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Association between polymorphism in the *JAK2* gene (*JAK2*/e20/*Rsa*I) and selected performance parameters in beef cattle

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The aim of the present study was to analyse the polymorphism in the *JAK2* gene in three beef cattle breeds (Angus, Hereford, and Limousin) in relation to performance traits. A total of 465 individuals were genotyped. The PCR-RFLP method was used to identify a silent mutation in exon 20 (dbSNP ID: rs110298451). Three genotypes (*AA, AG, GG*) were identified in all the tested breeds, with the *A* allele being the most frequent. In the Limousin breed, the most favourable traits were recorded for individuals with the *AA* genotype, i.e. higher birth weight (+ 2.1 kg), average daily gains (+68g) and weight at 210 days (+12.3 kg) compared to individuals with the *GG* genotype. Different results were obtained for the Hereford breed, where the *GG* genotype determined the highest birth weight (+1.3 kg), daily gains (+50 g) and body weight at weaning (+ 10.6 kg) in comparison with individuals of the *AA* genotype. In Angus cows, heterozygotes were characterized by the highest beefing abilities. There was no significant association between the *JAK2*/e20/*Rsa*I polymorphism and age at first calving. The results obtained in the present study did not indicate whether the analysed *JAK2*/e20/*Rsa*I polymorphism could be used in cattle selection. Therefore, it would be reasonable to perform additional association studies on larger numbers of recorded and genotyped animals

KEY WORDS: beef cattle / *JAK2* / tyrosine kinase / meat traits

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Tyrosine kinases are the main mediators of intracellular signaling pathways. This group also includes the family of cytoplasmic tyrosine kinases-JAK (Janus Kinase).

The primary structure of each JAK kinase consists of seven homologous domains designated JH1-JH7, from the carboxyl to the amino terminus [Baxter *et al.* 2005]. The C-terminal kinase domain (JH1) is responsible for proper enzymatic activity [He *et al.* 2005]. The JH2 domain (pseudokinase) acts as an inhibitor and regulator of the JH1 domain [Haan *et al.* 2010]. The SH2 homologous region (JH3 ÷ JH4 domains) is located in the central part of JAK2 proteins, binding directly to proteins interacting with JAK2. The N-terminal part of JAK2 is composed of the FERM domain (JH5 ÷ JH7), conserved among many cellular proteins. The described domain is involved in the localisation of JAK proteins in relation to the cell membrane and the cytoskeleton, including binding to target receptors (e.g. GHR) [Rane and Reddy 2000, Babon *et al.* 2014].

JAK2 kinase acts primarily as a part of the signal pathways of STAT (Signal Transducer and Activator of Transcription) proteins, so its most important function is to participate in the signal transmission of extracellular growth factors, cytokines and hormones [Baxter *et al.* 2005, He *et al.* 2005]. Mammals have strong JAK2 expression in many organs and tissues [Yamaoka *et al.* 2004]. Activation of JAK2 kinase initiates the process, as a result of which the STAT molecule is phosphorylated and then the active STAT molecules enter the cell nucleus, which results in the stimulation of target gene transcription [Sędzimirska 2007].

JAK2 activity is closely controlled by several mechanisms, including protein tyrosine phosphatases, such as SHP-1, PTP1B and CD45, and signal protein cytokine suppressors that bind JAK2, inhibit its catalytic activity and promote JAK2-mediated proteasomes [Klingmuller *et al.* 1995, Yasukawa *et al.* 1999, Myers *et al.* 2001, Irie-Sasaki *et al.* 2001, Ungureanu *et al.* 2002]. Activation induced by cytokine receptors is usually rapid and transient. The constitutive activity of *JAK2* may occur in various tumorigenic processes [Lacronique *et al.* 1997].

Janus kinases play a key role in cell signaling at the cytokine level, they catalyse protein phosphorylation and indirectly initiate transcription of target genes. They act through specific receptors (e.g. erythropoietin, interleukins, interferons), including the processes of immunity, growth or cell division. The kinase itself is activated by a ligand for cytokine receptors such as the growth hormone – GH-GHR [Argetsinger and Carter-Su, 1996]. Among JAKs, JAK2 is activated by more than two-thirds of known cytokine receptor ligands, including the growth hormone (GH), prolactin, erythropoietin and leptin, which makes it the most studied member of the JAK family [Herrington *et al.* 2000].

