Szkoła Główna Gospodarstwa Wiejskiego

w Warszawie

Instytut Nauk o Zwierzętach

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Wpływ wybranych czynników genetycznych i fizjologicznych na cechy użytkowości mlecznej i mięsnej bydła, ze szczególnym uwzględnieniem zmian o charakterze antyoksydacyjnym

Influence of selected genetic and physiological factors on milk and meat performance traits in cattle, with particular reference to antioxidant changes

Rozprawa doktorska

Doctoral thesis

Rozprawa doktorska wykonana pod kierunkiem:

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Spis treści

Streszczenie

Rozprawę doktorską stanowi zbiór sześciu opublikowanych i powiązanych tematycznie artykułów naukowych. Celem pracy była ocena wpływu deficytu energetycznego, strategii krzyżowania oraz systemów odchowu cieląt na właściwości antyoksydacyjne i stabilność oksydacyjną mleka oraz mięsa w odniesieniu do redukcji skutków chowu wsobnego w populacji bydła rasy polskiej holsztyńsko-fryzyjskiej (PHF).

Wysoka produkcja mleka u krów stanowiła przez wiele lat priorytet w programach hodowlanych, co zaowocowało znaczącym postępem genetycznym w zakresie cech produkcyjnych. Niemniej jednak, osiągnięcie wysokiego potencjału genetycznego wiąże się z rosnącymi wymaganiami fizjologicznymi zwierząt, zwłaszcza w okresie przejściowym. W tym kluczowym okresie, obejmującym czas przed porodem i w początkowej fazie laktacji, krowy często nie są w stanie pokryć zapotrzebowania energetycznego z paszy. Niedobór energii zmusza organizm do mobilizacji rezerw endogennych, co sprzyja rozwojowi ketozy – zaburzenia metabolicznego, które często inicjuje kaskadę kolejnych problemów zdrowotnych. Powszechne występowanie takich schorzeń jest efektem długotrwałej selekcji w kierunku poprawy cech produkcyjnych, w tym stosowania w rozrodzie wyłącznie zwierząt o najwyższej wartości hodowlanej. Wysoki poziom inbredu w populacji PHF doprowadził do obniżenia zmienności genetycznej oraz osłabienia cech użytkowych, w tym wydajności mlecznej i jakości mleka, a zwłaszcza zawartości białka i tłuszczu. Alternatywą mającą na celu ograniczenie lub całkowite wyeliminowanie skutków inbredu jest wykorzystanie krzyżowania międzyrasowego, a zwłaszcza wystąpienie związanego z krzyżowaniem efektu heterozji. Wystąpienie tego zjawiska przynosi korzyści związane ze wzrostem wartości cech zwłaszcza niskoodziedziczalnych cech funkcjonalnych, dzięki czemu wpływa bezpośrednio na jakość pozyskiwanego mleka. Krzyżowanie międzyrasowe można wykorzystać również do zwiększenia jakości mięsa w stadach bydła mlecznego poprzez wykorzystanie krzyżowania towarowego, gdzie krowy rasy HF kryte są nasieniem wyspecjalizowanych ras mięsnych. Mimo korzyści związanych z wykorzystaniem krzyżowania międzyrasowego nie należy całkowicie wyeliminować hodowli zwierząt czystorasowych, które powinny być podstawą hodowli. Potencjał tej rasy można wykorzystać do produkcji wysokiej jakości cielęciny poprzez odpowiednie żywienie oraz poprawę dobrostanu.

Celem doświadczenia pierwszego było określenie wpływu deficytu energetycznego na profil lipidowy mleka krów. W ramach badania analizowano próbki mleka oraz krwi pochodzące od 55 krów wieloródek i 50 krów pierwiastek, które podzielono na grupę zwierząt zdrowych oraz grupę krów ze zdiagnozowaną ketozą. Łącznie pobrano 315 próbek mleka (trzykrotnie od każdej krowy w odstępach tygodniowych, począwszy od 5. ± 2 dnia po porodzie) oraz 105 próbek krwi (w 5. ± 2 dniu po porodzie). W próbkach mleka analizowano skład podstawowy oraz profil kwasów tłuszczowych, natomiast w próbkach krwi oznaczano stężenie kwasu β-hydroksymasłowego (BHBA). Wyniki badania wykazały, że deficyt energetyczny w początkowym okresie laktacji istotnie ograniczał biosyntezę długołańcuchowych kwasów tłuszczowych, w tym izomerów skoniugowanych dienów kwasu linolowego (CLA). Obniżony poziom CLA w mleku krów dotkniętych deficytem energetycznym wskazywał na zmiany w aktywności enzymów odpowiedzialnych za syntezę, m.in. delta-9-desaturazy. Wyniki uzyskane w doświadczeniu pierwszym potwierdziły hipotezę, że deficyt energetyczny wpływa na ograniczenie syntezy CLA, co prowadzi do zmiany profilu lipidowego mleka.

Celem doświadczenia drugiego było zbadanie wpływu krzyżowania międzyrasowego na parametry użytkowe mleka. W ramach badania analizowano mleko pochodzące od 50 krów rasy polskiej holsztyńsko-fryzyjskiej (PHF) oraz 50 mieszańców PHF×SRB (szwedzka czerwona). Próbki mleka pobierano dziesięciokrotnie w trakcie laktacji, począwszy od pierwszego miesiąca po wycieleniu, co pozwoliło zgromadzić łącznie 1000 próbek. W mleku analizowano podstawowy skład chemiczny mleka oraz liczbę komórek somatycznych. Badania wykazały istotne różnice w wydajności oraz poziomie parametrów użytkowych mleka. Mieszańce PHF×SRB charakteryzowały się wyższą zawartością tłuszczu, białka oraz suchej masy w porównaniu do krów czystorasowych PHF, co sugeruje korzystny wpływ rasy SRB na jakość mleka. Wyższa zawartość tłuszczu, białka oraz suchej masy w mleku mieszańców PHF×SRB może wynikać z ich zdolności adaptacyjnej do zmiennych warunków środowiskowych oraz lepszej tolerancji na stres oksydacyjny charakterystyczny dla początkowego okresu laktacji. Wyniki badania potwierdzają hipotezę o pozytywnym wpływie krzyżowania międzyrasowego na jakość i skład chemiczny mleka, jednocześnie podkreślając potencjalne korzyści z jego zastosowania w praktyce hodowlanej. Uzyskane dane wskazują, że krzyżowanie międzyrasowe może być skutecznym narzędziem do poprawy parametrów użytkowych mleka w warunkach intensywnej produkcji.

W doświadczeniu trzecim celem było określenie wpływu krzyżowania międzyrasowego na właściwości antyoksydacyjne i stabilność oksydacyjną mleka. Do doświadczenia wybrano 60 krów podzielonych na dwie grupy: 30 krów rasy PHF oraz 30 krów mieszańców międzyrasowych PHF×SRB. Od zwierząt pobrano łącznie po 600 prób mleka oraz krwi, które pobierane były tego samego dnia, 10-krotnie w trakcie laktacji od 5± 2 dnia do 280 ±5 dnia laktacji. W przeprowadzonym badaniu w mleku oznaczono podstawowy skład chemiczny, zawartość białek serwatkowych oraz profil kwasów tłuszczowych. W próbkach krwi analizowano profil metaboliczny oraz całkowity potencjał antyoksydacyjny (TAS). Wyniki doświadczenia wykazały wyższą stabilność oksydacyjną mleka u mieszańców PHF×SRB w porównaniu do krów czystorasowych. Zwiększona aktywność enzymów antyoksydacyjnych w grupie mieszańców PHF×SRB może być efektem korzystnych mechanizmów adaptacyjnych, obejmujących efektywną detoksykację wolnych rodników oraz zmniejszoną podatność na uszkodzenia oksydacyjne. Wyniki potwierdzają hipotezę o korzystnym wpływie krzyżowania międzyrasowego na stabilność oksydacyjną mleka i zdolności antyoksydacyjne organizmu.

Celem czwartego doświadczenia było zbadanie wpływu genotypu oraz wieku pierwszego wycielenia na wybrane parametry rozrodu, profil metaboliczny oraz skład kwasów tłuszczowych mleka. Do analizy wybrano 60 krów, w tym 30 osobników rasy polskiej holsztyńsko-fryzyjskiej (PHF) oraz 30 mieszańców F1 (PHF×SRB – szwedzka czerwona). Zwierzęta podzielono na grupy w zależności od wieku pierwszego wycielenia: poniżej 24 miesięcy oraz powyżej 24 miesięcy. Każda z czterech grup liczyła 15 krów. W 35 ± 5 dniu laktacji pobrano próbki mleka i krwi. W mleku wykonano analizy składu podstawowego oraz profilu kwasów tłuszczowych, natomiast w próbkach krwi oznaczono parametry metaboliczne, w tym wskaźniki bilansu energetycznego. Dodatkowo, na podstawie dokumentacji hodowlanej, przeanalizowano parametry rozrodu, takie jak długość okresu międzyciążowego, wskaźnik zacieleń oraz długość okresu międzywycieleniowego. Uzyskane wyniki wykazały, że mieszańce F_1 odznaczały się wyższą stabilnością metaboliczną oraz mniejszą podatnością na wystąpienie ujemnego bilansu energetycznego w początkowym okresie laktacji w porównaniu do krów czystorasowych PHF. Zdolność do efektywnego wykorzystania energii w początkowym okresie laktacji może wpływać na wyższą wydajność mleczną oraz korzystniejszy profil kwasów tłuszczowych. Dodatkowo, lepsza adaptacja metaboliczna może przekładać się na korzystniejsze parametry rozrodu, w tym krótszy okres

międzyciążowy oraz wyższą skuteczność inseminacji. Wyniki te potwierdzają korzyści płynące z krzyżowania międzyrasowego, które przyczynia się do poprawy efektywności produkcyjnej i zdrowotności zwierząt w intensywnych systemach hodowlanych.

Celem doświadczenia piątego było określenie wpływu genotypu na potencjał antyoksydacyjny tkanki mięśniowej buhajów ras polska holsztyńsko-fryzyjska (PHF), limousine (LM) oraz mieszańców międzyrasowych PHF×LM. Do badania wybrano 62 buhaje, w tym 12 osobników rasy PHF, 25 rasy LM oraz 25 mieszańców PHF×LM. Po uboju pobrano próbki tkanki mięśniowej z mięśnia półbłoniastego, które następnie poddano analizie składu podstawowego, profilu kwasów tłuszczowych, zawartości bioaktywnych białek oraz witamin rozpuszczalnych w tłuszczach. Uzyskane wyniki potwierdziły hipotezę o wyższej zawartości bioaktywnych składników, w tym kwasów tłuszczowych omega-3, oraz wyższej aktywności enzymów antyoksydacyjnych w tkance mięśniowej buhajów mieszańców PHF×LM w porównaniu do zwierząt czystorasowych. Wzrost aktywności enzymów antyoksydacyjnych w mięśniach mieszańców F_1 wskazuje na korzystne efekty krzyżowania towarowego w kontekście poprawy mechanizmów obronnych przed stresem oksydacyjnym. Zwiększona aktywność systemu antyoksydacyjnego w tkance mięśniowej może wskazywać na lepszą ochronę przed uszkodzeniami oksydacyjnymi, co w konsekwencji może wpływać na poprawę jakości mięsa, jego trwałość oraz właściwości odżywcze, w tym zmniejszenie utleniania lipidów, co jest korzystne dla jakości sensorycznej i wartości prozdrowotnych produktu. Wyniki te potwierdzają, że krzyżowanie ras PHF i LM może stanowić efektywną strategię hodowlaną w kontekście poprawy jakości mięsa poprzez zwiększenie potencjału antyoksydacyjnego i ochrony przed stresem oksydacyjnym.

Celem ostatniego doświadczenia było określenie wpływu systemu odchowu na kształtowanie potencjału antyoksydacyjnego tkanki mięśniowej cieląt. Do badania wybrano dwie grupy po 15 cieląt każda. Pierwsza grupa była utrzymywana w kojcach, gdzie mleko było pobierane przez cielęta z automatów wyposażonych w smoczek, podczas gdy druga grupa była odchowywana przez mamki. W trakcie doświadczenia monitorowano stan zdrowia cieląt na podstawie obserwacji przeprowadzonych przez lekarza weterynarii, oceniano ich zachowanie oraz rejestrowano przyrosty masy ciała. Po zakończeniu 6-miesięcznego okresu odchowu, cielęta zostały poddane ubojowi, a następnie pobrano próbki tkanki mięśniowej z mięśnia półbłoniastego. W próbkach wykonano oznaczenia podstawowego składu, zawartości kwasów tłuszczowych, mioglobuliny, dialdehydu malonowego (MDA) oraz barwy mięsa. Wyniki

doświadczenia wykazały, że cielęta odchowywane przez mamki charakteryzowały się lepszą odpornością na stres oksydacyjny, mniejszą liczbą problemów zdrowotnych oraz wyższymi przyrostami masy ciała w porównaniu do cieląt utrzymywanych w kojcach. System odchowu cieląt w warunkach naturalnych, z mamką, sprzyjał lepszemu rozwojowi mechanizmów ochrony przed stresem oksydacyjnym, co przekładało się na korzystniejszy skład kwasów tłuszczowych w tkance mięśniowej oraz lepszą jakość mięsa. W tkance mięśniowej cieląt odchowywanych przez mamki zaobserwowano wyższą aktywność enzymów antyoksydacyjnych oraz niższy poziom MDA, co sugeruje mniejsze uszkodzenia oksydacyjne. Uzyskane wyniki potwierdzają hipotezę o istotnym wpływie systemu odchowu na kształtowanie potencjału antyoksydacyjnego tkanki mięśniowej cieląt, a także na stabilność oksydacyjną mięsa. Optymalizacja systemu odchowu cieląt w kierunku zminimalizowania stresu oksydacyjnego może mieć istotne znaczenie dla poprawy jakości mięsa, jego wartości odżywczych oraz trwałości.

Słowa kluczowe: czynniki genetyczne, czynniki fizjologiczne, użytkowość mleczna, użytkowość mięsna, potencjał antyoksydacyjny, bydło

Summary

The dissertation consists of six published and thematically related scientific articles. The aim of the dissertation was to evaluate the effects of energy deficit, crossbreeding strategies and calf rearing systems on antioxidant properties and oxidative stability of milk and meat in relation to reducing the effects of inbreeding in the Polish Holstein-Friesian (PHF) cattle population.

High milk production in cows has been a priority in breeding programs for many years, resulting in significant genetic progress in production traits. Nevertheless, achieving high genetic potential is associated with increasing physiological demands on animals, especially during the transition period. During this crucial period, which includes the time before parturition and in the early stages of lactation, cows are often unable to meet their energy requirements from feed. Energy deficiency forces the body to mobilize endogenous reserves, which promotes the development of ketosis - a metabolic disorder that often initiates a cascade of subsequent health problems. The widespread occurrence of such conditions is the result of long-term selection for improved production traits, including the use of only animals with the highest breeding value in reproduction. The high level of crossbreeding in the PHF population has led to a reduction in genetic variability and a weakening of functional traits, including milk yield and milk quality, especially protein and fat content. An alternative to reduce or completely eliminate the effects of crossbreeding is the use of crossbreeding, especially the occurrence of the crossbreeding-related heterosis effect. The occurrence of this phenomenon brings benefits related to an increase in the value of traits, especially low-inbreeding functional traits, thus directly affecting the quality of the milk obtained. Crossbreeding can also be used to increase meat quality in dairy herds through the use of commodity crossbreeding, where HF cows are covered with semen from specialized meat breeds. Despite the benefits related to the use of crossbreeding, the breeding of purebred animals, which should be the basis of breeding, should not be completely eliminated. The potential of this breed can be used to produce high-quality veal through proper nutrition and improved welfare.

The purpose of experiment one was to determine the effect of energy deficit on the lipid profile of cows' milk. The study analyzed milk and blood samples from 55 multiparous and 50 primiparous cows, which were divided into a group of healthy animals and a group of cows diagnosed with ketosis. A total of 315 milk samples (three times from each cow at weekly intervals, starting on the 5th ± 2 days after parturition) and 105

blood samples (on the 5th ± 2 days after parturition) were collected. The milk samples were analyzed for basal composition and fatty acid profile, while β-hydroxybutyric acid (BHBA) concentration was determined in blood samples. The results of the study showed that energy deficit during the early lactation period significantly reduced the biosynthesis of long-chain fatty acids, including isomers of conjugated linoleic acid dienes (CLA). Reduced levels of CLA in the milk of energy-deficient cows indicated changes in the activity of enzymes responsible for synthesis, including delta-9-desaturase. The results obtained in experiment one supported the hypothesis that energy deficit affects the reduction of CLA synthesis, leading to changes in the lipid profile of milk.

The purpose of experiment two was to study the effect of crossbreeding between breeds on milk performance parameters. The study analyzed milk from 50 Polish Holstein-Friesian (PHF) cows and 50 PHF×SRB (Swedish Red) hybrids. Milk samples were taken ten times during lactation, starting from the first month after calving, which allowed a total of 1,000 samples to be collected. The basic chemical composition of the milk and the number of somatic cells were analyzed in the milk. The study showed significant differences in yield and levels of milk functional parameters. PHF×SRB hybrids had higher fat, protein, and dry matter contents compared to purebred PHF cows, suggesting a favourable effect of the SRB breed on milk quality. The higher fat, protein, and dry matter content in the milk of PHF×SRB hybrids may be due to their adaptability to changing environmental conditions and better tolerance to oxidative stress characteristic of the early lactation period. The results of the study support the hypothesis that crossbreeding has a positive effect on milk quality and chemical composition while highlighting the potential benefits of its use in breeding practice. The data obtained indicate that crossbreeding can be an effective tool for improving milk performance under intensive production conditions.

In experiment three, the goal was to determine the effect of crossbreeding on the antioxidant properties and oxidative stability of milk. The experiment selected 60 cows divided into two groups: 30 PHF cows and 30 PHF × SRB crossbred hybrid cows. A total of 600 milk and blood samples each were taken from the animals, which were collected on the same day, 10 times during lactation, from 5±2 days to 280 ±5 days of lactation. In the study conducted, the basic chemical composition, whey protein content, and fatty acid profile were determined in milk. Metabolic profile and total antioxidant potential (TAS) were analyzed in blood samples. The results of the experiment showed higher oxidative stability of milk in PHF×SRB hybrids compared to purebred cows. The increased activity

of antioxidant enzymes in the PHF×SRB hybrid group may be the result of favourable adaptive mechanisms, including efficient detoxification of free radicals and reduced susceptibility to oxidative damage. The results support the hypothesis that crossbreeding has a beneficial effect on the oxidative stability of milk and the antioxidant capacity of the organism.

The purpose of the fourth experiment was to study the effect of genotype and age at first calving on selected reproductive parameters, metabolic profile, and milk fatty acid composition. Sixty cows were selected for analysis, including 30 individuals of the Polish Holstein-Friesian (PHF) breed and 30 F_1 hybrids (PHF×SRB - Swedish Red). The animals were divided into groups according to the age of first calving: under 24 months and over 24 months. Each of the four groups consisted of 15 cows. On the 35th ± 5th day of lactation, milk and blood samples were taken. Basic composition and fatty acid profile analyses were performed in the milk, while metabolic parameters, including energy balance indices, were determined in the blood samples. In addition, reproductive parameters such as the length of the inter-pregnancy period, the mating rate and the length of the inter-breeding period were analyzed on the basis of breeding records. The results showed that F_1 hybrids were characterized by higher metabolic stability and less susceptibility to the occurrence of negative energy balance during the early lactation period compared to purebred PHF cows. The ability to use energy efficiently during the early lactation period may influence higher milk yields and a more favourable fatty acid profile. In addition, better metabolic adaptation may translate into more favourable reproductive parameters, including a shorter inter-pregnancy period and higher insemination efficiency. These results confirm the benefits of crossbreeding, which contributes to improved production efficiency and animal health in intensive breeding systems.

The purpose of experiment five was to determine the effect of genotype on the antioxidant potential of muscle tissue of bulls of the Polish Holstein-Friesian (PHF), Limousin (LM) and PHF×LM crossbred hybrids. Sixty-two bulls were selected for the study, including 12 individuals of the PHF breed, 25 of the LM breed and 25 of the PHF×LM hybrids. After slaughter, muscle tissue samples were taken from the semimembranosus muscle, which was then analyzed for basal composition, fatty acid profile, bioactive protein content and fat-soluble vitamins. The results confirmed the hypothesis of a higher content of bioactive components, including omega-3 fatty acids, and a higher activity of antioxidant enzymes in the muscle tissue of PHF×LM hybrid bulls

compared to purebred animals. The increased activity of antioxidant enzymes in the muscles of F_1 hybrids indicates the beneficial effects of commodity crossbreeding in terms of improving defense mechanisms against oxidative stress. Increased activity of the antioxidant system in muscle tissue may indicate improved protection against oxidative damage, which may consequently improve meat quality, shelf life and nutritional properties, including reduced lipid oxidation, which is beneficial to the sensory quality and health-promoting values of the product. These results confirm that crossbreeding between PHF and LM breeds can be an effective breeding strategy for improving meat quality by increasing antioxidant potential and protection against oxidative stress.

The purpose of the latest experiment was to determine the effect of the rearing system on shaping the antioxidant potential of calf muscle tissue. Two groups of 15 calves each were selected for the study. The first group was kept in pens where milk was taken by the calves from automatic machines equipped with a teat, while the second group was reared by foster cows. During the experiment, the health of the calves was monitored based on observations by a veterinarian, their behaviour was evaluated, and weight gains were recorded. At the end of the 6-month rearing period, the calves were slaughtered, and muscle tissue samples were taken from the semimembranosus muscle. The samples were used to determine the basic composition, fatty acid content, myoglobulin, malondialdehyde (MDA) and meat colour. The results of the experiment showed that calves reared by sucklers had better resistance to oxidative stress, fewer health problems and higher weight gains compared to calves kept in pens. The system of rearing calves under natural conditions with a mom promoted better development of protection mechanisms against oxidative stress, which translated into more favorable fatty acid composition in muscle tissue and better meat quality. Higher activity of antioxidant enzymes and lower levels of MDA were observed in the muscle tissue of calves reared by mothers, suggesting less oxidative damage. The results support the hypothesis that the rearing system has a significant effect on shaping the antioxidant potential of calf muscle tissue, as well as on the oxidative stability of meat. Optimizing the calf rearing system to minimize oxidative stress may be important for improving meat quality, nutritional value and shelf life.

