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ZMIENNOŚĆ W SEKWENCJI KODUJĄCEJ GENU KINAZY JANUSOWEJ 2 (*JAK2*) W POWIĄZANIU Z CECHAMI UŻYTKOWOŚCI MIĘSNEJ I PARAMETRAMI ROZRODU WYBRANYCH RAS BYDŁA I OWIEC

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„Zmienność w sekwencji kodującej genu Kinazy Janusowej 2 (JAK2) w powiązaniu z cechami użytkowości mięsnej i parametrami rozrodu wybranych ras bydła i owiec”

[D-1] Padzik N., Szewczuk M. (2020) Association between polymorphism in the *JAK2* gene (*JAK2*/e20/*Rsa*I) and selected performance parameters in beef cattle. Anim. Sci. Pap. Rep. 38, 333-344 [załącznik nr 1].
IF_{2020}= 1,06; 40 pkt. $MNiSW_{2020}$

Mój wkład w powstawanie pracy polegał na udziale we wszystkich etapach badań, obejmujących: pobranie materiału, zaplanowanie i przeprowadzenie doświadczenia, gromadzenie danych, analiza uzyskanych wyników, redagowanie manuskryptu, korekta po recenzji.

Indywidualny wkład w publikację: 75%

[D-2] Padzik N., Szewczuk M., Ropka-Molik K. (2021) Distribution of *JAK2* genotypes across Suffolk and Pomeranian sheep. Small Rumin. Res. 194, 106282 [załącznik nr 2].
IF_{2021}= 1,89; 100 pkt. $MNiSW_{2021}$

Mój wkład w powstawanie pracy polegał na udziale we wszystkich etapach badań, obejmujących: pobranie materiału, zaplanowanie i przeprowadzenie doświadczenia, gromadzenie danych, analiza uzyskanych wyników, redagowanie manuskryptu, korekta po recenzji.

Indywidualny wkład w publikację: 70%

[D-3] Oster N., Szewczuk M., Zych S., Stankiewicz T., Błaszczyk B., Wieczorek-Dąbrowska M. (2023) Association between Polymorphism in the Janus Kinase 2 (*JAK2*) Gene and Selected Performance Traits in Cattle and Sheep. Animals 13, 2470 [załącznik nr 3].

IF_{2023}= 3,23; 100 pkt. $MNiSW_{2023}$

Mój wkład w powstawanie pracy obejmował: zaplanowanie i przeprowadzenie doświadczenia, gromadzenie danych, analizę uzyskanych wyników, redagowanie manuskryptu oraz korektę po recenzji.

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Spis treści

1. Streszczenie

Postęp hodowlany zwierząt gospodarskich opiera się o doskonalenie osobników w kolejno następujących po sobie pokoleniach. Ważnym elementem hodowli jest selekcja prowadząca do utrwalenia i udoskonalenia cech o wartości użytkowej. Obok doboru opartego na fenotypach, odkrycia genetyki molekularnej pozwoliły na udoskonalenie hodowli pod względem pożądanych genotypów. Dlatego też istnieje potrzeba poszukiwania i identyfikacji genów, których zmienność może mieć bezpośredni lub pośredni związek z cechami użytkowymi owiec i bydła utrzymywanych w kierunku mięsnym.

Kinaza Janusowa zalicza się do grupy białkowych kinaz tyrozynowych (PTK), biorących udział w katalizowaniu fosforylacji białek oraz determinujących szlaki sygnałowe pochodzące z różnych cytokin (m.in. hormon wzrostu, erytropoetyna, interleukina, interferon). Wraz z innymi genami tworzy oś somatotropową, w której to polimorfizmy są bezpośrednio i pośrednio związane z fenotypem, głównie w odniesieniu do składu i syntezy mleka, właściwości prozdrowotnych mięsa i tuszy oraz czynności rozrodczych.

Celem badań była walidacja i poszukiwanie nowych miejsc polimorficznych w genie *JAK2* u bydła i owiec, następnie określenie częstości występowania alleli i genotypów zidentyfikowanych miejsc polimorficznych w *JAK2,* oraz oszacowanie ewentualnego związku pomiędzy wybranymi wariantami genetycznymi, a cechami użytkowości mięsnej oraz niektórymi parametrami kształtującymi użytkowość rozrodczą bydła i owiec.

Za pomocą metod biologii molekularnej (PCR, sekwencjonowanie Sangera, PCR-RFLP, PCR-ACRS), analizowano łącznie 6 miejsc polimorficznych, tj. 3 u bydła (eksony 16, 20 i 23) i 3 u owiec (dwa w eksonie 6 i jedno w 24). Następnie dokonano analiz asocjacyjnych z wybranymi cechami użytkowości mięsnej i rozrodczej.

W pierwszej pracy [**D-1**], analizowano polimorfizm w eksonie 20 (rs110298451) genu *JAK2* u trzech ras bydła mięsnego: angus, hereford i limousin w zależności od cech związanych z mięsnością. W przypadku rasy limousin najkorzystniejsze cechy odnotowano u osobników z genotypem *AA*, tj. większą masę urodzeniową, większe średnie przyrosty dobowe oraz większą masą ciała w 210 dniu odchowu w porównaniu z osobnikami o genotypie *GG*. Odmienne wyniki uzyskano dla rasy hereford, u której genotyp *GG* decydował o największej masie urodzeniowej, przyrostach dziennych i masie ciała przy odsadzeniu w porównaniu z osobnikami o genotypie *AA*. U krów rasy angus heterozygoty charakteryzowały się największymi masami ciała i przyrostami dobowymi.

Zsekwencjonowanie prób PCR w pracy **D-2** pozwoliło na walidację istniejących miejsc polimorficznych w owczym genie *JAK2*. W przypadku pierwszego miejsca polimorficznego (rs160146162) zlokalizowanego w eksonie 6, genotyp *AA* występował rzadko u rasy pomorskiej (15%), natomiast u rasy suffolk nie został zidentyfikowany. Genotyp *GG* identyfikowano najczęściej u rasy suffolk, natomiast u rasy pomorskiej najczęściej występowały osobniki heterozygotyczne. W drugim miejscu polimorficznym (rs160146160), również zlokalizowanym w eksonie 6 u obu ras najczęściej identyfikowano genotyp *AG,* a najrzadziej *GG.* W przypadku trzeciego miejsca polimorficznego (rs429445187), zlokalizowanego w intronie 24 odnotowano największy udział heterozygot.

Trzecia publikacja [**D-3**] była rozszerzeniem oraz podsumowaniem wcześniejszych prac. Krowy rasy hereford i limousin o genotypie *AA* (e16/*Rsa*I) i *AA* (e23/*Hae*III) cechowały się najwyższą masą ciała i lepszymi przyrostami dobowymi ($P \leq 0,05$). W przypadku owiec, niezależnie od rasy, w badanych okresach największe masy ciała i przyrosty dobowe charakteryzowały osobniki o genotypach *AA* (e6/*Ear*I), *GG* (e6/seq) i *AA* (e24/*Hpy*188III) ($P \leq 0,01$). Te same osobniki rasy pomorskiej cechowały się również lepszą płodnością oraz przeżywalnością jagniąt ($P \leq 0,01$).

Przeprowadzone badania w pracy doktorskiej są pierwszymi analizami asocjacyjnymi dla wszystkich miejsc polimorficznych zidentyfikowanych w części kodującej genu *JAK2*. Polimorfizmy pojedynczych nukleotydów w genie *JAK2* mogą służyć jako markery genetyczne cech wzrostu i rozwoju oraz wybranych cech rozrodczych u przeżuwaczy, pod warunkiem, że w przyszłości badanie te zostaną rozszerzone o inne stada, jak również rasy i zostaną przeprowadzone dodatkowo analizy w układach haplotypów i/lub genotypów kombinowanych.

2. Abstract

The breeding progress of livestock is based on the improvement of individuals in successive generations. Selection is an important element of breeding to consolidate and improve features with utility value. In addition to selection based on phenotypes, discoveries in molecular genetics have allowed the refinement of breeding in terms of desired genotypes. Therefore, there is a need to search for and identify genes whose variability may be directly or indirectly related to the performance characteristics of sheep and cattle.

Janus kinase belongs to the group of protein tyrosine kinases (PTKs) involved in catalyzing the phosphorylation of proteins and determining signaling pathways from various cytokines (e.g. growth hormone, erythropoietin, interleukin, interferon). Together with other genes, it creates somatotropic axis in which polymorphisms are directly and indirectly related to the phenotype, mainly in relation to the composition and synthesis of milk, meat and carcass properties and reproduction.

The aim was to validate and search for new polymorphic sites in the *JAK2* gene in cattle and sheep. Another objective was to determine the allele frequencies and genotypes of polymorphic sites in *JAK2* and estimation of a possible relationship between selected genetic variants and meat performance characteristics and some parameters shaping the reproductive performance of cattle and sheep.

Using molecular biology methods (PCR, Sanger sequencing, PCR-RFLP, PCR-ACRS), a total of 6 polymorphic sites were analyzed, 3 in cattle (exon 16, 20 and 23) and 3 in sheep (two in exon 6 and one in 24). Then, association analyses were performed with selected meat and reproductive performance characteristics.

In the first paper [**D-1**], the polymorphism in exon 20 (rs110298451) of the *JAK2* gene was analysed in three breeds of beef cattle: Angus, Hereford and Limousine depending on production capacity. In the case of the Limousine breed, the most favorable features were noted in individuals with the *AA* genotype, i.e. higher birth weight, higher average daily gains and higher body weight at day 210 compared to individuals with the *GG* genotype. Different results were obtained for the Hereford breed, in which the *GG* genotype determined the highest birth weight, daily gains and body weight at weaning, compared to individuals with the *AA* genotype. In Angus cows, heterozygotes were characterized by the highest body weights and daily gains.

The sequencing of individuals in the **D-2** work allowed the validation of existing and the discovery of new polymorphic sites in the sheep *JAK2* gene. In the case of the first

polymorphic site (rs160146162) located in exon 6, the *AA* genotype was rare in the Pomeranian breed (15%) and not identified in the Suffolk breed. The *GG* genotype was most often identified in the Suffolk breed, while in the Pomeranian breed, heterozygous individuals were the most common. In the second polymorphic site (rs160146160), also located in exon 6, the *AG* genotype was most frequently identified in both breeds, and the *GG* genotype the least frequently. In the case of the third polymorphic site (rs429445187), located in intron 24, the highest share of heterozygotes was noted.

The third publication [**D-3**] was an extension and a summary of earlier work. Hereford and Limousine cows with *AA* (e16/*Rsa*I) and *AA* (e23/*Hae*III) genotypes were characterized by the highest body weight and better daily gains ($P \leq 0.05$). In the case of sheep, regardless of breed, in the studied periods, the highest body weight and daily gains were characterized by individuals with genotypes *AA* (e6/*Ear*I), *GG* (e6/seq) and *AA* (e24/*Hpy*188III) ($P \leq 0.01$). The same individuals of the Pomeranian breed were also characterized by better fertility and survival of lambs ($P \leq 0.01$).

These are the first association studies for all polymorphic sites identified in the coding part of the *JAK2* gene. Single nucleotide polymorphisms in the *JAK2* gene can serve as genetic markers of growth and development traits and selected reproductive traits in ruminants, if, for example, they are further studied in subsequent populations and analyzed in haplotype and/or combined genotype systems.

3. Wstęp

Wzrost liczby ludności skutkuje większym zapotrzebowaniem na produkty odzwierzęce. Bydło oraz owce w dużym stopniu przyczyniają się do globalnej produkcji żywności. Ponadto poprawa wydajności zwierząt oraz poszukiwanie mięsa wołowego oraz chudego mięsa owczego o właściwościach prozdrowotnych znajduje swoje zastosowanie w rosnących wymogach konsumentów, niosąc przy tym korzyści dla zdrowia ludzi (Cavanagh i in., 2010).

Biologiczna wydajność produkcji mięsa i mleka owczego jest niższa od analogicznego potencjału innych gatunków zwierząt hodowlanych. Zarówno zabiegi hodowlane, podobnie jak i wdrażanie nowych technologii nie dają możliwości całkowitego skompensowania tej dysproporcji, co ma swoje odbicie w wysokich cenach produktów owczarskich, a tym samym i zawężonym rynku zbytu. Utrzymująca się od lat niekorzystna sytuacja na rynku jagnięciny narzuca zachowanie i wdrożenia nowych niskonakładowych metod produkcji, funkcjonowanie w ramach struktur rolnictwa ekologicznego z wykorzystaniem trwałych użytków zielonych z przeznaczeniem na bazę paszową (Paraponiak i Wieczorek-Dąbrowska, 2017).

Obecność związków bioaktywnych w mięsie owczym, pozwala je określić mianem żywności funkcjonalnej, czyniąc ją przy tym odpowiednią dla dzieci, rekonwalescentów czy osób starszych. Mięso owcze jest m.in. źródłem wysokiej jakości biologicznej białka, mikro- i makroelementów, sprzężonego kwasu linolowego oraz L-karnityny. Obok żywienia, systemu utrzymania, płci czy standardu wagowego związanego z wiekiem, również genotyp zwierzęcia wpływa na wartość rzeźną oraz dietetyczną mięsa (Pietrzkiewicz i in., 2017).

Selekcja oparta na markerach genetycznych skupia się przede wszystkim na poprawie cech użytkowych zwierząt, takich jak: mleczność i mięsność. Cechy ilościowe warunkowane są wpływem wielu genów, stąd metoda doboru zwierząt oparta wyłącznie na fenotypie obarczona jest dużym błędem. Skuteczny postęp hodowlany możliwy jest dzięki selekcji opartej na genotypie (Esrafili Taze Kand Mohammaddiyeh i in., 2023). Podczas doboru zwierząt coraz częściej brane są pod uwagę również cechy rozrodcze. Cechy te mają bezpośrednie przełożenie na wydajność produkcyjną. Intensywność selekcji jest bowiem zależna od liczby cieląt i jagniąt urodzonych w ciągu roku oraz odstępu między pokoleniami. System produkcji zarówno mleka jak i mięsa, cechujący się nieregularną rozrodczością m.in. zbyt długim odstępem międzypokoleniowym, staje się nierentowny z punktu widzenia ekonomicznego (Mohammadi i in., 2022; Zamani i in., 2023). Wykorzystanie markerów

polimorfizmu pojedynczego nukleotydu (SNP) daje możliwość wyboru odpowiedniego genotypu i próbę z fenotypem, co przejawia się znaczącymi korzyściami hodowlanymi. Przykładem tego, mogą być programy hodowlane opracowane dla bydła mlecznego i mięsnego wdrażane w Ameryce Północnej i Europie, gdzie wykorzystanie osiągnięć genetyki molekularnej przyczynia się do rentowności gospodarstw i ciągłej poprawy wartości produkcyjnej zwierząt (Menezes i in., 2014).

Identyfikacja genów warunkujących fenotypowe przejawianie się cech ilościowych jest trudna ze względu na dużą liczbę genów zaangażowanych w powstanie cechy, co w efekcie daje niewielki efekt addytywny. Wiele badań skupia się na identyfikacji *loci* cech ilościowych (QTL - ang. Quantitative Trait Loci) w obrębie całego genomu bydła (w mniejszym stopniu owiec), istotnych z ekonomicznego punktu widzenia. QTL, to dające się łatwo zlokalizować markery genetyczne, które są najczęściej jedynie ściśle sprzężone z genami kontrolującymi interesujące nas cechy. W idealnych warunkach QTL mogą być włączane do programów selekcji wspomaganych markerami genetycznymi (MAS - ang. Marker Assisted Selection) (Bora i in., 2023). Zdaniem Sarma i Singh (2022) większość QTL ma bardzo niewielki, stąd trudny do oszacowania wpływ. Szansę na urzeczywistnienie w/w koncepcji dają tzw. mikromacierze (chipy DNA), które pozwalają na genotypowanie osobników jednocześnie w tysiącach miejsc polimorficznych danego genomu. Technikę, która umożliwia równoczesną identyfikację tysięcy SNP oraz określenie ich związku z daną cechą określa się badaniem asocjacyjnym całego genomu (ang. genome-wide association study; GWAS). Poszukiwanie *loci* cech ilościowych jest kosztowne i jak dotąd nie przyniosło spektakularnych rezultatów u bydła i owiec.

Pomimo wielu niekwestionowanych zalet, technika GWAS daje tylko orientacyjne wskazówki co do potencjalnych QTL, lecz nie wyjaśnia mechanizmu przyczynowo - skutkowego danego procesu biologicznego. Liczba znanych genów mających wpływ na poszczególne cechy ilościowe nie jest do końca ustalona, stąd konieczne jest ciągłe powtarzanie szacowania ewentualnych związków pomiędzy polimorfizmem potencjalnych genów, a cechami użytkowości. Ważne jest wówczas genotypowanie zwierząt w oparciu o nowo odkryte SNP, połączone z gromadzeniem informacji umożliwiających ich powiązanie z użytkowością (Eusebi i in., 2020). Stanowi to element kolejnej strategii określanej jako poszukiwanie genów kandydujących (ang. candidate genes) dla danej cechy ilościowej. Podejście to opiera się na wyborze genów, których produkty białkowe biorą bezpośredni udział w konkretnych procesach fizjologicznych (Hernández-Montiel i in., 2020).

Nadal istnieje potrzeba poszukiwania i identyfikacji genów, których zmienność może mieć bezpośredni lub pośredni związek z cechami użytkowości owiec i bydła utrzymywanych w kierunku mięsnym.