JAK2 autophosphorylation is an important step in signaling regulation, leading to kinase activation. Tyrosines 221, 570, 813, 1007 and 1008 have been identified as JAK2 autophosphorylation sites by mapping 2-D phosphopeptides after an *in vitro* kinase assay. Phosphospecific antibodies confirmed that the above-mentioned tyrosines are phosphorylated *in vivo* in overexpressed JAK2 and endogenous JAK2 activated by GH [Argetsinger *et al.* 2010], suggesting that JAK2 activation, either by overexpression

or GH stimulation, leads to similar tyrosine phosphorylation sites. Within the JAK2 kinase domain, there is a region that has significant sequence homology with the insulin receptor regulatory region and which contains two tyrosines, 1007 and 1008, being potential regulatory sites. Further studies confirmed that tyrosine 1007, which is located in the kinase domain activation loop, is necessary for full activation. Tyrosine 966 has been shown to bind to the SH3 domain containing a protein with a yet unknown function [Carpino *et al.* 2002]. Feng *et al.* [1997] emphasised the key role of JAK2 in the transduction of growth signals and differentiation derived from ligand-activated cytokine receptor complexes. Similar studies by Kurzer *et al.* [2004] showed that phosphorylation of tyrosine 813 was required for the SH2-B beta adapter protein, containing the SH2 domain, to bind JAK2 and increase the JAK2 and STAT5B activity.

In cattle, the *JAK2* gene is located on chromosome 8 and its length is 119.445 bp. Depending on the type of transcript, it consists of 24 or 25 exons separated in some fragments by long introns. Currently, ~3000 polymorphic sites have been detected within the bovine *JAK2* gene. The translation start codon (AUG) is located in exon 3 (https://www.ncbi.nlm.nih.gov/gene/525246).

The use of marker-assisted selection may significantly accelerate selection progress and affect the improvement of a specific group of traits (productive and reproductive), as well as provide better quality meat with health-promoting characteristics. Many studies have shown that numerous genes encoding elements of the somatotropic axis are associated with growth, development and some reproductive traits, which indicates their possible relationship with beef performance [Parmentier *et al*. 1999]. To date, no detailed analysis has been performed on the effect of mutations in the bovine Janus kinase 2 (*bJAK2*) gene on animal growth, development and selected reproductive parameters. The present study focuses on the search for a possible relationship between polymorphism in the Janus Kinase 2 gene (*JAK2*/e20/*Rsa*I) and selected production parameters in beef cattle. The polymorphism is located in exon 20 encoding the JH1 domain of JAK2 kinase, which is responsible for proper enzymatic activity.

Material and methods

A total of 465 blood samples were collected from cows of three breeds – Angus (A, n=168), Hereford (H, n=200) and Limousin (L, n=97), kept on the same farm in the West Pomeranian Province, Poland. The blood samples had been collected previously as part of other research projects. Genomic DNA was isolated from the blood using a MasterPure DNA Purification Kit Version II (Epicentre Technologies, USA) according to the manufacturer's instructions.

The primers for amplifying the bovine *JAK2* gene fragment were designed using the Primer3 program (http://primer3.ut.ee/). The rs110298451 (A→G) polymorphism in the *JAK2* gene was identified using the PCR-RFLP (Polymerase Chain Reaction-

Table 1. Detailed information on the analysed polymorphism

Gene region	Chromosomal location	Accession number*	Restriction enzyme	Primers (5' – 3')
Exon 20	39394036	c.2736A>G rs110298451	*Rsa*I	F: ATGGGCAACATACCAGCACT R: GCCGGTATGACCCTCTACAA

*According to ENSEMBL.

Restriction Fragment Length Polymorphism) method (Tab. 1). This is an example of a silent mutation at the third nucleotide of the lysine codon (AAA→AAG) at position 912 of the amino acid chain of the JAK2 protein.