Key words: cattle, genetic factors, physiological factors, dairy performance, meat performance, antioxidant potential

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Rozprawę doktorską stanowi zbiór sześciu opublikowanych i powiązanych tematycznie artykułów naukowych:

Publikacja 1. Solarczyk P., Gołębiewski M., Slósarz J., Puppel K. 2023. Interaction Between the Concentration of β-Hydroxybutyric Acid and the Content of Long-Chain Fatty Acids in the Early Stage of Lactation—Comparing Multiparous and Primiparous Cows. *Applied Sciences* 13(13), 7870. doi:10.3390/app13137870

(100 pkt. MNiSW; IF 2,500; cyt. wg WoS: 1)

Publikacja 2. Solarczyk P., Slósarz J., Gołębiewski M., Puppel K. 2021. A comparison between Polish Holstein-Fresian and F_1 hybrid Polish Holstein-Fresian × Swedish Red cows in terms of milk yield traits, *Mljekarstvo* 71(2), 141-150. doi:10.15567/mljekarstvo.2021.0207

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(140 pkt. MNiSW; IF 3,300; cyt. wg WoS: 1)

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Wykaz stosowanych skrótów

a* – zaczerwienienie

ADG – dzienny przyrost

AFC – wiek pierwszego wycielenia

AFI – wiek pierwszej inseminacji

b* – zażółcenie

BHBA – kwas β-hydroksymasłowy

BLG – β-laktoglobulina

BSA – albumina surowicy bydlęcej

C – nasycenie

Cas – kazeina

CFU – liczba kolonii bakterii

CLA – skoniugowane dieny kwasu linolowego

CLA9 – C18:2 cis9, trans11

CLA10 – C18:2 trans10, cis12

DHA – kwas dekozaheksaenowy

EPA – kwas eikopantaenowy

F/P – stosunek tłuszczu do białka

F_1 – pierwsze pokolenie mieszańców międzyrasowych

FA – kwasy tłuszczowe

FAME – estry metylowe kwasów tłuszczowych

GGTP – γ-glutamylotransferaza

GL – długość ciąży

GUS – Główny Urząd Statystyczny

h° – kąt barwy

HF – rasa holsztyńsko-fryzyjska

HO – odmiana czarno-biała rasy holsztyńsko-fryzyjskiej

IP – okres międzyciążowy

L* – jasność

LA – kwas linolowy C18:2 n-6

Lf – laktoferyna

LNA – kwas α-linolowy C18:3 n-3

LM – rasa limousine

Lp – laktoperoksydaza

LSM – średnie najmniejszych kwadratów

Lz – lizozym

MDA – aldehyd dimalonowy

MH – krowy wieloródki zdrowe

MK – krowy wieloródki z ketozą

MUFA – jednonienasycone kwasy tłuszczowe

NEB – ujemny bilans energetyczny

NEFA – nieestryfikowane kwasy tłuszczowe

OWUB – Ocena Wartości Użytkowej Bydła

PBC – okres międzywycieleniowy

PFHBiPM – Polska Federacja Hodowców Bydła i Producentów Mleka

PH – krowy pierwiastki zdrowe

PI – indeks zacieleń

PK – krowy pierwiastki z ketozą

PHF – rasa polska holsztyńsko-fryzyjska

PHF×LM – mieszaniec międzyrasowy krów rasy polskiej holsztyńsko-fryzyjskiej i buhajów rasy limousine

PHF×SRB – mieszaniec międzyrasowy krów rasy polskiej holsztyńsko-fryzyjskiej i buhajów rasy szwedzkiej czerwonej

PPD – okres przestoju poporodowego

PUFA – wielonienasycone kwasy tłuszczowe

RP HPLC – wysokosprawna chromatografia cieczowa w odwróconym układzie faz

RW – odmiana czerwono-biała rasy holsztyńsko-fryzyjskiej

SCE – składowe zwierciadła

SEM – błąd standardowy

SFA – nasycone kwasy tłuszczowe

SP – okres usługi

SRB – rasa szwedzka czerwona

TAS – całkowity status antyoksydacyjny

TBARS – kwas 2-tiobarbiturowy

TEO – 1,1,3,3-tetraetoksypropan

TMR – total mixed ration

TVA – kwas trans wakcenowy, C18:1 trans 11

UFA – nienasycone kwasy tłuszczowe

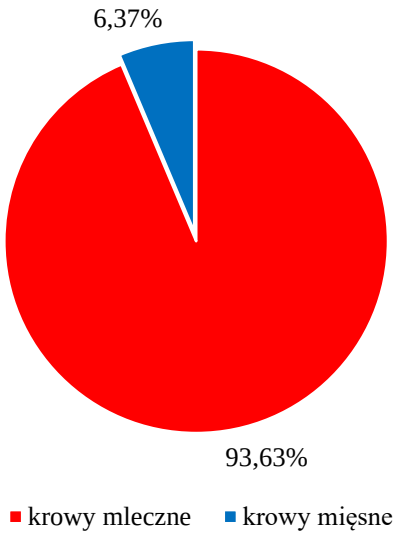
ZB – polska czarno-biała

Wp – białka serwatkowe

ZR – polska czerwono-biała

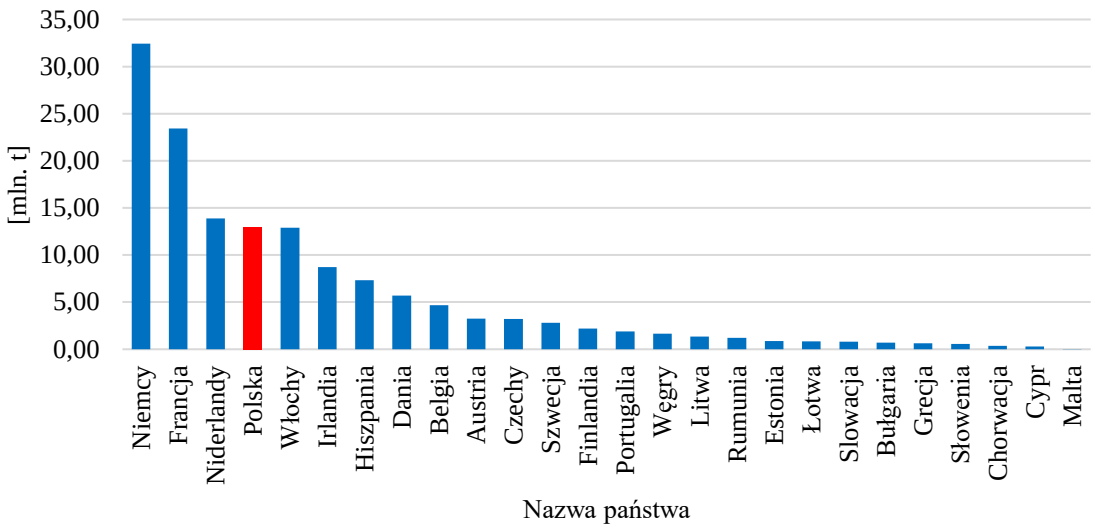
1. Wstęp

W produkcji zwierzęcej kluczowymi obszarami działalności są produkcja jaj, mięsa i mleka. Bydło jako jedno z głównych gatunków hodowlanych, odgrywa znaczącą rolę w dostarczaniu mleka i mięsa o wysokiej wartości odżywczej. W Polsce populacja bydła liczy 6,435 miliona sztuk, z czego 2,203 miliona stanowią krowy mleczne. W obrębie tej populacji dominują krowy mleczne, które stanowią 93,63% całości, podczas gdy krów mięsnych jest zaledwie 6,37%. W Polsce dominującym kierunkiem użytkowania bydła jest produkcja mleka, co odzwierciedla proporcjonalny udział krów mlecznych w ogólnym pogłowiu bydła [**Wykres 1**] (GUS, 2024).



Wykres 1. Struktura krów w Polsce w 2023 roku [opracowanie własne na podstawie (GUS, 2024)].

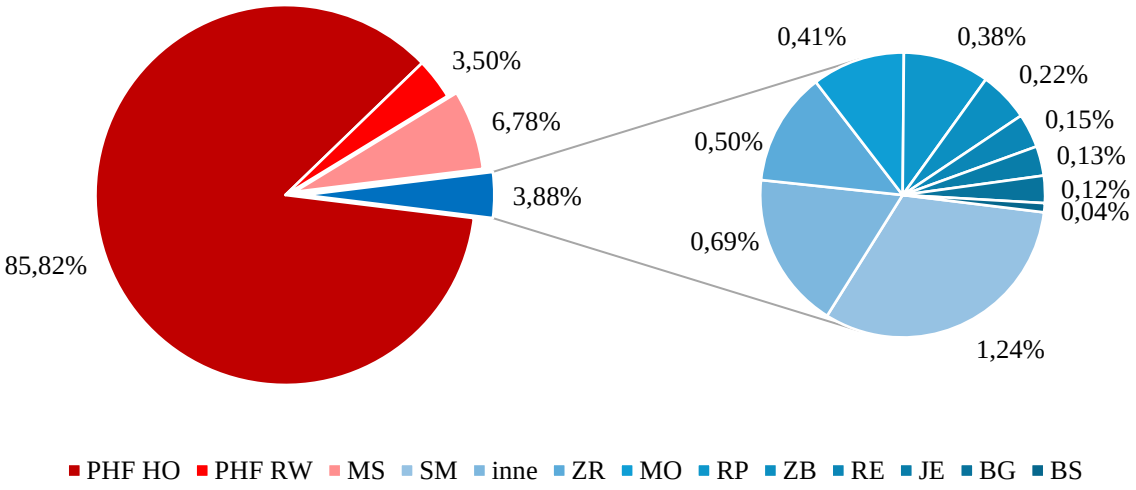
Znaczenie produkcji mleka w Polsce bez wątpienia potwierdza wysoka 4. pozycja pod tym względem w Unii Europejskiej z produkcją na poziomie prawie 13 milionów ton rocznie [**Wykres 2**] (CLAL, 2024).



Wykres 2. Produkcja mleka w poszczególnych krajach Unii Europejskiej w 2023 r. (CLAL, 2024).

Wysoka pozycja, jaką Polska zajmuje wśród producentów mleka, nie jest wypadkową jedynie liczebności krów, ale także ich wydajności. Jak podaje Polska Federacja Hodowców Bydła i Producentów Mleka (PFHBiPM) średnia wydajność mleczna krów w Polsce w 2023 roku wynosiła 7 596 kg (PFHBiPM, 2024) i była wyższa od wydajności z poprzedniego roku o 2,3%.

Produkcja mleka oparta jest przede wszystkim na krowach w typie mlecznym oraz kombinowanym, a dominującą rolę spełnia lokalna odmiana najbardziej popularnej na całym świecie rasy holsztyńsko fryzyjskiej (HF) Polska holsztyńsko fryzyjska (PHF) występująca w dwóch odmianach barwnych: czarno-białej (HO) oraz czerwono-białej (RW), co stanowi łącznie 89,32% pogłowia krów [**Wykres 2**] (PFHBiPM, 2024).



Wykres 3. Struktura rasowa krów mlecznych objętych Oceną Wartości Użytkowej Bydła (OWUB) w 2023 roku

Rasa PHF powstała w wyniku krzyżowania wypierającego krów rasy polskiej czarno-białej (ZB; dawniej nizinnej czarno-białej) oraz polskiej czerwono-białej (ZR; dawniej nizinnej czerwono-białej), które inseminowane były importowanym nasieniem buhajów rasy HF z Ameryki Północnej oraz Europy Zachodniej (Nowicki i in., 2011). Swoją popularność bydło rasy HF zawdzięcza wysokiemu potencjałowi produkcyjnemu, który jest wynikiem konsekwentnie prowadzonej przez wiele lat pracy hodowlanej w kierunku zwiększenia wydajności mleka. Osiągnięcie tego celu możliwe było dzięki rozwojowi i wykorzystaniu biotechnik rozrodu, polegających na zwiększeniu możliwości rozrodczych samic i samców, w tym przede wszystkim zastosowaniu inseminacji (Diskin, 2018; Moore i Hasler, 2017). Do rozrodu kierowane były tylko najlepsze osobniki pod względem wartości cech, a selekcja w określonym kierunku przebiegała w efektywny sposób dzięki czemu była skuteczna, a wartość hodowlana zwierząt uległa zwiększeniu, a wraz nią ich potencjał produkcyjny (Miglior i in., 2017; Moore i Hasler, 2017). Niestety wraz z wykorzystaniem w hodowli jedynie najlepszych osobników pod względem produkcyjnym oraz zwiększonymi możliwościami rozrodczymi, wybitne osobniki uzyskują dużą liczbę potomstwa, co prowadzi do ograniczenia puli genetycznej poprzez wzrost homozygotyczności i zmniejszenie bioróżnorodności w obrębie populacji (Crowe i in., 2018; Gutiérrez-Reinoso i in., 2022). Taki model hodowli sprzyja narastaniu współczynnika spokrewnienia, a w konsekwencji wzrostu inbredu, a także możliwości wystąpienia depresji inbredowej, która powoduje obniżenie wartości cech prowadząc do

obniżenia produkcji oraz zwiększenia częstotliwości problemów zdrowotnych u zwierząt (Doekes i in., 2019; Gutiérrez-Reinoso i in., 2022). Wraz z narastaniem homozygotyczności w obrębie populacji częściej ujawniają się także recesywne allele letalne i semiletalne, które w przypadku bydła posiadającego niską zdolność rozrodczą są cechą szczególnie niekorzystną (Fritz i in., 2013; VanRaden i in., 2011). Dodatkowo uwzględnienie w indeksach selekcyjnych jedynie cech związanych z produkcją mleka oraz jego składem podstawowym, spowodowało obniżenie wartości drugorzędowych, niskoodziedziczalnych cech funkcjonalnych takich jak zdrowotność, rozród oraz długowieczność (Miglior i in., 2017). Według danych PFHBiPM średnia długość życia krów populacji aktywnej wynosi 5,37 lat (PFHBiPM, 2024), a długość życia krów wpływa na efektywność ekonomiczną gospodarstw (Dallago i in., 2021). Wczesne brakowanie i wyższy wskaźnik śmiertelności u krów związany jest bardzo często związany z wyższą średnią wydajnością mleczną (Alvåsen i in., 2014), co bardzo często powoduje problemy zdrowotne eliminujące krowy z dalszej produkcji (Adamczyk i in., 2017). Głównymi przyczynami brakowania krów ze stada są problemy z rozrodem, kulawizny, zapalenie gruczołu mlekowego oraz choroby metaboliczne (Dallago i in., 2021). Te ostatnie stanowią duży problem we współczesnych gospodarstwa mlecznych. Najczęściej występującym schorzeniem z tej grupy jest ketoza, która może dotyczyć nawet 80% krów w stadzie (Gordon i in., 2013). Występuje zazwyczaj w tzw. okresie przejściowym obejmującym końcowy etap ciąży, wycielenie oraz początek laktacji. Jest to związane z dynamicznymi zmianami fizjologicznymi zaburzającymi homeostazę organizmu krów powodując rozwój stresu oksydacyjnego i obniżenie zdolności immunosupresyjnych o podłożu hormonalnym (Caixeta i Omontese, 2021; Horst i in., 2021; Raboisson i in., 2014). Do zaburzeń tych dochodzi ze względu na stres związany z porodem oraz zapoczątkowaniem produkcji siary i mleka, co powoduje zwiększone zapotrzebowanie na składniki odżywcze (Solarczyk i in., 2024). Skutkuje to powstaniem ujemnego bilansu energetycznego (NEB), dlatego krowy zaczynają wykorzystywać rezerwy tłuszczu, które zgromadziły w swoim ciele przekształcając go w energię i ciała ketonowe (Puppel i in., 2019). Dlatego też, ważnym aspektem jest odpowiedni sposób zarządzania stadem m.in. poprzez działania profilaktyczne i szybką diagnostykę (Mezzetti i in., 2021). Do diagnostyki wykorzystuje się testy do oznaczania poziomu ciał ketonowych, które są pośrednimi metabolitami zmobilizowanego tłuszczu. Ciała ketonowe powstają podczas ketogenezy zachodzącej w wątrobie w trakcie mitochondrialnej β-oksydacji długołańcuchowych kwasów tłuszczowych (FA) (White,

2015; Zhang i Ametaj, 2020). Niestety, wystąpienie NEB w okresie przejściowym jest bardzo często dopiero początkiem problemów, jakie hodowcy napotykają w przypadku krów mlecznych. Wraz z wystąpieniem NEB u krów pojawiają się również problemy z rozrodem, takie jak krótka, cicha ruja lub jej brak, co utrudnia wybór odpowiedniego czasu krycia krów. Ponadto ciąża często nie rozwija się, nawet jeśli dojdzie do zapłodnienia lub dochodzi do resorbcji lub zamierania zarodków we wczesnym etapie ich rozwoju. Konsekwencją wystąpienia NEB jest pogorszenie się wskaźników płodności tj. przestoju poporodowego, okresu usługi, indeksu zacieleń, okresu międzyciążowego i międzyocieleniowego (Sammad i in., 2022). Wystąpienie ketozy ma także odzwierciedlenie w jakości pozyskiwanego mleka od krów, którego skład chemiczny ulega zmianie (Puppel i in., 2019; Puppel i in., 2022; Puppel i in., 2017; Puppel i in., 2021; Solarczyk i in., 2023), ze względu na presję, jaką to schorzenie wywiera na szlaki metaboliczne w organizmie krowy (Solarczyk i in., 2024), a jest to szczególnie niekorzystne z punktu widzenia konsumentów.

Mleko uznawane jest za doskonały pokarm, w którego skład wchodzi ponad 250 różnych składników takich jak: wysokiej jakości białko, tłuszcz, laktoza, witaminy oraz mikro- i makroelementy (Foroutan i in., 2019). Wartość biologiczna białka mleka krowiego uznawana jest za jedną z najwyższych wśród białek pochodzenia zwierzęcego. Ze względu na pełny zestaw aminokwasów egzogennych w odpowiednich proporcjach, białko mleka krowiego charakteryzuje się wysoką przyswajalnością i doskonałą jakością odżywczą. Z tego względu mleko stanowi cenny surowiec w diecie człowieka, pełniąc istotną rolę w procesach metabolicznych oraz w zapewnieniu organizmowi niezbędnych składników odżywczych (Antunes i in., 2023; Solarczyk i in., 2024; Usman i Zeliha, 2020). Mleko krowie jest cennym źródłem energii, głównie dzięki zawartości tłuszczu, który składa się z ponad 400 różnych kwasów tłuszczowych. Kwasy te wykazują różnorodne właściwości bioaktywne, w tym działanie prozdrowotne, które ma istotny wpływ na zdrowie człowieka. Wśród nich znajdują się kwasy tłuszczowe o właściwościach przeciwzapalnych, wspierające układ immunologiczny, a także kwasy nasycone i nienasycone, które korzystnie wpływają na profil lipidowy oraz zdrowie układu sercowo-naczyniowego. Ponadto, tłuszcz mleczny jest źródłem izomerów sprzężonego kwasu linolowego (CLA), który wykazuje potencjalne działanie przeciwnowotworowe oraz wspomaga procesy metaboliczne organizmu (Solarczyk i in., 2024).

Alternatywą dla utrzymania wysokiej jakości mleka oraz optymalizacji cech funkcjonalnych bydła mlecznego jest zastosowanie krzyżowania międzyrasowego. Praktyka ta umożliwia wykorzystanie synergii pomiędzy genotypami różnych ras, co prowadzi do uzyskania potomstwa o lepszej kombinacji cech pożądanych, takich jak wydajność mleczna, jakość mleka, odporność na choroby oraz zdolności reprodukcyjne. Krzyżowanie międzyrasowe może również przyczyniać się do poprawy efektywności metabolicznej, zwłaszcza w kontekście adaptacji do zmieniających się warunków środowiskowych i produkcyjnych. Dodatkowo, zwiększona heterozygotyczność u mieszańców może prowadzić do poprawy zdrowotności stada, redukując wrażliwość na choroby oraz wspomagając stabilność biologiczną populacji. Ponadto, taka strategia hodowlana może przyczynić się do efektywnego zarządzania genotypową bioróżnorodnością w stadzie, co jest kluczowe dla długoterminowej stabilności oraz zrównoważonej produkcji mleka (Freyer i in., 2008; Heins i in., 2006a, 2006b). Metoda krzyżowania międzyrasowego, choć znana od wielu dekad, nie zyskała jeszcze szerokiego uznania w hodowli bydła mlecznego, w przeciwieństwie do innych gatunków zwierząt gospodarskich, w przypadku których stosowanie tej techniki jest powszechne od około 60 lat. W sektorach takich jak hodowla trzody chlewnej czy drobiu, krzyżowanie międzyrasowe stało się standardową praktyką w celu poprawy wskaźników takich jak przeżywalność, płodność, tempo wzrostu oraz odporność na patogeny. Pomimo dobrze udokumentowanych korzyści tej metody w kontekście zwiększenia efektywności produkcyjnej i zdrowotności zwierząt, w hodowli bydła mlecznego krzyżowanie międzyrasowe nie zostało jeszcze w pełni zaakceptowane, co może wynikać z ugruntowanej tradycji hodowlanej oraz obaw związanych z potencjalnymi trudnościami w utrzymaniu pożądanych cech fenotypowych i produkcyjnych w długoterminowej selekcji (McAllister, 2002; Sørensen i in., 2008). Metoda krzyżowania międzyrasowego sprzyja wystąpieniu efektu heterozji, który wynika z korzystnej interakcji genotypów rodzicielskich. Efekt ten jest rezultatem heterozygotyczności potomstwa, powstałej na skutek połączenia różnych alleli od rodziców różnych ras. W wyniku tego procesu, potomstwo może wykazywać lepsze cechy fenotypowe i funkcjonalne w porównaniu do rodziców, w tym poprawę wydajności produkcyjnej, zdrowotności, płodności oraz odporności na czynniki środowiskowe i choroby. Efekt heterozji jest szczególnie widoczny w populacjach, w których dochodzi do krzyżowania ras o różnych, komplementarnych cechach genetycznych, prowadząc do zwiększonej żywotności, wydolności organizmu oraz lepszej adaptacji do różnych warunków hodowlanych (Heins

i Hansen, 2012). Dodatkowym atutem krzyżowania jest możliwość przyspieszenia osiągnięcia pożądanego celu, a przede wszystkim uniknięcia wzrostu inbredu i zwiększenia różnorodności genetycznej. Według danych PFHBiPM w 2023 roku w Polsce populacja krów mieszańców międzyrasowych ras mlecznych wynosiła 6,78% populacji aktywnej [**Wykres 3**] (PFHBiPM, 2024).

Wysoki udział rasy PHF w pogłowiu bydła w Polsce sprawia, że stanowi ona podstawę w produkcji wołowiny. W tym celu wykorzystywane są zarówno czystorasowe buhajki, jak i wybrakowane ze stada krowy mleczne. Wołowina pozyskiwana z bydła rasy PHF budzi jednak pewne kontrowersje, szczególnie w kontekście jakości oraz jej konkurencyjności względem mięsa pozyskiwanego od wyspecjalizowanych ras w produkcji wołowiny, które cechują się wyższymi parametrami rzeźnymi, lepszą wydajnością i bardziej pożądanymi właściwościami organoleptycznymi mięsa (Solarczyk i in., 2020). Poprawa jakości wołowiny pochodzącej od bydła rasy PHF wymaga wdrożenia zaawansowanych strategii zarządzania stadem, które obejmują programy żywieniowe oraz selektywne metody hodowlane.

Zastosowanie krzyżowania towarowego krów PHF z rasami mięsnymi stanowi skuteczną metodę poprawy jakości mięsa, umożliwiając wykorzystanie najlepszych cech genetycznych obu grup. Krzyżowanie to może prowadzić do poprawy cech jakościowych, takich jak marmurkowatość czy profil kwasów tłuszczowych, co przyczynia się do poprawienia właściwości odżywczych i sensorycznych finalnego produktu. Takie podejście wpływa również na optymalizację właściwości organoleptycznych mięsa, co zwiększa konkurencyjność wołowiny w porównaniu do mięsa pochodzącego z wyspecjalizowanych ras mięsnych (Solarczyk i in., 2020). Natomiast inną alternatywą wykorzystania buhajków rasy PHF, jest produkcja cielęciny (Balzani i in., 2021). Europejczycy traktują cielęcinę jako przysmak i produkt dietetyczny. W Polsce w 2010 r. wyprodukowano około 20 t cielęciny, a średnie spożycie tego mięsa na mieszkańca wyniosło 335 g (Koreman). Cielęcina stanowi około 20% mięsa pochodzącego od bydła hodowanego w UE, z czego ponad 33% pochodzi ze stad mlecznych, w których większość, około 75%, pochodzi od cieląt płci męskiej(Sans i Fontguyon, 2009). Jakość cielęciny jest wynikiem skomplikowanej interakcji wielu czynników, w tym strategii żywieniowych, wieku uboju, aktywności fizycznej, poziomu stresu, warunków środowiskowych, genotypu, metod obróbki poubojowej, stanu zdrowia cieląt oraz oczekiwań konsumentów. Te elementy mają istotny wpływ na cechy organoleptyczne mięsa, takie jak tekstura, soczystość, smak oraz zawartość tłuszczu, a

także na jego wartość odżywczą. Właściwe zarządzanie każdym z tych aspektów, szczególnie w kontekście genetyki i żywienia, jest kluczowe dla uzyskania optymalnej jakości cielęciny, która spełnia zarówno wymagania rynkowe, jak i preferencje konsumentów (Meagher i in., 2019).

Podsumowując, rasa PHF w Polsce stanowi podstawę krajowej produkcji mleka i wołowiny, będąc efektem intensywnej selekcji genetycznej, wykorzystania biotechnologii rozrodu oraz intensyfikacji systemów produkcji mlecznej. Długoterminowe stosowanie tych strategii hodowlanych prowadzi jednak do narastającego deficytu zmienności genetycznej i wzrostu poziomu inbredu, co skutkuje ograniczeniem bioróżnorodności, spadkiem zdrowotności zwierząt oraz pogorszeniem wydajności produkcyjnej i jakości produktów zwierzęcych. W odpowiedzi na te wyzwania, liczne badania wskazują na korzyści płynące z wdrożenia strategii krzyżowania międzyrasowego jako metody łagodzącej skutki depresji inbredowej. Efekt heterozji, wynikający ze zwiększonej heterozygotyczności, redukuje negatywne skutki związane z inbredem, a także prowadzi do poprawy jakości produktów zwierzęcych, zarówno mleka, jak i mięsa. Ponadto, zwiększona heterozygotyczność uzyskana poprzez krzyżowanie przyczynia się do lepszej adaptacji zwierząt do zmieniających się warunków środowiskowych, co stanowi długoterminowy mechanizm poprawy zdrowotności. Dlatego też, implementacja strategii krzyżowania w hodowli PHF stanowi efektywną metodę poprawy zarówno jakości produkcji mleka i mięsa, jak i zdrowotności zwierząt. Zmniejsza również negatywne skutki wynikające z ograniczonej zmienności genetycznej i inbredu, stanowiąc tym samym długofalową odpowiedź na wyzwania współczesnej hodowli bydła mlecznego i mięsnego.