Oś somatotropowa jest głównym regulatorem zarówno fizjologii rozrodu, jak i metabolizmu ssaków (Bartke, 2022). Polimorfizm w genach osi somatotropowej jest bezpośrednio i pośrednio związany z fenotypem, głównie w odniesieniu do składu i syntezy mleka (Song et al. 2022), właściwości produkcyjnych mięsa i tuszy (Gerasimov i in., 2023) oraz czynności rozrodczych (Tait i in., 2018). Kinaza Janusowa będąca przedmiotem prezentowanych badań zalicza się do grupy białkowych kinaz tyrozynowych (PTK), biorących udział w katalizowaniu fosforylacji białek oraz determinujących szlaki sygnałowe pochodzące z różnych cytokin (m.in. hormon wzrostu, erytropoetyna, interleukina, interferon). U ssaków wyróżnia się kinazy: JAK1, JAK2, JAK3 oraz kinazę tyrozynową TYK2. Typowa kinaza JAK, to białko o stosunkowo dużej wielkości (ponad 1100 aminokwasów) o masie molekularnej 120-140 kDa. W zależności od gatunku, geny kodujące poszczególne kinazy umiejscowione są w różnych chromosomach (Garrido-Trigo i Salas, 2020).

Istnieje wiele badań potwierdzających wpływ mutacji w genie *JAK2* na cechy użytkowe krów mlecznych. Wykazano między innymi, że szlak JAK-STAT reguluje laktację oraz że PI3K/Akt w obrębie szlaku JAK-STAT ulega nadekspresji u krów w okresie laktacji (Brenaut i in., 2012). Analiza delecji genów u myszy udokumentowała ważną rolę sygnalizacji JAK-STAT w laktacji i rozwoju gruczołu mlecznego (Yamaji i in., 2013). Ponadto udokumentowano ważną rolę szlaku JAK-STAT w różnicowaniu krwinek i regulacji genu kazeiny podczas produkcji mleka. Prolaktyna wykorzystuje również sygnalizację JAK-STAT i reguluje laktację oraz rozród u ssaków (Khan i in., 2020). W badaniach Szewczuk (2015), stwierdzono wyraźny związek między polimorfizmem *JAK2*/e20/*Rsa*I, a cechami mleczności bydła. Analizowany SNP był związany z większą wydajnością mleka, białka i tłuszczu. W odniesieniu do cech związanych z użytkowością mięsną i rozrodem brak jest piśmiennictwa naukowego opisującego wpływ genu *JAK2* m.in. na masę ciała i przyrosty dobowe w różnych okresach życia zwierzęcia oraz płodność, plenność czy okres międzywycieleniowy.

U bydła gen *JAK2* zlokalizowany jest w chromosomie 8. Długość wynosi 119,455 pz. [źródło: http://www.ncbi.nlm.nih.gov/gene/525246]. W zależności od rodzaju transkryptu składa się z 24 lub 25 eksonów poprzedzielanych w niektórych miejscach długimi intronami. Obecnie w obrębie bydlęcego genu *JAK2* wykryto ~3000 miejsc polimorficznych [źródło: https://www.ncbi.nlm.nih.gov/snp/?term=JAK2+Bos+taurus]. U owiec gen ten jest

zlokalizowany w chromosomie 2. Długość mRNA wynosi 6897 pz. [źródło: https://www.ncbi.nlm.nih.gov/gene/101113909]. Składa się z 25 eksonów.

Coraz powszechniejsze stają się badania nad opracowaniem map genomu zwierząt hodowlanych, powiązanych z molekularnymi markerami genetycznymi, a cechami będącymi przedmiotem zainteresowania. Cechy wzrostu i rozwoju oraz dotyczące jakości tuszy i mięsa stają się coraz bardziej istotne w programach hodowli owiec i bydła ze względu na ich wartość ekonomiczną (Berry i in., 2022).

Dotychczas nie podjęto szczegółowych analiz wpływu mutacji w genie Kinazy Janusowej 2 (*JAK2*) na wzrost, rozwój oraz na wybrane parametry rozrodcze bydła i owiec. Badania z tego zakresu są pewnym novum w kontekście parametrów użytkowych bydła i owiec.

4. Hipoteza i cele badawcze

4.1 Cele:

- Walidacja istniejących w bazach danych i poszukiwanie nowych miejsc polimorficznych w stosunkowo słabo poznanym genie *JAK2* u bydła i owiec w oparciu o stada bydła mięsnego, jak również owiec o mięsno-wełnistym kierunku użytkowania.
- Określenie częstości występowania alleli i genotypów dla wykrytych miejsc polimorficznych w genie *JAK2:*
    - bydło: exon 16, rs210148032; exon 20, rs110298451; exon 23 rs211067160;
    - owce: exon 6, rs160146162; exon 6, rs160146160; exon 24, rs160146116.
- Oszacowanie związku pomiędzy wariantami genetycznymi zlokalizowanymi w części kodującej genu *JAK2*, wykrytymi w analizowanych stadach, a cechami użytkowości mięsnej oraz niektórymi parametrami kształtującymi użytkowość rozrodczą bydła i owiec.

4.2. Hipoteza

Ze względu na kluczową rolę jaką pełni *JAK2* w wewnątrzkomórkowym przekazywaniu sygnału hormonów z rodziny hormon wzrostu (GH)/prolaktyna (PRL), postawiono hipotezę, według której zmienność w genie *JAK2* może mieć związek z kształtowaniem cech użytkowości mięsnej oraz wybranych parametrów rozrodczych bydła i owiec.

5. Materiał i metody

Materiał do badań stanowiła krew obwodowa pobrana z żyły szyjnej zewnętrznej od krów rasy limousin, hereford i angus oraz od owiec rasy suffolk i pomorskiej do probówek próżniowych zawierających antykoagulant K_3EDTA. Izolacja została przeprowadzona za pomocą zestawu Master Pure Genomie DNA Purification Kit firmy Epicentre Technologies™ wg protokołu załączonego do zestawu przez producenta.

Sekwencje starterowe do metodyk własnych zaprojektowano w programie Primer3 w oparciu o sekwencje DNA i mRNA dostępne na stronach National Center for Biotechnology – NCBI (http://www.ncbi.nlm.nih.gov/nuccore/). Sekwencjonowanie uzyskanych fragmentów genów zlecono firmom Oligo (Instytut Biochemii i Biofizyki PAN, Warszawa) oraz Genomed S.A. (Warszawa). Część próbek zsekwencjonowano w Instytucie Zootechniki - Państwowym Instytucie Badawczym w Balicach, podczas odbywanego stażu naukowego. Analizy restrykcyjne wybranych fragmentów genów ustalano w oparciu o programy WebCutter 2.0 i NEBcutter 2.0.

Genotypy określono z zastosowaniem poniższych metod badawczych:

- łańcuchowa reakcja polimerazy (PCR, ang. polymerase chain reaction);
- sekwencjonowanie uzyskanych fragmentów DNA i analiza fluorogramów;
- polimorfizm długości fragmentów restrykcyjnych fragmentów DNA powstałych w wyniku trawienia za pomocą enzymów restrykcyjnych (PCR-RFLP, ang. restriction fragments length polymorphism);
- polimorfizm sztucznie utworzonych miejsc restrykcyjnych powstałych w wyniku amplifikacji DNA z użyciem modyfikowanych sekwencji starterowych, gdy brak było

enzymów rozpoznających natywną sekwencję (ACRS, ang. amplification-created restriction site).

Analizy zależności pomiędzy genotypami, a wybranymi cechami związanym z wzrostem i rozwojem zwierząt oraz reprodukcyjnymi przeprowadzono na podstawie danych uzyskanych z dokumentacji hodowlanej stad. Obliczenia statystyczne przeprowadzono przy użyciu ogólnego modelu liniowego (GLM) i wykonano w programie Statistica (10.0, 13.3 PL software package, Statsoft Inc. 2011, 2020).

6. Uzyskane wyniki

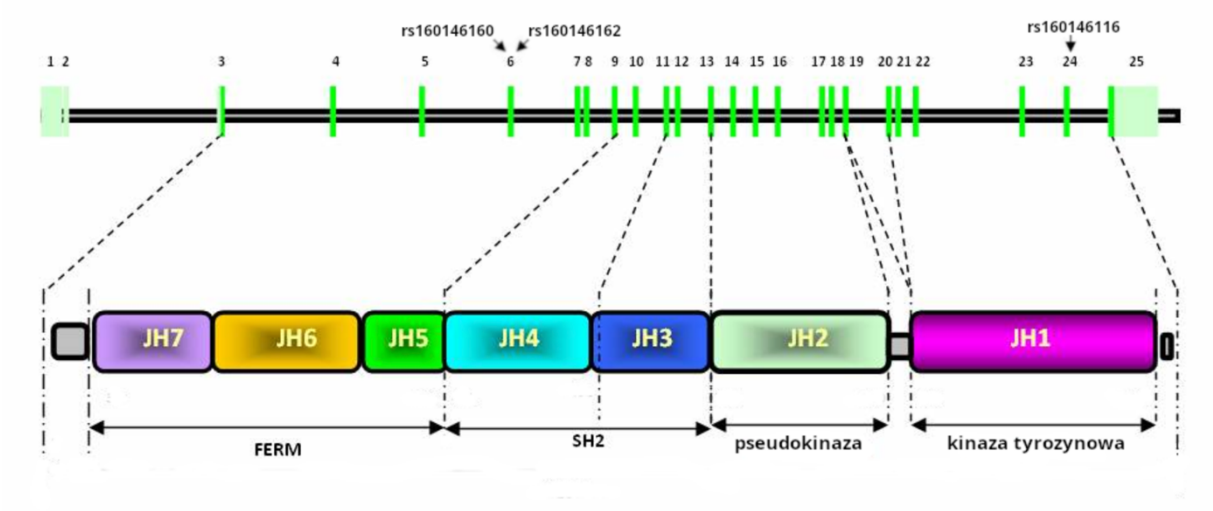
Wynikiem przeprowadzanych analiz była walidacja z potwierdzeniem występowania 6 miejsc polimorficznych (Tab. 1) w części kodującej genu *JAK2* u odpowiednio licznych stad owiec (Ryc. 1) i bydła (Ryc. 2). Nie potwierdzono istnienia 11 znanych miejsc polimorficznych (tabela 1 w sekcji „suplemmentary file" Animals publikacja [**D-3**]) oraz nie wykryto nowych, dotąd nie zdeponowanych. Dzięki utworzeniu baz danych osobników o określonych genotypach, możliwe było przeprowadzenie analiz asocjacyjnych pod względem wybranych parametrów użytkowych, związanych z mięsnością i rozrodczością zwierząt.

Tabela 1. Szczegółowe informacje dotyczące analizowanych polimorfizmów

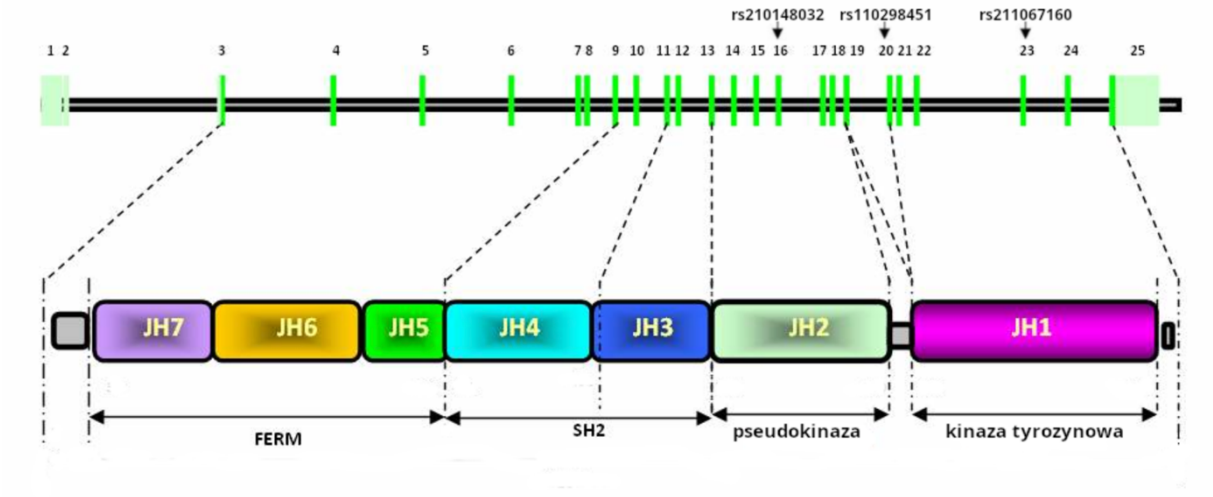
Region genu	Gatunek	N	Lokalizacja chromosomalnaNumer dostępu rs*	Typ mutacji Kodon Aminokwas	Metoda identyfikacji	Startery (5' – 3')
Ekson 16	*Bos taurus*	769	8:39400906 g.39400906A>G rs210148032	zmiany sensu ATT → GTT Ile 704 Val	PCR-ACRS /*Rsa*I	F: gggccctggacatactaagtg R: gtcttttggcaaaactgtaG
Ekson 20	*Bos taurus*	465	8:39394036 c.2736A>G rs110298451	cicha AAA → AAG Lys912	PCR-RFLP /*Rsa*I	F: atgggcaacataccagcact R: gccggtatgaccctctacaa
Ekson 23	*Bos taurus*	759	8:39383281 g.39383281A>G rs211067160	cicha CCA →CCG Pro1057	PCR-ACRS /*Hae*III	F: catatattgacaagagtaaaagccG R: tccccacctttcaaaacttc
Ekson 6	*Ovis aries*	333	2:78861360 g.73397955A>G rs160146162	cicha GAG → GAA Glu177	PCR-RFLP /*Ear*I	F: ttgaccttgttaaatgtatatgttctg R: ttgcataagaaaattacctgatagagc
Ekson 6	*Ovis aries*	333	2:78861417 g.72850917A>G rs160146160	cicha ACA → ACG Thr196	Sekwencjono-wanie Sangera	F: ttgaccttgttaaatgtatatgttctg R: ttgcataagaaaattacctgatagagc
Ekson 24	*Ovis aries*	333	2:78905481 g.73442093A>G rs160146116	cicha CTA →CTG Leu1082	PCR-RFLP /*Hpy*188III	F: tctgcttgaaattaaatgtaccaaa R: tcagtgaactgcataaactgacc

*Zgodnie z ENSEMBL;

N - liczba zgenotypowanych osobników



Rycina. 1 Organizacja genu, kolejność domen *JAK2* oraz przybliżona lokalizacja analizowanych miejsc polimorficznych u owiec (opracowanie własne na podstawie: http://www.uniprot.org/uniprot/E1BCP6)



Rycina. 2 Organizacja genu, kolejność domen *JAK2* oraz przybliżona lokalizacja analizowanych
miejsc polimorficznych u bydła (opracowanie własne na podstawie: http://www.uniprot.org/uniprot/E1BCP6)

7. Cykl publikacji

[**D-1**]

Wiele badań wskazuje, iż liczne geny kodujące elementy osi somatotropowej związane są ze wzrostem, rozwojem oraz z niektórymi cechami rozrodczymi, co wskazuje na ich ewentualny związek z użytkowością mięsną krów. Głównymi mediatorami wewnątrzkomórkowych szlaków sygnałowych są kinazy tyrozynowe. Do grupy tej należy również rodzina cytoplazmatycznych kinaz tyrozynowych - JAK (Janus Kinase). Wiedza ta oraz brak piśmiennictwa w opisywanym zakresie, dała podstawę do zbadania wpływu polimorfizmu w genie *JAK2* na kształtowanie się cech użytkowych bydła.

W oparciu o wyniki analizy molekularnej w pracy **D-1,** otwierającej prezentowany cykl publikacji określono strukturę genetyczną populacji trzech ras bydła dla układu *JAK2*/e20/*Rsa*I - częstość występowania genotypów i alleli w analizowanych stadach bydła a następnie oszacowano związek pomiędzy badanym polimorfizmem, a wybranymi cechami użytkowymi.

Łącznie pobrano 465 próbek krwi od krów trzech ras – angus (n=168), hereford (n=200) i limousin (n=97) utrzymywanych w tym samym gospodarstwie w województwie zachodniopomorskim. Metodę PCR-RFLP zastosowano do identyfikacji cichej mutacji w eksonie 20 (dbSNP ID: rs110298451).

We wszystkich stadach najczęściej identyfikowano allel A. U rasy limousin najkorzystniejsze cechy odnotowano u osobników z genotypem *AA*, tj. większą masę urodzeniową (+ 2,1 kg), średni-przyrost dobowy (+ 68 g) oraz masę ciała w 210 dniu (+ 12,3 kg) w porównaniu z osobnikami o genotypie *GG*. Odmienne wyniki uzyskano dla rasy hereford, u której genotyp *GG* warunkował największą masę urodzeniową (+1,3 kg), przyrosty dobowe (+50 g) i masę ciała przy odsadzeniu (+10,6 kg) w porównaniu z osobnikami o genotypie *AA*. U krów rasy angus heterozygoty charakteryzowały się największymi masami i przyrostami, jakkolwiek dla większości cech nie odnotowano statystycznie istotnych różnic. Nie stwierdzono również istotnej zależności między polimorfizmem *JAK2*/e20/*Rsa*I, a wiekiem pierwszego wycielenia.

[**D-2**]

W pracy **D-2** podjęto się określenia struktury genetycznej dwóch ras owiec. Analizie poddano 103 osobniki dwóch ras: pomorska (n=64) i suffolk (n=39), utrzymywanych w gospodarstwie ekologicznym w województwie zachodniopomorskim. Owce pomorskie utrzymywano w hodowli zachowawczej, przez co objęte zostały programem Ochrony Zasobów Genetycznych Zwierząt Gospodarskich.

W rezultacie, poprzez sekwencjonowanie w analizowanym genie potwierdzono występowanie trzech polimorfizmów.