PCR reaction mixtures contained the aforementioned genomic DNA, 2x 2 µl of PCR buffer with $(NH4)_2SO_4$, 2x 0.1 µl dNTP, 1x 2 µl $MgCl_2$ (FERMENTAS, ABO Gdansk, Poland), 1x 1 µl forward primer (10 pmol/µl) and 1x 1 µl reverse primer (10 pmol/µl) (IBB PAN, Warsaw, Poland), 0.8 µl x 5 units of Taq DNA polymerase (FERMENTAS, ABO Gdansk, Poland) and nuclease-free deionised water (Epicentre Technologies, Madison, USA) added to a total volume of 20 µl. The following PCR protocol was used: an initial denaturation (5 min at 94ºC); 33 cycles of: 94ºC for 50s, 60ºC for 1 min, 72ºC for 50s and a final extension at 72ºC for 7 min.

After the PCR reaction, the amplicons were subjected to the restriction enzyme treatment at +37°C for 4 hours. The mutation site was recognised by the *Rsa*I (GT/AC) enzyme (FERMENTAS, ABO Gdansk, Poland) and the digestion pattern was designed in Webcutter 2.0 (http://www.firstmarket.com/cutter/cut2.html). The product length was 178 bp (*A* allele: 113 + 65bp; *G* allele: 76 + 65 + 37bp). After digestion, 10 µl of each product were separated by electrophoresis in 2% ethidium bromide-stained agarose gels. The gels were visualised under UV and archived.

Associations between genotype and birth weight (BWT), weaning weight adjusted to 210 days of age (WWT_{210}), average daily gains from birth to weaning (ADG), age and body weight at first calving were analysed based on the data obtained from the official recordings.

Statistical calculations were performed using a General Linear Model (eqs. 1 and 2):

BWT, ADG, WWT_{210}

$$(1)\ y_{ijkl} = \boldsymbol{\mu} + G_i + BYS_j + s_k + e_{ijkl}$$

where:

y_{ijkl} – analysed trait;
µ – overall mean;

G_i – fixed effect of *JAK2* genotype (i=1, …3);

BYS_j – fixed effect of birth year/season (Limousin j=1,…15; Hereford k=1, …21; Angus k=1, …20);

s_k – random effect of sire (Limousin k=1,…21; Hereford k=30; Angus k=39);

e_{ijkl} – random error.

Age and body weight at first calving

$$(2)\ y_{ijklm} = \mu + G_i + s_j + CYS_k + e_{ijklm}$$

where:

y_{ijklm} – analysed trait;

μ – overall mean;

G_i – fixed effect of *JAK2* genotype (i=1, …3);

CYS_j – fixed effect of year/season of 1st calving (Limousin j=1,…24; Hereford j=1, …27; Angus k=1,…29);

s_k – random effect of sire (Limousin k=1,…21; Hereford k=30; Angus k=39);

e_{ijklm} – random error.

The differences between individual genotypes were examined using Duncan's test with the Bonferroni correction.

The chi-square test was used to verify the Hardy-Weinberg equilibrium in each population. Statistical analysis was performed using the STATISTICA programme (10.0 PL software package, Statsoft Inc. 2011).

Results and discussion

A specific PCR product of 178 bp was obtained. Digestion with the *Rsa*I restriction enzyme identified two alleles (*A* and *G*) of the *JAK2*/e20/*Rsa*I polymorphism. Based on the results of molecular analysis, the genetic structure of the population was determined, i.e. genotype and allele frequencies in the studied cattle herds (Tab. 2).

