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Identification of CXCL8c.105A>G and CXCL8c.210C>T polymorphism in Polish HF cattle

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Abstract

Background: Bovine chemokine C-X-C motif legend 8 (*CXCL8*) also known as interleukin 8 (IL8) is a chemotactic factor that attracts neutrophils, basophils, and T-cells, in re-

sponse to an inflammatory stimulus. The aim of this study was to investigate the novel single nucleotide polymorphisms (SNPs) located at the promoter region of *CXCL8* gene in Polish Holstein Friesian (HF) bulls.

Methods: Genotypic profiling of *CXCL8c*.105A>G and *CXCL8*c.210C>T SNP polymorphism were carried out by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) methods using *HpyCH4VI and RsaI* restriction enzymes. Polymorphism study was conducted on 151 randomly selected Polish HF bulls.

Results: The genotype frequencies of *CXCL8c*.105A>G SNPs polymorphism in the investigated Polish HF bulls were observed as 0.24%, 0.5% and 0.26% respectively, whereas, the genotype frequencies of *CXCL8*c.210C>T SNPs polymorphism were observed as 0.27%, 0.5% and 0.23% respectively. Overall, the allele frequencies were recorded higher for "G' allele for *CXCL8c*.105A>G and "C" allele and *CXCL8*c.210C>T SNP polymorphism. In both investigated SNP polymorphism, the state of genetic equilibrium was maintained in Polish HF bulls. Overall, the obtained results identified four haplotypes with the highest frequencies of CG (0.493%) and TA (0.476%) haplotypes, and the lowest frequencies of CA (0.02%) and TG (0.01%) haplotypes, respectively.

Conclusions: Study concludes that both polymorphism could be further investigated for the trait-associated studies in the breeding population of Polish HF cattle.

Keywords: *Interleukin 8*; *CXCL8*; polymorphism; genotypes; allele frequency; SNP; RFLP; Polish HF.

Introduction

As a major mediator of the inflammatory response, bovine chemokine *C-X-C motif legend 8* (*CXCL8*) gene is secreted by mononuclear macrophages, neutrophils, eosinophils, T lymphocytes, epithelial cells, and fibroblasts and functioned as a chemotactic factor by guiding the neutrophils to the site of infection [**1**]. In cattle, *CXCL8* is a chemotactic factor that attracts neutrophils, basophils, and T-cells, but not monocytes. In response to an inflammatory stimulus, *CXCL8* gene released from several cell types including neutrophil activation [**2**]. This gene is believed to play a major role in the pathogenesis of the several diseases, *viz.*, mastitis, systemic inflammatory response syndrome (SIRS), lower respiratory tract

infection bronchiolitis, caused by the respiratory syncytial virus (RSV) and other lung disease and septicemia which can cause large livestock losses [**3–4**]. The bovine *CXCL8* gene is located on chromosome 6 (BTA6) with 1485bp in length and encoded a protein of 101 amino acids. In past, several studies on bovine *CXCL8* gene polymorphism were investigated in the HF calves [**5**], Norwegian Red [**6**], Chinese HF cattle [**7**] and beef breeds [**8**] and dairy cattle [**9**]. Herein this paper, we investigate two single nucleotide polymorphisms (SNPs) polymorphism (*CXCL8c*.105A>G and *CXCL8*c.210C>T) located at the promoter region of bovine *CXCL8* gene in Polish Holstein Friesian (HF) bulls.

Material and methods

Animals: The study investigated 151 randomly selected Polish HF bulls with known breeding values (BVs) assessed from the 57 proven sires. The official results of the assessment of BVs of investigated bulls were obtained from the research institute of animal production, Balice, website (http://www.izookrakow.pl). The data was collected from the assessment of breeding bulls in the annual year report of 2017.

Methods

Genomic DNA isolation was performed using the commercial DNA isolation kit. For both *CXCL8c*.105A>G and *CXCL8*c.210C>T SNP polymorphism, the primer sequences were designed using the online PRIMER3 program (www.genome.wit.mit.edu). Primers with the following nucleotide sequence were used in the PCR reaction:

Forward primer sequence: 5 'GGTTTTAAACAAAAGCAATAGAAGTA3 '

Reverse primer sequence: 5'TATATCATCGTAAGAAGAGGGAAC3 '

The polymerase chain reaction of *CXCL8c*.105A>G and *CXCL8*c.210C>T SNP polymorphism was carried out on SansoQuest LabCycler 48 (LabVision AB, Sweden) using the first step of DNA denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 sec, the primer annealing temperature at 52°C for 30 sec and elongation at 72°C for 45 sec and finally the elongation synthesis at 72°C for 5 min. The PCR reaction was carried out a total volume of 25µl including, 2.5µl 1×PCR buffer, 4.0µl 0.2mM dNTP, 1.0µl of each primer, 1.5 µl mM $MgCl_2$, 0.2µl of *DNA Taq-polymerase* with an activity of 10 units/µl and 2µl of DNA template. The amplification results were checked in 1% agarose gel. The horizontal electrophoresis was performed at an electric field of 150 volts for 30 minutes.

The PCR-RFLP genotypic profiles of bovine *CXCL8c*.105A>G **(rs41255708)** and *CXCL8*c.210C>T **(rs110291328)** SNP polymorphism were performed using *HpyCH4V* (BioLabs, England) and the *RsaI* (BioLabs, England) restriction enzymes.