2. Hipotezy badawcze

Hipoteza 1. Deficyt energetyczny występujący w początkowym okresie laktacji skutkuje ograniczeniem lipogenezy długołańcuchowych kwasów tłuszczowych, w tym izomerów skoniugowanego kwasu linolowego, co może modulować profil lipidowy mleka.

Hipoteza 2. Mieszańce F_1 PHF×SRB charakteryzują się wyższymi parametrami użytkowymi mleka w porównaniu do parametrów uzyskiwanych u czystorasowych krów rasy PHF.

Hipoteza 3. Właściwości antyoksydacyjne oraz stabilność oksydacyjna mleka są wyższe u mieszańców F_1, PHF×SRB niż u krów rasy PHF.

Hipoteza 4. Heterozygoty F_1 pochodzące z krzyżowania PHF i SRB wykazują niższe predyspozycje do ujemnego bilansu energetycznego w początkowej fazie laktacji w porównaniu do krów czystorasowych PHF.

Hipoteza 5. Potencjał antyoksydacyjny tkanki mięśniowej buhajów jest wyższy u mieszańców F_1 pochodzących z krzyżowania krów rasy PHF z buhajami rasy limousine (LM) niż u buhajów czystorasowych PHF, osiągając wartości porównywalne do buhajów czystorasowych LM.

Hipoteza 6. System odchowu cieląt znacząco wpływa na kształtowanie się potencjału antyoksydacyjnego ich tkanki mięśniowej, co determinuje stabilność oksydacyjną cielęciny.

3. Cel i zakres pracy

Celem pracy była ocena wpływu deficytu energetycznego, strategii krzyżowania oraz systemów odchowu cieląt na właściwości antyoksydacyjne i stabilność oksydacyjną mleka oraz mięsa w odniesieniu do redukcji skutków chowu wsobnego w populacji bydła rasy polskiej holsztyńsko-fryzyjskiej. Wysoki poziom inbredu w populacji PHF prowadzi do obniżenia zmienności genetycznej oraz osłabienia cech użytkowych, w tym wydajności mlecznej i jakości mleka, a zwłaszcza zawartości białka i tłuszczu. Badania skupiły się na określeniu efektywności krzyżowania międzyrasowego jako strategii zwiększającej heterozygotyczność oraz łagodzącej efekty depresji inbredowej, co oceniono poprzez analizę wybranych parametrów użytkowych, właściwości antyoksydacyjnych oraz stabilności oksydacyjnej mleka. W pracy uwzględniono także interakcje badanych czynników z równowagą energetyczną i procesami metabolicznymi u bydła, które mogą wspierać poprawę jakości i trwałości produktów pochodzenia zwierzęcego (mleka i mięsa).

Zakres badań:

Doświadczenie 1. Analiza profilu lipidowego mleka krów rasy PHF, w tym zawartości długołańcuchowych kwasów tłuszczowych, z uwzględnieniem wpływu deficytu energetycznego, profilu metabolicznego oraz wieku zwierząt na biosyntezę kwasów tłuszczowych, w tym ich skład i izomeryzację (Publikacja 1).

Doświadczenie 2. Ocena parametrów użytkowych mleka krów rasy PHF oraz ich mieszańców F_1 PHF×SRB w kontekście równowagi energetycznej oraz adaptacji metabolicznej do warunków produkcyjnych (Publikacja 2).

Doświadczenie 3. Badanie potencjału antyoksydacyjnego i oksydacyjnej stabilności mleka, w tym aktywności enzymów antyoksydacyjnych oraz zawartości markerów stresu oksydacyjnego w zależności od genotypu krów mlecznych (Publikacja 3).

Doświadczenie 4. Ocena parametrów rozrodu, profilu metabolicznego oraz składu lipidowego mleka krów rasy PHF oraz ich mieszańców F_1 PHF×SRB, z uwzględnieniem wpływu wieku pierwszego wycielenia na mechanizmy regulacyjne (Publikacja 4).

Doświadczenie 5. Analiza potencjału antyoksydacyjnego tkanki mięśniowej buhajów rasy PHF, LM oraz mieszańców F_1 PHF×LM, z uwzględnieniem mechanizmów obrony przed stresem oksydacyjnym, oceny aktywności enzymów antyoksydacyjnych oraz stężenia markerów uszkodzeń oksydacyjnych w tkance mięśniowej (Publikacja 5).

Doświadczenie 6. Badanie potencjału antyoksydacyjnego tkanki mięśniowej oraz zdrowotności cieląt rasy PHF, w zależności od systemu odchowu, z uwzględnieniem wpływu rodzaju odchowu na rozwój mechanizmów ochrony przed stresem oksydacyjnym (Publikacja 6).

4. Metodyka badań

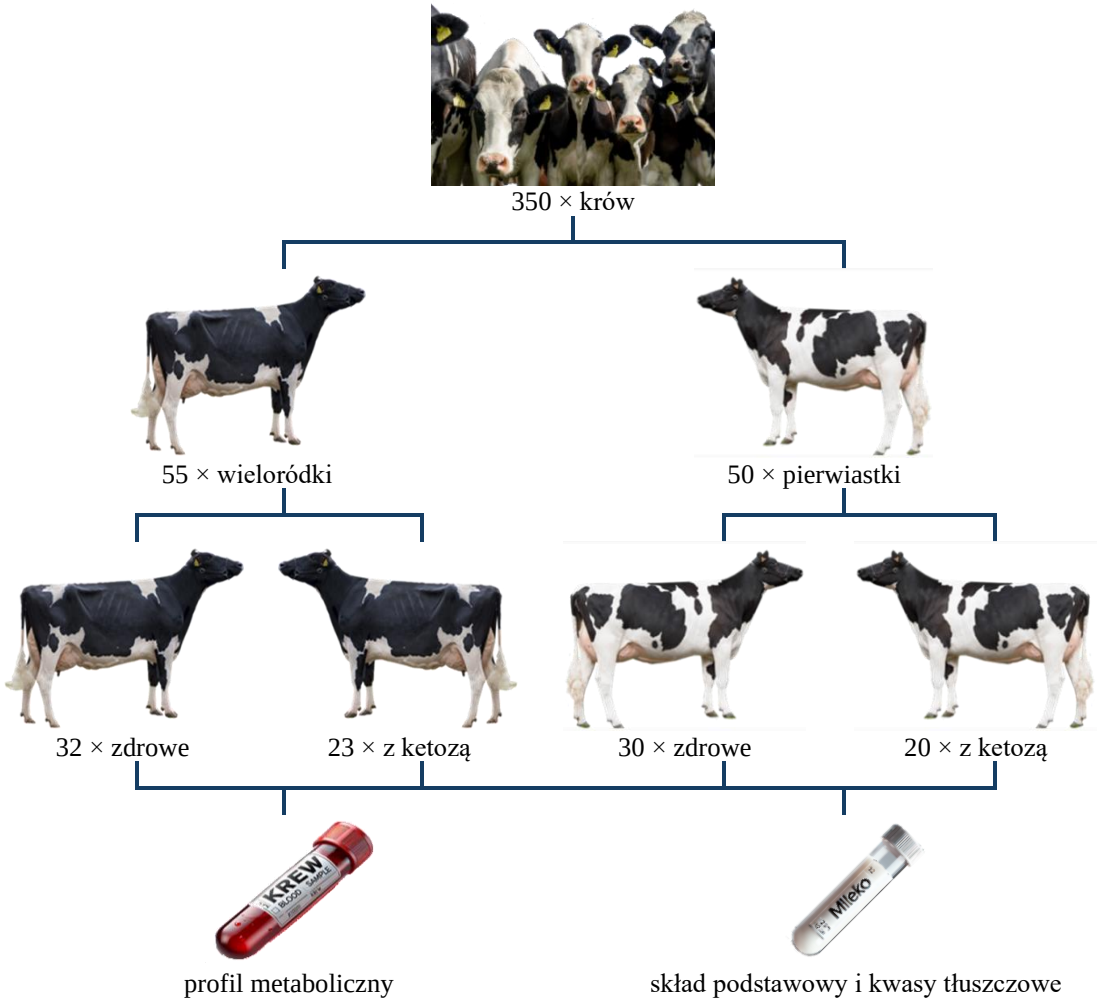
Doświadczenia 1 – 4 zrealizowano w Rolniczym Zakładzie Doświadczalnym Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie (RZD SGGW), gdzie utrzymywane jest stado bydła mlecznego liczące 350 krów o przeciętnej wydajności mlecznej przekraczającej 10 000 kg na laktację. Krowy są utrzymywane w systemie wolnostanowiskowym, a do doju wykorzystuje się halę typu „rybia ość". Zbilansowana dawka pokarmowa jest formułowana zgodnie z zaleceniami systemu INRA, uwzględniając intensywne potrzeby żywieniowe wysokowydajnych krów. Zwierzęta karmione są *ad libitum* dawką TMR, zawierającą kiszonkę z kukurydzy, kiszonkę z lucerny, CCM, śrutę poekstrakcyjną sojową i rzepakową oraz suplementy mineralne (kreda pastewna, sól, tlenek magnezu), co zapewnia kompleksowe pokrycie potrzeb pokarmowych oraz stabilność parametrów metabolicznych w kontekście intensywnej produkcji mlecznej.

Doświadczenie 1.

Analiza profilu lipidowego mleka krów rasy PHF, w tym zawartości długołańcuchowych kwasów tłuszczowych, z uwzględnieniem wpływu deficytu energetycznego, profilu metabolicznego oraz wieku zwierząt na biosyntezę kwasów tłuszczowych, w tym ich skład i izomeryzację (Publikacja 1).

Ze stada 350 krów do doświadczenia wybrano 55 krów wieloródek (w drugiej laktacji) oraz 50 pierwiastek, które następnie podzielono na 4 grupy krów: pierwiastki zdrowe (PH; n=30), wieloródki zdrowe (MH; n=32), pierwiastki z ketozą (PK; n=20) oraz wieloródki z ketozą (MK; n=23). Podziału na grupy dokonano na podstawie zawartości BHBA (krowy zdrowe 0,6-1,2 mmol/L, krowy z ketozą >1,2 mmol/L) we krwi. W badaniu uwzględniono jedynie krowy bez dysfunkcji układu lokomotorycznego oraz bez cech wskazujących na stany zapalne racic (np. *dermatitis digitalis*, *pododermatitis*) czy zapalenie gruczołu mlekowego (*mastitis*). Selekcja taka miała na celu wykluczenie wpływu czynników zdrowotnych, które mogłyby w sposób istotny wpłynąć na uzyskane wyniki dotyczące wskaźników produkcyjnych i metabolicznych, kluczowych dla oceny parametrów jakościowych mleka (**Schemat 1**).

Próbki mleka oraz krwi pobrano od zwierząt w 5. ± 2 dniu po wycieleniu. Pierwsze pobranie próbek odbyło się tego samego dnia, a kolejne próbki mleka były pobierane w odstępach tygodniowych przez dwa tygodnie, co łącznie dało 315 próbek mleka i 105 próbek krwi. Mleko pobierano w trakcie porannego oraz wieczornego doju, za pomocą mlekometrów do butelek o pojemności 250 mL, po czym próbki były mieszane, zapewniając reprezentatywność. Krew pobierano z żyły szyjnej zewnętrznej przy użyciu probówek o objętości 10 mL, zawierających EDTA jako antykoagulant, aby zapobiec krzepnięciu. Po pobraniu próbki krwi poddawano wirowaniu w wirówce laboratoryjnej (3500 rpm przez 10 minut) w celu oddzielenia osocza. Cały materiał biologiczny był transportowany do laboratorium Katedry Hodowli Zwierząt SGGW, gdzie próbki mleka i krwi poddano analizom biochemicznym, metabolicznym oraz ocenie parametrów jakościowych mleka.



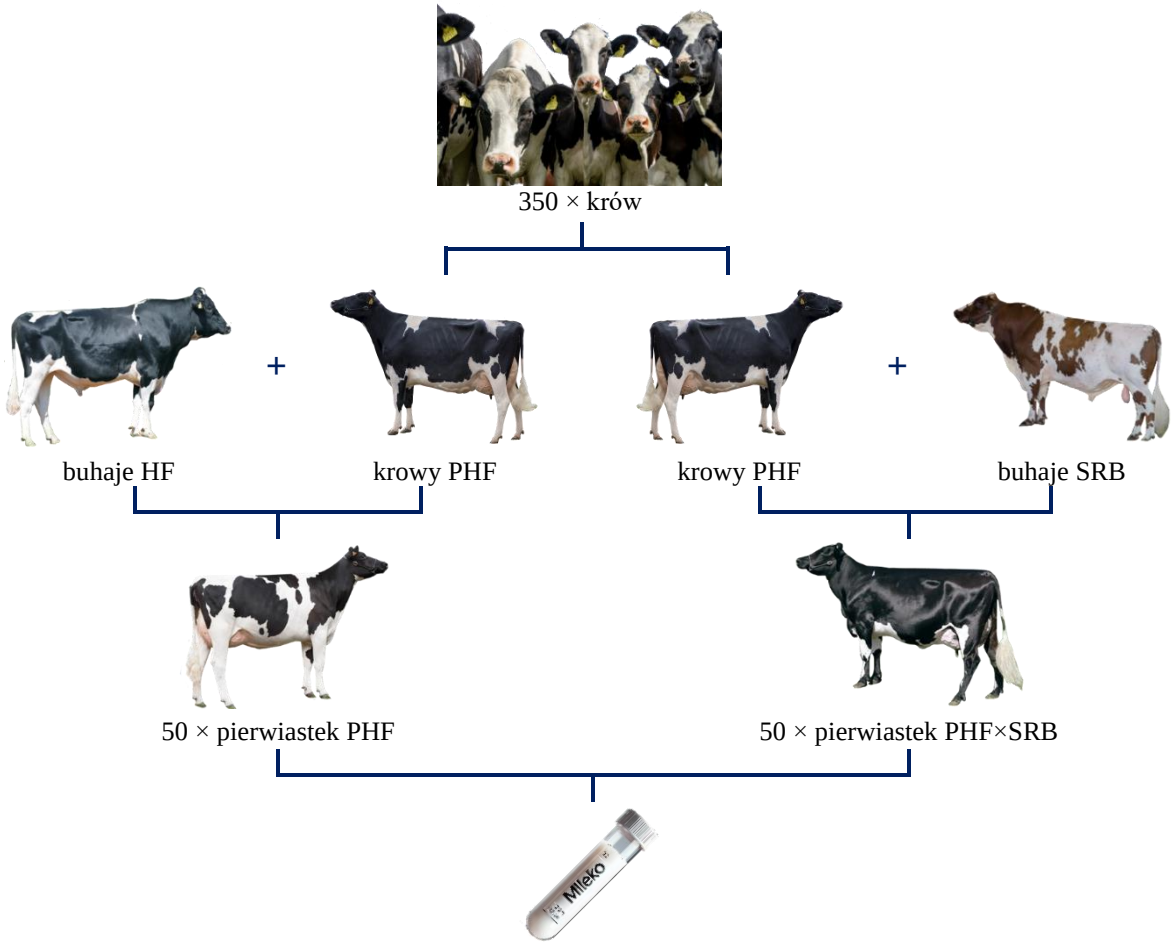
Schemat 1. Schemat doświadczenia 1

Doświadczenie 2

Ocena parametrów użytkowych mleka krów rasy PHF oraz ich mieszańców F_1 PHF×SRB w kontekście równowagi energetycznej oraz adaptacji metabolicznej do warunków produkcyjnych (Publikacja 2).

Ze stada 350 krów mlecznych, wybrano 50 pierwiastek rasy polskiej holsztyńsko-fryzyjskiej, które stanowiły grupę kontrolną, oraz 50 pierwiastek będących mieszańcami F_1, uzyskanymi z krzyżowania krów rasy PHF z buhajami rasy szwedzka czerwona (SRB), które stanowiły grupę doświadczalną. Zwierzęta zostały dobrane w sposób zapewniający jednorodność pod względem wieku, fazy laktacji oraz stanu zdrowotnego (**Schemat 2**).

Mleko od krów pobierano 10-krotnie w comiesięcznych interwałach, co dało łącznie 1000 prób. Mleko pobierano z porannego i wieczornego doju do butelek o pojemności 250 mL przy wykorzystaniu mlekometrów, a następnie transportowano do laboratorium Katedry Hodowli Zwierząt SGGW, gdzie przeprowadzono analizy laboratoryjne.

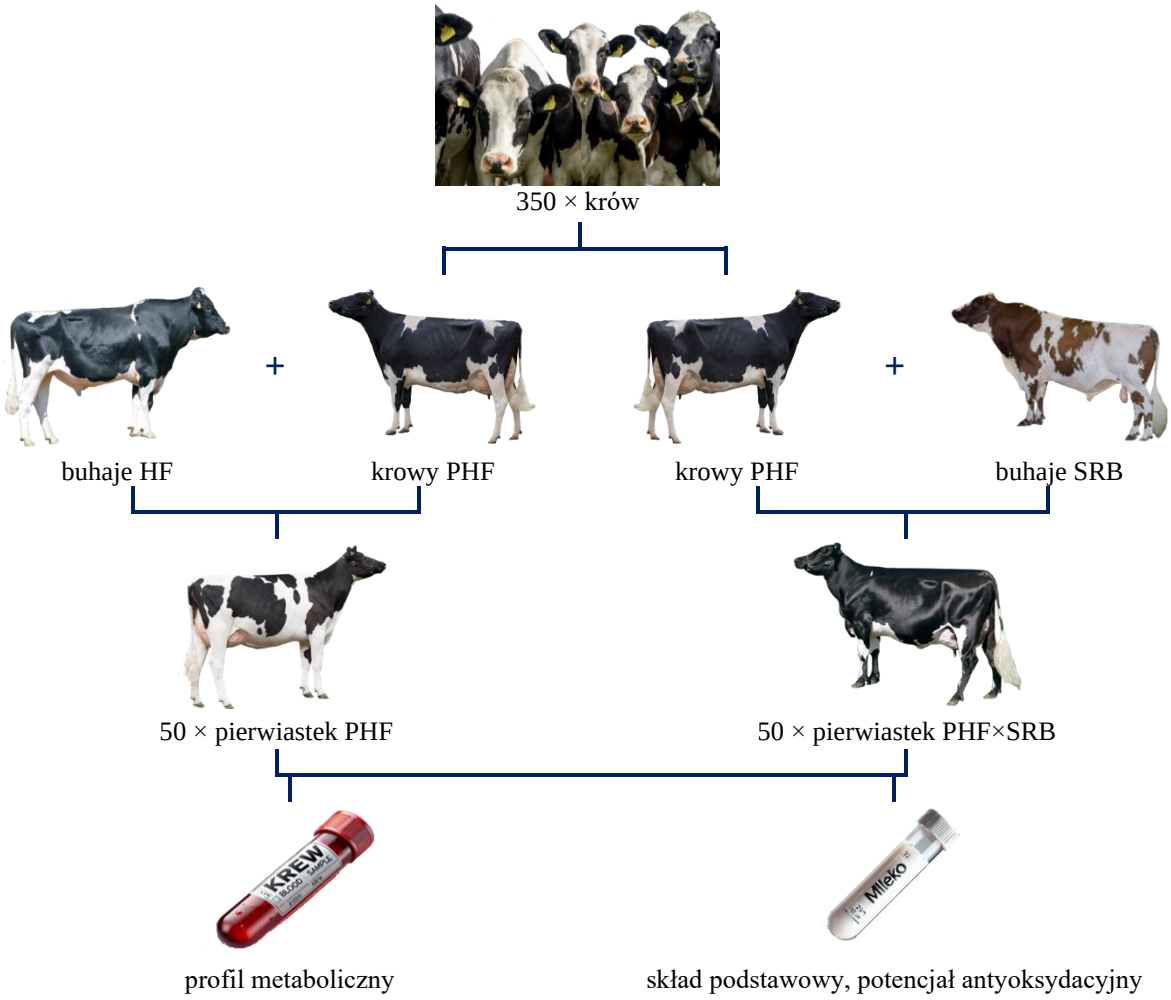


Schemat 2. Schemat doświadczenia 2.

Doświadczenie 3.

Badanie potencjału antyoksydacyjnego i oksydacyjnej stabilności mleka, w tym aktywności enzymów antyoksydacyjnych oraz zawartości markerów stresu oksydacyjnego w zależności od genotypu krów mlecznych (Publikacja 3).

Doświadczenie przeprowadzono na 60 krowach pierwiastkach. Grupa kontrolna składała się z 30 krów rasy polska holsztyńsko-fryzyjska, natomiast do grupy doświadczalnej zakwalifikowano 30 krów pierwiastek będących mieszańcami F_1 PHF×SRB. Krowy włączone do doświadczenia nie wykazywały objawów patologicznych, takich jak zapalenie racic, zaburzenia metaboliczne, czy zapalenie gruczołu mlekowego (*mastitis*). Stan metaboliczny krów był regularnie kontrolowany poprzez pomiar NEFA, BHBA, glukozy, a także analizę stosunku tłuszcz/białko (**Schemat 3**).



Schemat 3. Schemat doświadczenia 3

Materiał badawczy pobierano 10-krotnie od każdej krowy w miesięcznych odstępach czasowych, począwszy od 5. dnia po wycieleniu, aż do 280. ± 5 dnia laktacji. Próby mleka pobierano zarówno z porannego, jak i wieczornego udoju, które następnie mieszano co łącznie stanowiło 600 próbek mleka i 600 próbek krwi. Po pobraniu, próbki były transportowane do laboratorium Katedry Hodowli Zwierząt Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie, gdzie poddano je analizom biochemicznym i metabolicznym.

Doświadczenie 4.

Ocena parametrów rozrodu, profilu metabolicznego oraz składu lipidowego mleka krów rasy PHF oraz ich mieszańców F_1 PHF×SRB, z uwzględnieniem wpływu wieku pierwszego wycielenia na mechanizmy regulacyjne (Publikacja 4).