Table 2. Genotype and allele frequencies of *JAK2* locus in the analysed breeds

Genotypes	Hereford			Angus			Limousin		
	AA	*AG*	*GG*	*AA*	*AG*	*GG*	*AA*	*AG*	*GG*
N	90	74	36	129	20	19	34	41	22
Percentage of genotypes (%)	45	37	18	77	12	11	35	42	23
HWE significance p-value	0.0043	0.6350	0.3650	0.000	0.8274	0.1726	0.1634	0.5618	0.4382
Allele p(A) frequency	0.6350			0.8274			0.5618		
Allele p(G)frequency	0.3650			0.1726			0.4382		

In all the herds the *A* allele was identified most frequently. According to the NCBI nucleotide sequence of the *JAK2* gene in the Hereford breed (Gene ID: 525246) and other generally available sequences, adenine appears to be the major allele. Three genotypes were found in all the breeds, i.e. *AA*, *GG* and *AG*. The *AA* genotype was dominant in Hereford and Angus cattle. In the case of Limousin cattle, the highest frequency was found for heterozygotes. The lowest frequency in all the breeds was

recorded for the *GG* genotype. The genotype distribution in the Hereford and Angus breeds was not consistent with the Hardy-Weinberg (HWE) law (p-value: 0.0043 and 0.0000, respectively). The Limousin population was in the Hardy-Weinberg equilibrium (p-value = 0.1634).

The values of selected beef performance traits and age at first calving for all the tested breeds are presented in Table 3.

Table 3. Means of BWT (birth weight), ADG (average daily gains), WWT (weaning weight), age and body weight at first calving in cows with different *JAK2*/*Rsa*I genotypes for individual breeds (standard deviations in parentheses)

Breed	Genotype	n	BWT (kg)	ADG (g)	WWT $_{210}$ (kg)	Age at first calving (days)	Body weight at first calving (kg)
Hereford	*AA*	90	33.6 (0.45)	1057^{a} (16.21)	256.0^{a} (3.41)	992.58 (31.86)	570.1 (4.05)
	AG	74	33.8 (0.48)	1084 (16.43)	260.0 (3.47)	1068.94 (36.78)	571.5 (5.43)
	GG	36	34.9 (0.61)	1107^{a} (19.80)	266.6^{a} (4.51)	1054.04 (52.29)	576.2 (4.87)
Angus	*AA*	129	36.7 (0.27)	1000 (6.65)	249.7 (1.68)	1054.16 (16.85)	564.8 (2.77)
	AG	20	37.0 (0.67)	1007 (13.64)	252.9^{a} (5.24)	1132.00 (56.21)	568.0 (6.73)
	GG	19	36.2 (0.63)	992 (16.92)	241.3^{a} (3.60)	1071.20 (25.58)	563.7 (6.18)
Limousin	*AA*	34	35.8^{a} (0.76)	1098^{a} (19.98)	268.7^{a} (5.16)	1126.18 (32.69)	582.6^{ab} (3.04)
	AG	41	34.2 (0.77)	1057 (20.54)	259.7 (5.16)	1105.44 (22.95)	573.7^{a} (2.14)
	GG	22	33.7^{a} (0.82)	1030^{a} (24.32)	256.4^{a} (6.33)	1123.45 (49.87)	572.8^{b} (2.39)

^{ab}Means within columns bearing the same letters within breed differ significantly at $p \leq 0.05$.

A lower body weight at first calving in Angus cattle (+/- 560kg) in comparison with the other tested breeds (+/- 575kg) may be due to the fact that it is an early maturing breed. The first calving may occur at the age of 24 months. Age at first calving is a direct consequence of the moment when breeding of heifers begins. The measure is the degree of development most often expressed as the correct body weight at a certain age. Premature mating of heifers delays overall body development, while a delay leads to fatness, which in both cases usually results in calving difficulties [Lopes *et al.* 2016].

In the case of the Limousin breed the most favourable trait values were noted for individuals with the *AA* genotype, which had higher birth weight (+ 2.1 kg), average daily gains (+68 g) and weight at 210 days of age (+12.3 kg) compared to individuals with the *GG* genotype ($p \leq 0.05$). Different results were obtained for the Hereford breed, where the *GG* genotype was associated with the highest birth weight (+1.3 kg), daily gains (+50 g) and body weight at weaning (+10.6 kg) compared to individuals with the *AA* genotype ($p \leq 0.05$). In contrast, heterozygous Angus cows showed the highest beef performance, although the differences in relation to the other genotypes were non-significant. Statistically significant differences ($p \leq 0.05$) in body weight at 210 days (+11.6 kg) were shown only between heterozygotes and *GG* homozygotes.