Pierwszy (g.72850860G>A, rs160146162) i drugi (g.72850917A>G, rs160146160) wariant polimorficzny zlokalizowany był w tym samym eksonie 6 owczego genu *JAK2*. Trzeci SNP zlokalizowany był w intronie 24 (g.72895034G>A, rs429445187). W przypadku polimorfizmu pierwszego SNP, genotyp *AA* identyfikowany był najrzadziej u rasy pomorskiej (15%), natomiast u rasy suffolk nie wystąpił. Genotyp *GG* najczęściej identyfikowano u rasy suffolk (84,85%), natomiast u rasy pomorskiej odnotowano najwięcej osobników heterozygotycznych (45%). Rozkład alleli i genotypów polimorfizmu drugiego był podobny u obu ras. Zarówno u owiec rasy suffolk jak i pomorskiej przeważał genotyp *AG* (kolejno 54,5% i 46,7%). W trzecim miejscu polimorficznym odnotowano znaczącą przewagę heterozygot (pomorska 52,3% i suffolk 65,7%).

Według danych zaprezentowanych przez International Sheep Genome Consortium (https://www.ensembl.org/Ovis_aries/Variation/Population?db=core;r=2:7285036072851360;v=rs160146162;vdb=variation;vf=53623132#94_tablePanel), miejsce polimorficzne g.72850917A>G, zostało przebadane przez inne zespoły badawcze na 632 próbkach pochodzących od owiec różnych ras. U większości ras allel G przeważał, a frekwencja alleli kształtowała się następująco: G=0,8150 i A=0,185. W badaniach zaprezentowanych w niniejszej pracy również odnotowano znaczącą przewagę allelu G. Pozostałe miejsca polimorficzne g.72850860G>A, n=633; SNP g.72895034G>A, n=632) charakteryzowały się podobnie jak w pracy **D-2** przewagą frekwencji allelu A.

[D-3]

W trzeciej publikacji dokonano podsumowania i rozszerzenia badań dotyczących wpływu mutacji w genie *JAK2* na cechy użytkowe i parametry rozrodcze bydła i owiec. Opisano po raz pierwszy 3 SNP zlokalizowane w części kodującej genu *JAK2* (dwa dla bydła i 1 dla owiec), które nie były uwzględnione w pracach **D-1** i **D-2**. Wśród analizowanych polimorfizmów znajdowały się również dwa miejsca polimorficzne zlokalizowane w eksonie 6 owczego genu *JAK2*, opisane w publikacji **D-2**.

Łącznie pobrano 781 próbek krwi od trzech ras bydła, w tym hereford (n=276), angus (n=345) i limousin (n=160), natomiast od dwóch ras owiec pobrano łącznie 333 próbki krwi (138 owiec pomorskich i 195 owiec suffolk). Wszystkie zwierzęta utrzymywano na fermie zlokalizowanej w województwie zachodniopomorskim.

Analizą statystyczną objęto łącznie 5 miejsc polimorficznych. Dla bydła były to dwa miejsca polimorficzne zlokalizowane w eksonie 16 (rs210148032; p.Ile704Val, w obrębie pseudokinazy (JH2)) i eksonie 23 (cicha mutacja rs211067160, w obrębie domeny kinazy JH1), natomiast dla owiec, analizowano dwa miejsca polimorficzne w eksonie 6 (rs160146162 i rs160146160; kodujące domenę FERM) i jedno miejsce polimorficzne w eksonie 24 genu *JAK2* (rs160146116; domena kinazy JH1).

Badania asocjacyjne uzyskane dla bydła podobnie jak w pracy **D-1** były niejednoznaczne. Jakkolwiek, krowy rasy hereford i limousin o genotypie *AA* (e16/*Rsa*I) i *AA* (e23/*Hae*III) miały większą masę ciała i lepsze przyrosty dobowe ($P \leq 0,05$). Nie zaobserwowano wyraźnej tendencji w kształtowaniu się wybranych cech rozrodczych. W przypadku owiec, niezależnie od rasy, w badanych okresach największą masą ciała i przyrostami dobowymi charakteryzowały się osobniki o genotypie *AA* (e6/*Ear*I), *GG* (e6/seq) i *AA* (e24/*Hpy*188III) ($P \leq 0,01$). Te same osobniki rasy pomorskiej charakteryzowały się również lepszą płodnością i przeżywalnością jagniąt ($P \leq 0,01$).

Uzyskane w opublikowanych pracach (**D-1**, **D-2**, **D-3**) wyniki, stanowiące cykl publikacji spełniają założone w pracy cele badawcze, potwierdzając tym samym postawioną hipotezę badawczą mówiącą o tym, iż zmienność w genie *JAK2* może mieć związek z kształtowaniem cech użytkowości mięsnej oraz z wybranymi parametrami rozrodczymi bydła i owiec.

8. Stwierdzenia i wnioski

1. Nie wykryto nowych miejsc polimorficznych w częściach kodujących *JAK2*.
2. Potwierdzono i opisano występowanie 7 znanych miejsc polimorficznych w genie *JAK2* (3 SNP u bydła w części kodującej genu oraz 3 SNP u owiec również w części kodującej i dodatkowo 1 w intronie).
3. W badanych stadach bydła i owiec nie zidentyfikowano 11 znanych SNP w części kodującej genu *JAK2*, pierwotnie wybranych na poczet pracy doktorskiej (tabela 1 w sekcji „suplemmentary file" Animals publikacja [**D-3**]).
4. Stwierdzono wystąpienie związku pomiędzy zidentyfikowanymi wariantami genetycznymi, a cechami użytkowości mięsnej oraz niektórymi parametrami kształtującymi użytkowość rozrodczą bydła i owiec.
5. Niezależnie od rasy owiec, największymi masami ciała i przyrostami dobowymi charakteryzowały się osobniki o genotypach *AA* w układzie *JAK2*/e6/*Ear*I, *GG* w układzie *JAK2*/e6/seq i *AA* w układzie *JAK2*/e24/*Hpy*188III. Te same osobniki rasy pomorskiej cechowały się lepszą płodnością i przeżywalnością jagniąt.
6. W przypadku bydła uzyskano wyniki niejednoznaczne. W układzie polimorficznym *JAK2*/e20/*Rsa*I najkorzystniejsze parametry odnotowano dla bydła rasy limousine, gdzie genotyp *AA* związany był z większą masą urodzeniową, większymi średnimi przyrostami dobowymi oraz większą masą ciała w 210 dniu odchowu. Odmienne wyniki uzyskano dla rasy hereford, u której genotyp *GG* decydował o największej masie urodzeniowej, większych przyrostach dobowych oraz większej masie ciała przy odsadzeniu. U krów rasy angus heterozygoty charakteryzowały się najlepszymi cechami wzrostu i rozwoju. Krowy rasy hereford i limousin o genotypie *AA* w układzie *JAK2*/e16/*Rsa*I i o genotypie *AA* w układzie *JAK2*/e23/*Hae*III charakteryzowały się największą masą ciała i lepszymi przyrostami dziennymi, czego nie potwierdzono u rasy angus.
7. Niezależnie od analizowanego miejsca polimorficznego nie zaobserwowano wyraźnej tendencji w kształtowaniu się wybranych cech rozrodczych u bydła.
8. Wyniki prezentowanych badań mogą w przyszłości zostać wykorzystane przez hodowców w analizowanych gospodarstwach w prowadzeniu selekcji w stadach, jak również uwzględnione w programach hodowlanych dla bydła o mięsnym kierunku użytkowania i owiec mięsno-wełnistych. Istnieje zatem konieczność kontynuowania

badań z tego zakresu na większych liczebnie stadach bydła i owiec z uwzględnieniem innych ras.

9. Podsumowanie

Zastosowanie nowoczesnych technik molekularnych pozwala zidentyfikować warianty synonimiczne i niesynonimiczne, a także te, które wpływają na składanie pre-mRNA. Algorytmy bioinformatyczne mogą posłużyć jako narzędzie do oceny efektu zidentyfikowanych zmian. Należy jednak podkreślić, że wyniki takich testów są jedynie prognostyczne, a dokładny efekt konkretnej mutacji należy zweryfikować w badaniach funkcjonalnych, stąd podjęto się analiz asocjacyjnych prezentowanych polimorfizmów z cechami użytkowymi bydła i owiec. Bez wątpienia JAK2 pełni kluczową rolę w przekazywaniu sygnału wewnątrz komórki. Wszelkie zmiany w składaniu genu *JAK2* oraz przepisaniu informacji genetycznej mogą w pewnym stopniu decydować o efektywności takiego sygnału, jakkolwiek autorka zdaje sobie sprawę z poligenicznej kontroli kształtowania cech związanych z użytkowością mięsną i rozrodem bydła i owiec, stąd też należy ostrożnie podchodzić do formułowania końcowych wniosków.

Podsumowanie walidacji znanych miejsc polimorficznych w pracach [**D-1**] – [**D-3**] zawarte jest w tabeli 1 w sekcji „suplemmentary file" Animals publikacja [**D-3**].

Można założyć, że wyniki powyższych badań, o ile zostaną potwierdzone przez innych autorów na niezależnych populacjach, mogą w przyszłości zostać uwzględnione w programach hodowlanych bydła o mięsnym kierunku użytkowania i owiec o użytkowości mięsno wełnistej i przełożyć się na korzyści ekonomiczne gospodarstw.

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12. Wykaz załączników

12.1. Publikacja [**D-1**] Association between polymorphism in the *JAK2* gene (*JAK2*/e20/*Rsa*I) and selected performance parameters in beef cattle

12.2. Publikacja [**D-2**] Distribution of *JAK2* genotypes across Suffolk and Pomeranian sheep

12.3. Publikacja [**D-3**] Association between polymorphism in the Janus Kinase 2 (*JAK2*) gene and selected performance traits in cattle and sheep

12.4. Oświadczenia autorów

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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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Short communication

Distribution of *JAK2* genotypes across Suffolk and Pomeranian sheep

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ABSTRACT

In recent years, the improvement of sheep production traits has met with great interest from breeders. A significant effect of the *JAK2* gene encoding Janus kinase 2 on growth and reproductive parameters in different animal species has been confirmed. The aim of the present study was to identify new polymorphic sites in the ovine *JAK2* gene. Three single nucleotide polymorphisms (SNPs) have been identified using the Sanger sequencing method. The first and the second variant were located in exon 6 (R1 = g.72850860 G > A; rs160146162; R2 = g.72850917A > G; rs160146160). The third SNP was located in intron 22 proximal to exon 23 (R3 = g.72895034 G > A; rs429445187). A total of 98 individuals from two breeds (Pomeranian, n = 64 and Suffolk, n = 39) were genotyped. They were kept on an organic farm located in the West Pomeranian Province. In the case of the g.72850860 G > A polymorphism, the A allele and the AA genotype were the least frequent in the Pomeranian breed. Fifteen percent of animals had the AA genotype, whereas this genotype was not detected in the Suffolk breed. The GG genotype was the most frequent in the Suffolk breed (84.85 %), while heterozygotes were predominant in the Pomeranian breed (45 %). Allele and genotype frequencies of the g.72850917A > G polymorphism were similar in both breeds, in which the AG genotype was predominant (54.5 % and 46.7 % for Suffolk and Pomeranian, respectively). Also, allele and genotype frequencies of an intronic polymorphism were similar in both breeds, in which heterozygotes were the most common (52.3 % and 65.7 % for Pomeranian and Suffolk, respectively). The results of the present study could be used for the further association analysis of selected performance traits in connection with the described polymorphisms.

1. Introduction

JAK2 kinase belongs primarily to the signaling pathways of STAT (Signal Transducer and Activator of Transcription) proteins, through which it is involved in the signal transmission of extracellular growth factors, cytokines and hormones (Baxter et al., 2005). The primary structure of each JAK kinase consists of seven homologous domains designated JH1-JH7, from the carboxy to the amino terminus (He et al., 2005). The C-terminal kinase domain (JH1) is responsible for proper enzymatic activity, while the JH2 domain (pseudokinase) acts as an inhibitor and regulator of the aforementioned JH1 domain. The SH2 homologous region (JH3 ÷ JH4 domains) is located in the central part of the JAK2 proteins, binding directly to proteins that interact with JAK. The N-9 terminal region of JAK consists of the FERM domain (JH5 ÷ JH7), which is conserved among many cellular proteins and involved in the location of JAK in relation to the cell membrane and the cytoskeleton, including binding to target receptors, e.g. GHR (Babon et al., 2014). The constitutive activity of JAK2 may occur in various tumoral processes (Lacronique et al., 1997). Alternatively, a JAK2 inhibitor may bind to additional regulatory proteins. High JAK2 expression was found in many mammalian organs and tissues (Yamaoka et al., 2004). A typical JAK kinase is a relatively large protein (over 1100 amino acids) with a molecular weight of 120−140 kDa. Depending on the species, genes encoding individual kinases are located on different chromosomes (Rane et al., 2000). The ovine Janus Kinase gene is located on chromosome 2, consists of 24 exons and is 6897 bp-long (https://www.ensembl.org/Ovis_aries/Gene). Although numerous polymorphic sites have already been deposited in various databases, there are currently no studies on the polymorphisms within the *JAK2* gene in Pomeranian and Suffolk sheep.

Cattle research indicates a relationship between *JAK2* polymorphism and dairy performance of various breeds (Szewczuk, 2015) as well as susceptibility to mastitis (Usman et al., 2015). In recent years, the improvement of production traits in sheep has met with great interest from breeders. The use of marker-assisted selection may result in a significant increase in selection response and improvement of a specific

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group of traits (productive and reproductive ones) as well as a better quality and health-promoting value of meat. The aim of the present study was to identify new polymorphic sites within the ovine *JAK2* gene and to determine their frequency in two sheep breeds, i.e. Suffolk and Pomeranian, representing different production types. The results of the present study could be used for the further association analysis of selected performance traits in connection with the described polymorphisms.

2. Material and methods

A total of 98 individuals from two breeds (Pomeranian, n = 64 and Suffolk, n = 39), kept on an organic farm located in the West Pomeranian Province, were subjected to preliminary analysis. Pomeranian sheep is one of endangered livestock breeds, which is included in the *Program for the Protection of Livestock Genetic Resources* in Poland. In spring and summer, the animals remained on pasture, while in winter, they were kept in paddocks. Biological material (peripheral blood) from all tested animals was collected from the external jugular vein for the purpose of the previous studies. Sample collection has been approved by the Local Commission for Animal Experiments in Poznań (Resolution No. 20/2018). The indigenous Pomeranian sheep, which is a variation of the Polish long-haired sheep, is a basic breed in the sheep population of north-western Poland, mainly found in the Pomeranian Province, and the second most numerous sheep breed included in the program for the protection of genetic resources. On the other hand, the second breed is a leading one in the British Isles, with a history of almost 200 years. It evolved from the mating of Norfolk horn ewes with Southdown rams (Paraponiak, 2015).

2.1. DNA isolation

Genomic DNA from whole blood was isolated using the MasterPure™DNA Purification kit for Blood Version II, according to the manufacture's protocol and verified by the spectrophotometric method using NanoDrop 2000 (Thermo Scientific, Life Technology, USA).

2.2. SNP

Three polymorphic sites were selected for analysis (Table 1)

2.3. Sequencing

All of the three polymorphisms were genotyped using the Sanger sequencing method at the National Research Institute of Animal Production in Balice (Cracow, Poland) in accordance with the accredited test protocols (Accreditation Certificate of Testing Laboratory no. AB 1587). The PCR products were obtained using AmpliTaq Gold® 360 Master Mix (Applied Biosystems, Thermo Fisher Scientific, USA) according to the protocol at the annealing temperature of 57 °C. Next, the PCR products were purified with the EPPiC Fast (A&A Biotechnology, Poland) kit and sequencing PCR was performed using the BigDye™ Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems™, Thermo-Fisher Scientific). After purification with the BigDye XTerminator™ Purification Kit (Applied Biosystems™, Thermo-Fisher), the amplicons were sequenced using the 3500 Genetic Analyzer (Applied Biosystems™) according to the manufacturer's protocol.

Table 1
Detailed information about the analyzed polymorphisms.

Gene region	Chromosomal location	Accession number (rs#)	Primers
exon 6 (R1)	2:72850860	g.72850860G>A	F: TTGACCTTGTTAA
		rs160146162	ATGTATATGTTCTG
exon 6 (R2)	2:72850917	g.72850917A>G	R: TTGCATAAGAAAAT
		rs160146160	TACCTGATAGAGC
intron 22 (R3)	2:72895034	g.72895034G>A	F: TCTGCTTGAAAT
		rs429445187	TAAATGTACCAAA R: TCAGTGAACTGCA TAAACTGACC

2.4. Data analysis

The results were analyzed using the Chromas sequence analysis program (https://chromas-lite.software.informer.com/2.1/). The obtained sequences were compared with the reference sequence using the BLAST (Basic Local Alignment Search Tool) program (https://blast.ncbi.nlm.nih.gov).

Hardy-Weinberg equilibrium was verified and allelic frequencies were calculated using the Court Laboratory HW calculator (Court's 2005–2008 online calculator).

3. Results

Three single nucleotide polymorphisms (SNPs) were identified in all individuals (Table 1). The first and the second variant were located in exon 6 [R1 = g.72850860 G > A; rs160146162; R2 = g.72850917A > G; rs160146160 (Fig.1)]. Both mutations in exon 6 were silent (synonymous variants). The third SNP was located in intron 22 proximal to exon 23 [R3 = g.72895034 G > A; rs429445187 (Fig. 1)]. Due to their location, the discovered polymorphisms may affect JAK2 transcription (https://www.ensembl.org/Ovis_aries/Gene).

3.1. Allele and genotype frequency of the g.72850860 G > A (R1) SNP

The g.72850860 G > A polymorphic site was analyzed in 60 Pomeranian and 33 Suffolk individuals (Table 2). Genotype and allele frequencies were significantly different between breeds (p-value <0.0001). The AA genotype frequency in the Pomeranian breed was 15 %, while no individual of this genotype was identified in the Suffolk breed. The GG genotype occurred most frequently in the Suffolk breed (84.85 %), while heterozygotes predominated in the Pomeranian breed (45 %). In both breeds, the A allele proved to be dominant and its frequency ranged between 92 % and 62 %. Both populations were in Hardy-Weinberg equilibrium.