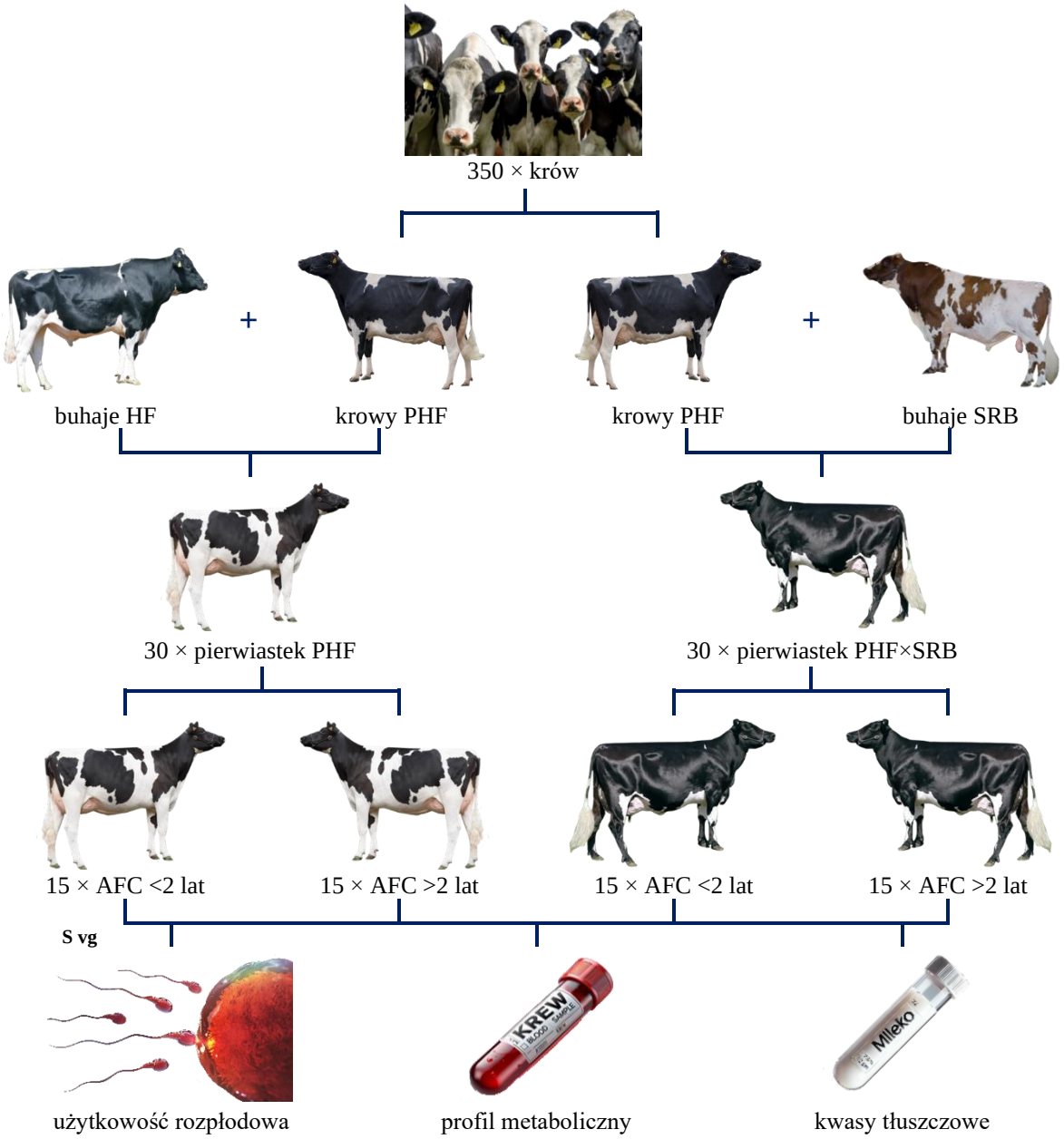
Doświadczenie przeprowadzono na 60 pierwiastkach wybranych z grupy 350 krów mlecznych. Do grupy kontrolnej zakwalifikowano 30 pierwiastek rasy polskiej

holsztyńsko-fryzyjskiej (PHF), natomiast do grupy doświadczalnej wybrano 30 pierwiastek mieszańców międzyrasowych F_1, uzyskanych w wyniku krzyżowania krów rasy PHF z buhajami rasy szwedzkiej czerwonej (PHF×SRB). Zwierzęta podzielono na cztery grupy w zależności od wieku pierwszego wycielenia, przy czym każda grupa liczyła 15 zwierząt: pierwiastki PHF wycielone w wieku poniżej dwóch lat (<2 lat PHF; średni wiek AFC 23,3 miesiąca), pierwiastki PHF wycielone w wieku powyżej dwóch lat (>2 lat PHF; średni wiek AFC 25,9 miesiąca), pierwiastki F_1 PHF×SRB wycielone w wieku poniżej dwóch lat (<2 lat PHF×SRB; średni wiek AFC 23,2 miesiąca) oraz pierwiastki F_1 PHF×SRB wycielone w wieku powyżej dwóch lat (>2 lat PHF×SRB; średni wiek AFC 24,8 miesiąca) (

Schemat *4*).

Pobieranie próbek mleka i krwi przeprowadzono w 35. ± 5 dniu po wycieleniu, od wszystkich zwierząt biorących udział w badaniu. Mleko pobierano z doju porannego oraz wieczornego do jednorazowych butelek o pojemności 250 mL przy użyciu mlekometrów, a następnie mieszano. Krew pobierano z żyły szyjnej zewnętrznej za pomocą probówek o objętości 10 mL, zawierających EDTA jako antykoagulant. Po pobraniu materiału biologicznego, próbki zostały niezwłocznie przewiezione do laboratorium Katedry Hodowli Zwierząt SGGW w celu dalszych analiz.

W celu uzyskania danych dotyczących wskaźników rozrodu, skorzystano z dokumentacji hodowlanej, obejmującej następujące informacje: datę urodzenia zwierzęcia, datę pierwszej inseminacji, datę skutecznej inseminacji, datę pierwszego wycielenia, datę pierwszej inseminacji po wycieleniu, datę skutecznej inseminacji po wycieleniu, liczbę dawek inseminacyjnych zastosowanych do pokrycia krowy oraz datę kolejnego wycielenia. Na podstawie tych danych wyliczono następujące wskaźniki: wiek pierwszej inseminacji (AFI), indeks zacieleń (PI), okres usługi (SP), długość ciąży (GL), wiek pierwszego wycielenia (AFC), okres przestoju poporodowego (PPD), okres międzyciążowy (IP) oraz okres międzywycieleniowy (PBC).



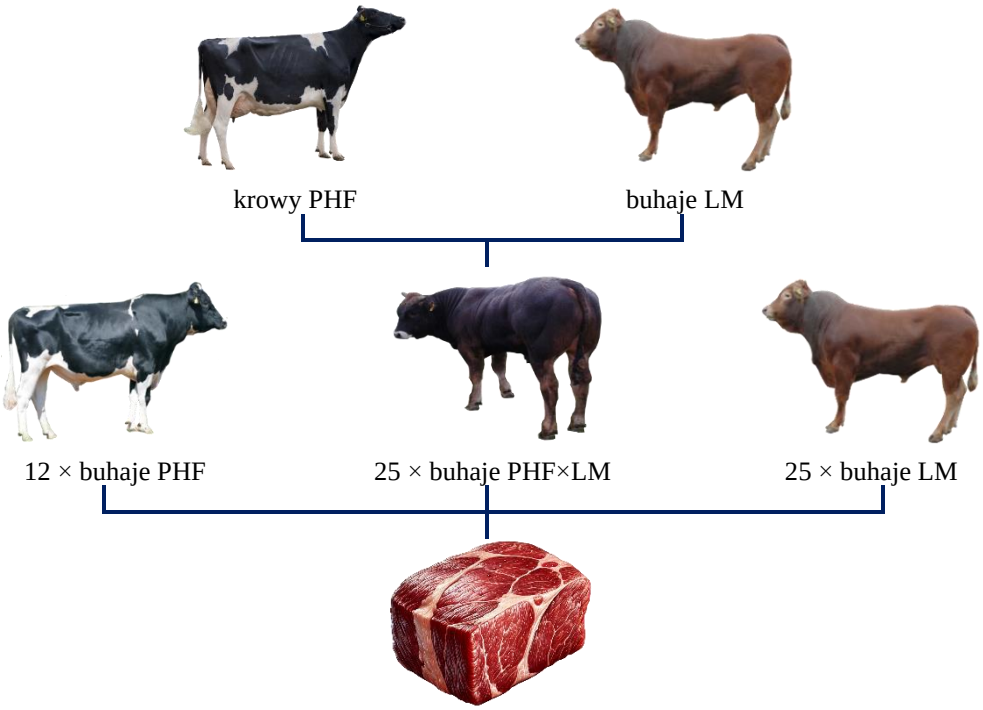
Schemat 4. Schemat doświadczenia 4.

Doświadczenie 5.

Analiza potencjału antyoksydacyjnego tkanki mięśniowej buhajów rasy PHF, LM oraz mieszańców F_1 PHF×LM, z uwzględnieniem mechanizmów obrony przed stresem oksydacyjnym, oceny aktywności enzymów antyoksydacyjnych oraz stężenia markerów uszkodzeń oksydacyjnych w tkance mięśniowej (Publikacja 5).

Doświadczenie przeprowadzono w gospodarstwie położonym w województwie warmińsko-mazurskim, specjalizującym się w opasie bydła. W okresie opasu zwierzęta były żywione dawką TMR *ad libitum*, zapewniającą optymalne warunki żywieniowe,

zgodne z wymaganiami produkcyjnymi bydła mięsnego. W badaniu uczestniczyły 62 buhaje: 25 rasy limousine (LM), 12 rasy polska holsztyńsko-fryzyjska (PHF) oraz 25 mieszańców F_1, pochodzących z krzyżowania krów rasy PHF z buhajami rasy LM (PHF×LM) (**Schemat 5**).



Schemat 5. Schemat doświadczenia 5.

Ubój zwierząt nastąpił w wieku 21-24 miesięcy. Następnie tusze były schładzane przez 24h w temperaturze 2-4°C i po tym okresie pobierane były równolegle do osi mięśnia próbki mięśnia półbłoniastego (*musculus semimembranosus*) w ilości 300 g. Po uboju dokonywano pomiaru masy tuszy ciepłej, następnie zostały one przewiezione do laboratorium Katedry Hodowli Zwierząt SGGW. Ubój oraz obróbka poubojowa tusz przeprowadzana była zgodnie z rozporządzeniem Rady Europy (WE) nr 1099/2009 z dnia 24 września 2009 r.

Doświadczenie 6.

Badanie potencjału antyoksydacyjnego tkanki mięśniowej oraz zdrowotności cieląt rasy PHF, w zależności od systemu odchowu, z uwzględnieniem wpływu rodzaju odchowu na rozwój mechanizmów ochrony przed stresem oksydacyjnym (Publikacja 6).

Doświadczenie przeprowadzono w ekologicznym gospodarstwie rolnym zlokalizowanym w Wyczechowie, w którym wszystkie praktyki hodowlane były zgodne z wytycznymi Rozporządzenia Parlamentu Europejskiego i Rady Unii Europejskiej 2018/848 z dnia 30 maja 2018 r. dotyczącymi produkcji ekologicznej i znakowania produktów ekologicznych. W gospodarstwie tym stosowano metody hodowlane, żywieniowe oraz zarządzanie stadem, które spełniały rygorystyczne normy ekologiczne, z uwzględnieniem dbałości o dobrostan zwierząt oraz ochronę środowiska (Regulation, 2007).

Schemat 6. Schemat doświadczenia 6

Do doświadczenia wybrano 30 cieląt, które podzielone zostały na dwie grupy - kontrolną, w której cielęta karmione były dwa razy dziennie mlekiem pochodzącym od krów z tego gospodarstwa przy użyciu automatycznych podajników ze smoczkiem, natomiast cielęta z grupy doświadczalnej posiadały stały dostęp do dwóch krów mamek. Wszystkie zwierzęta miały stały dostęp do siana. W tym samym czasie utrzymywano w grupach po 5 cieląt, a doświadczenie realizowano w 3 powtórzeniach (**Schemat 6**).

Mamki biorące udział w doświadczeniu musiały charakteryzować się silnym instynktem macierzyńskim oraz powodzeniem w odchowie cieląt w poprzednich laktacjach, a ich mleko musiało charakteryzować się liczbą bakterii poniżej 100 000 CFU oraz liczbą komórek somatycznych poniżej 200 000/1 mL.

Cielęta ważone były przy użyciu systemu CalmScale, który usytuowany był w obszarze pojenia bydła i wykorzystywał znaczniki RFID zwierząt oraz anteny do precyzyjnej ich identyfikacji oraz zapisu danych. Dane dotyczące masy zwierząt wraz z innymi istotnymi informacjami były rejestrowane i udostępniane do analizy. Średni dzienny przyrost (ADG) obliczono na podstawie różnicy masy od dnia 0 (masa w dniu

rozpoczęcia doświadczenia) i dzieląc ją przez liczbę dni od dnia 0 do dnia uboju, zapewniając kluczowy wskaźnik do oceny wzrostu cieląt.

Ocena stanu zdrowia zwierząt przeprowadzana była codziennie przez lekarza weterynarii, który dokumentował występowanie biegunki, kaszlu i kataru. Wraz z tymi obserwacjami wdrożono standardowe środki zapobiegawcze ukierunkowane na choroby wirusowe i infekcje pasożytnicze.

Dane behawioralne były zbierane jako średnie z 5-godzinnych okresów obserwacji przeprowadzanych co miesiąc w ciągu pierwszych 6 miesięcy życia cieląt. W analizie zachowania cieląt opartej na etogramie, zachowania zostały wstępnie podzielone na trzy główne kategorie: aktywne, spoczynkowe i nieprawidłowe. Aktywne zachowania obejmowały ruch, zachowania spoczynkowe zaś brak ruchu, a nieprawidłowe zachowania obejmowały takie działania, jak: ssanie lub lizanie innych cieląt (jama gębowa, uszy, pępek, ogon i moszna) oraz ssanie lub lizanie przedmiotów w kojcach. Całkowita częstotliwość obejmowała sumę zachowań takich jak ssanie lub lizanie przedmiotów oraz sumę zachowań obejmujących ssanie lub lizanie innych cieląt na godzinę.

Po osiągnięciu przez cielęta odpowiedniego wieku (6 miesięcy), zostały one poddane ubojowi, a ich tusze schładzano przez 24 godziny w temperaturze 2-4°C, aby ułatwić odpowiednią konserwację mięsa. Po okresie chłodzenia z każdej tuszy cielęcej pobrano próbki mięsa o masie 300 g z mięśnia półbłoniastego (*musculus semimembranosus*) wycięte równolegle do osi mięśnia.

Metody analityczne

W pobranych materiale biologicznym zostały oznaczone następujące analizy:

Doświadczenie 1, 2, 3, 4

Analizę podstawowego składu chemicznego mleka, w tym zawartości tłuszczu, białka, kazeiny oraz laktozy, przeprowadzono za pomocą spektroskopii w podczerwieni z transformacją Fouriera (FTIR) przy użyciu analizatora MilkoScan FT 120 (Foss Electric, Hillerød, Dania). Do przygotowania krzywych kalibracyjnych dla analizatora wykorzystano mleko referencyjne, dostarczone przez Krajowe Centrum Hodowli Zwierząt.

Doświadczenie 1, 2, 3

Jakość cytologiczną mleka, mierzoną liczbą komórek somatycznych (LKS), określono za pomocą cytometrii przepływowej, stosując analizator Somacount 150 (Bentley, Warszawa, Polska).

Doświadczenie 3

Zawartość α-laktoalbuminy, β-laktoglobuliny, lizozymu, laktoferyny, albuminy surowicy bydlęcej oraz laktoperoksydazy w mleku oznaczono za pomocą wysokosprawnej chromatografii cieczowej (RP-HPLC), korzystając z aparatu Agilent 1100 Series (Agilent Technologies, Waldbronn, Niemcy). Analiza ilościowa wykona została za pomocą kalibracji przy użyciu wzorców witamin firmy Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA).

Doświadczenie 5

Oznaczanie zawartości anseryny, karnozyny, tauryny, koenzymu Q10, kreatyniny i kreatyny przeprowadzono przy użyciu wysokosprawnej chromatografii cieczowej z odwróconą fazą (RP-HPLC), z użyciem chromatografu Agilent 1100 Series (Agilent Technologies, Waldbronn, Niemcy). Separację przeprowadzono w temperaturze pokojowej, z zastosowaniem gradientu rozpuszczalnika na kolumnie Jupiter C18 300A (Phenomenex, Torrance, CA, USA), zgodnie z metodyką Łukasiewicz i in. (Łukasiewicz i in., 2018). Analiza ilościowa wykona została za pomocą kalibracji przy użyciu wzorców witamin firmy Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA).

Doświadczenie 5

Podstawowy skład chemiczny mięsa określono za pomocą analizatora Food Scan™ (Foss Electric, Hillerød, Dania).

Doświadczenie 6

Tłuszcz śródmięśniowy ekstrahowano z 10 g mięśni przy użyciu mieszaniny chloroformu i metanolu w stosunku 2:1. Kwasy tłuszczowe zostały następnie przekształcone w estry metylowe kwasów tłuszczowych (FAME) w katalizowanym zasadą procesie transestryfikacji przy użyciu metanolanu sodu. Kwasy tłuszczowe rozdzielono przy użyciu chromatografu gazowego model TRACE GC, w którym zastosowano wysokopolarną kolumnę kapilarną ze stopionej krzemionki o długości 100 m, średnicy 25 mm i grubości warstwy 0,25 m. Identyfikację związków FAME

przeprowadzono przy użyciu detektora płomieniowo-jonizacyjnego (FID). Warunki chromatografii gazowej i proces identyfikacji związków FAMEbyły zgodnie ze standardową procedurą (Natalello i in., 2019).

Doświadczenie 6

Ocena stabilności oksydacyjnej świeżego mięsa została przeprowadzona zgodnie z metodyką Natalello i in. (2019). Z każdej próbki mięśnia przygotowano trzy plastry mięsa o grubości 2 cm i przechowywano je na tackach styropianowych owiniętych trzema warstwami domowej folii spożywczej. Przechowywanie odbywało się w ciemności w temperaturze 4°C przez trzy okresy: zero dni (2 godziny dojrzewania), cztery i siedem dni. Po każdym okresie przechowywania, jeden z trzech plastrów był wykorzystywany do pomiaru barwy. Do oceny stabilności koloru wykorzystano spektrofotometr Minolta CM 2022, skonfigurowany w trybie wyłączenia składowych zwierciadlanych (SCE). Pomiary przeprowadzono przy oświetleniu A i 10 standardowym obserwatorze. Na powierzchni mięsa wykonano trzy nienakładające się na siebie pomiary i obliczono średnią wartość. Pomiary barwy, w tym L* (jasność), a* (zaczerwienienie), b* (zażółcenie), C (nasycenie) i h° (kąt barwy), zostały zarejestrowane w przestrzeni kolorów CIE L* a* b*.

Doświadczenie 6

Analiza utleniania lipidów obejmowała pomiar stężenia substancji reaktywnych kwasu 2-tiobarbiturowego (TBARS) po zakończeniu każdego okresu przechowywania (Natalello i in., 2020). Stężenie dialdehydu malonowego (MDA) w każdej próbce określono przy użyciu krzywej kalibracyjnej przygotowanej z TEP (1,1,3,3-tetraetoksypropan) w wodzie destylowanej w stężeniach od 5 do 65 nmol/4 ml.

Doświadczenie 6

Stężenie mioglobiny określono zgodnie z opisaną metodyką 2 g mięśni homogenizowano przy użyciu homogenizatora tkankowego Heidolph Diax 900 pracującego z prędkością 9500 obrotów na minutę z buforem fosforanowym. Zhomogenizowane próbki poddano następnie wirowaniu przy 6800 g w temperaturze 4°C przez 15 minut, a następnie przefiltrowano przez bibułę Whatman 541. Przefiltrowany supernatant skanowano za pomocą spektrofotometru UV/VIS UV-1601 i mierzono absorbancję przy 525 nm. Stężenie mioglobiny obliczono na podstawie pomiarów absorbancji (Krzywicki, 1982).

Doświadczenie 1, 4

Oznaczenie zawartości NEFA, BHBA, glukozy, białka całkowitego, albuminy, kreatyniny oraz γ-glutamylotranspeptydazy, przeprowadzono przy wykorzystaniu analizatora biochemicznego BS800M (PZ Cormay, Warszawa, Polska).

Doświadczenie 4

Całkowity status antyoksydacyjny osocza krwi oceniano za pomocą zestawu ELISA (Randox Laboratories) do oznaczania Total Antioxidant Status (TAS), przy użyciu analizatora NanoQuant Infinite M200 Pro (Tecan Austria GmbH, Grödig, Austria):

HX-FeIII - metmioglobina, X- [FeIV = 0] - ferryloglobina, ABTS® - 2,2-azino-di [3-etylobenzotiazolinosulfonian] (materiały RANDOX). mmol/L definiuje stężenie TAS

$HX\text{-}Fe^{III} + H_2O_2$à $X\text{-}[Fe^{IV}=0] + H_2O$

$ABTS^{®} + X\text{-}[Fe^{IV}=0]$ à $ABTS^{®+} + HX\text{-}Fe^{III}$

Wykonano ekstrakcję tłuszczu mlekowego wg metody Röse-Gottlieba dzięki której uzyskano tłuszcz stanowiący podstawę do przeprowadzenia następujących analiz laboratoryjnych:

Doświadczenie 4, 5

Oznaczanie zawartości witamin rozpuszczalnych w tłuszczu i β-karotenu wykonano za pomocą wysokosprawnej chromatografii cieczowej z odwróconym układzie faz RP-HPLC Agilent 1100 (Agilent Technologies, Waldbronn, Germany) i kolumny Zorbax Eclipse XDB C8. Analiza ilościowa wykona została za pomocą kalibracji przy użyciu wzorców witamin firmy Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA).

Doświadczenie 1, 3, 4, 5

Oznaczanie zawartości kwasów tłuszczowych wykonano przy wykorzystaniu chromatografu gazowego Agilent 7890 GC (Agilent Technologies, Waldbronn, Germany) i kolumny Varian Select FAME. Metylacja kwasów tłuszczowych została przeprowadzona metodą trans-estryfikacji (EN ISO 5509). Identyfikację kwasów tłuszczowych wykonano na podstawie względnego czasu retencji w stosunku do kwasu palmitynowego. Analiza ilościowa wykona została za pomocą kalibracji przy użyciu wzorców witamin firmy Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA).

Analiza statystyczna

Doświadczenie 1

Uzyskane dane poddano analizie statystycznej przy użyciu pakietu IBM SPSS 22.0. Rozkład składu chemicznego mleka oraz wybranych kwasów tłuszczowych sprawdzono przy użyciu testu Shapiro-Wilka. Do określenia wpływu fazy laktacji na skład chemiczny mleka oraz poziom wybranych kwasów tłuszczowych wykorzystano jednoczynnikową analizę wariancji ANOVA. Zmiany zawartości kwasów tłuszczowych w odniesieniu do poziomu BHBA we krwi oraz fazy laktacji sprawdzono za pomocą wieloczynnikowej analizy wariancji. Między czynnikami uwzględniono tylko interakcje statystycznie istotne ($p \leq 0,05$). Poziom istotności został określony po przeprowadzeniu wstępnych analiz statystycznych. Do porównania wielu zmiennych zastosowano test LSD Fishera.

Doświadczenie 2

Uzyskane dane zostały poddane analizie statystycznej przy użyciu pakietu IBM SPSS 6.0. Rozkład składu chemicznego mleka sprawdzono przy zastosowaniu testu Shapiro-Wilka. W celu ustalenia wpływu genotypu na skład chemiczny mleka oraz LKS zastosowano jednoczynnikową analizę wariancji ANOVA, natomiast zmiany w koncentracji podstawowych składników mleka w odniesieniu do genotypu i etapu laktacji określono za pomocą wieloczynnikowej analizy wariancji. Korelacja Pearsona została wykorzystana do ilościowego określenia stopnia zależności liniowej między zmiennymi.

Doświadczenie 3

Dane poddano kompleksowej kompilacji statystycznej, wykorzystując analizę wariancji (ANOVA) za pomocą metody najmniejszych kwadratów ułatwionej przez oprogramowanie PS IMAGO PRO 10.0. Istotne różnice między średnimi grupowymi określono za pomocą statystyki F. Charakterystyki rozkładu białka serwatkowego, kwasów tłuszczowych i składu witamin zbadano za pomocą testu Shapiro-Wilka.

Doświadczenie 4

Do analizy statystycznej zastosowano analizę wariancji ANOVA z użyciem metody najmniejszych kwadratów do porównania średnich grupowych. Przed przeprowadzeniem analizy wariancji sprawdzono, czy dane są zgodne z rozkładem normalnym przy zastosowaniu testu Shapiro-Wilka. Wszystkie zmienne dały wartości p większe niż 0,05, co potwierdza, że normalność nie została naruszona. Jednorodność wariancji:

przeprowadzono test Levene'a na jednorodność wariancji, zapewniając, że wariancje w grupach były równe ($p > 0,05$).

Doświadczenie 5

Uzyskane dane poddano analizie statystycznej przy użyciu jednoczynnikowej analizy wariancji ANOVA, gdzie czynnikiem stałym była rasa. Istotne średnie zostały rozdzielone przy użyciu testu Duncana (przy $p < 0,05$). Rozkład składników bioaktywnych sprawdzono za pomocą testu Shapiro-Wilka. Wszystkie testy zostały wykorzystane przy użyciu pakietu IBM SPSS 23. Dane przedstawiono jako średnie najmniejszych kwadratów (LSM) z błędem standardowym średniej.

Doświadczenie 6

Dane doświadczalne z oceny wydajności opasowej cieląt zostały przeanalizowano za pomocą procedury GLM Repeated Measures Procedure by SAS, ver.9. Zawartość tłuszczu, kwasów tłuszczowych i mioglobiny oszacowano przy użyciu jednoczynnikowej analizy wariancji. Do oceny danych dotyczących stabilności oksydacyjnej (kolor i utlenianie lipidów w czasie przechowywania) wykorzystano procedurę MIXED firmy SAS, ver. 9.0. Różnice między średnimi zostały ocenione przy użyciu testu Tukeya dla porównań wielokrotnych. Analizę chi-kwadrat zastosowano do oceny statusu choroby (chory lub nie chory) w odniesieniu do przydziału do grupy (grupa kontrolna vs. grupa eksperymentalna).

5. Omówienie głównych wyników badań

Doświadczenie 1.

Analiza profilu lipidowego mleka krów rasy PHF, w tym zawartości długołańcuchowych kwasów tłuszczowych, z uwzględnieniem wpływu deficytu energetycznego, profilu metabolicznego oraz wieku zwierząt na biosyntezę kwasów tłuszczowych, w tym ich skład i izomeryzację.