Similar trends were observed for body weight at first calving in heifers. However, significant differences ($p \leq 0.05$) were noted only in the Limousin breed, where again individuals with the *AA* genotype were heavier compared to heterozygous (-8.9 kg)

and homozygous *GG* (-9.8 kg) individuals. There was no significant relationship between the *JAK2*/e20/*Rsa*I polymorphism and age at first calving.

Based on the results published by other authors regarding the crystal structure of *JAK2* kinase domains, it can be assumed that even small changes in tyrosine surrounding regions activated in response to the GH signal may be necessary for the JAK2 molecule to assume maximum active conformation [Argetsinger *et al.* 2010].

The rs110298451 polymorphism does not directly affect the amino acid sequence. This synonymous/silent mutation may only be strictly associated with another causative mutation in the *JAK2* gene or some molecular mechanisms, rendering the synonymous mutation "non-silent". The proper functioning of cells within the muscle tissue, including myocytes, requires high efficiency and accuracy of translation and transcription. The redundancy of the genetic code provides some margin of error. However, selection between alternative synonymous codons ("common" and "rare") for the same amino acid residue (in this case, lysine K912 located next to an important tyrosine Y913) may also affect the timing and protein folding during translation probably due to a different abundance of the tRNA molecules for each codon in the cell [Kimchi-Sarfaty *et al.* 2007, Gingold and Pilpel 2011]. Consequently, if amino acid transport to the ribosome is delayed or accelerated, translation is carried out at a much slower or faster rate. Then the incorporation of lysine K912 into a polypeptide chain would occur several times slower or faster. Codon usage may also influence mRNA stability and, if the rare mRNA molecule is relatively unstable, it can be rapidly degraded by enzymes in the cytoplasm [Angov 2011]. Moreover, synonymous substitution in the exon sequence may affect the accuracy and rate of translation [Drummond and Wilke 2008] or the way, in which e.g. the unprocessed *JAK2* mRNA is spliced, arranged [Parmley *et al.* 2006] and transported from the nucleus to the cytoplasm [Smith *et al*. 2007]. Recent studies have highlighted the importance of splicing disorders in the etiology of hereditary diseases [Abramowicz and Gos 2018].

A method that provides a simultaneous identification of thousands of SNPs and the determination of their relationships with production and reproduction traits is referred to as the genome-wide association study (GWAS). Identifying SNPs that can be responsible for some variation in quantitative traits may significantly improve individual selection in the future. For example, Snelling *et al.* [2010] confirmed the existence of 231 SNPs overlapping with quantitative trait loci (QTLs) previously described by other authors. The tests were performed on beef cattle and the examined traits included mainly beef and reproductive parameters. Other GWAS have shown the effect of the *JAK-STAT* signaling pathway on nutrition efficiency, with an indication of polymorphic sites located within or adjacent to the *CNTFR, OSMR* and *GHR* genes [Richard and Stephens 2014, Abo-Ismail *et al.* 2018]. The SNPs described by these authors have contributed to the significant genetic variability of the studied traits, and therefore can be potentially used or tested in order to select cattle for the desired values of meat parameters. The *CNTFR* gene, similarly as the *JAK2* gene, is located on chromosome 8. Considering the location of the *JAK2* gene and its involvement in

the *JAK2/STAT* signaling pathway as a GH-GHR signaling element, it can be assumed that this gene can also have a significant impact on the development of selected quantitative traits.

There are no data in the available literature on the relationship between polymorphism within bovine *JAK2* and meat parameters. However, the GH is suggested to play an important role in improving beef parameters, as it binds to the growth hormone receptor that activates the *JAK2* pathway. The activated *JAK2* in turns induces STAT5 [Ali *et al.* 2019].

To date, many reports have pointed to the existence of statistically significant relationships between the *GH* gene polymorphism and meat performance parameters in cattle [Hai *et al*. 2009, Ishag *et al*. 2010, Su *et al*. 2012].