3.2. Allele and genotype frequency of the g.72850917A > G (R2) SNP

The g.7285091A > G polymorphic site was analyzed in 60 Pomeranian and 33 Suffolk individuals (Table 2). No significant differences in genotype or allele frequencies were observed between the tested breeds. The AG genotype was predominant in both breeds (54.5 % and 46.7 % for Suffolk and Pomeranian, respectively). The GG genotype was the least frequent (12.2 % and 13.3 % for Suffolk and Pomeranian, respectively). The G allele had the highest frequency in both breeds, which were in Hardy-Weinberg equilibrium.

3.3. Allele and genotype frequency of the g.72895034 G > A (R3) SNP

The g.72895034 G > A polymorphism was analyzed in 63 Pomeranian and 35 Suffolk individuals (Table 2). Allele and genotype frequencies were similar in both breeds, in which heterozygotes were the most frequent (52.3 % and 65.7 % for Pomeranian and Suffolk, respectively). In both breeds, the GG genotype was the least frequent, while the A allele was predominant. The population of Pomeranian sheep was in Hardy-Weinberg equilibrium, whereas that of Suffolk sheep was not (p = 0.0361).

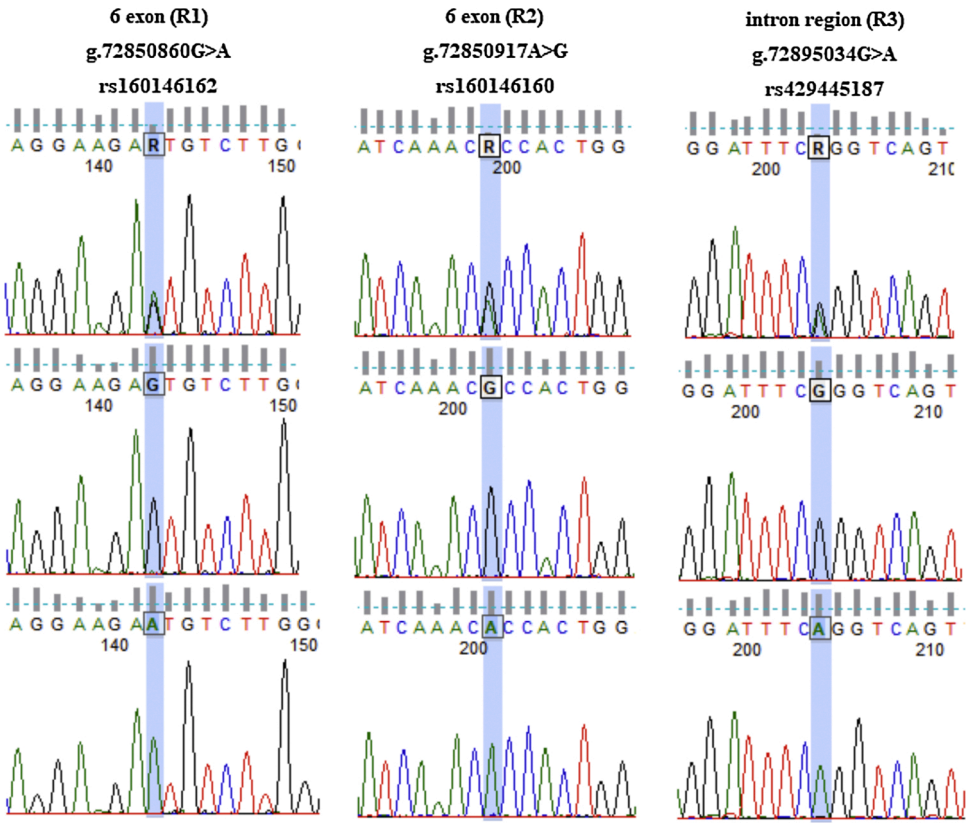

Fig. 1. Electrophoretic analysis of SNP variants.

Table 2
Genotype and allele frequencies within the breeds under study.

Genotypes	Pomeranian sheep			Suffolk		
	AA	AG	GG	AA	AG	GG
exon 6 (R1) g.72850860G>A rs160146162						
N	9	27	24	–	5	28
Genotype percentage	15	45	40	–	15.15	84.85
HWE significance	p-value	0.7566		p-value	0.6377	
Allele frequency	A=0.3750			A=0.0757		
	G=0.6250			G=0.9242		
	AA	AG	GG	AA	AG	GG
exon 6 (R2) g.72850917A>G rs160146160						
N	24	28	8	11	18	4
Genotype percentage	40	46.7	13.3	33.3	54.5	12.2
HWE significance	p-value	0.9704		p-value	0.4136	
Allele frequency	A=0.6333			A=0.6060		
	G=0.3666			G=0.3939		
	AA	AG	GG	AA	AG	GG
intron region (R3) g.72895034G>A rs429445187						
N	20	33	10	9	23	3
Genotype percentage	31.8	52.3	15.9	25.7	65.7	8.6
HWE significance	p-value			p-value		
Allele frequency	A=0.5793	0.5532		A=0.5857	0.0361	
	G=0.4206			G=0.4142		

N – sample size.

4. Discussion

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

Many studies have indicated that numerous genes encoding elements of the somatotropic axis are associated with growth, development and reproductive traits, which suggests their possible relationship with quantitative traits in sheep (Parmentier et al., 1999). The analyzed exon 6 is a part of the FERM domain (JH5-JH7), which is conserved across many proteins. It has been proven that this region is involved in the

location of JAK in relation to the cell membrane and the cytoskeleton, including binding to target receptors, e.g. GHR (Babon et al., 2014). The polymorphism identified in intron 22 is located in the JH1 domain of JAK2. This domain is situated at the C-terminus of the kinase and is responsible for its proper enzymatic activity (Haan et al., 2010; Rane et al., 2000).

Most studies on JAK2 expression have involved human subjects. One report described the essential role of the V617 F point mutation within Janus kinase 2 in thrombocythemia. This mutation occurs in 60–70 % of patients suffering from this disease and is located in the domain acting as an inhibitor (Dziedziczenia et al., 2007). Another work on *JAK2* polymorphism examined the effect of many mutations of this gene on myeloproliferative syndromes. A panel of 13 mutations was described, nine of which were located in the pseudokinase domain and the remaining four in the linker (SH2) (Zhao et al., 2009). Topkaya et al. (2014) reported that the mutation can be detected by restriction enzyme digestion. He et al. (2005), in their study on mice, confirmed the effect of Janus kinase 2 on the growth hormone receptor stability.

The analyzed polymorphisms have previously been identified and annotated in the NCBI and Ensembl databases and are included in the IlluminaOvineHDSNP genotyping chip. According to the International Sheep Genome Consortium, the g.72850860 G > A (R1) polymorphic site has so far been tested on 632 samples from different sheep breeds (however, excluding Suffolk or Pomeranian ones). In most breeds, the G allele predominated and the allele frequencies were 0.815 and 0.185 (for the G and A allele, respectively). A significant predominance of the G allele was also noted in the cited study. The g.72850917A > G (R2) polymorphic site was genotyped in 633 individuals, while the g.72895034 G > A SNP (R3, intron variant) in 632 ones (again, excluding Suffolk or Pomeranian breeds). For both SNPs, a higher frequency of allele A (R2: A = 0.776 and G = 0.224; R3: A = 0.544 and G = 0.456) was observed. These results are consistent with the aforementioned reports, since the predominance of the A allele has also been found (https://www.ensembl.org/Ovis_aries/Variation/Population).

Pomeranian sheep are characterized by exceptional adaptability to harsh/demanding environmental conditions, high resistance and low requirements in terms of housing and feeding. They also show a great ability to use natural feed resources. This breed is recommended for keeping in small flocks, well adapted to local, environmental conditions of lowland areas, which is of key importance to organic production. Their advantage is gentleness and outstanding maternal ability (Brzostowski et al., 2005; Paraponiak, 2015; Milewski, 2018). Due to a drastic decrease in population size (3% of the state recorded in 1985), the Institute of Animal Production - the National Research Institute in Krakow included Pomeranian sheep in the Genetic Resources Conservation Program in 2005. The main goal of the program is to increase the size of a typical purebred population of ewes of the foundation stock, stabilize and preserve breed standard and genetic variability. In 2019, the program involved 103 flocks comprising a total of 8444 individuals (http://owce.bioroznorodnosc.izoo.krakow.pl) (Anon, 2020).

Suffolk sheep are well-adapted to organic, semi-intensive livestock production systems in terms of body weight gains and meat performance. An organic system does not facilitate a full exploitation of the fattening potential of these sheep, due to, e.g., limited concentrate feeding. Nevertheless, lower productivity translates into a higher quality of raw material (especially for health-related traits), while reducing production costs by pasture feeding, which can be an interesting alternative for producers (Paraponiak, 2015) (Breed Information: History. Suffolk Sheep Society. Accessed May 2019).

The analyzed SNPs are non-synonymous variants and include one intronic polymorphism, but according to other authors, they can also significantly affect the function of the studied gene and indirectly influence individual's phenotype. Currently, it is known that introns are functionally active elements of gene and genome function (Jo et al., 2015). They encode intronic regulatory elements involved in assembly, transcription and recombination events, including gene mobility (Chorev et al., 2012). This means that not only the preserved non-coding sequences function as regulatory elements, but also variable regions can be adaptively active (Sjakste et al., 2011). Mullen et al. (2011) showed that the SNPs found in the intronic region of the bovine *IGF1* gene may have a significant impact on animal production parameters (milk production and growth traits). Sternberg et al. (1988) found that the first intron of the mouse muscle creatine kinase gene may contain a positive regulatory element. Splicing mutations usually cause errors during the linking process and can lead to inaccurate intron removal and thus changes in an open reading frame (Abramowicz et al. (2018)). Recent studies have highlighted the abundance and importance of splicing mutations in the etiology of hereditary diseases (Vaz-Drago et al., 2017). The use of modern techniques has identified synonymous and non-synonymous variants as well as intronic mutations that affected pre-mRNA splicing (Chabot et al., 2016). Bioinformatics algorithms can be used as a tool to assess the effect of identified changes (Desmet et al., 2009). However, it should be emphasized that the results of such tests are only prognostic and the exact effect of a particular mutation should be verified in functional studies (Fredericks et al., 2015). Therefore, the SNPs examined in this paper may also be causal and affect selected production traits.

5. Conclusion

present study reports preliminary results on the variability within the coding and non-coding sequences of the *JAK2* gene encoding kinase involved in multiple signaling pathways. The study confirmed the presence of three selected polymorphic sites in the ovine *JAK2* gene both in the standard Suffolk meat breed and the indigenous Pomeranian breed. Due to the favorable distribution of genotypes and their inclusion in commercial microchips, selected polymorphic sites can be used in future association studies on traits related to growth, development and reproduction in sheep.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Article

Association between Polymorphism in the Janus Kinase 2 (*JAK2*) Gene and Selected Performance Traits in Cattle and Sheep

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Simple Summary: An important element of livestock breeding is the selection that leads to the consolidation and improvement of the traits with utility value. The use of single-nucleotide polymorphism (SNP) markers offers the opportunity to select the appropriate genotype linked to the phenotype, which manifests itself in significant breeding benefits. Polymorphic sites within the genes coding the somatotropic axis are directly and indirectly associated with the phenotype, particularly concerning the production properties of growth and carcass as well as reproductive function. This study analyzed the effect of selected polymorphisms in the Janus Kinase 2 (*JAK2*) gene on performance characteristics of cattle and sheep. The impact of the mutations on the development of selected traits, which are important from the economic standpoint, was evaluated. Such studies can be utilized to enhance the growth characteristics of cattle and sheep, thereby facilitating the development of more profitable and sustainable breeding methods.

Abstract: The Janus Kinase 2 (JAK2) tyrosine kinase is an essential component of signal transduction of the class II cytokine receptors, including the growth hormone receptor. Therefore, it may play a crucial role in the signaling pathway of the somatotropic axis, which influences growth, development, and reproductive traits in ruminants. For this purpose, for three breeds of cattle (Hereford, Angus, and Limousin; a total of 781 individuals), two polymorphic sites located in exon 16 (rs210148032; p.Ile704Val, within pseudokinase (JH2)) and exon 23 (silent mutation rs211067160, within JH1 kinase domain) were analyzed. For two breeds of sheep (Pomeranian and Suffolk; 333 individuals in total), two polymorphic sites in exon 6 (rs160146162 and rs160146160; encoding the FERM domain) and one polymorphic site in exon 24 of the *JAK2* gene (rs160146116; JH1 kinase domain) were genotyped. In our study, the associations examined for cattle were inconclusive. However, Hereford and Limousin cattle with genotypes *AA* (e16/*Rsa*I) and *AA* (e23/*Hae*III) tended to have the highest body weight and better daily gains ($p \leq 0.05$). No clear tendency was observed in the selected reproductive traits. In the case of sheep, regardless of breed, individuals with the *AA* (e6/*Ear*I), *GG* (e6/seq), and *AA* (e24/*Hpy*188III) genotypes had the highest body weights and daily gains in the study periods ($p \leq 0.01$). The same individuals in the Pomeranian breed also had better fertility and lamb survival ($p \leq 0.01$). To the best of our knowledge, these are the first association studies for all these polymorphic sites. Single-nucleotide polymorphisms in the *JAK2* gene can serve as genetic markers for growth and selected reproductive traits in ruminants given that they are further investigated in subsequent populations and analyzed using haplotype and/or combined genotype systems.

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1. Introduction

Genomic selection using single-nucleotide polymorphisms (SNP) is revolutionizing livestock breeding. The primary advantage of genomic selection, in comparison to traditional pedigree-based methods, is the ability to obtain individuals with the desired phenotype and performance traits in the initial generation of selection. There are many studies confirming the effectiveness of the above-mentioned selection. Furthermore, a strong correlation between phenotypic data and predicted genomic values has been consistently demonstrated [1,2].

The biological foundation underlying the development of livestock performance characteristics is rooted in the elements comprising the somatotropic axis [3]. The somatotropic axis in the postnatal life stimulates cell hyperplasia and hypertrophy, thus contributing to the further growth of organs and tissues [4]. Proper growth requires the growth hormone (GH) secreted by the pituitary gland to bind to its specific receptor and activate a complex signaling cascade. The GH receptor (GHR) is initially produced as a dimer and then transported unliganded to the cell surface. Binding of GH to the GHR dimer results in a conformational change of the dimer, activation of intracellular Janus Kinase 2 (JAK2), and phosphorylation of the signal transducer and activator of transcription 5B (STAT5B). The phosphorylated STAT5B dimers are then translocated into the nucleus, where they transcriptionally activate various genes, including insulin-like growth factor 1 (*IGF1*), *IGF* binding protein 3, and the acid-labile subunit (*ALS*) [5]. The presence of two JAK2 molecules results in the transphosphorylation and activation of one JAK2 by the other, which in turn leads to the phosphorylation of downstream GHR tyrosine residues. Phosphorylated and dimerized GHR triggers a multiple-signal cascade translocated to the nucleus, where STATs or other non-receptor tyrosine kinases bind to a specific site in the DNA sequence, activating target genes [6].

The primary structure of JAK2 kinase consists of seven homologous domains (Figure 1), designated JH1–JH7, from the carboxyl terminus to the amino terminus [7]. The C-terminal kinase (JH1) domain is responsible for the correct enzymatic activity, while the JH2 domain (pseudokinase) acts as an inhibitor and regulator of the aforementioned JH1 domain. The SH2 homologous region (JH3–JH4 domains) is located in the center of JAK2 proteins, binding directly to JAK-interacting proteins. The N-terminal part of the JAK is built by the FERM domain (JH5–JH7; F for 4.1 protein, E for ezrin, R for radixin, and M for moesin). The FERM domain is highly conserved among many cellular proteins and is involved in the localization of JAKs in relation to the cell membrane and cytoskeleton–including binding to target receptors, e.g., GHR [8]. Constitutive JAK2 activity can occur in various neoplastic processes [9]. Alternatively, JAK2 inhibition may be associated with additional regulatory proteins. A typical JAK kinase is a relatively large protein (more than 1100 amino acids) with a molecular weight of 120–140 kDa. Depending on the species, the genes encoding the individual kinases are located on different chromosomes [10].

The cytokine-activated kinase (JAK) and signal transducer and activator of transcription (STAT) signaling pathway serves as a crucial link between proteins within the cell, directly influencing processes such as cell proliferation, apoptosis, mammary gland development, lactation, and immune responses. This pathway plays a vital role in transmitting information from cell surface receptors to the cell nucleus, ultimately regulating gene expression through transcription [11].

There are many studies confirming the influence of Janus Kinase 2 on the performance characteristics of dairy cows. Among other things, it has been shown that the JAK-STAT pathway regulates lactation and that PI3K/Akt within the JAK-STAT pathway is overexpressed in lactating cows [12]. Analysis of gene deletions in mice has documented the important role of JAK-STAT signaling in lactation and mammary gland development [13].

Moreover, an important role of the JAK-STAT pathway in blood cell differentiation and casein gene regulation during milk production has been documented [14]. Prolactin also uses JAK-STAT signaling and regulates lactation and reproduction in mammals [15]. In the research conducted by Szewczuk [16], a clear relationship was found between *JAK2/e20/Rsa*I polymorphism and milk-yield traits. The SNP was associated with higher milk, protein, and fat yield. With regard to growth and reproductive traits, there is a lack of literature describing the influence of the *JAK2* gene on, among other things, body weight and daily gains at different periods of the animal's life as well as fertility, prolificacy, or calving interval. The only studies in this area are the results presented by Padzik and Szewczuk [17], which also involved the bovine *JAK2*/e20/*Rsa*I polymorphic site. In contrast, with regard to the ovine JAK2 gene, a preliminary study by Padzik et al. [18] only focused on allele and genotype frequencies and did not consider animal performance traits.