Wyniki doświadczenia zostały opublikowane w publikacji:

Solarczyk P., Gołębiewski M., Slósarz J., Puppel K. 2023. Interaction Between the Concentration of β-Hydroxybutyric Acid and the Content of Long-Chain Fatty Acids in the Early Stage of Lactation—Comparing Multiparous and Primiparous Cows. *Applied Sciences* 13(13), 7870.

Kwas C18:1 trans11 (TVA) stanowi dominujący kwas tłuszczowy o konfiguracji trans obecny w mleku. Zawartość TVA w mleku krów pierwiastek (PH) oraz wieloródek (MH) wykazywała stabilność w całym okresie badania. U zwierząt ze zdiagnozowaną ketozą (MK, PK) wykazane zostały zmiany w stężeniu TVA, zależne od fazy laktacji oraz statusu metabolicznego zwierząt. W pierwszym tygodniu laktacji (5–7 dzień), najwyższa koncentracja TVA wykazana została w mleku krów PH oraz MH, natomiast w grupie MK stwierdzono niższą zawartość tego kwasu. W odniesieniu do grupy MK, zawartość TVA była wyższa o 0,212 g u krów pierwiastek oraz o 0,161 g u krów wieloródek. W drugim tygodniu laktacji (8–14 dzień) zaobserwowano obniżenie zawartości TVA w mleku krów PH, MH oraz MK. U krów pierwiastek z ketozą (PK) stwierdzono wzrost stężenia TVA o 0,15 g. W trzecim tygodniu laktacji (15–21 dzień), najwyższa koncentracja TVA wystąpiła w mleku krów MK, które również wykazały największy wzrost stężenia tego kwasu (0,493 g). W grupie PK zaobserwowano najniższą wartość TVA. Wyniki przeprowadzonego badania wskazują na istotną zmienność w zawartości kwasu trans-wakcenowego w mleku krów w zależności od fazy laktacji oraz statusu metabolicznego. W szczególności, zaobserwowane różnice w koncentracji TVA mogą wynikać z zaburzeń metabolicznych towarzyszących ketozie. W grupie pierwiastek (PH) i wieloródek (MH), gdzie nie występowały oznaki ketozy, stężenie TVA było stabilne, co może sugerować prawidłowy metabolizm lipidowy. Natomiast u krów ze zdiagnozowaną ketozą (MK, PK), zmniejszona zawartość TVA we wczesnych fazach laktacji może wynikać z zaburzonego metabolizmu tłuszczów. Warto podkreślić, że w drugim tygodniu laktacji zaobserwowano spadek zawartości TVA u krów PH, MH i MK, co może być efektem zmieniającego się profilu metabolicznego w odpowiedzi na zmienne warunki energetyczne, w tym deficyt energetyczny występujący w tym okresie laktacji. Interesującym wynikiem jest wzrost zawartości TVA o 0,15 g u krów pierwiastek z ketozą (PK), co może sugerować reakcję adaptacyjną organizmu na zmiany w metabolizmie lipidów w odpowiedzi na zmiany hormonalne związane z ketozą.

Kwas linolowy (C18:2 n-6; LA) jest najczęściej spożywanym kwasem omega-6 w diecie człowieka. Zawartość LA w mleku krów pierwiastek obniżała się w trakcie trwania doświadczenia; ponadto, zawartość LA, zarówno u zdrowych, jak i chorych krów pierwiastek, była wyższa w pierwszym tygodniu laktacji niż u krów wieloródek. Zawartość LA u krów wieloródek była dość zmienna. U MH zawartość LA wzrosła w drugim tygodniu laktacji i uległa obniżeniu w trzecim tygodniu. Najniższy poziom LA ze wszystkich grup zwierząt zaobserwowano w pierwszych dwóch tygodniach

doświadczenia u krów MK. Ponadto zaobserwowano obniżenie zawartości LA w drugim tygodniu w porównaniu z pierwszym tygodniem laktacji, podczas gdy w trzecim tygodniu nastąpił znaczny wzrost zawartości LA w mleku tych krów, który wyniósł 0,905 g/100 g tłuszczu – wartość ta była najwyższą wartością odnotowaną wśród grup doświadczalnych.

Ze względu na swoje właściwości prozdrowotne ważną rolę w diecie człowieka odgrywają także skoniugowane dieny kwasu linolowego (CLA; C18:2 cis9, trans11 – CLA9; C18:2 trans10, cis12 – CLA10).

W pierwszym tygodniu laktacji najwyższą zawartość CLA9 zaobserwowano w mleku PH i MH, a najniższą w mleku PK i MK. W szczególności zawartość CLA9 w mleku MH była wysoka i wynosiła aż 0,833 g/100 g tłuszczu, podczas gdy w mleku PH wynosiła 0,684 g/100 g tłuszczu. Zawartość CLA9 w mleku krów PH w pierwszym tygodniu laktacji była o 32,75% wyższa niż w mleku PK, podczas gdy w mleku MH była o 67,7% wyższa niż w mleku MK. W drugim tygodniu laktacji najwyższą zawartość CLA9 stwierdzono w mleku PH, pomimo obniżenia w porównaniu z pierwszym tygodniem, następnie w mleku PK, w którym zawartość CLA9 wzrosła. Jednak należy zwrócić uwagę, że nastąpiło dość duże obniżenie zawartości CLA9 w mleku MH, które wynosiło 55%. Najniższy poziom CLA9 w drugim tygodniu laktacji ponownie zaobserwowano w mleku krów MK, chociaż i tak zawartość ta była wyższa o 21% w porównaniu z pierwszym tygodniem. W trzecim tygodniu laktacji po raz kolejny najwyższą zawartość CLA9 zaobserwowano w mleku krów MH, u których nastąpił wzrost zawartości o 21,5% w porównaniu z poprzednim tygodniem. Z kolei w przypadku krów PH zawartość CLA9 obniżyła się, podczas gdy u krów MK poziom wzrósł, choć nadal był najniższy wśród zwierząt objętych doświadczeniem.

W trakcie doświadczenia mleko MH charakteryzowało się najwyższą zawartością CLA10, jednak w drugim tygodniu zaobserwowano obniżenie zawartości o 15%, a w trzecim tygodniu laktacji o kolejne 19% w porównaniu z poprzednimi tygodniami. Wysoki poziom CLA10 zaobserwowano również u krów PH w pierwszych dwóch tygodniach laktacji, choć jego zawartość obniżyła się o 25,4% i ponownie o 66% w porównaniu do poprzednich tygodni, co oznacza, że w trzecim tygodniu laktacji odnotowano najniższą zawartość CLA10 w mleku krów PH. Znacznie niższe wartości, zwłaszcza na początku laktacji, zaobserwowano u krów ze zdiagnozowaną ketozą. Zawartość CLA10 u tych zwierząt, w porównaniu do grup zdrowych, była o 319% niższa u krów pierwiastek, a o 1 018% niższa w przypadku krów wieloródek. W drugim

tygodniu laktacji nadal występowała istotna różnica w zawartości CLA10 między krowami zdrowymi a tymi, u których zdiagnozowano ketozę, choć wartości te były już niższe u zwierząt zdrowych. Z kolei zawartość CLA10 wzrosła u zwierząt z ketozą a różnica wynosiła 161% dla krów pierwiastek i 233% dla krów wieloródek. W trzecim tygodniu laktacji zawartość CLA10 była najwyższa u wieloródek zdrowych i tych z ketozą, podczas gdy obniżenie zawartości CLA10 zaobserwowano u krów pierwiastek. Warto zauważyć, że nastąpiło znaczne obniżenie zawartości CLA10 u zdrowych zwierząt, który w trakcie całego doświadczenia wynosił 74,7% u krów pierwiastek i 39,8% u krów wieloródek, natomiast wzrost zawartości o 81,25% nastąpił u krów wieloródek z ketozą.

Wyniki przeprowadzonych badań wskazują na istotne zmiany w koncentracji długołańcuchowych kwasów tłuszczowych, szczególnie kwasu linolowego (C18:2 n-6; LA) oraz skoniugowanych dienów kwasu linolowego (CLA), w zależności od stanu metabolicznego, fazy laktacji oraz grupy doświadczalnej. W mleku pierwiastek (PH) oraz wieloródek (MH) wykazany został stopniowy spadek zawartości LA w czasie trwania eksperymentu, co może wskazywać na adaptacyjne mechanizmy metaboliczne regulujące równowagę lipidową w odpowiedzi na zwiększone zapotrzebowanie energetyczne organizmu w pierwszej fazie laktacji. Takie zmiany mogą wynikać z mobilizacji tłuszczu z tkanki tłuszczowej, a także z osłabienia aktywności biosyntezującej gruczołu mlekowego w warunkach zwiększonego stresu metabolicznego. U krów ze zdiagnozowaną ketozą (MK, PK), zawartość LA w mleku wykazała większą zmienność w porównaniu do grupy zwierząt zdrowych, co może sugerować zaburzenia w metabolizmie lipidów oraz deficyt energetyczny wynikający z upośledzonej funkcji wątroby i zaburzeń w szlakach biosyntezujących kwasy tłuszczowe. Obniżenie poziomu LA, szczególnie w pierwszym i drugim tygodniu laktacji, sugeruje ograniczoną zdolność organizmu do skutecznej mobilizacji rezerw tłuszczowych, co jest charakterystyczne dla stanu ketozy. Wzrost koncentracji LA w trzecim tygodniu laktacji w grupie MK, mimo iż zauważalny, pozostawał niższy w porównaniu do grupy zdrowych zwierząt, co może wskazywać na niepełną adaptację organizmu do zmienionych warunków metabolicznych. W odniesieniu do skoniugowanych dienów kwasu linolowego, zawartość CLA9 oraz CLA10 również wykazywała istotne zmiany w zależności od grupy zwierząt oraz fazy laktacji. Wysoka zawartość CLA9 w mleku krów PH i MH w pierwszym tygodniu laktacji wskazuje na aktywność enzymatyczną, szczególnie izomerazy CLA, która konwertuje kwas linolowy do formy skoniugowanej. Obniżenie

stężenia CLA9 w mleku krów z ketozą może być wynikiem upośledzenia aktywności enzymów zaangażowanych w ten proces, co jest konsekwencją dysfunkcji metabolicznych związanych z zaburzeniem metabolizmu tłuszczów. Wzrost zawartości CLA9 w trzecim tygodniu laktacji w grupie krów z ketozą sugeruje możliwość częściowej kompensacji tej dysfunkcji, jednak wartości te nadal pozostawały niższe w porównaniu do grup zwierząt zdrowych, co może wskazywać na ograniczoną zdolność organizmu do produkcji tych izomerów w wyniku przewlekłego deficytu energetycznego. Zawartość CLA10, również uzyskiwana w wyniku izomeryzacji CLA9, była najwyższa w mleku krów MH, co sugeruje dużą aktywność enzymów uczestniczących w tym procesie w grupie zdrowych zwierząt. Jednak obniżenie koncentracji CLA10 w mleku krów z ketozą może być związane z zaburzeniem metabolizmu lipidów, szczególnie w kontekście zmniejszenia efektywności syntez i metabolizmu kwasów tłuszczowych. Istotna różnica w zawartości CLA10 między grupą zdrowych krów a zwierzętami z ketozą, szczególnie w drugim i trzecim tygodniu laktacji, może świadczyć o upośledzeniu szlaków biosyntezujących CLA oraz ich izomerów, co jest konsekwencją przewlekłego stresu metabolicznego, który upośledza zdolności enzymatyczne i skutkuje obniżeniem produkcji kwasów tłuszczowych o właściwościach prozdrowotnych.

Doświadczenie 2.

Ocena parametrów użytkowych mleka krów rasy PHF oraz ich mieszańców F1 PHF×SRB w kontekście równowagi energetycznej oraz adaptacji metabolicznej do warunków produkcyjnych.

Wyniki doświadczenia zostały opublikowane w publikacji:

Solarczyk P., Slósarz J., Gołębiewski M., Puppel K. 2021. A comparison between Polish Holstein-Friesian and F_1 hybrid Polish Holstein-Friesian × Swedish Red cows in terms of milk yield traits, *Mljekarstvo* 71(2), 141-150.

Użytkowość mleczna krów stanowi kluczowy element określający efektywność ekonomiczną gospodarstw mlecznych. W badaniach własnych dzienna produkcja mleka w pierwszej laktacji była istotnie wyższa o 14,61% u krów rasy PHF w porównaniu do mieszańców PHF×SRB. Zawartość białka, tłuszczu i suchej masy w mleku była istotnie wyższa w mleku mieszańców PHF×SRB w porównaniu do mleka krów rasy PHF. Niższa zawartość poszczególnych składników w mleku krów rasy PHF jest związana z efektem rozcieńczenia, który jest wynikiem większej produkcji mleka. Na wyższą zawartość

białka, tłuszczu i suchej masy u mieszańców PHF×SRB wpływa również rasa szwedzka czerwona, która charakteryzuje się wyższą zawartością poszczególnych składników mleka, co związane jest z wieloletnią pracą hodowlaną prowadzoną w tym kierunku. Na podstawie uzyskanych wyników w doświadczeniu dla stosunku tłuszczu do białka (F/P) można stwierdzić, że dawka pokarmowa dla obu grup zwierząt została skomponowana w sposób prawidłowy, a zwierzęta nie wykazywały zaburzeń metabolicznych ze względu na średnią wartość stosunku tłuszcz białko: 1,26 dla PHF i 1,29 dla PHF×SRB.

Wydajność mleczna krów stanowi ważne kryterium oceny efektywności produkcji ferm mlecznych związanych z prowadzoną pracą hodowlaną oraz żywieniem zwierząt. Krowy rasy PHF biorące udział w doświadczeniu miały dłuższą o 63,47 dni laktację w porównaniu do krów mieszańców. Krótsza laktacja u mieszańców międzyrasowych może wskazywać na efektywną zdolność do osiągnięcia równowagi metabolicznej po ciąży, co w konsekwencji przyspiesza wystąpienie pierwszej rui po porodzie. W związku z dłuższą laktacją oraz wyższą dzienną produkcją mleka wydajność mleczna krów rasy PHF jest istotnie wyższa (o 2919,4 kg), a mleko charakteryzuje się niższą zawartością poszczególnych składników użytkowych (tłuszcz -0,50%, białko -0,28%, sucha masa -0,74%), decydujących o jego jakości technologicznej, w porównaniu z mlekiem mieszańców PHF×SRB. Jednak ilość pozyskanego surowca, mimo niższej koncentracji składników, skutkuje wyższą wydajnością mleka od krów rasy PHF (tłuszcz +76,11 kg, białko +73,7 kg, sucha masa +313,97 kg).

Podsumowując, wyniki badań jednoznacznie wskazują, że krzyżowanie ras PHF i SRB stanowi efektywną strategię poprawy jakości mleka, szczególnie w zakresie zawartości białka, tłuszczu oraz suchej masy, przy jednoczesnym zachowaniu wysokiej wydajności mlecznej. Efekt heterozji, obserwowany u mieszańców PHF×SRB, prowadzi do wyższej koncentracji składników odżywczych w mleku, szczególnie białka i tłuszczu, w porównaniu do krów czystorasowych rasy PHF. Podwyższenie koncentracji białka i tłuszczu w mleku stanowi korzystny aspekt zarówno pod względem jakościowym, jak i ekonomicznym, w kontekście produkcji mleka o wyższej wartości odżywczej.

Doświadczenie 3.

Badanie potencjału antyoksydacyjnego i oksydacyjnej stabilności mleka, w tym aktywności enzymów antyoksydacyjnych oraz zawartości markerów stresu oksydacyjnego w zależności od genotypu krów mlecznych.

Wyniki doświadczenia zostały opublikowane w publikacji:

Solarczyk P., Slósarz J., Gołębiewski M., Natalello A., Musati M., Luciano G., Priolo A., Puppel K. 2024. The Influence of Crossbreeding on the Composition of Protein and Fat Fractions in Milk: A Comparison Between Purebred Polish Holstein Friesian and Polish Holstein Friesian × Swedish Red cows. *Nutrients* 16(21), 3634.

Uzyskane wyniki badań wskazują na istotny wpływ efektu heterozji wynikającego z krzyżowania ras PHF i SRB na skład chemiczny mleka, jego wartość odżywczą oraz profil funkcjonalny. Krzyżowanie tych dwóch ras wykazuje pozytywne efekty w zakresie poprawy zawartości białka, tłuszczu oraz suchej masy w mleku, przy jednoczesnym zachowaniu wysokiej wydajności mlecznej. Heterozja jako zjawisko polegające na wyższej wydajności fenotypowej mieszańców w porównaniu do rodziców czystorasowych, prowadzi do poprawy jakości mleka, co jest zgodne z literaturą dotyczącą korzystnego wpływu genotypu na składniki odżywcze w mleku. Zawartość białka w mleku krów mieszańców PHF×SRB była wyższa o 7,62% w porównaniu do krów rasy PHF, co może wynikać z genotypu rasy szwedzkiej czerwonej, która charakteryzuje się wyższą zawartością białka w mleku. Dodatkowo, heterozja prowadzi do korzystniejszych wyników jakościowych u mieszańców, co może być powiązane z wyższą zdolnością do syntezowania specyficznych laktoprotein mleka, takich jak białka serwatkowe (WP) i kazeina (Cas). Wyższa koncentracja WP u mieszańców PHF×SRB sugeruje korzystniejszy profil białkowy, który może mieć istotne znaczenie w kontekście wartości odżywczej mleka. W mleku czystorasowych krów rasy PHF, Casstanowiła 84,76% białka całkowitego, podczas gdy u krów mieszańców PHF×SRB stanowiła 82,15%. Zawartość WP była o około 26% niższa w mleku czystorasowych PHF w porównaniu z mlekiem krów mieszańców PHF×SRB. Co ciekawe mimo wyższej zawartości WP, zawartość lizozymu (Lz) była o około 17,19% niższa w mleku krów mieszańców niż w mleku krów rasy PHF. Poziom laktoferyny (Lf) wykazywał również wyraźnie niższy poziom w mleku krów mieszańców o około 55,26% niż w mleku krów rasy PHF. Natomiast odwrotna zależność wykazana została w przypadku kształtowania

się BSA, której zawartość była o około 27,78% wyższa w mleku krów mieszańców PHF×SRB w porównaniu z mlekiem krów rasy PHF. Ponadto poziom BLG w mleku krów mieszańców był o około 45% wyższy niż u krów rasy PHF. Wreszcie, aktywność Lp była o około 14,71% wyższa w mleku krów mieszańców w porównaniu z mlekiem krów PHF. Różnice w profilu białkowym mleka między rasą PHF a mieszańcami PHF×SRB wynikają z genotypowych różnic w składzie białek, które mogą być efektem zarówno selekcji, jak i zjawiska heterozji. Mleko krów rasy PHF charakteryzuje się wyższym udziałem kazeiny. Z kolei w mleku mieszańców PHF×SRB stwierdzono wyższy poziom białek serwatkowych, w tym Lf, Lz oraz BSA, co może wynikać z większej ekspresji genów związanych z syntezą białek serwatkowych. Heterozja poprawia efektywność metaboliczną organizmu, co może prowadzić do zwiększonej syntezy białek o charakterze ochronnym i immunologicznym w mleku mieszańców. Ponadto, różnice w zawartości WP mogą świadczyć o odmiennych mechanizmach regulacji syntezy białek.

Mleko mieszańców PHF×SRB wykazało wyższą zawartość krótko- i średniołańcuchowych SFA, w tym kwasu masłowego (C4:0), kwasu kaprylowego (C10:0), kwasu laurynowego (C12:0) i kwasu mirystynowego (C14:0), w porównaniu z mlekiem krów rasy PHF. Różnice te były statystycznie istotne, wskazując na wynikającą z genotypu poprawę syntezy tych specyficznych SFA. Natomiast mleko krów rasy PHF miało wyższą zawartość kwasu stearynowego (C18:0), co sugeruje, różne szlaki metaboliczne, które wpływają na syntezę długołańcuchowych kwasów tłuszczowych. Zaobserwowane rozbieżności sugerują, że krzyżowanie wpływa na profil FA mleka, wzmacniając niektóre FA związane z właściwościami odżywczymi i funkcjonalnymi. Porównanie zawartości nienasyconych kwasów tłuszczowych (UFA) u krów czystorasowych PHF i krów mieszańców PHF×SRB ujawniło znaczące różnice związane z genotypem. Mieszańce wykazywały wyższe stężenie kwasu palmitooleinowego (C16:1), podczas gdy krowy PHF wykazywały znacznie wyższe poziomy kwasu C18:1 c9 i kwasu wakcenowego (C18:1 t11). Ponadto zawartość PUFA była wyższa w mleku PHF, z wyższymi poziomami kwasu linolowego (C18:2 c9, c12 n-6), kwasu α-linolenowego (C18:3 n-3) i CLA, co sugeruje korzystniejszy profil UFA u krów rasy PHF. Odwrotnie, było w przypadku kwasu γ-linolenowego (C18:3 n-6) i niektórych długołańcuchowych kwasów tłuszczowych n-6, gdzie mieszańce PHF×SRB wykazywały podwyższony poziom, co wskazuje na zróżnicowany metabolizm FA między dwoma genotypami. Mimo, iż różnice kwasów typu trans nie były istotne statystycznie, to ogólne

wyniki sugerują, że genotyp znacząco wpływał na zawartość UFA, co ma potencjalnie wpływ na właściwości odżywcze i funkcjonalne mleka.

Potencjał antyoksydacyjny mleka był istotnie wyższy w mleku krów czystorasowych PHF, o 14,0%, w porównaniu z mlekiem krów mieszańców PHF×SRB. Dodatkowo, stężenie witaminy E było wyższe o 28,3% w mleku krów rasy PHF. Podobna tendencja wykazana została również dla witamin D (15,3%) i K (10,1%). Wyższy poziom witamin E, D i K w mleku krów rasy PHF może być wynikiem bardziej efektywnego magazynowania tych składników w organizmach zwierząt tej rasy. Rasa PHF, znana z wysokiej wydajności mlecznej, charakteryzuje się bardziej efektywnymi mechanizmami akumulacji antyoksydantów, co jest powiązane z lepszym wykorzystaniem składników odżywczych.

W kontekście wyników uzyskanych w niniejszym badaniu, różnice w profilu witaminowym i potencjale antyoksydacyjnym mleka mogą mieć istotne znaczenie dla jakości mleka oraz prozdrowotnych właściwości produktów mlecznych. Chociaż heterozja wykazuje korzystny wpływ na użytkowość mleczną, w tym na poprawę parametrów użytkowych mleka, to w przypadku niektórych składników, takich jak witaminy, może prowadzić do pewnych rozbieżności. Zatem, w zależności od pożądanych cech mleka, krzyżowanie międzyrasowe może wymagać dalszej optymalizacji, uwzględniającej zarówno użytkowość mleczną, jak i zawartość składników antyoksydacyjnych, szczególnie witamin i substancji bioaktywnych.

Doświadczenie 4.

Ocena parametrów rozrodu, profilu metabolicznego oraz składu lipidowego mleka krów rasy PHF oraz ich mieszańców F1 PHF×SRB, z uwzględnieniem wpływu wieku pierwszego wycielenia na mechanizmy regulacyjne.

Wyniki doświadczenia zostały opublikowane w publikacji:

Solarczyk P., Gołębiewski M., Slósarz J., Natalello A., Musati M., Menci R., Sakowski T., Tucki K., Puppel K. 2024. Effect of Age at First Calving on the Reproduction Parameters, Metabolic Profile, and Fatty Acid Composition of Polish Holstein Friesian (PHF) and Crossbreds PHF × Swedish Red (SRB) cattle. *Metabolites* 14(11) 583.

Wysokie koszty produkcji skłaniają hodowców do poszukiwania rozwiązań mających wymierny wpływ na efektywność produkcji. Jednym z bardziej kosztownych zabiegów w gospodarstwach mlecznych jest odchów jałówek na remont stada, dlatego

działania skupiają się nad poszukiwaniem rozwiązań, które będą optymalizować ten proces. Jednym z nich jest skrócenie okresu odchowu, tak aby zwierzęta wchodziły w okres reprodukcyjny w optymalnym momencie. Badania wykazały istotne różnice w wieku osiągnięcia dojrzałości hodowlanej między jałówkami rasy PHF a mieszańcami PHF×SRB, której wyznacznikiem był wiek pierwszej inseminacji. Jałówki PHF×SRB inseminowane po raz pierwszy były wieku 434,9 dni, podczas gdy rasy PHF miały średni wiek pierwszej inseminacji wynoszący 454,5 dni ($p < 0,001$). Wykazana istotna różnica wskazuje na wpływ genotypu na wiek pierwszej inseminacji. Jednakże, mimo osiągnięcia wcześniejszej dojrzałości, PHF×SRB wymagały o 2,41% większej liczby dawek inseminacyjnych, aby inseminacja była skutecznaczego konsekwencją był wyższy indeks zacieleń i dłuższy okres usługi o 4,31% (średnio o 14 dni) w porównaniu do PHF. W odniesieniu do długości ciąży, krowy PHF×SRB miały średnią długość ciąży wynoszącą 280,9 dni, co stanowiło dłuższy okres o 0,21% w porównaniu do krów PHF (280,3 dni). Mimo wcześniejszego osiągania dojrzałości hodowlanej przez PHF×SRB, pozostałe wyniki nie wykazały jednoznacznego wpływu krzyżowania międzyrasowego na wybrane wskaźniki rozrodu.