Many authors have studied the impact of polymorphism in the *JAK2* gene on parameters related to milk traits. Szewczuk [2015] analysed the relationship between the polymorphic site described in the present study (*JAK2*/e20/*Rsa*I) and milk performance of various dairy cattle breeds. Individuals with the *GG* genotype were characterised by higher milk yields, protein and fat contents than those with the *AA* genotype. Further research by Usman *et al.* [2015] on the Chinese Holstein cattle breed described the relationship between the *JAK-STAT* polymorphism and the immune response in clinical cases of mastitis. The identified polymorphic sites can serve as potential genetic markers in the selection of mastitis-free dairy cattle. Single nucleotide polymorphisms in the *JAK2* and *DGAT1* (diacylglycerol acyltransferase) genes for dairy cattle production traits and mastitis were also studied by Khan *et al.* [2019]. A significant relationship ($p<0.05$) was demonstrated between milk fat percentage, somatic cell count (SCC), serum cytokine levels (e.g. interleukin 6 or interferon gamma) and at least one or more analysed SNPs. Ali *et al.* [2019] suggested that *JAK2* may be an important candidate gene and the tested SNPs may be useful genetic markers of production and mastitis-related traits. In SNP1 (*G>A*, rs379754157), the *GG* genotype was significantly ($p < 0.01$) associated with higher SCC, while SNP2 (*A>G*, rs134192265) and SNP3 (*A>G*, rs110298451) were significantly ($p<0.01$) associated with a higher percentage of lactose compared to the other genotypes.

Most reports on *JAK2* expression originate from human studies. One of the available papers describes the *V617F* point mutation within human Janus Kinase 2 (*hJAK2*) in a condition called essential thrombocythemia. This mutation occurs in 60–70% of patients with this disease and is located in the domain acting as an inhibitor [Dziedczenia and Kuliczkowski 2007]. Another study on the *hJAK2* gene polymorphism examined the effect of many mutations in this gene on myeloproliferative syndromes. A total of 13 mutations were described, nine of which were located in the pseudokinase domain and the other four in the linker (SH2) [Zhao *et al.* 2009]. He *et al.* [2005] in their study on mice confirmed the effect of Janus Kinase 2 on the growth hormone receptor stability. In the presence of *JAK2, GHR* expression was significantly increased on the surface of target cells.

In beef cattle breeding, daily body weight gains are of great importance, which is related to the amount of purchased raw material. Depending on the animal genetic potential (breed, individual variability), on-farm feed resources, cultivation area and the availability of pastures an appropriate grazing system is selected, taking into account also animal welfare and aspects related to environmental protection, including greenhouse gas emissions. This principle was directly implemented on the farm where the research was conducted. The beef cattle included in this experiment came from a cooperative farm (field cultivation, five breeds of beef cattle, dairy cattle, pigs, and poultry) and were raised under a semi-intensive system with a large acreage of pastures, based on the farm's own feed base, which was associated with lower labour intensity and maintenance costs (pasture with access to a shelter all year round).

The European Union (EU) is the third largest beef producer in the world. The future of the European beef industry depends on meeting the challenge of its sustainability by protecting food safety, ensuring nutritional quality and palatability, protecting the environment and animal welfare, while maintaining sustainable land use and landscape quality [Hocquette *et al.* 2018]. The EU is one of the most efficient beef producers, as demonstrated by its relatively low greenhouse gas production. Different beef production systems show a significant variation in total emissions [de Vries and de Boer 2010] and the composition of individual greenhouse gases, which determines their impact on the climate [Pierrehumbert and Eshel 2015]. Greenhouse gas emissions in farms can be reduced by implementing appropriate management practices, proper manure storage or selecting the right diet [Pattey *et al.* 2005]. Improving quantitative characteristics related to beef and reproduction can have a positive effect on the profitability of farming and reduction of greenhouse gas emission [Haas *et al.* 2017].

Due to the lack of literature on the discussed subject in relation to beef cattle, it was impossible to compare the obtained results with those of other authors. The present study is a preliminary one. In order to determine whether the analysed *JAK2*/e20/*Rsa*I polymorphism could be included in future selection schemes for beef cattle, haplotype analysis of *JAK2* should be carried out, which could possibly be extended to combinations of genotypes for different polymorphic sites located in the genes encoding the so-called somatotropic axis.

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