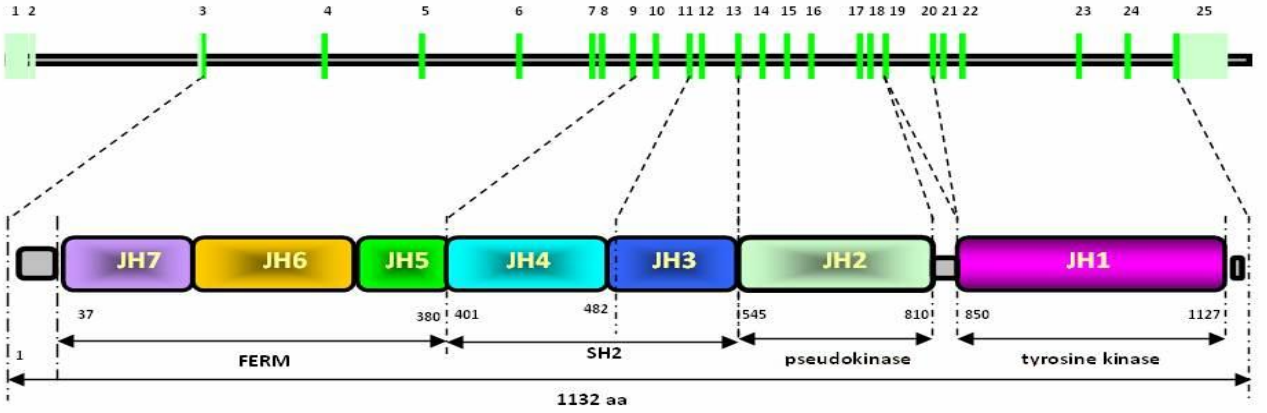

Figure 1. The primary structure of JAK2 kinase (own elaboration).

The aim of the present study was to analyze the possible relationship between polymorphisms in the Janus Kinase 2 (*JAK2*) gene and selected growth and reproductive traits in cattle and sheep. Attention was primarily focused on gene fragments encoding crucial domains that determine JAK2 kinase activity.

2. Material and Methods

2.1. Animals

A total of 781 blood samples were collected from three breeds of cattle, including Hereford (n = 276), Angus (n = 345), and Limousin (n = 160). In regard to sheep, a total of 333 blood samples of two breeds were collected (138 Pomeranian and 195 Suffolk sheep). All animals were kept on a single farm located in West Pomeranian Province, Poland. All animals were kept in a pasture and indoor system. Feeding was carried out in accordance with the standards accepted for these species [19], based on grass and other roughage and concentrates depending on the season. The animals had constant access to water and salt licks.

2.2. DNA Isolation

DNA was extracted using a MasterPure DNA Purification Kit Version II (Epicentre Technologies, Madison, WI, USA) from 3 mL of blood collected into tubes containing EDTA (IMPROVACUTER® K3 EDTA, Guangzhou, China). Briefly, this kit uses a rapid desalting process (red and white cell lysis solutions followed by protein precipitation solution) to remove contaminating macromolecules and avoid toxic organic solvents. Rinsing twice with 70% ethanol and resuspending the DNA in TE buffer (10 mM Tris-HCl (pH 7.5), 1 mM EDTA) allows samples to be stored for many months at <−20 °C.

2.3. Sequencing

Three fragments of the bovine *JAK2* gene (covering whole exons 3, 16, and 23) and four fragments of the ovine *JAK2* gene (exons 6, 12, 13, and 24) were selected for sequencing (Table S1 included in the Supplementary Materials). Each of the selected exons potentially contained a minimum of two known polymorphic sites (according to Ensembl–the ARS-UCD1.2 assembly (cow) and the Oar_rambouillet_v1.0 assembly (sheep)). For each exon, 10 pooled samples consisting of 10 individuals were sequenced (Genomed, Warsaw, Poland). Fluorograms were analyzed using Chromas ver. 2.6.6. (Technelysium Pty Ltd., Sydney, Australia). Only the polymorphic sites that had three or more heterozygote-specific results after sequencing of the pooled samples were selected for further genotyping. An additional consideration was the type of mutation detected (order of importance: missense, synonymous, intronic), and all of the detected SNPs had to be present within all breeds under analysis.

2.4. Bovine JAK2 Gene Polymorphism

Because the most used commercial enzymes were not available for the native sequence, genotyping of both polymorphisms detected within the three breeds under study was carried out using a polymerase chain reaction–artificially created restriction site (PCR-ACRS) method (Table 1). Primers were designed using Primer3web ver. 4.1.0 online software (https://primer3.ut.ee/) (accessed on 22 July 2021), and correct enzyme digestions after the introduction of a single-nucleotide mismatch were confirmed online by WebCutter v. 2.0 (http://heimanlab.com/cut2.html) (accessed on 22 July 2021) and NEBcutter V2.0 (http://nc2.neb.com/NEBcutter2/) (accessed on 22 July 2021).

Table 1. Characterization of selected single-nucleotide polymorphisms within bovine *JAK2* gene.

Primer Sequence (5′ → 3′)	Polymorphism Position ** and Type	Codon and Amino Acid(s) ***	PCR-ACRS (Restriction Enzyme, Cleavage Site, and Genotypes)
JAK2e16mF gggccctggacatactaagtg	rs210148032 g.39400906A>G	A<u>T</u>T → G<u>T</u>T	*Rsa*I (gtˆac) PCR amplicon 211 bp *AA* 190 bp + 21 bp
JAK2e16mR gtcttttggcaaaactgta<u>G</u> *	Missense	p.Ile704Val	*AG* 211 bp + 190 bp + 21 bp *GG* 211 bp (no cut)
JAK2e23mF catatattgacaagagtaaaagcc<u>G</u> *	rs211067160 g.39383281A>G	CC<u>A</u> → CC<u>G</u>	*Hae*III (ggˆcc) PCR amplicon 211 bp *AA* 211 bp (no cut)
JAK2e23mR tccccacctttcaaaacttc	Synonymous	p.Pro1057	*AG* 211 bp + 185 bp + 26 bp *GG* 185 bp + 26 bp

* Mismatch is underlined (ACRS-PCR); ** position according to: RefSeq NC_037335.1 Chromosome 8 ARS-UCD1.2 Primary Assembly; *** amino acids abbreviations: Ile, isoleucine; Val, valine; Pro, proline.

2.5. Ovine JAK2 Gene Polymorphism

After analysis of the sequencing data, two polymorphic sites in the ovine *JAK2* gene were selected and genotyped using a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. Unfortunately, it was not possible to select an optimal methodology for the third SNP (rs160146160). Therefore, all samples were individually sequenced (Genomed, Warsaw, Poland) (Table 2).

Table 2. Characterization of selected single-nucleotide polymorphisms within ovine *JAK2* gene.

Primer Sequence (5′ → 3′)	Polymorphism Position * and Type	Codon and Amino Acid **	PCR-RFLP (Restriction Enzyme, Cleavage Site, and Genotypes)
oJAK2e6F ttgaccttgttaaatgtatatgttctg	rs160146162 g.73397955A>G Synonymous	GA<u>A</u> → GA<u>G</u> p.Glu177	*Ear*I (CTCTTC(1/4)^) PCR amplicon 280 bp *AA* 280 bp (no cut) *AG* 280 bp + 170 bp + 110 bp *GG* 170 bp + 110 bp
oJAK2e6R ttgcataagaaaattacctgatagagc	rs160146160 g.73398012G>A Synonymous	AC<u>G</u> → AC<u>A</u> p.Thr196	PCR amplicon 280 bp not applicable (sequencing)
oJAK2e24F tctgcttgaaattaaatgtaccaaa oJAK2e24R tcagtgaactgcataaactgacc	rs160146116 g.73442093A>G Synonymous	CT<u>A</u> → CT<u>G</u> p.Leu1082	*Hpy*188III (TC^NNGA) PCR amplicon 261 bp *AA* 205 bp + 56 bp *AG* 205 bp + 137 bp + 68 bp + 56 bp *GG* 137 bp + 68 bp + 56 bp

* Position according to: RefSeq NC_056055.1 Chromosome 2 ARS-UI_Ramb_v2.0 Primary Assembly; ** amino acids abbreviations: Glu, glutamic acid; Thr, threonine; Leu, leucine.

2.6. PCR Conditions

The PCR reaction was carried out with a total volume of 25 µL of the solution mixture consisting of 12.5 µL 2X PCR Master Mix (Thermo Scientific, ABO, Gdansk, Poland), 0.1 µL of primers (each at concentration of 10 pmol/mL), 2 µL of genomic DNA, and nuclease-free water up to 25 µL. Amplification was performed using Biometra TGradient thermal Cycler (Analytik, Jena, Germany). The first run was at 95 °C for 5 min, followed by subsequent 33 cycles of 95 °C × 30 s, 60 °C × 45 s (all primers), and 72 °C × 45 s and the last run at 72 °C for 5 min (final elongation). The yield and specificity of PCR products were evaluated by electrophoresis in 2% agarose gels (BASICA LE GQT, Prona, ABO, Gdansk, Poland) with ethidium bromide, viewed under UV light and scored in a gel documentation system.

2.7. Digestion

In regard to the first polymorphism within the bovine *JAK2* gene (rs210148032), amplicons (211 bp) were digested with 5U of the *Rsa*I restriction enzyme (37 °C/3 h; MBI Fermentas, ABO, Gdansk, Poland), which recognized one GT↓AC sequence within the PCR product. The second bovine polymorphic site (rs211067160) was also characterized by an amplicon of 211 bp. (However, it relates to a different region of the gene; Table 1) In this case, the *Hae*III restriction enzyme recognized one polymorphic site containing this SNP (5 U at 37 °C/3 h; MBI Fermentas, ABO, Gdansk, Poland). A polymorphism within the 280 bp fragment of exon 6 of the ovine *JAK2* gene was detected using the *Ear*I restrictase (also 5 U, 37 °C/3 h; MBI Fermentas, ABO, Gdansk, Poland). The ovine variant within exon 24 was differentiated using the *Hpy*188III restriction endonuclease (5 U at 37 °C/3 h; New England BioLabs Inc., Ipswich, MA, USA). Electrophoretic separation and visualization were identical to that described above.

2.8. Statistical Analysis

Analyses of the association between genotype and selected growth and reproductive traits were carried out on the basis of data obtained from the breeding records of individual herds maintained by the Polish Union of Meat Cattle Breeders and Producers and the Polish Union of Sheep Farmers. For cattle, the following traits were analyzed: birth weight (BWT), weaning weight adjusted to 210 days of age (WWT_{210}), average daily gain from birth to weaning (ADG), age and body weight at first calving, and calving interval. Traits studied in sheep included body weight at 2, 30, and 56 days of age; average daily gain from 2 to 56

and 30 to 56 days of age; and body weight at mating. Additionally, fertility, prolificacy, and lamb survival were analyzed.

Statistical analysis was performed using the STATISTICA software (13.3 PL software package, Statsoft Inc., Kraków, Poland, 2020). The differences between particular genotypes were evaluated with Duncan's test. Statistical calculations were performed using a general linear model (GLM).

The following statistical models were used:

- (Growth traits—cattle: BWT, ADG, and WWT_{210}; sheep: BW2D, BW30D, BW56D, BWM, $ADG_{2–56}$, and $ADG_{30–56}$)

(1) $Y_{ijklm} = \mu + G_i + s_j + BYS_k + [LS_l] + e_{ijklm}$ where Y_{ijklm} is the analyzed trait; μ the overall mean; G_i the fixed effect of *JAK2* genotype (i = 1, . . .3); s_j the random effect of sire (Hereford j = 1, . . .31; Angus j = 1, . . .39; Limousin j = 1, . . .24; Pomeranian j = 1, . . .3; Suffolk j = 1, . . .5); BYS_k the fixed effect of birth year/season (Hereford k = 1, . . .20; Angus k = 1, . . .20; Limousin k = 1, . . .20; Pomeranian k = 1, . . .16; Suffolk k = 1, . . .16); $[LS_l]$ the fixed effect of litter size of mother (only in sheep: 1 single, 2 twins), and e_{ijklm} the random error.

- (Cattle: age and body weight at first calving as well as calving interval)

(2) $Y_{ijkl} = \mu + G_i + s_j + CYS_k + e_{ijkl}$ where Y_{ijkl} is the analyzed trait; μ the overall mean; G_i the fixed effect of *JAK2* genotype (i = 1, . . .3); s_j the random effect of sire (Hereford j = 1, . . .30; Angus j = 1, . . .39; Limousin j = 1, . . .21); CYS_k the fixed effect of year/season of 1st calving (Hereford k = 1, . . .22; Angus k = 1, . . .27; Limousin k = 1, . . .21); and e_{ijkl} the random error.

- (Only sheep: fertility, prolificacy, and lamb survival)

(3) $Y_{ijkl} = \mu + G_i + LS_j + LY_k + e_{ijkl}$ where Y_{ijkl} is the analyzed trait; μ the overall mean; G_i the fixed effect of *JAK2* genotype (i = 1, . . .3); LS_j the fixed effect of litter size (1 single, 2 twins); LY_k the fixed effect of lambing year (Pomeranian k = 1, . . .6; Suffolk k = 1, . . .8); and e_{ijkl} the random error.

The chi-square test was used to verify whether each population was in Hardy–Weinberg equilibrium.

3. Results

3.1. Sequencing

After a total of 100 individuals from three breeds of cattle were sequenced, two polymorphic sites were identified in the coding region of the bovine *JAK2* gene (Figure 2). In the case of the sheep *JAK2* gene, three polymorphic sites were previously identified and described by Padzik et al. [17].

3.2. Bovine JAK2 Frequencies

A total of 769 individuals from three breeds of cattle were genotyped for the *JAK2*/e16/*Rsa*I polymorphism (Table 3; Figures S1 and S2 included in the Supplementary Materials). Regardless of breed, the largest number of individuals was identified with the *AA* genotype (p.Ile704 variant; 58.3–59.4%), followed by heterozygotes (33–34.4%). In contrast, individuals with the *GG* genotype (p.Val704 variant; 7.25–8.41%) were rarely identified. For this reason, the A allele was most commonly observed (75%, regardless of breed). The genotype frequencies of Hereford and Limousin cows were consistent with HWE (*p*-value 0.25 and 0.24, respectively), while the distribution of genotypes in the herd of Angus cows was inconsistent with HWE (*p*-value = 0.03).

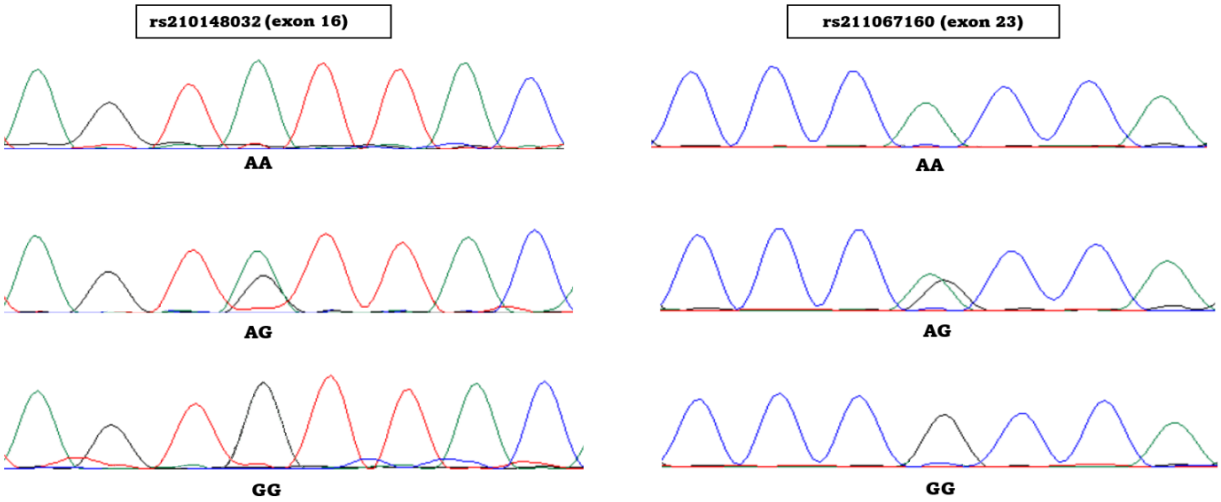

Figure 2. Chromatograms for two polymorphic sites identified within the bovine *JAK2* gene. green—adenine, black—guanine, red—thymine, blue—cystosine.

Table 3. The number and frequency of the *JAK2*/e16/*Rsa*I and the *JAK2*/e23/*Hae*III genotypes and alleles in the three bovine breeds under study.

Breed		*JAK2*/e16/*Rsa*I			Total	allele	
		AA	*AG*	*GG*		*A*	*G*
Hereford	*n*	161	95	20	276	0.7554	0.2446
	Frequency	0.5833	0.3442	0.0725	1.0000		
Angus	*n*	195	110	28	333	0.7508	0.2492
	Frequency	0.5856	0.3303	0.0841	1.0000		
Limousin	*n*	95	53	12	160	0.7594	0.2406
	Frequency	0.5937	0.3313	0.0750	1.0000		
Breed		*JAK2*/e23/*Hae*III			Total	allele	
		AA	*AG*	*GG*		*A*	*G*
Hereford	*n*	165	83	23	271	0.7620	0.2380
	Frequency	0.6088	0.3063	0.0849	1.0000		
Angus	*n*	61	132	152	345	0.3681	0.6319
	Frequency	0.1768	0.3826	0.4406	1.0000		
Limousin	*n*	28	72	43	143	0.4476	0.5524
	Frequency	0.1958	0.5035	0.3007	1.0000		

n, number of individuals.