Po wycieleniu krowy można było podzielić na grupy związane z wiekiem pierwszego wycielenia. Mieszańce PHF×SRB obu grup (<2: 78,5 dnia, >2: 86,2 dnia) wykazywały krótszy okres przestoju poporodowego w porównaniu z grupami krów rasy PHF (<2: 97,5 dnia, >2: 87,7 dnia) (wartość $p \leq 0,001$). Krótszy okres przestoju poporodowego podkreśla zdolność do regeneracji organizmu po ciąży oraz powrotu do homeostazy organizmu, na co prawdopodobnie miał wpływ genotyp zwierząt. Indeks zacieleń, u mieszańców PHFxSRB (<2: 2,27, >2: 1,54) wykazywał niższe wartości dla krów wycielonych w wieku powyżej dwóch lat w stosunku do krów PHF (<2: 2,09, >2: 2,43) (wartość $p \leq 0,001$), co może potwierdzać przypuszczenie związane z lepszą zdolnością regeneracyjną mieszańców w porównaniu z krowami rasy PHF, ale także z mniejszą mobilizacją energii do produkcji mleka. Okres usługi był porównywalny we wszystkich kategoriach wiekowych obu grup (<2: 59,8 dni, >2: 62,9 dni dla PHF; <2: 37,1 dni, >2: 28,9 dni dla PHF×SRB) ($p = 0,224$), co wskazuje na podobną dynamikę czasową niezależnie od genotypu, chociaż mieszańce PHF×SRB miały tendencję do krótszego okresu usługi. Podobnie, okres międzyciążowy był zbliżony w obu grupach wiekowych i genotypowych (<2: 157,3 dni, >2: 150,6 dni dla PHF; <2: 115,5 dni, >2: 115,1 dni dla PHF×SRB) ($p = 0,556$), co sugeruje, że genotyp nie wpływał istotnie na długość tego okresu, choć u mieszańców międzyrasowych zaobserwowano tendencję do krótszego okresu

międzyciążowego. Częstotliwość wycieleń, która jest ważnym wskaźnikiem efektywności zarządzania stadem, była spójna we wszystkich grupach (p = 0,878). Mimo to, mieszańce PHF×SRB miały tendencję do krótszego okresu międzywycieleniowego niż krowy rasy PHF. Z kolei, okres ciąży wykazał istotne różnice pomiędzy grupami. Mieszańce PHF×SRB miały średnią długość ciąży odpowiednio 283,3 dnia (<2) i 278,2 dnia (>2), podczas gdy krowy PHF miały średnią długość ciąży wynoszącą 280,5 dnia (<2) i 280,0 dnia (>2) (p≤0,001). Ta rozbieżność sugeruje, że krzyżowanie międzyrasowe może wpływać na długość ciąży, prawdopodobnie poprzez złożoną interakcję genetyczną, co ma potencjalne implikacje dla strategii zarządzania stadem.

Duże znaczenie w chowie bydła mlecznego ma stan zdrowia zwierząt, który oddziałuje nie tylko na ilość produkowanego mleka, ale także na jego jakość oraz na zdolności rozrodcze. Wśród krów mlecznych największym problemem wydają się być choroby o podłożu metabolicznym, w tym przede wszystkim ketoza, która może dotyczyć nawet 80% wszystkich krów. Ketoza, będąca efektem zaburzenia bilansu energetycznego w początkowej fazie laktacji, prowadzi do mobilizacji zapasów tłuszczu, a jej diagnostyka opiera się na analizie ciał ketonowych oraz poziomie NEFA (niezestryfikowane kwasy tłuszczowe), uwalnianych z tkanki tłuszczowej w wyniku katabolizmu. Badanie poziomu NEFA u krów PHF i mieszańców PHF×SRB wskazało na istotne różnice. Mieszańce międzyrasowe PHF×SRB charakteryzowały się istotnie niższym poziomem NEFA w porównaniu z PHF. Wartość NEFA była o 70% wyższa u krów PHF niż u mieszańców, co może wskazywać na większą ilość mobilizowanej tkanki tłuszczowej z organizmu krów. Najważniejszym i najczęściej stosowanym wskaźnikiem do diagnozowania ketozy jest powstający z NEFA w czasie estryfikacji BHBA. Obie grupy mieszańców międzyrasowych PHF×SRB wykazywały niższe poziomy BHBA w porównaniu do krów rasy PHF. Szczególnie wysoki poziom BHBA występował u krów rasy PHF, które wycieliły się w wieku powyżej 2 lat, co może wskazywać na większe prawdopodobieństwo wystąpienia ketozy oraz większą mobilizację tkanki tłuszczowej do wytworzenia energii niezbędnej do życia krów. Glukoza, to kolejny ważny czynnik odzwierciedlający równowagę metaboliczną i dobrostan bydła ujawniający specyficzne różnice dla właściwego genotypu w różnych grupach wiekowych. Mieszańce PHF×SRB wykazywały znacznie niższe poziomy glukozy w porównaniu do obu kategorii wiekowych krów rasy PHF. Zaobserwowane różnice procentowe wynoszące -1,81% dla bydła w wieku pierwszego wycielenia <2 lat i bardziej wyraźne -6,99% dla bydła w wieku pierwszego wycielenia >2 lat. Różnice statystyczne uzyskanych wyników (p < 0,001)

określają wpływ specyficznych czynników genetycznych dla genotypów wykorzystanych w doświadczeniu w modulowaniu procesów metabolicznych, które znacząco wpływają na homeostazę glukozy w ocenianych grupach bydła. Przechodząc do poziomów białka, kluczowego wskaźnika stanu fizjologicznego, porównanie między grupami PHF i PHF×SRB wykazało istotne różnice. Mieszańce PHF×SRB charakteryzowały się znacznie niższym poziomem białka w obu kategoriach wiekowych. Różnice pomiędzy grupami doświadczalnymi wynosiły -5,65% dla krów wycielonych w wieku <2 lat i -2,97% dla krów wycielonych w wieku >2 lat (p = 0,016), co określa możliwy wpływ rasy na metabolizm białek. Albuminy, czyli białka osocza regulujące równowagę osmotyczną i transport niektórych hormonów i FA wykazują potencjalne różnice w wykorzystaniu składników odżywczych i stanu zdrowia zwierząt. Analiza poziomu albuminy wskazała znaczące różnice między grupami PHF i PHF×SRB. Mieszańce PHF×SRB miały znacznie wyższe poziomy albuminy w obu kategoriach wiekowych niż bydło rasy PHF. Zaobserwowane różnice procentowe wynoszące +6,42% dla krów wycielonych w wieku <2 lat i +13,75% dla krów wycielonych w wieku >2 lat, podkreślają potencjalny wpływ genotypu na metabolizm, wpływając na dynamikę powstawania. Analiza poziomu kreatyniny ponownie ujawniła znaczące różnice między grupami PHF i PHF×SRB. U mieszańców PHF×SRB konsekwentnie odnotowano znacznie wyższe poziomy kreatyniny w obu kategoriach wiekowych w porównaniu z bydłem rasy PHF. Zaobserwowane różnice wynosiły +4,23% dla krów wycielonych w wieku <2 lat i znaczące +27,93% dla wycielonych w wieku >2 lat, co wskazuje na potencjalny wpływ czynników genetycznych pracę nerek.

Gamma-glutamylotransferaza (GGTP) jest enzymem występującym głównie w wątrobie, ale także w innych narządach, takich jak nerki i trzustka. Jako marker biochemiczny, GGTP jest powszechnie stosowana w diagnostyce zaburzeń funkcji wątroby oraz chorób układu żółciowego. Enzym ten bierze udział w metabolizmie glutationu, a jego podwyższony poziom jest często związany z zaburzeniami w funkcjonowaniu hepatocytów i dróg żółciowych. Aktywność GGTP wykazała istotne różnice między krowami rasy PHF a mieszańcami PHF×SRB. W grupie mieszańców PHF×SRB zaobserwowano istotnie obniżoną aktywność GGTP w porównaniu do krów rasy PHF w obu analizowanych grupach wiekowych. Różnice procentowe wyniosły -20,94% w przypadku krów wycielonych w wieku poniżej 2 lat oraz -35,47% u krów wycielonych po 2. roku życia, co zostało potwierdzone statystycznie (p=0,015). Wykazane różnice sugerują, że czynniki genotypowe mogą wpływać na aktywność

enzymów wątrobowych, w tym na regulację poziomu GGTP, co może mieć konsekwencje dla procesów metabolicznych i homeostazy oksydacyjnej organizmu.

Bydło rasy PHF wycielone w wieku <2 lat wykazywało wyższe poziomy C6:0 (1,868 g/100 g) w porównaniu do obu grup PHF×SRB (1,548 g/100 g dla <2 i 1,600 g/100 g dla >2). Różnica ta była istotna statystycznie (p=0,001). W przypadku C10:0, wystąpiła znacząca różnica w poziomach między grupami PHF i PHF×SRB cielącymi się w wieku >2 lat, gdzie mleko mieszańców PHF×SRB charakteryzowało się wyższym poziomem (2,745 g/100 g vs. 2,167 g/100 g), z istotnością wynoszącą 0,024. Podobny trend zaobserwowano w przypadku C12:0, mleko mieszańców PHF×SRB wycielonych w wieku >2 lat miało wyższy poziom (3,058 g/100 g) w porównaniu z mlekiem krów rasy PHF (2,800 g/100 g), a różnica ta była statystycznie istotna (p=0,001). Warto zwrócić uwagę na to, że C16:0 nie wykazał wyższego poziomu w mleku krów mieszańców PHF×SRB wycielonych w wieku >2 lat (32,148 g/100 g) w porównaniu z zawartością w mleku krów rasy PHF (32,195 g/100 g), wynik ten nie wykazywał różnicy statystycznie istotnej (p=0,107). Przechodząc do C18:0, nie zaobserwowano znaczących różnic między grupami PHF i PHF×SRB dla żadnej kategorii wiekowej, z p=0,587. Jednak zawartość C20:0 wykazała znaczną różnicę u krów wycielonych w wieku <2 lat. Krowy mieszańce PHF×SRB wycielone w wieku <2 lat wykazywały znacznie niższy poziom (0,072 g/100 g) w porównaniu do krów rasy PHF (0,243 g/100 g), co wskazuje istotność statystyczna na poziomie p <0,001. Zawartość izomerów sprzężonego kwasu linolowego, CLA c9, tr11 i CLA tr10, c12, również wykazywały znaczące różnice. Oba te izomery miały niższy poziom w mleku obu grup mieszańców PHF×SRB w porównaniu do mleka krów rasy PHF, niezależnie od kategorii wiekowej, z wartościami p odpowiednio <0,001 i 0,059. Wreszcie, C22:0, FA o stosunkowo niskiej zawartości w mleku. Poziom C22:0 różnił się znacznie między grupami, szczególnie u krów wycielonych w wieku >2 lat (p = 0,006).

Doświadczenie 5.

Analiza potencjału antyoksydacyjnego tkanki mięśniowej buhajów rasy PHF, LM oraz mieszańców F1 PHF×LM, z uwzględnieniem mechanizmów obrony przed stresem oksydacyjnym, oceny aktywności enzymów antyoksydacyjnych oraz stężenia markerów uszkodzeń oksydacyjnych w tkance mięśniowej.

Wyniki doświadczenia zostały opublikowane w publikacji:

Solarczyk P., Gołębiewski M., Slósarz J., Łukasiewicz M., Przysucha T., Puppel K. 2020. Effect of Breed on the Level of the Nutritional and Health-Promoting Quality of Semimembranosus Muscle in Purebred and Crossbred Bulls. *Animals* 10(10), 1822.

O wartości biologicznej i odżywczej mięsa decyduje zawartość białka w mięśniu. Wyższą zawartość białka w tkance mięśniowej stwierdzono u mieszańców rasy PHF×LM, która wynosiła 22,41 g/100 g mięsa, natomiast najniższą w tkance mięśniowej buhajów rasy PHF, wynoszącej 19,4 g/100 g mięsa. Uzyskane wartości wykazały, że rasa miała istotny wpływ na zawartość białka w tkance mięśniowej (p-value 0,000).

Badania własne wykazały, że tkanka mięśniowa buhajów rasy LM charakteryzowała się najniższym poziomem tłuszczu (1,85 g/100 g), natomiast najwyższym tkanka rasy PHF (2,95 g/100 g mięsa). Kolejnym ważnym składnikiem mięsa jest kolagen. Najwyższą zawartość kolagenu stwierdzono u buhajków rasy PHF, która wynosiła 592,94 mg/100 g mięsa, a najniższą u mieszańców PHF×LM (492,24 mg/100 g).

Prozdrowotna jakość mięsa zależy od zawartości nasyconych kwasów tłuszczowych (SFA). Najwyższy poziom SFA, wynoszący 49,74 g/100 g, stwierdzono w tkance mięśniowej buhajów rasy PHF. Należy podkreślić, że rasa ta charakteryzowała się najwyższą zawartością wszystkich badanych kwasów tłuszczowych: kwasu laurynowego (C12:0; 0,09 g/100 g), kwasu palmitynowego (C16:0; 27,47 g/100 g) i kwasu stearynowego (C18:0; 27,47 g/100 g). Poziomy wyżej wymienionych kwasów tłuszczowych w tkance mięśniowej buhajów rasy LM i mieszańców PHF×LM były zbliżone, szczególnie w przypadku kwasów C12:0, C14:0 i C16:0. Istotną różnicę stwierdzono w przypadku zawartości kwasu C18:0, gdzie u buhajów rasy LM poziom wynosił 18,40 g/100 g, a u mieszańców PHF×LM 16,87 g/100 g. Najniższą całkowitą zawartość SFA (43,99 g/100 g) zaobserwowano u buhajków mieszańców PHF×LM. Podczas analizy próbek okazało się, , że stężenie SFA i C18:0 było istotnie zależne od genotypu zwierząt (p-value 0,000). Innymi kwasami tłuszczowymi, które wpływają na wartość odżywczą i prozdrowotną wołowiny są jednonienasycone kwasy tłuszczowe (MUFA) i wielonienasycone (PUFA). Głównymi kwasami PUFA n-3 w wołowinie są kwasy α-linolenowy (18:3 n-3), eikozapentaenowy (20:5 n-3) i dokozaheksaenowy (22:6 n-3

Pierwszym z analizowanych składników lipidowych był kwas wakcenowy (C18:1 trans-11; TVA), odpowiedzialny za wydajność tkanek i narządów. Jego najwyższy poziom stwierdzono w tkance mięśniowej mieszańców PHF×LM (1,31 g/100 g), a

najniższy u buhajów rasy PHF (0,83 g/100 g). Najwyższą zawartość LA, 8,70 g/100 g, stwierdzono w mięsie pozyskanym od buhajów rasy LM. Nieco niższy poziom stwierdzono u mieszańców PHF×LM (7,97 g/100 g), a najniższy u PHF (6,24 g/100 g). W tkance mięśniowej buhajów rasy LM oraz mieszańców PHF×LM odnotowano odpowiednio zawartość 3,26 g/100 g i 3,59 g/100 g, natomiast w tkance mięśniowej PHF wynosiła 2,59 g/100 g. Badania wykazały, że na zawartość CLA istotny wpływ miała rasa. Kwas α-linolowy (C18:3 n-3; LNA) odgrywa kluczową rolę w glikolizie wątrobowej, lipogenezie *de novo* i regulacji kwasów tłuszczowych. Najwyższy poziom LNA stwierdzono w tkance mięśniowej mieszańców PHF×LM, a wynosił on 0,71 g/100 g, natomiast najniższy był u buhajów rasy PHF i wynosił 0,49 g/100 g. Innymi analizowanymi kwasami były kwas eikozapentaenowy i dokozaheksaenowy, które wspomagają funkcjonowanie układu nerwowego. Stwierdzono, że zarówno EPA, jak i DHA występują w największej ilości w mięsie mieszańców (0,65 g/100 g i 0,16 g/100 g), a najmniej stwierdzono w mięsie rasy PHF (0,42 g/100 g i 0,07 g/100 g).

Bioaktywne dipeptydy, w tym karnozyna i anseryna, są ważnymi składnikami wołowiny wpływającymi na jej wartość prozdrowotną. Anseryna (β-alanylo-L-(N-metylo) histydyna) jest pochodną metylokarnozyny. Jest to dipeptyd składający się z -alaniny i L-(N-metylo) histydyny. Występuje głównie w mięśniach szkieletowych i mózgu, a w organizmach ssaków działa jako przeciwutleniacz. Najwyższy poziom anseryny (83,64 mg/100 g) stwierdzono w mięsie rasy LM, a najniższy w mięsie rasy PHF (61,22 mg/100 g). Zawartość karnozyny w mięśniach mieszańców była najwyższa i wynosiła 492,36 mg/100 g, a najniższy poziom odnotowano w mięśniach rasy PHF i wynosił on 387,3 mg/100 g. Tauryna należy do aminokwasów i powszechnie występuje w tkankach zwierzęcych. Brak tauryny w diecie powoduje zmniejszenie liczby leukocytów oraz zdolności neutrofili do wybuchu tlenowego i fagocytozy. Najwyższy poziom tauryny, 48,99 mg/100 g, stwierdzono u mieszańców PHF×LM, a najniższy w mięsie rasy PHF, 34,28 mg/100 g. Koenzym Q10, zwany również ubichinonem, jest związkiem występującym w każdej komórce organizmu i odgrywa w niej kluczową rolę. Obniżenie poziomu koenzymu Q10 sprzyja rozwojowi chorób powstających m.in. w wyniku działania reaktywnych form tlenu, np. chorób układu krążenia czy nowotworów. Najwyższy poziom koenzymu Q10, 2,67 mg/100 g, stwierdzono w tkance mieszańców PHF×LM, a najniższy w mięsie rasy PHF, 1,87 mg/100 g.

Badania wykazały, że na stężenie β-karotenu i α-retinolu istotny wpływ miał rodzaj rasy. Badanie wykazało prawie dwukrotnie wyższy poziom β-karotenu w grupie

mieszańców PHF×LM w porównaniu do grupy PHF. Najwyższy poziom α-tokoferolu, stwierdzono u mieszańców PHF×LM 4,76 μg/g, a najniższy w mięsie PHF, 1,61 μg/g. Można zatem stwierdzić, że zwiększony poziom α-tokoferolu w tkankach chronił je przed powstającymi przebarwieniami i utlenianiem lipidów. Należy podkreślić, że jakość tkanki mięśniowej pozyskanej od zwierząt biorących udział w doświadczeniu jest zróżnicowana, na co znacząco wpływa genotyp zwierząt.

Doświadczenie 6.

Badanie potencjału antyoksydacyjnego tkanki mięśniowej oraz zdrowotności cieląt rasy PHF, w zależności od systemu odchowu, z uwzględnieniem wpływu rodzaju odchowu na rozwój mechanizmów ochrony przed stresem oksydacyjnym.

Wyniki doświadczenia zostały opublikowane w publikacji:

Solarczyk P., Sakowski T., Gołębiewski M., Slósarz J., Grodkowski G., Grodkowska K., Biondi L., Lanza M., Natalello A., Puppel K. 2023. The Impact of Calf Rearing with Foster Cows on Calf Health, Welfare, and Veal Quality in Dairy Farms. *Agriculture* 13(9) 1829.

Zdrowotność jest bardzo ważnym czynnikiem pływającym na odchów zwierząt, u cieląt najczęściej spotykanymi problemami zdrowotnymi są: biegunki, kaszel oraz katar. W badaniach własnych, biegunka występowała zdecydowanie częściej u zwierząt z grupy kontrolnej, w której cielęta mleko pobierały z automatów wyposażonych w smoczki w porównaniu do cieląt odchowywanych przez mamki. Podobna sytuacja dotyczyła przypadków wystąpienia kataru oraz kaszlu.

Pomiary masy ciała wykonano sześciokrotnie w trakcie trwania doświadczenia w odstępach czterotygodniowych od dnia urodzenia do dnia uboju cieląt.

Początkowa masa ciała cieląt była porównywalna w obu grupach, wynosząc średnio 43,36 kg dla grupy kontrolnej i 43,64 kg dla grupy eksperymentalnej, w której cielęta były karmione przez mamki. W czwartym tygodniu życia przeprowadzono drugi pomiar masy ciała. W grupie kontrolnej średnia masa cieląt wynosiła 58,76 kg, co oznaczało przyrost o 15,4 kg, czyli 33,44% w stosunku do masy urodzeniowej. W grupie doświadczalnej średnia masa wzrosła do 62,21 kg, co stanowiło przyrost o 18,58 kg (42,58%) względem wartości początkowej. Kolejny pomiar, wykonany w ósmym tygodniu doświadczenia, wykazał, że masa cieląt w grupie kontrolnej osiągnęła średnio 84,21 kg, co stanowiło wzrost o 40,85 kg, czyli 94,21% w stosunku do masy

urodzeniowej. W grupie eksperymentalnej masa ciała cieląt wyniosła 92,57 kg, co odpowiadało wzrostowi o 48,93 kg (112,12%). Różnica średniej masy ciała cieląt pomiędzy grupą kontrolną a eksperymentalną wyniosła -8,36 kg ($p \leq 0,05$). Czwarty pomiar masy ciała, wykonany w 12. tygodniu życia, wykazał, że średnia masa cieląt w grupie kontrolnej wynosiła 114,00 kg, co stanowiło wzrost o 70,64 kg (162,92%) względem masy urodzeniowej. Cielęta w grupie eksperymentalnej osiągnęły w tym okresie średnią masę 130,71 kg, co oznaczało wzrost o 87,07 kg (199,52%). Odnotowano istotną różnicę między średnią masą cieląt w obu grupach wynoszącą -16,71 kg ($p \leq 0,01$). W 16. tygodniu życia przeprowadzono piąty pomiar masy ciała. W grupie kontrolnej średnia masa wynosiła 144,86 kg, co stanowiło przyrost o 101,5 kg (234,09%) w stosunku do wartości początkowej. W grupie eksperymentalnej średnia masa osiągnęła 161,36 kg, co odpowiadało wzrostowi o 117,72 kg (269,75%). Różnica masy między grupami wyniosła -16,5 kg ($p \leq 0,01$). Ostateczny pomiar masy ciała przeprowadzono w dniu uboju. Średnia masa cieląt w grupie kontrolnej wynosiła 208,43 kg, co stanowiło wzrost o 165,07 kg (380,43%) w porównaniu do masy początkowej. W grupie eksperymentalnej cielęta osiągnęły średnią masę 245,36 kg, co oznaczało przyrost o 201,72 kg (462,24%) względem masy urodzeniowej. W dniu uboju różnica masy ciała pomiędzy grupą kontrolną a eksperymentalną wynosiła -36,93 kg, co było istotne statystycznie ($p \leq 0,01$).

Od początku trwania eksperymentu zaobserwowano istotne różnice w dziennych przyrostach masy ciała cieląt między grupami badawczymi, przy czym cielęta w grupie eksperymentalnej osiągały wyższe przyrosty masy ciała niż te w grupie kontrolnej. W czwartym tygodniu różnica w dziennym przyroście masy wynosiła -0,14 kg ($p \leq 0,05$). W ósmym tygodniu różnica ta utrzymywała się na tym samym poziomie -0,14 kg, przy wyższym poziomie istotności ($p \leq 0,01$). W dwunastym tygodniu wzrosła do -0,19 kg ($p \leq 0,01$), natomiast w szesnastym tygodniu wynosiła -0,13 kg ($p \leq 0,01$). W dniu uboju dzienna różnica w przyroście masy ciała osiągnęła wartość -0,20 kg ($p \leq 0,01$). Uzyskane wyniki potwierdzają wyższe tempo wzrostu cieląt w grupie utrzymywanej przez mamki, co przełożyło się na istotnie wyższą masę ciała w dniu uboju.

Duże znaczenie w ocenie wartości odżywczej mięsa ma skład podstawowy. W tym doświadczeniu zawartość białka w tkance mięśniowej cieląt z grupy kontrolnej wynosiła 31,24 g i była o 2,16 g niższa ($p \leq 0,01$) niż w tkance mięśniowej od zwierząt z grupy doświadczalnej, gdzie uzyskano 33,4 g.

