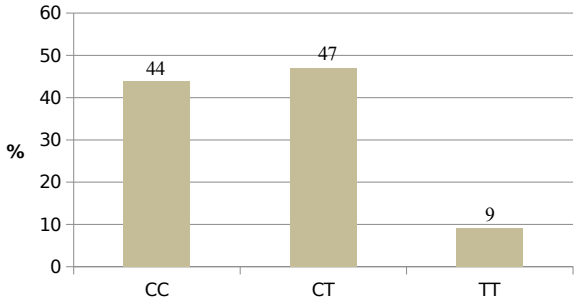

Fig. 1. Frequencies of *leptin* genotype in HF cows

Given the significant differences observed across various studies, it is necessary to conduct research on a much larger sample of Polish HF cattle to draw definitive conclusions.

Table 1 shows the basic indices for productivity of cows in first, second and third 305-day lactation, depending on the leptin genotype.

The highest milk yield in the first lactation (10509 kg) was recorded for *TT* cows and the lowest (9714 kg) for the *CC* cows. After calculating VCM and ECM still the cows with the *TT* genotype were the most efficient (VCM = 12331, ECM = 10612) and the differences in comparison to the other groups were significantly higher than in case of the actual performance. The differences between the actual performance, VCM and ECM between the analysed cattle groups were statistically significant ($P \leq 0.01$ and $P \leq 0.05$). The highest milk yield in the first lactation in the cows with the *TT* genotype was correlated with the highest fat yield (362 kg) and protein yield (345 kg). The highest yield of these milk compounds in the *TT* cows was a result of their highest percentage content in the milk (3.50% of fat and 3.32% of protein).

The results obtained in the first lactation were not entirely confirmed in the second lactation. The actual milk yield in all allelic leptin groups were similar (10740 kg for *CC* to 10826 kg for *CT*). Also the protein yield was on a similar level in all groups, which was a result of the similar percentage content of protein (3.27–3.33%). However, in the *TT* cattle the fat yield was about 30–40% lower compared to other groups. This was a result of lower fat percentage content in milk in this group (3.28%) than in the other groups (3.64% and 3.59%).

In the third 305-days lactation again the most efficient for milk yield, VCM and ECM was the group of cows with the *TT* leptin genotype. At the same time, their milk had the lowest mean percentage fat content (2.87%) and protein (3.31%). The highest fat percentage (3.71%) and

Table 1. Milk, fat and protein yield in the first, second and third 305-day lactation with regard to the cattle *leptin* genotype.

Lactation	Genotypes	Milk yield, kg	VCM, kg	ECM, kg	Fat yield, kg	Protein yield, kg	Fat content, %	Protein content, %
		x̄ ±SD						
I	CC, n = 44	9715^{Aa} ±1659	11365^{Aa} ±1771	9793^{Aa} ±1588	334 ±64.8	318 ±49.9	3.46 ±0.51	3.29 ±0.16
	CT, n = 47	10028^{a} ±1741	11669^{ab} ±1748	10004^{ab} ±1510	336 ±57.5	329 ±52.0	3.40 ±0.53	3.29 ±0.17
	TT, n = 9	10510^{A} ±1392	12332^{Ab} ±1107	10613^{Ab} ±974	362 ±48.9	346 ±32.1	3.50 ±0.69	3.31 ±0.29
II	CC, n = 39	10740 ±1483	12660 ±1550	10988 ±1386	382 ±63.2	352 ±43.6	3.59 ±0.57	3.28 ±0.19
	CT, n = 40	10826 ±1720	12945 ±1826	11194 ±1594	391 ±64.1	360 ±52.7	3.65 ±0.52	3.33 ±0.22
	TT, n = 9	10772 ±2155	12304 ±1708	10539 ±1670	348 ±66.7	349 ±49.7	3.28 ±0.67	3.27 ±0.22
III	CC, n = 33	11473 ±1866	13245 ±1967	11585 ±1748	401 ±68.2	367 ±55.3	3.52a ±0.46	3.21 ±0.18
	CT, n = 30	10848 ±1673	12511 ±2590	11136 ±1427	397 ±45.9	341 ±94.4	3.71b ±0.55	3.31 ±0.20
	TT, n = 8	13472 ±436	14454 ±1243.5	12406 ±427	387 ±6.4	416 ±50.9	2.87ab ±0.14	3.08 ±0.28

A – significance between the values for $P \leq 0.01$.
a, b – significance between the values for $P \leq 0.05$.

protein percentage (3.31%) was noted in heterozygotic cows.