More diverse results between the three breeds of cattle in terms of genotype and allele frequencies were obtained for the *JAK2*/e23/*Hae*III polymorphism (Table 3) after testing 759 individuals. In the case of the Hereford breed, as in the previous SNP, the *AA* genotype was most frequently identified (60.9%), followed by heterozygotes (30.6%). Hereford cows with the *GG* genotype were rarely identified (8.5%). This distribution was not consistent with HWE (p-value = 0.01). The opposite situation was noted for the Angus breed. The most numerous were cattle with the *GG* genotype (44%) and slightly less often heterozygous individuals (38.3%). Individuals with the *AA* genotype constituted only 17.7% of the population of Angus cows. This distribution was also not consistent with HWE (p-value = 0.00). The distribution of genotypes in the herd of Limousin cows was different. Half of the herd consisted of heterozygous individuals (50.3%). There were also numerous cows with the *GG* genotype (30.1%). The *AA* genotype was the least frequently identified (19.6%). The distribution was consistent with HWE (p-value = 0.83). The above results were reflected in allele frequencies. The A allele clearly dominated only in the

Hereford breed (76.2%) and the G allele in the Angus herd (63.2%). The allele frequency in the Limousin herd was more even (allele A accounted for 44.8% of cases and allele G for 55.2%).

3.3. Bovine JAK2/e16/RsaI Associations

In the case of the polymorphism in exon 16 (rs210148032, p.Ile704Val) (Table 4), the birth weight of Hereford and Limousin calves with the *AA* genotype (p.Ile704) was significantly higher than that of the individuals with the *GG* genotype (p.Val704; +1.8 kg and +2.6 kg, respectively; $p \leq 0.05$). However, for the Angus breed, the opposite results were reported, with *GG* individuals born heavier by 2.2 kg compared to *AA* individuals ($p \leq 0.05$). A similar situation was noted for the weaning weight (WWT_{210}). Hereford and Limousin calves with the *AA* genotype were significantly heavier compared to the *GG* genotypes (+11.1 kg ($p \leq 0.05$) and +27.2 kg ($p \leq 0.01$), respectively). For the Angus breed, the differences in WWT_{210} were nonsignificant. In addition, although only for the Hereford breed, there were significant differences in average daily gains (ADG) ($p \leq 0.01$). Individuals with the frequent *AA* genotype had higher ADG compared to both heterozygotes (+132 g) and individuals with the rare *GG* genotype (+165 g). For the other breeds, the differences in ADG were statistically nonsignificant. The observed trends were maintained until the first calving. The body weight of the cow at the first calving differed significantly depending on the identified genotype. In the case of Hereford, individuals with the *AA* genotype were significantly heavier than both heterozygous individuals (+17.9 kg, $p \leq 0.05$) and individuals with the rare *GG* genotype (+28.8 kg, $p \leq 0.01$). In contrast, Limousin cows with the *AA* and *AG* genotypes had an almost identical average body weight at first calving (~610 kg) and were significantly different ($p \leq 0.05$) from the *GG* genotypes, which had an average body weight 29 kg lower than the other cows.

Table 4. The effect of *JAK2*/e16/*Rsa*I genotypes on the growth performance and selected reproductive traits in cows.

Breed	Genotype	Birth Weight (kg)	Average Daily Gain (g)	Weaning Weight (kg)	Age at First Calving (Days)	Body Weight at First Calving (kg)	Calving Interval (Days)
Hereford	*AA*	34.7 ^{a} (0.36)	1091 ^{AB} (13.11)	262.2 ^{a} (2.85)	1039 ^{A} (28.20)	589.4 ^{Aa} (2.11)	499 ^{ab} (16.06)
	AG	34.0 (0.33)	959 ^{A} (14.84)	254.3 (3.50)	975 ^{a} (31.00)	571.5 ^{a} (5.90)	420 ^{a} (13.57)
	GG	32.9 ^{a} (0.51)	926 ^{B} (16.60)	251.1 ^{a} (6.97)	813 ^{Aa} (37.69)	560.6 ^{A} (6.79)	399 ^{b} (16.36)
Angus	*AA*	36.2 ^{a} (0.30)	994 (7.76)	247.1 (1.87)	1076 (19.20)	564.6 (3.33)	416 ^{Aa} (2.02)
	AG	37.3 (0.39)	1011 (8.13)	251.6 (2.50)	1057 (26.91)	562.3 (3.81)	403 ^{ab} (2.23)
	GG	38.4 ^{a} (0.59)	1012 (8.58)	255.5 (7.89)	1011 (25.83)	574.5 (8.46)	389 ^{Ab} (1.22)
Limousin	*AA*	35.2 ^{a} (0.41)	1001 (23.62)	275.5 ^{A} (3.22)	1077 (14.55)	610.8 ^{a} (10.27)	469 (21.51)
	AG	34.2 (0.57)	1001 (35.12)	261.3 (3.61)	1045 (14.83)	610.3 ^{b} (13.77)	479 (27.96)
	GG	32.6 ^{a} (0.65)	954 (10.35)	248.3 ^{A} (6.19)	1090 (15.67)	581.2 ^{ab} (5.69)	466 (6.69)

The means in the columns marked with the same letters within the same breed differ significantly at: small letters, $p \leq 0.05$; capitals, $p \leq 0.01$. Numbers in parentheses are the standard errors of the means.

Some relationships were also noted for selected reproductive traits of cattle, although they were statistically confirmed only for Hereford and Angus breeds. Hereford individuals with the *GG* genotype, despite having the significantly lowest birth weight and body weight

in later periods, as described above, had the lowest average age of first calving (~27 months) compared to the other genotypes, with the difference between the *GG* genotype and the heterozygous *AG* genotype being 162 days (i.e., ~5.5 months difference, $p \leq 0.05$) and between *GG* and the most common *AA* genotype being as much as 226 days (i.e., ~7.5 months difference, $p \leq 0.01$). In the case of the Angus and Limousin breeds, no significant differences were found because the age of the first calving was similar, occurring at around 33–36 months of age for cows of both breeds. Cows with the *GG* genotype also had a shorter calving interval. Within the Hereford breed, the differences were as much as 100 days compared to cows with the *AA* genotype ($p \leq 0.05$). Also, heterozygotes in the *JAK2*/e16/*Rsa*I polymorphism for the Hereford breed had a 79-day shorter calving interval compared to cows with the *AA* genotype ($p \leq 0.05$). The calving interval of Angus cows was even more diverse within the genotypes. As with Hereford, cows with *GG* genotype had a 27-day shorter calving interval compared to cows with *AA* genotype ($p \leq 0.01$) and 14 days shorter than heterozygous cows ($p \leq 0.05$). Heterozygotes also had a shorter calving interval by 13 days compared to *AA* cows ($p \leq 0.05$). In the case of the Limousin breed, there were no significant differences in the calving interval of cows with the different *JAK2*/e16/*Rsa*I genotypes. Irrespective of breed and genotype, all calvings were single and uncomplicated.

3.4. Bovine JAK2/e23/HaeIII Associations

Regardless of breed, there was no association between the silent mutation in exon 23 (rs211067160) and birth weight of calves (Table 5). In the case of weaning weight in the Hereford and Limousin populations, individuals with *AA* genotype were heavier by 16.4 kg and 14.3 kg, respectively, compared to individuals with *GG* genotype ($p \leq 0.05$) and had better average daily weight gains of 154 g ($p \leq 0.01$) and 91 g, respectively ($p \leq 0.05$). In addition, heterozygous Hereford cows also had higher ADG than *GG* cows (+112 g; $p \leq 0.01$). However, Limousin cows with the previously preferred *AA* genotype had 36.8–38.3 kg lower body weight at first calving compared to cows with the other genotypes ($p \leq 0.05$). For other breeds, no statistical differences in body weight at the first calving were noted.

Similarly to the previously discussed polymorphic site, the age of first calving in Angus and Limousin cows (from 34 to 36 months) did not depend on *JAK2*/e23/*Hae*III genotypes in contrast to Hereford cows, for which, again, individuals with the rare *GG* genotype had significantly earlier age at first calving (~29 months) than *AA* cows (~34 months) ($p \leq 0.05$). At the same time, cows of this breed, also with the *GG* genotype, had a 90-day shorter calving interval compared to the *AA* genotype ($p \leq 0.05$). In the population of Limousin cows, the most numerous heterozygotes had a significantly longer calving interval by 90 days than cows with the rarest *AA* genotype ($p \leq 0.05$).

For the Angus breed, no statistically significant differences were observed between *JAK2*/e23/*Hae*III genotypes for the growth and reproductive traits. Additionally, it was noted that regardless of breed or genotype, all births were single and occurred without complications or human assistance.

Table 5. The effect of *JAK2*/e23/*Hae*III genotypes on the growth performance and selected reproductive traits in cows.

Breed	Genotype	Birth Weight (kg)	Average Daily Gain (g)	Weaning Weight (kg)	Age at First Calving (Days)	Body Weight at First Calving (kg)	Calving Interval (Days)
Hereford	*AA*	34.5 (0.34)	1060 ^{A} (12.86)	260.8 ^{a} (2.53)	1020 ^{a} (27.83)	584.9 (3.26)	487 ^{a} (15.87)
	AG	34.1 (0.38)	1018 ^{B} (24.03)	257.9 (4.95)	988 (37.85)	575.6 (4.05)	430 (14.72)
	GG	33.9 (1.03)	906 ^{AB} (16.51)	244.4 ^{a} (3.47)	874 ^{a} (51.07)	571.9 (7.08)	397 ^{a} (16.73)
Angus	*AA*	37.16 (0.71)	1005 (14.27)	249.6 (3.66)	1065 (25.94)	559.4 (5.42)	423 (4.37)
	AG	37.40 (0.42)	1017 (11.25)	250.8 (2.92)	1021 (25.59)	563.9 (4.72)	400 (1.71)
	GG	36.3 (0.29)	993 (7.37)	248.4 (1.99)	1084 (20.98)	566.0 (3.28)	410 (2.08)
Limousin	*AA*	35.5 (0.77)	1058 ^{a} (17.42)	274.5 ^{a} (4.38)	1075 (18.39)	580.5 ^{ab} (3.13)	413 ^{a} (28.82)
	AG	34.2 (0.40)	990 (32.90)	271.3 (3.91)	1078 (15.23)	618.8 ^{a} (12.95)	504 ^{a} (27.69)
	GG	35.2 (0.73)	967 ^{a} (36.69)	261.2 ^{a} (4.13)	1056 (25.25)	617.3 ^{b} (17.77)	483 (32.79)

The means in the columns marked with the same letters within the same breed differ significantly at: small letters, $p \leq 0.05$; capitals, $p \leq 0.01$. Numbers in parentheses are the standard errors of the means.

3.5. Ovine JAK2 Frequencies

For three polymorphic sites, 138 Pomeranian and 195 Suffolk individuals were genotyped (Table 6; Figures S3 and S4 included in the Supplementary Materials).

Table 6. The number and frequency of genotypes and alleles for three different *JAK2* polymorphisms among two sheep breeds under study.

Breed		*JAK2*/e6/*Ear*I			Total	allele	
		AA	*AG*	*GG*		*A*	*G*
Pomeranian	*n*	32	58	48	138	0.4420	0.5580
	Frequency	0.2319	0.4203	0.3478	1.0000		
Suffolk	*n*	29	43	123	195	0.2590	0.7410
	Frequency	0.1487	0.2205	0.6308	1.0000		
Breed		*JAK2*/e6/seq			Total	allele	
		AA	*AG*	*GG*		*A*	*G*
Pomeranian	*n*	55	59	24	138	0.6123	0.3877
	Frequency	0.3986	0.4275	0.1739	1.0000		
Suffolk	*n*	49	110	36	195	0.5333	0.4667
	Frequency	0.2513	0.5641	0.1846	1.0000		
Breed		*JAK2*/e24/*Hpy*188III			Total	allele	
		AA	*AG*	*GG*		*A*	*G*
Pomeranian	*n*	43	67	28	138	0.5544	0.4456
	Frequency	0.3116	0.4855	0.2029	1.0000		
Suffolk	*n*	49	128	18	195	0.5795	0.4205
	Frequency	0.2513	0.6564	0.0923	1.0000		

n, number of individuals.

In the case of the first polymorphic site (*JAK2*/e6/*Ear*I), for the Pomeranian breed, numerous occurrences of individuals of each genotype were noted, with the largest number of individuals being heterozygous (42%), followed by sheep with the *GG* genotype (34.8%), and slightly less often, individuals with *AA* genotype were identified (23.2%). Allele frequencies were similar (A, 44.2%; G, 55.8%). The genotype distribution was consistent with HWE (p-value = 0.08). However, in the flock of Suffolk sheep, the *GG* genotype (63%) and the G allele (74%) clearly dominated, and the distribution of genotypes was not consistent with HWE (p-value < 0.01).

In the case of the second polymorphic site (*JAK2*/e6/seq), heterozygotes (POM 42.7% and SUF 56.4%) were the most numerous in both breeds, followed by individuals with the *AA* genotype (39.9% and 25.1%, respectively) and with the *GG* genotype (17.4% and 18.5%, respectively). The A allele occurred more frequently than the G allele (61.2% vs. 53.3%). The distribution of genotypes in both populations was consistent with HWE (p-value = 0.24 for the Pomeranian breed and p-value = 0.06 for the Suffolk breed).

The polymorphism in exon 24 (*JAK2*/e24/*Hpy*188III) was also characterized by a slight dominance of heterozygous individuals (POM 48.6% and SUF 65.6%), followed by sheep with the *AA* genotype (31.2% and 25.1%, respectively) and the least frequent being sheep with the *GG* genotype (20.3% and 9.2%, respectively). The frequencies of the A allele were similar (55.4% and 57.8%). The distribution of genotypes in the Pomeranian sheep population was consistent with HWE (p-value = 0.84) and inconsistent with HWE in the Suffolk sheep herd (p-value < 0.01).

3.6. Ovine JAK2 Associations—Growth Traits

At the early stages of animal life, highly significant differences depending on the genotype were observed for most of the analyzed growth performance traits and for each polymorphic site (Table 7). The exception was the subsequent body weight at mating, which was significant only for the Suffolk breed.

Sheep with the *AA* genotype for the *JAK2*/e6/*Ear*I polymorphic site were significantly heavier than the other sheep on day 2 (+0.3–0.7 kg for Pomeranian and +0.7–0.8 kg for Suffolk), on day 30 (+1.2–1.3 kg; only Suffolk), and on the 56th day of life (Pomeranian +1.2–2.2 kg and Suffolk 2.8–3.6 kg) and at mating (+4.1 kg; only Suffolk), especially from the worst-performing individuals with the *GG* genotype ($p \leq 0.01$) compared to heterozygous sheep and sheep with the *AA* genotype (Pomeranian +16–27 g and Suffolk +40–53 g) between 2 and 56 days of age. For Suffolk sheep, between 30 and 56 days of age, even higher differences in average gains were noted (+62–90 g).

Table 7. Means and standard errors (in parentheses) of weights and gains traits in sheep with different *JAK2* genotypes of three polymorphic sites.

	Breed	Genotype	Body Weight at 2 Days (kg)	Body Weight at 30 Days (kg)	Body Weight at 56 Days (kg)	Average Daily Gains between 2–56 Days (g)	Average Daily Gains between 30–56 Days (g)	Body Weight at Mating (kg)
JAK2/e6/*Ear*I	Pomeranian	*AA*	4.3 ^{AB} (0.04)	n/a	19.3 ^{AB} (0.19)	278 ^{AB} (3.52)	n/a	54.4 (0.43)
		AG	4.0 ^{BC} (0.03)	n/a	18.1 ^{BC} (0.15)	262 ^{Ba} (2.78)	n/a	53.8 (0.28)
		GG	3.6 ^{AC} (0.02)	n/a	17.1 ^{AC} (0.18)	251 ^{Aa} (3.20)	n/a	53.2 (0.43)
	Suffolk	*AA*	4.8 ^{AB} (0.05)	14.2 ^{AB} (0.13)	23.5 ^{AB} (0.21)	347 ^{AB} (3.44)	359 ^{AB} (7.24)	62.0 ^{AB} (0.55)
		AG	4.1 ^{B} (0.02)	13.0 ^{B} (0.19)	20.7 ^{Ba} (0.25)	307 ^{Ba} (4.39)	297 ^{Ba} (9.86)	58.9 ^{B} (0.39)
		GG	4.0 ^{A} (0.03)	12.9 ^{A} (0.15)	19.9 ^{Aa} (0.18)	294 ^{Aa} (2.96)	269 ^{Aa} (4.95)	57.9 ^{A} (0.26)

Table 7. *Cont.*

	Breed	Genotype	Body Weight at 2 Days (kg)	Body Weight at 30 Days (kg)	Body Weight at 56 Days (kg)	Average Daily Gains between 2–56 Days (g)	Average Daily Gains between 30–56 Days (g)	Body Weight at Mating (kg)
JAK2/e6/seq	Pomeranian	*AA*	3.8 ^{A} (0.04)	n/a	17.9 ^{A} (0.18)	261 ^{A} (3.16)	n/a	53.8 (0.31)
		AG	3.9 ^{B} (0.04)	n/a	17.7 ^{B} (0.16)	256 ^{B} (2.73)	n/a	54.2 (0.34)
		GG	4.3 ^{AB} (0.05)	n/a	19.4 ^{AB} (0.24)	280 ^{AB} (4.01)	n/a	54.9 (0.53)
	Suffolk	*AA*	4.1 ^{A} (0.06)	12.7 ^{A} (0.25)	19.8 ^{A} (0.34)	292 ^{A} (5.32)	275 ^{A} (8.56)	58.4 ^{A} (0.54)
		AG	4.1 ^{B} (0.03)	13.1 ^{a} (0.14)	20.3 ^{B} (0.17)	301 ^{B} (2.93)	278 ^{B} (5.79)	58.4 ^{B} (0.25)
		GG	4.6 ^{AB} (0.06)	13.7 ^{Aa} (0.20)	22.5 ^{AB} (0.32)	333 ^{AB} (5.29)	338 ^{AB} (8.67)	60.2 ^{AB} (0.59)
JAK2/e24/*Hpy*188III	Pomeranian	*AA*	4.2 ^{AB} (0.05)	n/a	19.2 ^{AB} (0.20)	279 ^{AB} (3.83)	n/a	54.4 (0.43)
		AG	3.9 ^{AC} (0.04)	n/a	17.8 ^{AC} (0.13)	258 ^{AC} (2.14)	n/a	53.8 (0.28)
		GG	3.6 ^{BC} (0.05)	n/a	16.8 ^{BC} (0.15)	244 ^{BC} (2.98)	n/a	53.2 (0.43)
	Suffolk	*AA*	4.5 ^{AB} (0.06)	14.1 ^{AB} (0.16)	22.7 ^{AB} (0.26)	338 ^{AB} (4.07)	332 ^{AB} (9.04)	60.1 ^{Aa} (0.54)
		AG	4.1 ^{AC} (0.02)	13.0 ^{AC} (0.12)	20.2 ^{AC} (0.15)	298 ^{AC} (2.46)	278 ^{AC} (4.99)	58.7 ^{Ba} (0.23)
		GG	3.7 ^{BC} (0.05)	11.4 ^{BC} (0.40)	17.7 ^{BC} (0.35)	260 ^{BC} (5.98)	243 ^{BC} (11.28)	54.9 ^{AB} (0.46)

The means in the columns marked with the same letters within the same breed differ significantly at: small letters, $p \leq 0.05$; capitals, $p \leq 0.01$. n/a: for the Pomeranian breed, such data were not recorded.