The HpyCH4V endonuclease (BioLabs, England) recognized polymorphism at the nucleotide sequence 5'TGCA3' where (BioLabs, England) polymorphism replacing cytosine (A) with thymine (G) and creating the 5TGCG3' sequence. Whereas, the *Rsa I* endonuclease recognized polymorphism at the nucleotide sequence 5'GTAC3', where (BioLabs, England) polymorphism replacing cytosine (C) with thymine (T) and creating the 5'GTAT3' sequence.

Statistical analysis: The genotypes and alleles frequencies of *CXCL8c*.105A>G and *CXCL8*c.210C>T SNP polymorphism were determined by chi-square test using the statistical package of STATISTICA 12.0, StatSoft Polska (https://www.statsoft.pl/nowa-wersja-statistica-12-pl/) to verify the consistency of the expected and observed frequency of genotypes and alleles. The state of genetic equilibrium was assessed using the standard mathematical formula of Hardy-Weinberg law.

Results and discussion

In this study, promoter sequence of *CXCL8* gene (GenBank, NM_173925.2) with *CXCL8c*.105A>G and *CXCL8*c.210C>T SNPs polymorphism were amplified. The SNPs Polymorphism in both examined sites were determined using the *HpyCH4V* and *RsaI* restriction enzymes (**Figure 1**).

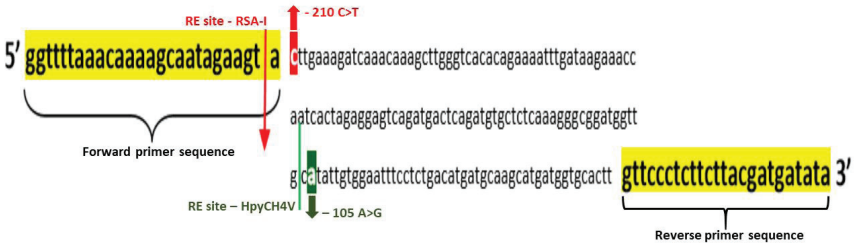

Figure 1. A fragment of the promoter region of bovine *CXCL8* gene showing *CXCL8c*.105A>G and *CXCL8*c.210C>T SNP polymorphism

The genotypic profile of *CXCL8*c.210C>T SNP polymorphism were identified three genotypes. The CC homozygotes were identified by the presence of 25bp and 177bp, TT homozygotes were characterized by the presence of 202bp, while heterozygotes CT genotypes were identified by the presence 202bp, 177bp and 25bp, respectively.

Similarly, the genotypic profile of *CXCL8c*.105A>G SNP polymorphism were identified three genotypes. The AA homozygotes were identified by the presence of 130bp and double bands 29bp and 14bp, GG homozygotes were characterized by the presence of 173bp and 29bp, while heterozygotes AG genotypes were identified by the presence 173bp, 130bp and double bands 29bp and 14bp, respectively.

The genotype and allele and frequencies of *CXCL8* gene polymorphism revealed the highest genotypic frequencies in both SNPs in comparison to homozygotes genotypes (**Table 1**). Overall, the allele frequencies were recorded higher for "C' and "G" alleles for *CXCL8c*.105A>G and *CXCL8*c.210C>T SNPs polymorphism.

Table 1. The genotype and allele frequencies of *CXCL8* gene polymorphism in Polish HF bulls

***CXCL8* gene polymorphism**	**n**	**Genotype frequency**			**Allele frequency**	
CXCL8c.105A>G	151	AA	AG	GG	A	G
		0.24	0.5	0.26	0.49	0.51
*CXCL8*c.210C>T		CC	CT	TT	C	T
		0.27	0.5	0.23	0.52	0.48

The chi-square analysis revealed that in both *CXCL8c*.105A>G and *CXCL8*c.210C>T SNP polymorphism were in the state of genetic equilibrium (**Table 2**).

Table 2. The chi-square analysis of *CXCL8* gene polymorphism in the investigated Polish HF bulls

CXCL8 **gene polymorphism**	**Chi-square**	**Genotype frequencies**			**p value**
CXCL8c.105A>G	Genotypes	AA	AG	GG	
	Observed	37	75	39	0.103
	Expected	37.5	75	37.5	0.95
*CXCL8*c.210C>T	Genotypes	CC	CT	TT	
	Observed	40	76	35	0.04
	Expected	41	75	35	0.98

Moreover, study identified four haplotypes in the investigated Polish HF bulls. The highest frequencies were observed for the CG and TA haplotypes, while lowest frequency was noted for the CA and TG (Table 3). In general, only two haplotypes predominantly existed in the investigated Polish HF bulls with the frequencies of 0.493% and 0.476% respectively (**Table 3**).

Table 3. Haplotypes frequency of *bovine CXCL8* gene in the investigated Polish HF bulls.

n	Haplotype frequencies			
	CA	CG	TA	TG
151	0.02	0.493	0.476	0.01

In past, the *CXCL8c*.105A>G and *CXCL8*c.210C>T SNPs polymorphism were investigated in cattle [**7,10**]. According to *CXCL8c*.105A>G SNP polymorphism study, Chen *et al.* [**7**] indicated that cows with the GG genotype were characterized by the lowest number of somatic cells in milk compared to cows with the AG and AA genotypes. However, according to *CXCL8*c.210C>T SNP polymorphism study [**10**], the presence of the C allele influences the cAMP binding protein and transcription factors, while the presence of the T allele influences the binding of LIM domains mediating protein. However, other study suggested that the promoter region of *CXCL8* gene was upregulated in postpartum uterine inflammation in cows [**11**].

Conclusions: Study concludes that both polymorphism could be further investigated for the trait-associated studies in the breeding population of Polish HF cattle.

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