In scientific literature the data concerning the influence of a particular *leptin* polymorphism on the cattle productivity traits is scarce. Some polymorphisms have been linked to traits associated with meat and dairy performance in cattle, but the results are inconclusive. Relationships have been observed related to carcass fat content and degree of meat marbling, as well as to daily weight gains, which are extremely important in assessing the fattening efficiency of beef cattle [Buchanan et al. 2002, Schenkel et al. 2005]. Corva et al. [2009] concluded that in the meat cattle (Brahman, Angus, Nelore) the variations of *leptin* seem to have virtually no influence on cows' productivity.

The search for a link between milk yield and other traits related to cattle dairy performance and reproduction in connection with the leptin gene has also been addressed by other researchers [Liefers et al. 2002, Buchanan et al. 2003, Komisarek and Antkowiak 2007, Kulig et al. 2009, Komisarek 2010, Kulig et al. 2010], but their results are inconclusive. Świtoński [2005] claims likewise. On basis of these and other research it seems that the cows with *TT* genotype have higher indices for all of the analysed productivity traits, but only in the first lactation. In the remaining two lactations the results were no so unequivocal. Also, too small number of individuals in some of the groups (*TT* genotype in the third lactation) does not permit drawing specific conclusions without confirmation in further research.

Table 2 shows the number of culled cows in particular leptin genotype groups together with the reasons for culling. The problem of cow missing is a very important issue from the farmer's point of view, as it affects the economics of the farm [Kerslake et al. 2018]. The causes of cow missing may be random, or may be due to the health of the animals. For many years, the trend of shortening the lifespan and useful life of dairy cows has continued [Strzałkowska et al. 2014]. The useful life of cows in Poland on average does not exceed six years. Cows are eliminated from herds prematurely [Sawa 2011, Boulton et al. 2017].

Cows with the CT genotype were missing most often (17 individuals, which accounted for 36% of this group), while the TT homozygote group was the least frequent – only one individual was missing, which accounted for 11% of this group of cows. The largest number of animals (12%) were missing due to fertility disorders, which often occurs on large farms keeping high-yielding PHF cows. Decreased fertility was the most common reason for missing heterozygous CT (8 cases) and homozygous CC (4 cases) individuals.

Another reason for eliminating cows from the herd was limb disease. Two cows each with the CT and CC genotypes and one cow with the TT genotype were culled. Other reasons for culling (metabolic diseases, post-partum arrears, random accidents and liver steatosis) were found even less frequently, and no TT homozygotes were removed from the herd because of them. Based on the results in Table 2, it can be assumed that animals with

Table 2. *LEP* gene polymorphisms and causes of culling in PHF CB cows up to the third lactation

	Genotype									Total	
	CC [44]			CT [47]			TT [9]				
	n	%[1]	%[2]	n	%[1]	%[2]	n	%[1]	%[2]	n	%
Cause for culling											
Metabolic disease	3	27.27	75.00	1	5.88	25.00	–	–	–	4	13.79
Udder diseases	–	–	–	3	17.66	100.0	–	–	–	3	10.34
Limb disease	2	18.18	20.0	2	11.76	20.0	1	–	10.0	5	17.24
Fertility disorders	4	36.37	33.33	8	47.06	66.67	–	–	–	12	41.38
RMF	1	9.09	50.0	1	5.88	50.0	–	–	–	2	6.90
Fatty liver	1	9.09	100.0	–	–	–	–	–	–	1	3.45
Random event	–	–	–	2	11.76	100.00	–	–	–	2	6.90
Lactation											
I	5	45.46	41.67	7	41.18	58.33	–	–	–	12	41.38
II	4	36.36	40.00	6	35.29	60.00	–	–	–	10	34.48
III	2	18.18	28.57	4	23.53	57.14	1	100.0	14.29	7	24.14
Total culled cows	11			17			1			29	

%[1] – in the genotype; %[2] – in the cause of culling or lactation.

Table 3. The relation between cattle disease and their genotype for *leptin*

Disease	Genotype						Total
	CC [44]		CT [47]		TT [9]		
	n	%	n	%	n	%	
Mastitis	9	20.45	5	10.47	2	22.22	16
Endometrtis	17	38.64	10	21.28	3	33.33	30
Placenta retain	5	11.36	3	6.38	1	11.11	9
Hoof infection	2	4.55	1	2.13	0	–	3
Ovarian cycts	1	2.27	2	4.26	0	–	3
Ketosis	4	9.09	3	6.38	0	–	7
Hypocalcaemia	1	2.27	0	–	0	–	1
Total	39	x	24	x	6	x	69

the TT genotype are more resistant to diseases of various aetiologies that cause culls. Only one individual with this genotype was cull during the study period. A different relationship between LEP polymorphisms and disease susceptibility is indicated by Chebel et al [2008]. These authors found that CT heterozygotes had the lowest risk of developing at least one clinical health disorder.