In the case of the second polymorphic site (*JAK2*/e6/seq) in both breeds, sheep with the *GG* genotype had the greatest body weights on day 2 (+0.4–0.5 kg), day 30 (+0.6–1 kg; only Suffolk), and 56 days of age (Pomeranian +1.5–1.7 kg and Suffolk +2.2–2.7 kg) and daily gains for the period from 2 to 56 days of age (Pomeranian +19–24 g and Suffolk +32–41 g) and for the period from 30 to 56 days of age (+60–63 g; Suffolk only) ($p \leq 0.01$) compared to heterozygotes and *AA* genotypes. For the Suffolk breed, the weight of sheep with the *GG* genotype was also higher by 1.8 kg during mating than in sheep with the other genotypes ($p \leq 0.01$).

Polymorphism within exon 24 (*JAK2*/e24/*Hpy*188III) was also characterized by highly significant associations ($p \leq 0.01$), where individuals with the *AA* genotype had the highest body weights compared to heterozygotes and individuals with the *GG* genotype on the 2nd day of life (Pomeranian +0.3–0.6 kg and Suffolk +0.4–0.8 kg) as well as on day 30 (+1.1–2.7 kg; Suffolk only) and on day 56 (Pomeranian +1.4–2.4 kg and Suffolk 2.5–5 kg). It was similar with daily gains both in the period from 2 to 56 days (Pomeranian +21–35 g and Suffolk +40–78 g) and in the period from 30 to 56 days (+54–89 g; Suffolk breed only). Also, during mating, sheep with the *AA* genotype were characterized by a higher weight than the other individuals (+1.4–5.2 kg; only in the Suffolk breed).

3.7. Ovine JAK2 Associations-Reproductive Traits

Table 8 presents the values of reproduction traits for Pomeranian and Suffolk sheep as well as lamb rearing in relation to three polymorphic sites located in the *JAK2* sheep gene. Genetic origin differentiated the average values of all the analyzed reproductive characteristics of mothers. In the case of the polymorphism in exon 6 (*JAK2*/*Ear*I), the highest fertility was shown by heterozygous Pomeranian sheep (95.8%), while the lowest (82.34%) was found in sheep with the *GG* genotype (82.34%). Statistically significant differences ($p \leq 0.01$) were noted between individuals with the *GG* genotype and individuals

that were homozygous *AA* (+11.96%) and heterozygous *AG* (+13.54%). In the case of the Suffolk breed, the highest fertility rate was shown in heterozygous individuals (94.41%) and the lowest in *AA* individuals (91.03%). However, no statistically significant differences were found in Suffolks. The prolificacy was similar among the genotypes within the breeds; however, statistically significant differences were noted in the Suffolk breed, where individuals with the *GG* genotype were characterized by the lowest prolificacy in comparison to individuals with the *AA* genotype ($p \leq 0.01$) and heterozygotes ($p \leq 0.05$).

Regardless of breed, the best lamb survival rates were noted for heterozygous sheep (94.28% Pomeranian; 96.34% Suffolk). The lowest lamb survival rates in sheep of both breeds were found in *GG* individuals, which was confirmed statistically ($p \leq 0.01$).

For the second polymorphic site (*JAK2*/e6/seq), there were clear differences between breeds. In Pomeranian sheep, the *GG* genotype was associated with the highest fertility (+7.52% *AG*, $p \leq 0.05$; +9.53% *AA*, $p \leq 0.01$), while in Suffolk sheep, similar fertility was observed in individuals with *AA* and *AG* genotypes, which significantly ($p \leq 0.05$) differed from mothers with the *GG* genotype (+4.28–4.4%).

The prolificacy of sheep in both groups, regardless of the genotype, did not exceed 1.18 and was similar. No statistically significant differences were observed. The Pomeranian sheep with the *GG* genotype reared the largest percentage of lambs (97.68%) compared to other mothers, and statistical differences were shown only for mothers with the *AA* genotype (+11.5%; $p \leq 0.01$). Different relationships were confirmed statistically ($p \leq 0.01$) in Suffolk sheep; the lamb survival was higher (92.09%) when ewes had the AG genotype than when the ewes had the GG genotype (difference +11.27%).

The relationship of the *JAK2*/e24/*Hpy188*III genotypes with fertility and lamb survival showed that, regardless of breed, sheep with *AA* genotype were characterized by the highest values of the analyzed traits ($p \leq 0.01$; $p \leq 0.05$). The difference in fertility between mothers with the *AA* genotype and individuals with the *GG* genotype was nearly 14% in Pomeranian sheep and 6% in Suffolk sheep, while the differences in lamb survival were approximately 12% for Pomeranian sheep and 19.5% for Suffolk sheep. In the case of the prolificacy, statistical differences were demonstrated in the Pomeranian breed, where individuals with the *GG* genotype differed significantly from the other individuals (0.14–0.19; $p \leq 0.01$).

Table 8. Influence of genotypes on reproductive performance of Pomeranian and Suffolk sheep breeds.

	Breed	**Genotype**	**Fertility (%)**	**Prolificacy (*n*/ewe)**	**Lamb Survival (%)**
JAK2/e6/*Ear*I	Pomeranian	*AA*	94.30 ^{A} (2.23)	1.15 (0.05)	93.55 ^{A} (2.21)
		AG	95.88 ^{B} (1.45)	1.11 (0.04)	94.28 ^{B} (1.67)
		GG	82.34 ^{AB} (2.70)	1.18 (0.05)	81.38 ^{AB} (3.86)
	Suffolk	*AA*	91.03 (2.89)	1.19 ^{A} (0.06)	95.40 ^{A} (1.92)
		AG	94.41 (1.11)	1.18 ^{a} (0.05)	96.34 ^{B} (1.48)
		GG	94.40 (1.66)	1.09 ^{Aa} (0.02)	84.52 ^{AB} (2.00)

Table 8. *Cont.*

	Breed	Genotype	Fertility (%)	Prolificacy (*n*/ewe)	Lamb Survival (%)
JAK2/e6/seq	Pomeranian	*AA*	88.30 ^{A} (2.35)	1.11 (0.04)	86.20 ^{A} (2.88)
		AG	90.31 ^{a} (2.05)	1.18 (0.04)	89.69 (2.64)
		GG	97.83 ^{Aa} (1.59)	1.13 (0.05)	97.68 ^{A} (1.66)
	Suffolk	*AA*	94.62 ^{a} (1.77)	1.13 (0.04)	87.06 (2.84)
		AG	94.74 ^{b} (1.11)	1.14 (0.03)	92.09 ^{A} (1.53)
		GG	90.34 ^{ab} (2.47)	1.06 (0.03)	80.82 ^{A} (4.23)
JAK2/e24/*Hpy*188III	Pomeranian	*AA*	95.17 ^{A} (1.76)	1.13 ^{A} (0.04)	95.77 ^{A} (1.66)
		AG	93.48 ^{B} (1.79)	1.08 ^{B} (0.03)	90.66 (1.93)
		GG	81.33 ^{AB} (3.74)	1.27 ^{AB} (0.08)	84.00 ^{A} (4.46)
	Suffolk	*AA*	95.05 ^{a} (1.44)	1.15 (0.03)	92.64 ^{A} (2.43)
		AG	94.17 (1.13)	1.11 (0.03)	89.44 ^{B} (1.66)
		GG	88.89 ^{a} (3.81)	1.11 (0.05)	73.15 ^{AB} (5.43)

The means in the columns marked with the same letters within the same breed differ significantly at: small letters, $p \leq 0.05$; capitals, $p \leq 0.01$. Numbers in parentheses are the standard errors of the means.

4. Discussion

Selection based on genetic markers focuses primarily on improving the performance characteristics of animals, such as milk yield and growth performance. The rate of genetic improvement can be increased by genotype-based selection [20]. Fertility traits are also increasingly taken into consideration during animal selection, as these traits directly influence production efficiency. The intensity of selection depends on factors such as the number of calves or lambs born per year and the interval between generations. In a production system that involves both milk and meat production, irregular reproduction patterns, such as long generation interval, can lead to economic unprofitability [21]. The use of SNP markers offers the possibility of selecting the appropriate genotype/haplotype or a combination of genotypes with a related phenotype, resulting in significant breeding benefits. An example of this is found in dairy and beef cattle-breeding programs in North America and Europe, where the use of molecular genetics contributes to the profitability of farms and the continuous improvement of production values [22].

4.1. GWAS Studies

Most of the target performance traits in livestock are polygenic, which are primarily suitable for testing using the genome-wide association study (GWAS) method [23]. Keogh et al. [24] performed GWAS (Charolaise and Limousin breeds) for SNPs involved in the reproductive potential of these breeds (calving interval, calving difficulty, and calf mortality) and selected production-related traits. The highest signals for carcass weight were noted for BTA2 (dominant effect of the myostatin gene) and BTA6 (non-SMC condensin I complex, subunit G (*NCAPG*)/ligand-dependent nuclear receptor corepressor-like (*LCORL*) locus). The ovine *JAK2* gene, located on the *Ovis aries* chromosome 2 (similarly to the ovine myostatin gene), does not appear directly as a strong signal in GWAS studies in sheep. In

contrast, one region on OAR6 (13 SNPs) was associated with body weight (*NCAPG/LCORL* locus, similar to cattle on BTA6) [25]. An important methodological limitation of GWAS studies is the inclusion in the analyses of only frequent gene variants, i.e., SNPs that occur in more than 5% of individuals in the population, and indicating only strong signals above the conventional threshold line. Weaker signals that may be missed by GWAS analysis can be identified and described using traditional quantitative trait locus (QTL) mapping, as long as these signals are associated with genes involved in complex biological pathways and processes [26].

4.2. Physiological Implications

Body weight is the most crucial indicator of growth and development in cow and sheep production. It directly and indirectly affects meat and wool production as well as the reproduction of animals [27]. Growth regulation is controlled by multiple signaling pathways that change throughout the life of the animal. Doubtlessly, the somatotropic axis (GH/IGF-I along with their receptors and many signaling proteins) is involved in these complex signaling pathways. This pathway is also essential for cell growth, differentiation, and development, in particular for the modulation and amplification of gonadotropin, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) activity during follicle growth in the ovary [28]. After birth, skeletal muscle development is controlled by the classical Wnt (portmanteau word formed by combining "Wingless" and "Integration-1") signaling pathway, while the nonclassical Wnt signaling pathway mainly mediates the self-renewal and the growth of muscle fibers [29]. There is also evidence that the JAK2/STAT3 pathway mediates the regulation of muscle satellite cell differentiation [30] and regulates the expression of genes related to skeletal muscle development and energy metabolism, especially affecting the expression of the *MyoD* and *Myf5* genes [31]. The IL-6/JAK2/STAT3 pathway may be a principal mediator in denervated skeletal muscle atrophy [32]. The JAK2-STAT5 pathway is activated by the pulsatile release of GH in response to acute aerobic exercise in human skeletal muscle [33]. Even a single amino acid variation in the JAK2 molecule can carry severe consequences. In humans, most patients with polycythemia vera (PV), one-half of the cases of essential thrombocythemia (ET), and other myeloproliferative neoplasms (MPNs) cases possess an JAK2 p.V617F mutation (exon 12) [34] leading to constitutive activity of the JAK2 gene-encoded tyrosine kinase. Identical mutations of the *JAK2* gene and consequences occur in dogs [35]. Of the five SNPs analyzed in this manuscript, only one (rs210148032 in exon 16 of the bovine *JAK2* gene) may have analogous physiological implications. However, there is a lack of research in this area. A more in-depth analysis is necessary in relation to genetic implications.

4.3. Genetic Implications

A total of five polymorphic sites (two in cattle and three in sheep) located in different regions of the *JAK2* gene were analyzed. To our knowledge, this is the first attempt to assess the relationship between these SNPs and selected cattle and sheep production traits. They are an example of two different types of gene polymorphism, which may determine their different partial influence on the observed differences in shaping growth performance and reproductive characteristics in these animal species.

The polymorphic site rs210148032 in exon 16 of the bovine *JAK2* gene is a typical example of a missense mutation (the first letter of the codon). This determines the replacement of the isoleucine amino acid with valine at position 704 of the amino acid chain in the JH2 domain (i.e., pseudokinase) of active JAK2. Such a change may therefore determine the function of JAK2, as the JH2 domain directly regulates the activity of the tyrosine kinase JH1 domain. The rate at which amino acids are seen to mutate to other residues in homologous proteins has been extensively studied [36]. The isoleucine–valine substitution (as well as leucine–valine and leucine–isoleucine) is quite common in nature. The severity of such amino acid substitution is estimated at the levels 2–4 (http://www.insilicase.com/Web/SubstitutionScore.aspx) (accessed on 14 May 2023),

where, for example, tryptophan is highly conserved and rarely changes to any other amino acid (level 17). Alanine, valine, isoleucine, and leucine are closely related in physicochemical properties because they are small, hydrophobic residues similar to methionine [37]. Being hydrophobic, isoleucine/valine prefers to be buried in protein hydrophobic cores. This may partly explain the fact that ambiguous results were obtained in the three cattle breeds. Hereford and Limousin cows with the p.Ile704 were always the heaviest and had the best weight gains. However, Angus cows with the rare p.Val704 had higher weights, although for most traits, this was not statistically confirmed. Rather, this SNP may be linked to other SNPs of greater importance, and the significance of the change of these particular A/G nucleotides can be explained in a similar way as for silent mutations.

The second polymorphic site in exon 23 of the bovine *JAK2* gene (rs211067160) and both SNPs in exon 6 (rs160146162 and rs160146160) as well as in exon 24 (rs160146116) of the sheep *JAK2* gene are examples of silent mutations. Generally, those SNPs do not result in alteration of gene expression, and there are no changes in the function and structure of the mature protein. However, even a simple A/G change may result in posttranscriptional changes such as alterations in mRNA splicing and stability, followed by nucleocytoplasmic export through the nuclear pore complex (NPC) by receptor-mediated and Ran-GTP gradient-dependent active transport [38]. In addition, synonymous codons may influence ribosome occupancy time and thus can increase or decrease elongation rates during translation [39]. The location of the *JAK2*/e23/*Hae*III polymorphism is extremely important (codon for p.Pro1057), as it is the central part encoding the JH1 tyrosine kinase domain with the conserved tyrosine sites in JAK2: p.Tyr1007 and p.Tyr1008. Similarly, in sheep, a p.Leu1082 residue also in the JH1 domain (encoded, among others, by exon 24 where *JAK2*/*Hpy*188III SNP is positioned) may also play a crucial role in the function of kinase. The stability of this fragment determines the ability to transmit a signal inside the target cell after activation of the receptor–ligand, which is usually a member of the gp^{130} receptor family and class II cytokine–receptor family (such as interleukin 3 receptor family, erythropoietin receptor (EPO), growth hormone (GH) receptor, prolactin receptor, and thrombopoietin (TPO) receptor) [40]. Another silent A/G mutation (rs110298451) located in exon 20 of the bovine *JAK2* gene was described by Padzik and Szewczuk [17] at the third nucleotide of the lysine codon (AA<u>A</u> → AA<u>G</u>) at position 912 (p.K912) of the mature amino acid chain (also JH1 domain with tyrosine kinase activity). The influence of the polymorphism on growth traits varied depending on the breed: for the Hereford breed, the *GG* genotype was favored; for the Angus breed, the heterozygous genotype; and for the Limousin breed, the *AA* genotype. Therefore, synonymous SNP within exons encoding the JH1 kinase domain may be associated with other causative mutations in the *JAK2* gene or a completely different gene involved in developing growth performance traits in cattle. In sheep, two SNPs located in exon 6 (codons for p.Glu177 and p.Thr196) were analyzed. This exon encodes a fairly conservative FERM domain, which primarily determines the binding of the JAK2 molecule to the target receptor (receptor association). However, apart from binding to the receptor, this domain plays a role in the overall organization of the tyrosine kinase structure and its subsequent activation/deactivation: a third level of regulation of kinase activity [41]. Sheep of both breeds with a combination of *AA*/*GG*/*AA* genotypes of SNPs located in exon 6 and exon 24 appear to be heavier and have better daily gains compared to other individuals. Since these are the first association studies for these SNPs, the results cannot be compared to studies by other authors. Therefore, this observation needs to be confirmed statistically in separate analyses considering haplotypes or genotype combinations.