Drugim ocenianym parametrem była zawartość tłuszczu, która była o 0,13 g niższa w tkance mięśniowej zwierząt w grupie doświadczalnej (w porównaniu do cieląt w grupie

kontrolnej) i wynosiła 1,88 g, podczas gdy u cieląt w grupie kontrolnej 2,01 g ($p \leq 0,05$). Jeśli chodzi o frakcję tłuszczową, można wyróżnić tłuszcz około mięśniowy i tłuszcz śródmięśniowy. W wynikach uzyskanych z tkanki mięśniowej zawartość tłuszczu śródmięśniowego była również wyższa u cieląt z grupy kontrolnej, różnica wynosiła 0,37 g ($p \leq 0,01$).

W przeprowadzonym badaniu oznaczono zawartość 17 nasyconych kwasów tłuszczowych oraz ich całkowitą sumę w tkance mięśniowej cieląt. Wszystkie analizowane kwasy tłuszczowe wykazywały istotnie wyższą zawartość w grupie doświadczalnej w porównaniu do grupy kontrolnej ($p \leq 0,01$). Wśród analizowanych kwasów tłuszczowych najwyższy poziom odnotowano dla kwasu palmitynowego (C16:0), którego średnia zawartość wyniosła 21,86 g w grupie kontrolnej oraz 24,66 g w grupie doświadczalnej, co stanowiło wzrost o 2,8 g. Drugim pod względem ilościowym kwasem tłuszczowym był kwas stearynowy (C18:0), którego zawartość wynosiła 11,27 g w grupie kontrolnej oraz 12,58 g w grupie doświadczalnej, co stanowiło różnicę 1,31 g. Na trzeciej pozycji pod względem zawartości znajdował się kwas mirystynowy (C14:0), z wartością 3,26 g w grupie kontrolnej i 3,96 g w grupie doświadczalnej, co stanowiło wzrost o 0,70 g. Stężenia pozostałych nasyconych kwasów tłuszczowych w obu grupach nie przekraczały 1 g.

Całkowita zawartość kwasów tłuszczowych z rodziny SFA w grupie kontrolnej stanowiła 37,15 g, podczas gdy w grupie doświadczalnej osiągnęła 42,39 g, co oznacza, że była o 5,24 g wyższa niż w grupie cieląt utrzymywanych w kojcach ($p \leq 0,01$).

Wśród jednonienasyconych kwasów tłuszczowych najwyższym poziomem charakteryzował się kwas oleinowy (C18:1 cis9). Zawartość tego kwasu w grupie kontrolnej wynosiła 28,69 g, natomiast w grupie doświadczalnej 24,04, przy czym różnica między nimi to aż -4,65 ($p \leq 0,01$). Drugim kwasem tłuszczowym z najwyższą zawartością był C16:1 cis 9 (3,17 g w grupie kontrolnej i 2,85 g w grupie doświadczalnej (o 0,32 g mniej) ($p \leq 0,01$). Zawartość pozostałych kwasów z rodziny MUFA była niższa niż 1 g.

Całkowita zawartość kwasów MUFA dla cieląt w grupie kontrolnej wynosiła 36,35 g i była na wyższym poziomie (5,2 g ($p \leq 0,01$)) niż w grupie doświadczalnej, gdzie uzyskano 31,15 g.

Najwyższą zawartością w grupie kwasów wielonienasyconych charakteryzował się kwas linolowy C18:2 cis9 cis12. Zawartość tego kwasu w grupie kontrolnej wynosiła 5,23 g, natomiast w grupie doświadczalnej 5,75 g i różniła się o 0,52 g ($p = 0,822$).

Drugim pod względem zawartości kwasem tłuszczowym był kwas arachidonowy C20:4 n-6, którego zawartość w tłuszczu cieląt utrzymywanych w kojcu wynosiła 2,07 g, natomiast w grupie cieląt z krowami zastępczymi 2,65 g i była o 0,58 g wyższa niż w grupie cieląt w kojcu ($p \leq 0,01$). Wartość pozostałych kwasów PUFA wynosiła poniżej 1 g w 100 g tłuszczu.

Suma kwasów PUFA w grupie kontrolnej wynosiła 9,67 g, natomiast w grupie doświadczalnej 11,32 g (różnica to 1,65 g) ($p \leq 0,05$). Jeśli chodzi o grupę kwasów tłuszczowych zaliczanych do PUFA n-6, ich zawartość w grupie kontrolnej wynosiła 8,55 g, natomiast w grupie doświadczalnej 10,06 g i była wyższa o 1,51 g ($p \leq 0,05$). PUFA n-3 w grupie doświadczalnej wynosił 0,88 g, podczas gdy w grupie kontrolnej wynosił 1,07 g, więcej o 0,19 g ($p \leq 0,01$). Stosunek n-6 do n-3 był wyższy w grupie kontrolnej i wynosił 9,52, podczas gdy w grupie cieląt z krowami zastępczymi wynosił 8,95, o 0,57 mniej ($p \leq 0,05$).

Zawartość mioglobiny w pobranych tkankach z obu grup była na podobnym poziomie: w grupie cieląt w kojcu 0,54 mg, podczas gdy w grupie cieląt z krowami zastępczymi było to 0,53 mg, różnica zaledwie 0,01 mg ($p \leq 0,725$).

Badania zmiany zawartości MDA podczas przechowywania tkanki mięśniowej zostały wykonane w trzech odstępach czasowych. Pierwsza analiza została przeprowadzona 24 godziny po uboju. Wartość MDA podczas pierwszego pomiaru dla cieląt w grupie kontrolnej wynosiła 0,82 mg, podczas gdy w grupie cieląt doświadczalnej 0,22 mg (o 0,6 mg) ($p \leq 0,01$). Drugiego pomiaru MDA dokonano w siódmym dniu po uboju; poziom MDA u cieląt z grupy kontrolnej wzrósł o 3,73 mg do wartości 4,55 mg, co stanowi wzrost o 454,88%. W grupie kontrolnej wzrósł o 3,06 mg do wartości 3,28 mg, co stanowi wzrost o 1390,91%. Wartość MDA była wyższa w grupie kontrolnej niż w grupie doświadczalnej, a różnica ta w siódmym dniu po uboju między grupami wynosiła 1,27 mg ($p \leq 0,01$).

Zawartość mioglobiny w tkankach mięśniowych cieląt z obu grup utrzymywała się na zbliżonym poziomie: w grupie kontrolnej wynosiła 0,54 mg, a w grupie doświadczalnej 0,53 mg, co oznacza różnicę zaledwie 0,01 mg ($p \leq 0,725$). Analiza zmian poziomu MDA w tkance mięśniowej podczas przechowywania przeprowadzona została w trzech punktach czasowych. Pierwszy pomiar wykonano 24 godziny po uboju; poziom MDA w grupie kontrolnej wynosił 0,82 mg, podczas gdy w grupie doświadczalnej wynosił 0,22 mg, co wskazuje na różnicę 0,6 mg ($p \leq 0,01$). Drugi pomiar MDA wykonano w siódmym dniu po uboju, w którym odnotowano istotny wzrost poziomu tego

wskaźnika oksydacji lipidów. W grupie kontrolnej poziom MDA wzrósł o 3,73 mg, osiągając wartość 4,55 mg, co oznacza wzrost o 454,88%. W grupie doświadczalnej poziom MDA wzrósł o 3,06 mg, osiągając wartość 3,28 mg, co stanowi wzrost o 1390,91%. W siódmym dniu po uboju poziom MDA pozostawał wyższy w grupie kontrolnej niż w grupie doświadczalnej, a różnica między grupami wynosiła 1,27 mg ($p \leq 0,01$).

W ramach eksperymentu oceniono także barwę tkanki mięśniowej cieląt, wykonując analizy w trzech terminach: 24 godziny, cztery dni oraz siedem dni po uboju. Dwadzieścia cztery godziny po uboju parametry jasności (L*), zaczerwienienia (a*), zażółcenia (b*), nasycenia i pigmentacji (C*) oraz kąta barwy (h°) wykazywały podobne wartości w obu grupach badanych – zarówno u cieląt z grupy kontrolnej (utrzymywanych w kojcach), jak i u cieląt odchowywanych przez krowy zastępcze. W czwartym dniu po uboju wartości L*, a* i b* również były porównywalne między grupami. Zauważono jednak, że wartość nasycenia (C*) była wyższa w grupie doświadczalnej, podczas gdy kąt barwy (h°) przyjął niższą wartość niż w grupie kontrolnej. Analiza zmian między pomiarami wykazała tendencję wzrostową dla parametrów L* i h°, podczas gdy wartości pozostałych parametrów uległy obniżeniu. W siódmym dniu po uboju wartości L* i b* utrzymywały się na porównywalnym poziomie w obu grupach, a wartość C* pozostawała stabilna. Parametr a* osiągnął jednak wyższe wartości w grupie doświadczalnej, a wartość h° pozostała niższa niż w grupie kontrolnej, podobnie jak cztery dni po uboju. W odniesieniu do zmian zachodzących między kolejnymi pomiarami stwierdzono wzrost wartości a*, b* i C*, przy jednoczesnym spadku wartości L* i h°.

6. Wnioski

Na podstawie przeprowadzonych badań sformułowane zostały następujące wnioski:

1. Deficyt energetyczny w początkowej fazie laktacji prowadzi do ograniczenia lipogenezy, w tym syntezy długołańcuchowych kwasów tłuszczowych oraz izomerów skoniugowanych dienów kwasu linolowego.
2. Krzyżowanie ras PHF z SRB, prowadzące do zwiększenia heterozygotyczności, wykazuje korzystny wpływ na parametry użytkowe mleka. Mieszańce F_1 charakteryzują się wyższą zawartością tłuszczu i białka w porównaniu do czystorasowych krów PHF, co wskazuje na poprawę efektywności metabolicznej, lepsze wykorzystanie energii oraz wyższy potencjał produkcyjny.

3. Potencjał antyoksydacyjny mleka u mieszańców F_1, uzyskanych z krzyżowań PHF z SRB, wykazuje wyższą aktywność enzymów antyoksydacyjnych, co skutkuje obniżeniem poziomu markerów stresu oksydacyjnego.
4. Krzyżowanie PHF z SRB poprawia równowagę energetyczną u krów, redukując predyspozycje do wystąpienia ujemnego bilansu energetycznego w początkowej fazie laktacji. Lepsze zarządzanie energią przez heterozygoty F_1 przyczynia się do zmniejszenia ryzyka rozwoju chorób metabolicznych, takich jak ketoza, a tym samym poprawia zdrowotność zwierząt i wydajność mleczną w całym okresie laktacyjnym.
5. Mieszańce F_1 uzyskane z krzyżowania PHF z buhajami rasy limousine wykazują wyższy potencjał antyoksydacyjny w tkance mięśniowej, co wpływa na poprawę jakości mięsa. Zwiększona aktywność enzymów antyoksydacyjnych prowadzi do lepszej stabilności oksydacyjnej mięsa, zmniejszając ryzyko jego pogorszenia w wyniku reakcji oksydacyjnych, co jest istotne z punktu widzenia jakości mięsa i jego trwałości.
6. System odchowu cieląt stanowi istotny czynnik w kształtowaniu potencjału antyoksydacyjnego ich tkanki mięśniowej. Optymalizacja warunków odchowu wpływa na lepszą aktywność enzymów antyoksydacyjnych, co przekłada się na poprawę stabilności oksydacyjnej cielęciny i zmniejszenie jej podatności na uszkodzenia oksydacyjne.
7. Krzyżowanie międzyrasowe stanowi skuteczną metodę poprawy jakości produktów zwierzęcych poprzez redukcję negatywnych skutków inbredu. Zwiększona heterozygotyczność sprzyja poprawie parametrów produkcyjnych oraz zdrowotnych zwierząt, co ma bezpośredni wpływ na jakość mleka i mięsa.

Wyniki badań wskazują na istotny wpływ krzyżowania oraz optymalizacji systemu odchowu cieląt na poprawę jakości produktów zwierzęcych, w tym mleka i mięsa, przez modyfikację potencjału antyoksydacyjnego i stabilności oksydacyjnej. Heterozygotyczność uzyskana dzięki takim strategiom hodowlanym przyczynia się do poprawy wydajności, jakości i zdrowotności zwierząt, stanowiąc skuteczną metodę w kontekście dążenia do poprawy jakości produktów pochodzenia zwierzęcego.

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8. Publikacje stanowiące rozprawę doktorską wraz z oświadczeniami

„Wpływ wybranych czynników genetycznych i fizjologicznych na cechy użytkowości mlecznej i mięsnej bydła, ze szczególnym uwzględnieniem zmian o charakterze antyoksydacyjnym”

Rozprawę doktorską stanowi zbiór sześciu opublikowanych i powiązanych tematycznie artykułów naukowych:

Publikacja 1. Solarczyk P., Gołębiewski M., Slósarz J., Puppel K. 2023. Interaction Between the Concentration of β-Hydroxybutyric Acid and the Content of Long-Chain Fatty Acids in the Early Stage of Lactation—Comparing Multiparous and Primiparous Cows. *Applied Sciences* 13(13), 7870. doi:10.3390/app13137870

(100 pkt. MNiSW; IF 2,500; cyt. wg WoS: 1)

Publikacja 2. Solarczyk P., Slósarz J., Gołębiewski M., Puppel K. 2021. A comparison between Polish Holstein-Fresian and F_1 hybrid Polish Holstein-Fresian × Swedish Red cows in terms of milk yield traits, *Mljekarstvo* 71(2), 141-150. doi:10.15567/mljekarstvo.2021.0207

(40 pkt. MNiSW; IF 1,111; cyt. wg WoS: 3)

Publikacja 3. Solarczyk P., Slósarz J., Gołębiewski M., Natalello A., Musati M., Luciano G., Priolo A., Puppel K. 2024. The Influence of Crossbreeding on the Composition of Protein and Fat Fractions in Milk: A Comparison Between Purebred Polish Holstein Friesian and Polish Holstein Friesian × Swedish Red cows. *Nutrients* 16(21), 3634. doi:10.3390/nu16213634

(140 pkt. MNiSW; IF 4,800; cyt. wg WoS: 0)

Publikacja 4. Solarczyk P., Gołębiewski M., Slósarz J., Natalello A., Musati M., Menci R., Sakowski T., Tucki K., Puppel K. 2024. Effect of Age at First Calving on the Reproduction Parameters, Metabolic Profile, and Fatty Acid Composition of Polish Holstein Friesian (PHF) and Crossbreds PHF × Swedish Red (SRB) cattle. *Metabolites* 14(11) 583. doi: 10.3390/metabo14110583

(100 pkt. MNiSW; IF 3,400; cyt. wg WoS: 0)

Publikacja 5. Solarczyk P., Gołębiewski M., Slósarz J., Łukasiewicz M., Przysucha T., Puppel K. 2020. Effect of Breed on the Level of the Nutritional and Health-Promoting Quality of Semimembranosus Muscle in Purebred and Crossbred Bulls. *Animals* 10(10), 1822. doi:10.3390/ani10101822

(100 pkt. MNiSW; IF 2,323; cyt. wg WoS: 7)

Publikacja 6. Solarczyk P., Sakowski T., Gołębiewski M., Slósarz J., Grodkowski G., Grodkowska K., Biondi L., Lanza M., Natalello A., Puppel K. 2023. The Impact of Calf Rearing with Foster Cows on Calf Health, Welfare, and Veal Quality in Dairy Farms. *Agriculture* 13(9) 1829. doi:10.3390/agriculture13091829

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Interaction between the Concentration of β-Hydroxybutyric Acid and the Content of Long-Chain Fatty Acids in the Early Stage of Lactation—Comparing Multiparous and Primiparous Cows

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Abstract: The aim of the experiment was to study the relationship between the age of cows, blood BHBA content, and CLA isomer (C18:2 cis9,trans11, CLA9; C18:2 trans10,cis12, CLA10) content during the first three weeks post-partum. For the experiment, 105 cows were selected from the entire herd and assigned to one of four groups: healthy primiparous (PH), healthy multiparous (MH) or ketotic primiparous (PK), ketotic multiparous (MK) based on their symptoms, and blood serum BHBA concentrations at 5 ± 2 days post-partum. Milk and blood samples were taken from the animals for a period of three weeks at weekly intervals on the same day. High levels of ketone bodies inhibit the activity of acetyl-CoA, thus decreasing the transport of acetyl-CoA, which may result in a decrease in CLA9 and CLA10 synthesis. Studies have shown that the age of the cows was an additional factor in determining the formation of CLA isomer levels during the early stage of lactation. The CLA9 content in the milk of PH cows in the first week of lactation was 32.75% higher than that of PK milk, while in MH milk, it was 67.7% higher than that of MK milk. The CLA10 content in the milk PH, when compared to the healthy groups, was 319% lower for primiparous cows. In summary, different reference limits in CLA9 and CLA10 content should be considered in the diagnosis of ketosis, taking into account, among other things, parity.

Keywords: milk fat; fatty acids; ketosis; CLA; BHBA

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1. Introduction

The worldwide growth in population has caused a continuous increase in the demand for protein from both plant and animal sources. This forecasted increase in demand also applies to milk, the global production of which continues to grow [1]. Milk is a valuable source of nutritional compounds and is especially rich in bioactive compounds [2–4]. It is considered a complete food, having more than 250 different constituents, such as complete protein, fat, lactose, micro- and macronutrients, and water- and fat-soluble vitamins [3,5]. The composition of milk is the result of the actions of many factors, among which should be mentioned genetic factors (breed, individual characteristics), environmental factors (nutrition, climatic conditions, season), and physiological factors (the age of the cow, stage of lactation, health status) [6–10]. One of the most sensitive and complex milk fractions is fat, which affected by all the above-mentioned factors.

This fraction consists of about 400 different fatty acids (FA) [11,12]. Milk fat is a heterogeneous emulsion, up to 99% of which is absorbed in the gastrointestinal tract [4,13], and made up of small highly dispersed fat globules. The largest proportion of cow's milk fat, i.e., 95.8–98.3%, is made up of triacylglycerols, which consist of glycerol and fatty acids, which fill the interior of the fat globules. The fat globule envelope is made up of diacylglycerols, monoacylglycerols, phospholipids, free fatty acids, total cholesterol, cholesterol in esterified form, and fat-soluble vitamins [14,15].

The process of milk FA formation varies: some milk is synthesized by the rumen's micro-organisms (i.e., bacteria and protozoa), while other milk is synthesized in the mammary gland [16]. FAs that have more than 16 carbon atoms are either derived from the cow's food and absorbed during digestion or mobilized from the cow's fat reserves. The acids derived from the fat reserves are transported as non-esterified fatty acids (NEFAs) and captured from plasma [17–19]. Of all the FAs present in milk, about 14% are considered to be unique to milk [16]. FAs of different structures (i.e., carbon chain length, degree of saturation, and configuration) have very different effects on human health [20–22]. The best-known FA with properties for promoting health is C18:2 cis9,trans11. The profiles of milk fatty acids are correlated with energy balance in dairy cows, and selected milk fatty acids can be considered as biomarkers for ketosis [10].

The increase in milk yields has resulted in the emergence of adverse phenomena in dairy herds that cause deterioration in the health and fertility of the animals. During the transition period (the period of late pregnancy and early lactation), there is stress associated with the intense hormonal changes accompanying the birth of a calf and the entry into lactation. Stress is usually associated with a decrease in appetite. At the time of rapidly increasing nutritional needs associated with milk secretion, reduced feed intake leads to a negative energy balance and mineral and vitamin deficiencies [10,23]. Ketosis affects more than 80% of cows in the herd. Cows are 3 to 8 times more likely to have displacement of the digestive tract. They have a two-fold increased risk of placental retention and threefold increased risk of uterine inflammation. They are as much as 6 times more likely to have cystic ovaries. During the transition period (late pregnancy and early lactation), there is stress associated with the intense hormonal changes accompanying the birth of the calf and the entry into lactation. In the case of some diseases, e.g., post-partum paralysis, there is a risk of cow death, while other diseases, e.g., ketosis or acidosis, cause significant production losses, such as reduced milk yield, deterioration of the chemical composition of milk and higher somatic cell count, deterioration of reproductive indices, the occurrence of hoof problems (laminitis), and, as a result of these issues, higher veterinary and herd repair costs [9]. Therefore, it is far better for breeders to take measures to prevent the occurrence of these diseases. An additional difficulty in the control of metabolic diseases is their subclinical states, especially in the case of subclinical ketosis and acidosis. The lack of unambiguous syndromes associated with the diseases means that they often go unnoticed, resulting in measurable economic losses on a herd-wide scale and over a long period of time. Ketone bodies are a group of organic compounds that are intermediate metabolites of fat. They include acetone (formed via spontaneous decarboxylation of acetoacetate), acetoacetic acid (in the form of anion-acetoacetate), and β-hydroxybutyric acid (in the form of anion-β-hydroxybutyrate; BHBA). The first step in ketogenesis involves the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA. Acetyl-CoA is also the substrate for CLA9 synthesis [10]. The hypothesis that we want to verify assumes that differences in the formation of CLA9 and CLA10 may be indicative of metabolic disorders in the bodies of cows. However, we hypothesize that the age of the cows will be an additional factor in determining the formation of different CLA isomers levels during the early stage of lactation, because primiparous cows need extra energy for growth [24]. The aim of the experiment was to study the relationship between the ages of cows, blood BHBA content, and CLA (different isomers) content during the first three weeks post-partum.

2. Materials and Methods

The experiment was implemented at the Agricultural Experimental Station of the University of Life Sciences, where about 350 dairy cows with an average yield of more than 10,000 kg of milk per 305-day lactation are kept in a free-stall barn. The animals are fed TMR twice daily ad libitum. The ingredient composition of the TMR (kg/day DM) was as follows: maize silage, 12.00; alfalfa silage, 4.00; corn silage, 2.00; soybean meal, 2.20; pasture ground chalk, 0.20; salt, 0.05; rapeseed meal, 1.80; and magnesium oxide, 0.06. Cows were fed twice a day. For the experiment, 105 cows (55 multiparous cows that were in their second

lactation, as well as 50 primiparous cows) were selected from the entire herd and assigned to one of four groups: healthy primiparous (PH; 30 cows), healthy multiparous (MH 32 cows) or ketotic primiparous (PK; 20 cows), ketotic multiparous (MK; 23 cows) based on their symptoms, and blood serum BHBA concentrations at 5 ± 2 days post-partum; each group was then kept separately. Dry matter intake (DMI) was determined by weighing remaining orts. Body condition score (BCS) was assessed via the BCS-5 method described by Edmonson et al. [25]. Symptoms of ketosis were as follows: reduced feed intake, poor body condition score, and BHBA > 1.2 mmol/L.

Milk and blood samples were taken from the animals for a period of three weeks at weekly intervals on the same day. All samples were taken at the following time intervals: between the fifth and seventh days of lactation for the first collection, the eighth and fourteenth days of lactation for the second collection, and the fifteenth and twenty-first days of lactation for the third collection. The basic composition of the milk and the fatty acids content were determined for the collected milk samples, while BHBA levels were determined via blood samples.

The milk samples (250 mL) were obtained from each cow using milk samplers (from morning and evening milking) and mixed. Blood samples (10 mL) were taken from each cow via jugular vein puncture using tubes (Vacuette, Kremsmünster, Austria) containing potassium-EDTA (K3EDTA, 1.8 g/L of blood) as an anticoagulant. Blood samples were centrifuged at 1800× *g* at 4 °C for 15 min, and the supernatant was immediately transported to the Veterinary Centre of WULS for the analysis of blood plasma metabolites (BHBA).

2.1. Chemical Analysis

The basic parameters of the milk—fat, protein, and casein content—were determined through automated infrared analysis using a MilkoScan FT 120 analyzer (Foss Electric, Hillerød, Denmark).

The level of BHBA was determined using a BS800M biochemical analyzer (PZ Cormay, Warsaw, Poland).

Fatty acid methylation was performed according to the trans-esterification method EN ISO 5509 [26]. Individual fatty acids were identified in crude fat using an Agilent 7890A GC (Agilent, Waldbronn, Germany) according to Puppel et al. [9]. Each peak was identified using pure methyl ester standards: FAME Mix RM–6, Lot LB 68242; Supelco 37 Comp. FAME Mix, Lot LB 68887; Methyl linoleate, Lot 094K1497; and CLA Conjugated (9Z, 11E), Lot BCBV3726 (Supelco, Bellefonte, PA, USA).

2.2. Statistical Analysis

The obtained data were statistically analyzed using the IBM SPSS 22.0 package [27]. The distribution of the milk chemical composition and selected fatty acids was checked using the Shapiro–Wilk test. MANOVA analysis was used to establish the influence of the lactation phase on the milk's chemical composition and the level of selected fatty acids. The changes in the concentration of selected fatty acids in regard to BHBA level in the blood and lactation stages were established via multivariate analysis.