Table 3 shows data concerning the frequency of cattle disease and its correlation with the *leptin* polymorphism. The most illnesses were noted in the cows with the *CC* genotype (39). Less diseases were recorded in the *CT* group (24) and the least in the *TT* group (only 6). The most common diseases were mastitis, endometritis and placenta retain.

The heterozygote group had the smallest percentage of diseased pieces in relation to their total number (51.1%) compared to the TT and CC homozygous groups (66.7 and 88.6%, respectively). Diseases caused by microorganisms (mastitis and endometritis) predominated in each group. The group of cows with the TT genotype had the lowest percentage of diseases of metabolic origin such as ketosis, placental retention, and hypocalcemia. Chebel et al. [2008], on the other hand, showed that the TT genotype predisposed cows to the occurrence of digestive displacement.

Leptin is considered as link between the neuroendocrine and immune systems [Carlton et al. 2012]. Pro-inflammatory actions of leptin in the immune system act as a potent enhancer of immune functions [Behnes et al. 2012]. Leptin can act on different immune cells, specifically by promoting activation of monocytes or macrophages and natural killer (NK) cells, inducing chemotaxis of neutrophils and degranulation of basophils, among other functions [Abella et al. 2017]. Various roles played by leptin to modulate innate and adaptive immunity have been recently reviewed by Mukherjee et al. [2023]. In present study serodiagnostic potentiality of goat, fish and mithun leptin was explored [Alam et al. 2014a, 2014b, Joardar et al. 2017]. Recently, Yadav et al. [2020] screened genomic region of leptin gene (loci g. 92450765 G>A) with anobjective to find the association of genotypes with fertility and production traits. The animals harboring GG genotype were observed to be more susceptible to reproductive disorders.

Mukherjee et al. [2023] reports that since the discovery of leptin, research has shown that adipose tissue plays a key role in regulating metabolic and immune functions, and there is undoubtedly more to be discovered, especially in the context of livestock biology, including dairy cows. Its contribution as a hormone that acts in multiple organs, including the hypothalamus, pituitary, pancreas and gonads, has now been shown to play a key role in fat deposition, reproductive fitness and disease resistance. The complex circuitry of leptin varies by cell type, which is as yet poorly understood. Translational studies have not been conducted with information on the genomic and proteomic features of leptin. Future research efforts should focus on determining the machinery of leptin in different cell types and its role in maintaining energy homeostasis, so that it can be effectively manipulated to increase production from livestock and design leptin-based therapeutic agents.

CONCLUSIONS

The *TT* genotype cattle group significantly exceeded ($P \leq 0.01$ and $P \leq 0.05$) the other cattle groups in actual performance, ECM and VCM, as well as in fat and protein yield and content but only in the first lactation. In the remaining analysed lactations, the results were not so unequivocal and require further studies.

There has been no significant *leptin* impact found on the occurrence of diseases in HF cattle, but only certain tendencies. The least percentage of pathogenic disease (mastitis and endometritis) was recorded in the *CT* cat-

tle group and the least metabolic diseases were noticed in the *TT* group.

ACKNOWLEDGEMENTS

The study was financed from funds allocated to maintenance of there search potential of the research units.

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ZWIĄZEK POLIMORFIZMU GENU LEPTYNY Z CECHAMI PRODUKCYJNYMI I ZDROWOTNOŚCIĄ KRÓW RASY POLSKIEJ HOLSZTYŃSKO-FRYZYJSKIEJ

STRESZCZENIE

Celem pracy była analiza zależności pomiędzy polimorfizmem genu *LEP* a cechami produkcyjnymi i funkcjonalnymi (zdrowotnością) bydła mlecznego rasy polskiej holsztyńsko-fryzyjskiej odmiany czarno-białej. Badania przeprowadzono na terenie województwa zachodniopomorskiego. Wykazano, że krowy o genotypie *TT* przewyższały istotnie ($P \leq 0.01$; $P \leq 0.05$) pozostałe grupy genotypowe krów w pierwszej laktacji pod względem wydajności rzeczywistej i przeliczeniowej (VCM i ECM) w produkcji mleka, a także wydajności tłuszczu i białka oraz zawartości tych składników w mleku. Najniższy odsetek występowania chorób drobnoustrojowych (*mastitis*, *endometritis*) zaobserwowano u krów z genotypem *CT*, natomiast grupa krów z genotypem *TT* odznaczała się najmniejszym odsetkiem chorób metabolicznych.

Słowa kluczowe: leptyna, bydło mleczne, cechy wydajności i zdrowia, choroby, rasa holsztyńsko-fryzyjska

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