4.4. Involvement of JAK2 in the Formation of Reproductive Traits

The optimal level of reproduction in beef cattle herds is closely tied to the potential economic benefits, which largely rely on beef production [42,43]. Two of the important indicators determining the fertility of cows are the age of the first calving and the length of the calving interval. Earlier age at first calving reduces the cost of rearing heifers through

earlier conception, which is influenced by body weight and condition of the cows [44], while the extension of the rearing period of heifers increases costs incurred by breeders [45,46].

Regardless of the polymorphic location, Hereford cows of the *GG* genotype calved the earliest and had the shortest calving interval compared to the *AA* genotype. In Angus cows, statistically significant differences were observed only for the *JAK2*/e16/*Rsa*I polymorphism and the calving interval, where, once more, individuals with the *GG* genotype had a shorter calving interval. Cows with a short birth intervals are usually characterized by the best fertility and the highest reproductive efficiency [45]. The length of the period in question is more important in the case of dairy cattle, while in the case of beef production, attention is paid to the calving rhythm, which means that breeders keep cows with regular calving intervals for further breeding [45]. The results obtained in this paper are difficult to relate to the studies of other authors in the context of the analyzed polymorphisms and the discussed reproductive traits. The only work on a similar subject is the study by Padzik and Szewczuk [17], which identified a silent mutation in exon 20 (dbSNP ID: rs110298451) in the *JAK2* gene in the same breeds of cattle. The age of cows at first calving was later (average days: Limousin: 1118, Hereford 1038, and Angus 1085, respectively). Moreover, the authors did not find a significant relationship between *JAK2*/e20/*Rsa*I polymorphism and age at first calving.

Reproductive traits in sheep have low heritability. Therefore, traditional selection by phenotype results in small annual genetic progress [47]. Identification of candidate genes is one strategy for improving these traits. These genes directly or indirectly affect fertility [48,49], prolificacy [50,51], and lamb rearing [51,52], which are of great importance and may contribute to increasing the rate of genetic improvement of these traits. It should be remembered that polygenic traits are traits that are constantly dispersed, referring to the existence of many genes that help in the expression of various gene traits (it is selective). Environmental elements, including animal stress, also significantly affect gene expression [27,53]. Therefore, in our study, the observations were made on the same farm, with the same management and feeding system for both sheep breeds. The sheep spent most of the year on pasture, where the nutritional properties of forage change depending on the season, which may affect gene expression. Metzler-Zebeli et al. [54] stated that different feeding levels can alter gene expression. Sheep grazing, stimulated by hot summers and cold winters, can also alter gene expression [55].

Because no reports are available in the literature on the relationships between the three polymorphic sites analyzed in our study and performance and reproduction traits of sheep, it is not possible to contrast and compare our results with those of other authors. Therefore, we discussed our results in terms of their economic importance and their influence on the genetic improvement of sheep.

In the case of polymorphisms located in exons 6 (*JAK2*/*Ear*I) and 24 (*JAK2*/*Hpy*188III) in the group of sheep with the *GG* genotype, a certain tendency was observed associated with the deterioration of fertility and lower rearing of lambs, which is also associated with the economic aspect of production [56,57].

An inverse relationship was found in sheep of the autochthonous Pomeranian breed in the case of the *JAK2*/e6/seq polymorphism, where the fertility and lamb survival in ewes of the *GG* genotype exceeded 97%, which indicates excellent reproduction. Suffolk ewes with the *GG* genotype were also characterized by high fertility (90.34%). It is assumed that the value of the indicator can be considered satisfactory when it is 90%, good if it exceeds 95%, and very good when it is close to 100% [58]. Preliminary analyses showed that ewes with the AA and AG genotypes had better fertility and lamb survival, apart from Pomeranian sheep in the case of the *JAK2*/e6/seq polymorphic site. Irrespective of the analyzed polymorphic site, the *GG* ewes reared fewer lambs than other mothers (except for sheep of the Pomeranian breed *JAK2*/e6/seq). The values of the index oscillate between 73.15–84.52%, which means that the losses of lambs in the analyzed groups of mothers with *GG* genotypes exceeded the acceptable level of 5% [59–61]. In these groups of sheep, there were lambs from twin pregnancies, which, according to Clune et al. [59], may be

less developed and quite often show reduced viability immediately after birth [60], which delays or even prevents proper colostrum intake [62]. As a result, deaths may occur in the first hours of life. Losses during the rearing period may also be due to the lack of colostrum or its poorer quality and insufficient milk yield of mothers. For this reason, lambs are often fed with preparations containing powdered colostrum and, in further rearing, with milk replacers [61]. Therefore, it should be considered that greater care provided by breeders may improve lamb-rearing results in this breed of sheep. The selection and elimination of individuals with the *GG JAK2*/e6/seq genotype should also be considered.

Lamb survival is an important element of sheep production closely related to the prolificacy and profitability of production. The prolificacy of sheep in both breed groups was similar, regardless of the genotype, and did not exceed 1.18. The prolificacy of Suffolk sheep was relatively low (1.06–1.19). The results for PZO indicate it has good potential in terms of prolificacy [63].

The assumed profitability of the production of lambs for slaughter occurs from at least 1.5 lambs raised from the mother [64], which in practice is difficult in the case of native breeds, such as Pomeranian sheep, except for prolific breeds. This trait in sheep depends to a large extent on, e.g., the breed, nutrition, and age of the ewes [57,64]. Taking into account the possibilities and predispositions of the analyzed sheep breeds in the context of higher prolificacy, attention should be paid to the nutrition of ewes before and during breeding [65], which, according to Łozicki [66], could increase the number of maturing ova and, consequently, improve fertility by 10–30%. In addition, the introduction of biotechnological methods in reproduction, namely the stimulation of the functioning of the reproductive system but also the induction of superovulation, according to Skliarov et al. [67], suggests the possibility of a significant improvement in this indicator.

5. Conclusions

The phenotypic characteristics of cattle and sheep are the result of a complex interaction of many genetic and environmental factors, which usually act simultaneously, and it is difficult to determine the degree of influence of each of them. Therefore, early identification of genetic features in young animals enables more effective selection management and effective breeding in the herd. Production indicators, such as, body weight, daily gains of lambs and calves at different stages of their lives, as well as reproductive indices, are important factors that are crucial for successful ruminant production and thus translate into profitability of production. Identification of genomic regions and biological pathways that contribute to the understanding of variability in body weight traits and reproductive traits is important for selection purposes. Therefore, the identification of a number of genes with a moderate and low impact on the traits discussed in the paper will allow the use of genetic-marker-assisted selection for in-breed selection in order to achieve higher body weight, daily gains, and better fertility and rearing of lambs and calves in various housing and feeding conditions. The results obtained in the study are ambiguous, and additional association studies are needed on other herds and within different breeds of cattle and sheep. For this reason, an analysis of haplotypes and/or combined genotypes of the *JAK2* gene and selected polymorphic sites located in the genes encoding the somatotropic axis should be performed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ani13152470/s1, Figure S1: Electrophoretic separation of restriction fragments resulting from digestion with the RsaI restriction enzyme (ACRS-PCR; bovine JAK2/e16/RsaI polymorphism); lane 1 (M), mass standard pUC19 DNA/MspI; lane 2, AG genotype; lane 3, AA genotype; lane 4, GG genotype; Figure S2: Electrophoretic separation of restriction fragments resulting from digestion with the HaeIII restriction enzyme (ACRS-PCR; bovine JAK2/e23/HaeIII polymorphism); lane 1 (M), mass standard pUC19 DNA/MspI; lane 2, AG genotype; lane 3, AA genotype; lane 4, GG genotype; Figure S3: Electrophoretic separation of restriction fragments resulting from digestion with the EarI restriction enzyme (PCR-RFLP; ovine JAK2/e6/EarI polymorphism) polymorphic site; lane 1 (M), mass standard pUC19 DNA/MspI; lane 2, AG geno-

type; lane 3, AA genotype; lane 4, GG genotype; Figure S4: Electrophoretic separation of restriction fragments resulting from digestion with the Hpy188III restriction enzyme (PCR-RFLP; ovine JAK2/e24/Hpy188III polymorphism); lane 1 (M), mass standard pUC19 DNA/MspI; lane 2, AG genotype; lane 3, AA genotype; lane 4, GG genotype; Table S1: A summary of the JAK2 gene fragments used for DNA sequencing and subsequent identification of polymorphic sites.

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Table S1 A summary of the *JAK2* gene fragments used for DNA sequencing and subsequent identification of polymorphic sites

Primer (5’ > 3’)	Tm (°C)	Product size (bp)	sequence	possible SNP	status
LEFT PRIMER gaaggataacttaccagtgtcactttc	59.90	352	*Bos taurus* exon 3 (highlighted in blue) 1 atgctatcag tgaagacttc tttatatatg **aaggataact taccagtgtc actttc**attt 61 gtaactggtt tctcttacag gcaaatgttc tgaaaacgac tctgcatggg aatggcttgc 121 cttacaatga cagaaatgga **A**ggaacatcc acatcccctg tacatcagaa tggtgatatt 181 tctggaaatg ctaactctgt gaagcaaata gatccagtcc tacaggtcta tct**T**ta**T**cat 241 tcccttggga acgctgaggg ggatt**a**tctg cagtttctaa c**T**ggagagta tgttgctgaa 301 gagatctgta ttgctgcttc taaagcttgt ggtaagtatt aaaaaacagt gttt**ttcttc** 361 **ttattaacat atgcttggtt t**attatactc taacacaatg tacctgtgta a	rs377943180 synonymous A>G p.Glu12	not detected
				rs458668038 synonymous T>G p.Leu43	not detected
RIGHT PRIMER aaaccaagcatatgttaataagaagaa	58.07			rs463147606 synonymous T>C p.Tyr44	not detected
				rs383317698 synonymous T>G p.Thr59	not detected
LEFT PRIMER gggccctggacatactaagtg	60.75	265	*Bos taurus* exon 16 (highlighted in green) 1 **gggccctgga catactaagt g**ctcaaatat ttgtgggtta atatttgaat gtttatgcaa 61 ttaattttaa tag**gaagaaa aaacccttat tcatgggaat gtgtg**C**gcca aaaatattct** 121 **tcttatcaga gaagaagaca ggaagacagg aaatcctcct ttcatcaaac ttagtgatcc** 181 **tggcattagt A**ttacagttt tgccaaaaga ca**g**taagttc aacaggaatc aaatttaact 241 ttatt**aacct ttgcttggaa agagg**tataa aaatcatgct gttaattttt ctcaaacact	rs210330018 synonymous C>T p.Cys675	not detected
RIGHT PRIMER cctctttccaagcaaaggtt	58.44			rs210148032 missense A>G p.Ile704Val	detected and described as *JAK2*/e16/*Rsa*I
LEFT PRIMER aaatcaagagttggcacatcaa	59.61	222	*Bos taurus* exon 23 (highlighted in dark grey) 1 ggta**aaatca agagttggca catcaa**gtaa ctcttttaaa tatattacag gtatgcacca 61 gaatcactga cagagagcaa gttttctgtg gcttcagatg tttggagctt tggagtggtt 121 ct**G**tatgaac ttttcacata tattgacaag agtaaaagcc c**A**ccagcggt cagtatgctt 181 tttgtttact ttcaattttt ttttt**aacat gagaaaagcg tttcga**aaga ataatagtaa	rs461568961 synonymous G>T p.Leu1044	not detected
RIGHT PRIMER tcgaaacgcttttctcatgtt	59.87			rs211067160 synonymous A>G p.Pro1057	detected and described as *JAK2*/e23/*Hae*III

Primer (5’ > 3’)	Tm (°C)	Product size (bp)	sequence	possible SNP	status
LEFT PRIMER ttgaccttgttaaatgtatatgttctg	57.85	280	*Ovis aries* exon 6 (highlighted in pink) 1 gagttgacct tgttaaatgt atatgttctg aaaattatgc tatagattaa aatataataa 61 tagattaaaa tgtgatagtg aaacttaagt attttcttct atattttgtt tttactttag 121 tggcggcatg atttttttaca tggatggata aaagtacccg tgactcatga aacacaggaa 181 gaAtgtcttg ggatggcagt gttagatatg atgagaatag ccaaagaaaa gaatcaaacG 241 ccactggaca tctatagctc tatcaggtaa ttttcttatg caaatccata tgagtatgac	rs160146162 synonymous A>G p.Glu177	detected and described as *JAK2*/e6/*Ear*I
RIGHT PRIMER ttgcataagaaaattacctgatagagc	60.36			rs160146160 synonymous G>A p.Thr196	detected and described as *JAK2*/e6/seq
LEFT PRIMER ctcagtgtgttttgattttatgtata	54.71	253	*Ovis aries* exon 12 (highlighted in light grey) 1 ctcagtgtgt tttgatttta tgtatattttt ttcattcatt ctatcctttt tttaaacaga 61 caaatcaaac cttctagtct tcagaaccaa tggtgtttct Gatgtaccaa cctcaccaac 121 tttacaaagg cataataaTg tgaaccaaat ggtgttccac aaaattagaa atgaagattt 181 ggtatttgta agtcaataga tgctgattat tgtctttttg tctttttaaa caactgttct 241 tcattttcta ccc	rs592691324 missense G>C p.Asp519His	not detected
RIGHT PRIMER gggtagaaaatgaagaacagttg	57.01			rs1094682814 missense T>G p.Asn531Lys	not detected
LEFT PRIMER ggacatatttcctaattcccaca	59.11	220	*Ovis aries* exon 13 (highlighted in yellow) 1 ttggacatat ttcctaattc ccacattttc ttccatcctt aatcattctt tctttttttt 61 ttttttttt ttttggttaa agaatgaaag ccttggccaa ggaacttta caaaaatttt 121 taaaggtatA agaagagaaa taggagacTa tggtcagctg catgaaacag aagttctttt 181 aaaagttctg gataaagcac atagaaacta ttcagaggtt tgtatattct ttatataatt	rs416867672 missense A>G p.Ile563Met	not detected
RIGHT PRIMER caaacctctgaatagtttctatgtgc	59.66			rs1086855307 missense T>C p.Tyr570His	not detected
LEFT PRIMER tctgcttgaaattaaatgtaccaaa	59.17	261	*Ovis aries* exon 24 (highlighted in red) 1 ttgagaattc tgcttgaaat taaatgtacc aaaaatatgt cattgaaaag tgggtttgcg 61 tttcaggaat ttatgcgtat gattggcaat gacaaacaag gacagatgat cgtgtttcat 121 ttgatagaac tcctAaagaa taatggaaga ttacCgagac cagatggatg cccagatgag 181 gtaacaaaaa tttttttttat ccacagtaat catgcatttt ctttctcttt ttacccaagg 241 atttcAggtc agtttatgca gttcactgaa ct	rs160146116 synonymous A>G p.Leu1082	detected and described as *JAK2*/e24/ *Hpy*188III
RIGHT PRIMER tcagtgaactgcataaactgacc	59.30			rs1094390234 missense C>T p.Pro1089Leu	not detected
				rs429445187 intronic A>G	detected

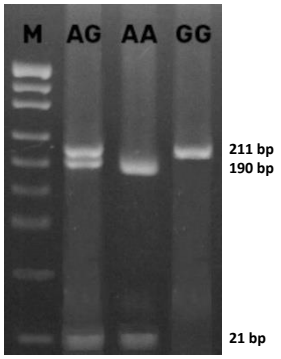

Figure S1. Electrophoretic separation of restriction fragments resulting from digestion with the *Rsa*I restriction enzyme (ACRS-PCR; bovine *JAK2*/e16/*Rsa*I polymorphism); lane 1 (M) - mass standard pUC19 DNA/*Msp*I; lane 2 - *AG* genotype; lane 3 - *AA* genotype; lane 4 - *GG* genotype.

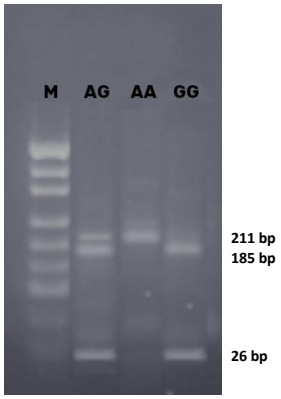

Figure S2. Electrophoretic separation of restriction fragments resulting from digestion with the *Hae*III restriction enzyme (ACRS-PCR; bovine *JAK2*/e23/*Hae*III polymorphism); lane 1 (M) - mass standard pUC19 DNA/*Msp*I; lane 2 - *AG* genotype; lane 3 - *AA* genotype; lane 4 - *GG* genotype.

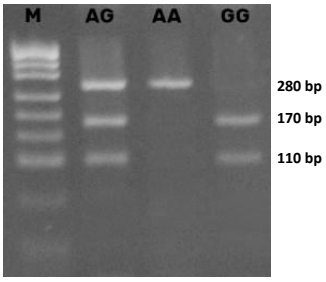

Figure S3. Electrophoretic separation of restriction fragments resulting from digestion with the *Ear*I restriction enzyme (PCR-RFLP; ovine *JAK2*/e6/*Ear*I polymorphism) polymorphic site; lane 1 (M) - mass standard pUC19 DNA/*Msp*I; lane 2 - *AG* genotype; lane 3 - *AA* genotype; lane 4 - *GG* genotype.

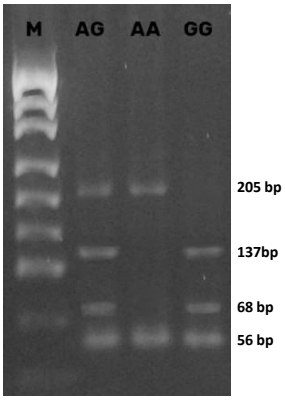

Figure S4. Electrophoretic separation of restriction fragments resulting from digestion with the *Hpy*188III restriction enzyme (PCR-RFLP; ovine *JAK2*/e24/*Hpy*188III polymorphism); lane 1 (M) - mass standard pUC19 DNA/*Msp*I; lane 2 - *AG* genotype; lane 3 - *AA* genotype; lane 4 - *GG* genotype.

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