The following statistical model was used:

$$Y = \mu + A_i + B_j + C_k + (A \times B)_{ij} + (A \times C)_{ik} + (B \times C)_{jk} + e_{ijkl} \quad (1)$$

where μ–mean, A_i—treatment effect (1st week of lactation; 2nd week of lactation; 3rd week of lactation), B_j—BHBA concentration (0.6–1.2 mmol/L; >1.2 mmol/L), C_k—parity effect (primiparous, multiparous), A × B—interaction between treatment effect and BHBA concentration, A × C—interaction between treatment effect and parity, B × C—interaction between week of BHBA concentration and parity, and e_{ijkl}—random error. Only interactions between factors whose influence was statistically significant ($p \leq 0.05$) were considered. The level of significance was determined after performing preliminary statistical analyses. For multivariable comparison, Fisher's LSD test was applied.

Only interactions between factors of which the influence was statistically significant ($p \leq 0.05$) were considered. The level of significance was determined after performing preliminary statistical analyses. For multivariable comparison, Fisher's LSD test was applied.

3. Results

Table 1 shows the changes in daily milk production and basic milk composition over the course of the experiment. Daily milk production increased over successive weeks of lactation, while the casein, protein, and fat levels in the milk decreased. The greatest changes in the basic composition of milk were seen between the first (days 5–7 of lactation) and second (days 8–14 of lactation) intakes, with a 1.27% decrease in fat, 0.37% decrease in protein, and 0.18% decrease in casein. The highest increase in milk production, i.e., 2.15 kg, occurred between the second (days 8–14 of lactation) and third (days 15–21 of lactation) collections.

Table 1. Chemical composition and daily milk production. Data were presented as least-squares means with the standard error of the mean. Treatment effect: 1st week of lactation; 2nd week of lactation; 3rd week of lactation. Means marked with the same letters differ significantly at lowercase letters, $p \leq 0.05$; uppercase letters, $p \leq 0.01$.

Component	Treatment Effect			SEM
	1	2	3	
Milk yield [kg]	28.15 ^{a}	28.71 ^{b}	30.86 ^{a,b}	1.596
Casein [%]	2.73 ^{A,B}	2.45 ^{A,c}	2.39 ^{B,c}	0.036
Protein [%]	3.36 ^{A,B}	2.99 ^{A,c}	2.85 ^{B,c}	0.046
Fat [%]	5.23 ^{A,B}	3.96 ^{A,C}	3.62 ^{B,C}	0.196
Fat/Protein	1.56 ^{A,B}	1.32 ^{A,C}	1.27 ^{B,C}	0.121

Table 2 contains data on the content of selected milk fatty acids per 100 g of fat, which were sourced during the experiment. The results indicated that the highest selected milk fatty-acid content was registered for C18:2 n-6 (LA); the next highest content was C18:1 trans11 (TVA), followed by CLA9 and CLA10. From the results, it was evident that there was an increase in the TVA and LA acid content due to the fact that the first increase in TVA content occurred between the second (days 8–14 of lactation) and third (days 15–21 of lactation) intakes; however, in the case of LA, the increase was observed throughout the experiment. In relation to the CLA isomers, the situation looked different. The CLA9 content decreased in the second week compared to the first week of lactation, but in the third week, there was an increase in this acid content. CLA10, as with TVA, remained at the same level during the first two weeks of lactation, while its content decreased in the third week, in contrast to TVA.

Table 2. Content of selected fatty acids [g/100 g fat]. Data were presented as least-squares means with the standard error of the mean. Treatment effect: 1st week of lactation; 2nd week of lactation; 3rd week of lactation. Means marked with the same letters differ significantly at lowercase letters, $p \leq 0.05$; uppercase letters, $p \leq 0.01$.

Fatty Acid	Treatment Effect			SEM
	1	2	3	
C18:1 trans11	1.01 ^{a}	1.01 ^{b}	1.12 ^{a,b}	0.044
C18:2 n-6	2.11 ^{a}	2.14 ^{b}	2.17 ^{a,b}	0.462
C18:2 cis9, trans11	0.51 ^{A,B}	0.44 ^{A}	0.46 ^{B}	0.019
C18:2 trans10, cis12	0.04	0.04	0.03	0.004

Table 3 shows the results of the average β-hydroxybutyric acid (BHBA) content. This acid is one of the markers for diagnosing diseases of metabolic origin. The BHBA content in

the blood of animals that participated in the experiment varied, though it was characterized by the first week of lactation having the highest level (days 5–7 of lactation), followed by a decrease in content value, which then remained at a similar level during the second and third weeks of the experiment, indicating that there was no problem related to metabolic diseases in the herd. However, compiling the results of the whole herd never fully reflects the real situation in the herd—as shown by the results in Figures 1–4, this herd indeed had ketosis.

Table 3. BHBA content in blood depending on the week of lactation [mmol/L]. Data were presented as least-squares means with the standard error of the mean. Treatment effect: 1st week of lactation; 2nd week of lactation; 3rd week of lactation. Means marked with the same letters differ significantly at lowercase letters, $p \leq 0.05$; uppercase letters, $p \leq 0.01$.

		Treatment Effect		
		1	**2**	**3**
BHBA	LSM	0.93 ^{AB}	0.67 ^{A}	0.68 ^{B}
	SEM	0.072	0.066	0.057

BHBA—β-hydroxybutyric acid.

Figure 1 shows the results of the C18:1 trans11 content in cows' milk per 100 g of fat, which depends on the age of the cows and the blood BHBA content. The TVA content remained at a similar level throughout the experiment in healthy primiparous (PH) and multiparous (MH) cows, while the results were quite different in animals diagnosed with ketosis (MK, PK). During the first week of lactation, PH and MH milk had by far the highest TVA content, while the lowest content was found in multiparous cows with ketosis (MK). Compared to animals whose blood results indicated ketosis, TVA content was 0.212 g higher in primiparous females and 0.161 g higher in multiparous females. In the second week, there was a decrease in TVA content in PH, MH, and MK cows, while the primiparous animals with ketosis (PK) experienced a 0.15-gram increase in TVA content. In the third week of lactation, the highest TVA content was found in milk from MK cows, where the highest increase in TVA content was also observed, amounting to 0.493 g; the lowest value was observed in PK cows.

Figure 2 shows the C18:2 n-6 content depending on the age of the cows and the BHBA content in blood. The results showed quite large fluctuations in this fatty acid's content across the different groups of animals participating in the experiment. LA content in the milk of primiparous cows showed a similar trend of decreasing content over the course of the experiment; in addition, the LA content, for both healthy and diseased primiparous cows, was higher in the first week of lactation than that in multiparous cows. LA content in multiparous cows was quite variable. In MH cows, the LA content increased in the second week of lactation and decreased in the third week; however, in MK cows, the lowest LA level of all animal groups was observed for the first two weeks of the experiment. In addition, a decrease in LA content was observed in the second week compared to the first week of lactation, while in the third week, there was a significant increase in LA content in the milk of these cows, which totaled 0.905 g/100 g fat—this figure was the highest value recorded among the experimental groups.

Figure 3 shows the results of C18:2 cis9,trans11 content according to the age of cows and the BHBA content in blood. In the first week of lactation (days 5–7), the highest CLA9 content was observed in PH and MH milk, with the lowest content found in PK and MK milk. In particular, the CLA9 content in MH milk was high, amounting to as much as 0.833 g/100 g of fat, while in PH milk, the respective figure was 0.684 g/100 g of fat. The CLA9 content in the milk of PH cows in the first week of lactation was 32.75% higher than that in PK milk, while in MH milk, the respective figure was 67.7% higher than that in MK milk. In the second week of lactation (days 8–14), the highest CLA9 content was found in PH milk, despite experiencing a decrease compared to the first week, and in PK milk, the CLA9 content of which had increased. On the other hand, there was a rather large

reduction in the CLA9 content in MH milk, which experienced a fall of up to 55%. The lowest level of CLA 9 in the second week of lactation was again observed in the milk of MK cows, though this figure represented an increase of 21% compared to the first week. In the third week of lactation (days 15–21), once again, the highest CLA9 content was observed in the milk of MH cows, which experience an increase of 21.5% compared to the previous week. In contrast, in the case of PH cows, the CLA9 content decreased, while the level in MK cows increased, albeit still recording the lowest level of this component among the animals included in the experiment.

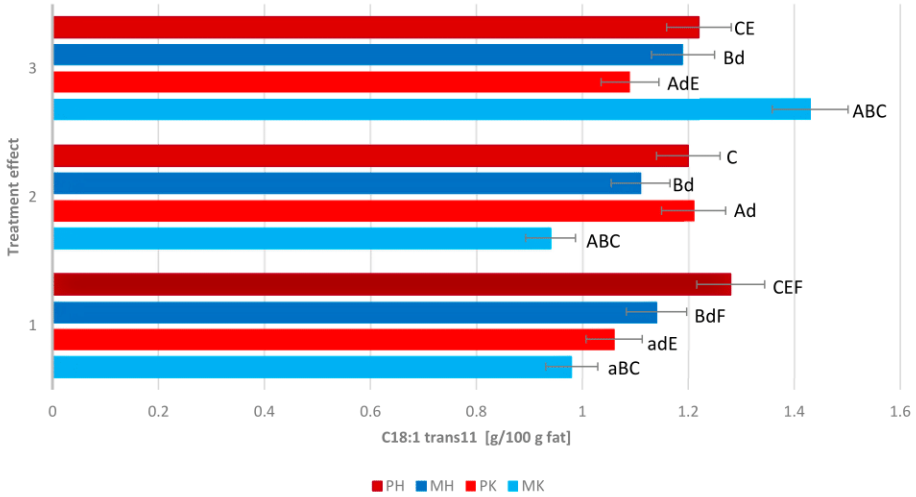

Figure 1. C18:1 trans11 [g/100 g fat] content depending on the BHBA level and the age of cows. Data were presented as least squares means with the standard error of the mean. Statistical differences between groups at $p \leq 0.01$ and collections at $p \leq 0.01$. Means marked with the same letters differ significantly at lowercase letters, $p \leq 0.05$; uppercase letters, $p \leq 0.01$. Groups: healthy primiparous (PH); healthy multiparous (MH); ketotic primiparous (PK); ketotic multiparous (MK). Treatment effect: 1st week of lactation; 2nd week of lactation; 3rd week of lactation.

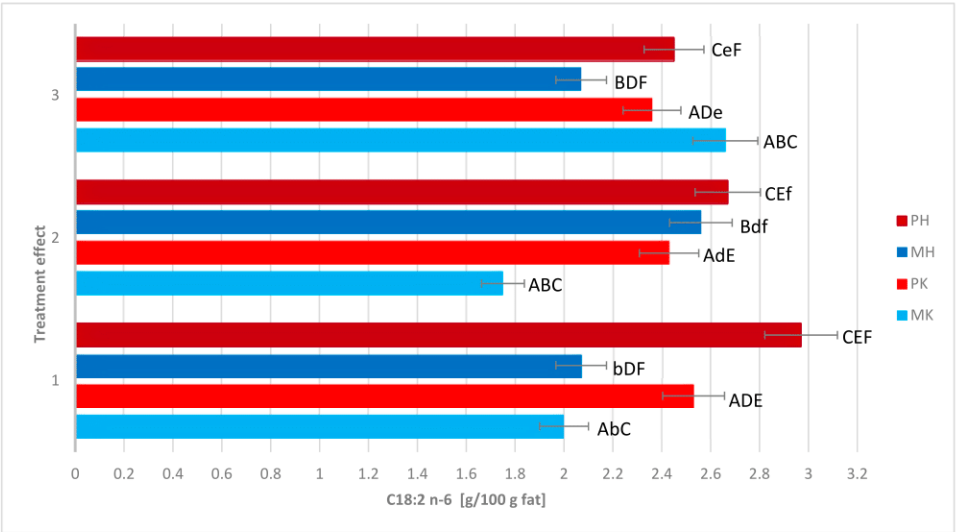

Figure 2. C18:2 n-6 [g/100 g fat] content depending on the BHBA level and the age of cows. Data were presented as least squares means with the standard error of the mean. Statistical differences between groups at $p \leq 0.01$ and collections at $p \leq 0.01$. Treatment effect: 1st week of lactation; 2nd week of lactation; 3rd week of lactation. Means marked with the same letters differ significantly at lowercase letters, $p \leq 0.05$; uppercase letters, $p \leq 0.01$.Groups: healthy primiparous (PH); healthy multiparous (MH); ketotic primiparous (PK); ketotic multiparous (MK).

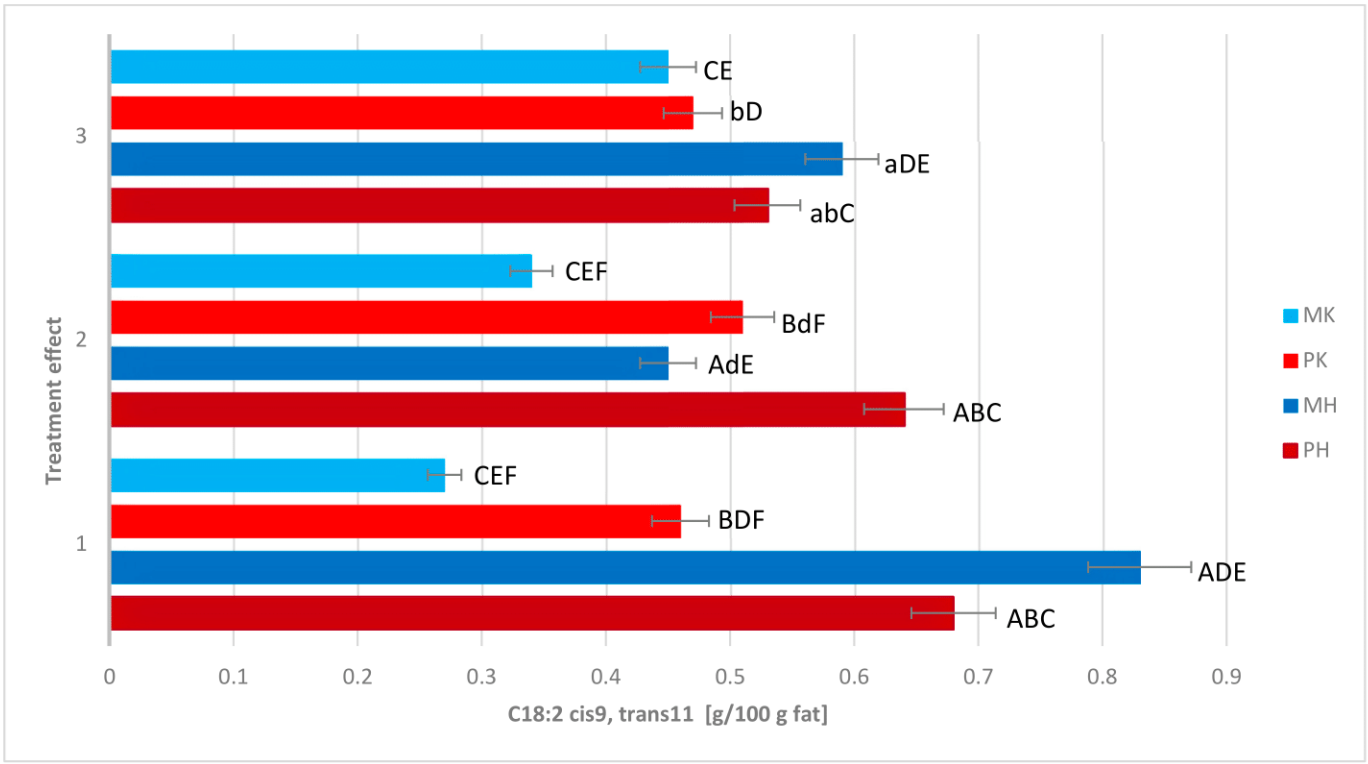

Figure 3. C18:2 cis9, trans11 [g/100 g fat] content depending on the BHBA level and age of the cows. Data were presented as least squares means with the standard error of the mean. Statistical differences between groups at $p \leq 0.01$ and collections at $p \leq 0.01$. Treatment effect: 1st week of lactation; 2nd week of lactation; 3rd week of lactation. Means marked with the same letters differ significantly at lowercase letters, $p \leq 0.05$; uppercase letters, $p \leq 0.01$. Groups: healthy primiparous (PK), healthy multiparous (MK); ketotic primiparous (PH); ketotic multiparous (MH).

In Figure 4 presents the results of the C18:2 trans10, cis-12 acid content in relation to the age of the animals and the BHBA content in blood. MH milk had the highest CLA10 content over the course of the experiment; however, a reduction of 15% was observed in the second week, along with another reduction of 19% in the third week of lactation, compared to those of each preceding week. High levels of CLA10 were also observed in PH cows during the first two weeks of lactation, though the content decreased by 25.4% and again by 66% compared to the preceding weeks, meaning that the third week of lactation recorded the lowest CLA10 content in milk of PH cows. Significantly lower values, especially at the beginning of lactation, were observed in cows diagnosed with ketosis. The CLA10 content in these animals, when compared to the healthy groups, was 319% lower for primiparous cows, as well as 1.018% lower for multiparous cows. In the second week of lactation, there was still a significant difference in CLA10 content between healthy cows and those diagnosed with ketosis, though these values were already lower in the healthy animals, amounting to 161% for primiparous cows and 233% for multiparous cows; in contrast, its content increased in animals with ketosis. In the third week of lactation, the CLA10 content was highest for multiparous healthy animals and those with ketosis, while a decrease in CLA10 content was seen in primiparous cows. Notably, there was a significant decrease in CLA10 content in healthy animals, which, over the course of the entire experiment, was 74.7% in primiparous cows and 39.8% in multiparas cows; an 81.25% increase in content for multiparas cows with ketosis was also recorded.

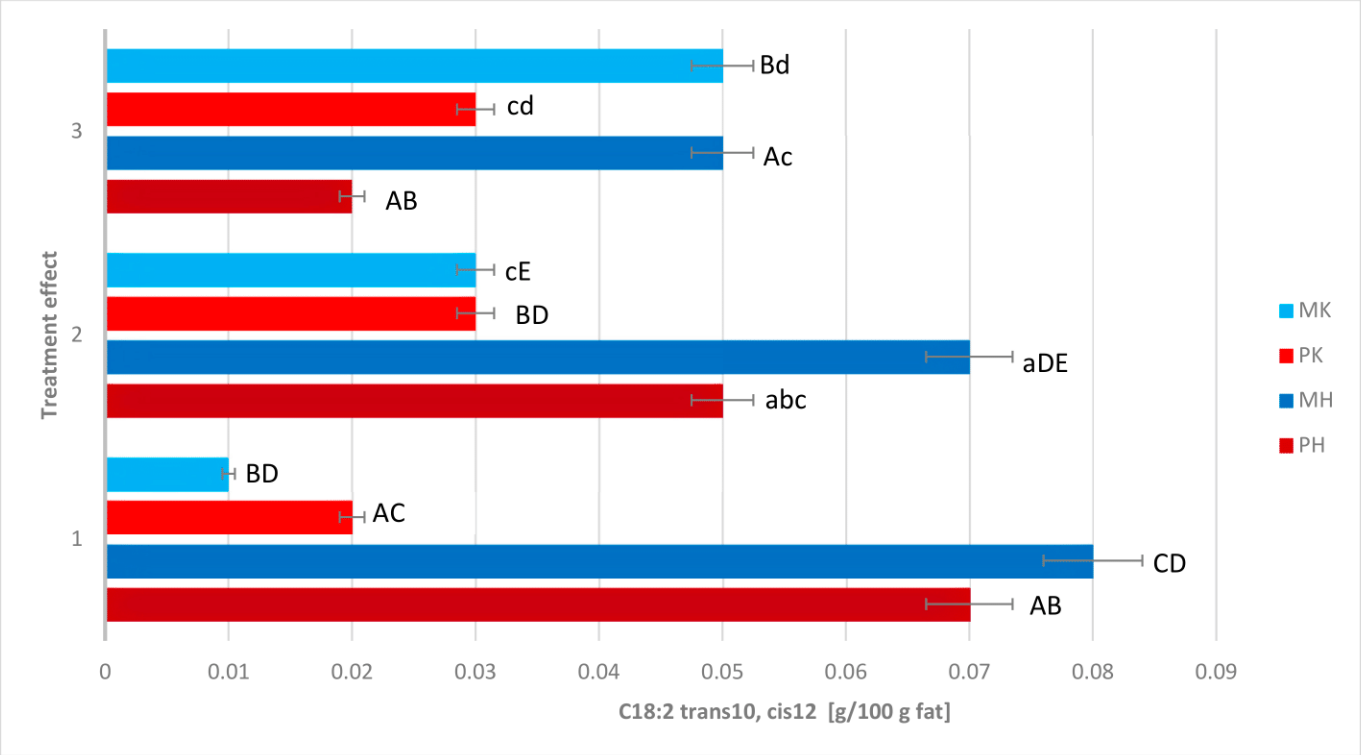

Figure 4. C18:2 trans10, cis12 [g/100 g fat] content depending on the BHBA level and age of the cows. Data were presented as least squares means with the standard error of the mean. Statistical differences between groups at $p \leq 0.01$ and collections at $p \leq 0.01$. Treatment effect: 1st week of lactation; 2nd week of lactation; 3rd week of lactation. Means marked with the same letters differ significantly at lowercase letters, $p \leq 0.05$; uppercase letters, $p \leq 0.01$. Groups: healthy primiparous (PK); healthy multiparous (MK); ketotic primiparous (PH); ketotic multiparous (MH).

4. Discussion

The experiment analyzed the first three weeks of lactation. There was a sharp increase in milk production during the early stage of lactation, which continued until the so-called peak of lactation was reached. In the results, it was possible to observe an increase in milk yield while, at the same time, seeing a decrease in the content of milk components (Table 1) [8,28–31]. Heuer et al. [32] reported that changes in gross composition of milk were useful risk predictors of energy balance in early lactation, e.g., fat/protein ratio > 1.4 and milk fat > 4.8%. Additionally, Čejna and Chládek [33] demonstrated that the optimum fat/protein ratio was 1.2–1.4, while values higher than 1.4 were connected to energy deficiency and subclinical ketosis. The highest fat/protein ratio (1.56) was demonstrated in the first week of lactation (Table 1), which indicated a negative energy balance during the first lactation period. Changes occurring in the basic composition of milk at the beginning of were related to several factors, the first of which, as reported by Puppel et al. [29], was the production of colostrum, which occurred at the beginning of lactation in all female mammals and differed significantly in composition from milk. Another factor may have been the changes in feed intake being provided to animals during the drying-out period and immediately after parturition. Yet another issue was the so-called dilution effect, which is associated with increased milk production [8]. All changes in the basic composition of milk should be analyzed in detail by farmers, because it is here that the first signals indicating an emerging problem in dairy cows that have a poorly balanced feed intake, which can lead to the occurrence of diseases of metabolic origin, can be observed [10,29].

At the beginning of lactation, a negative energy balance (NEB) is very often observed in dairy cows. This observation is associated with the cows' energy requirements for milk production not being sufficiently covered by their feed intake [34–36]. As a result,

the animals mobilize the stored fat in their bodies, which is converted, during lipolysis, into fatty acids that are the precursors to non-saturated fatty acids, namely palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1) [37]. The NEFAs formed during lipolysis adversely affect the metabolism of the liver, which, due to the large amount of NEFAs, uses them to produce β-hydroxybutyric acid, disrupting the metabolism of the compounds formed in the liver [38]. This outcome is the cause of ketosis, which, according to van der Drift et al. [38], is one of the most commonly diagnosed conditions in dairy cattle and can affect up to 60% of the cows in a herd. One of the main markers used to diagnose this condition is BHBA in the blood [39,40], the results of which, for our study, are shown in Table 3. The BHBA content in the blood of animals that participated in the experiment varied, though it was characterized by the first week of lactation having the highest level, followed by a decrease in content value. BHBA is also a precursor to the synthesis of milk fat in the mammary gland; this fact was also confirmed by the obtained results, in which high fat content and high BHBA in the blood were observed during the first week of lactation [10,29,41].

Recently, a rather popular research direction has been the determination of the effect of ketosis on selected milk components [10,29,31,35–38,41,42]. Due to the correlation between high biomarkers for diagnosing ketosis and high milk fat content, the study of fatty acid content is considered to be one of the most frequently chosen research areas related to the effect of ketosis on milk composition [42]. The conducted experiment also confirmed the theses of several other research teams [29,31,42], pointing out the key role of fatty acids in metabolism, especially those acids that are largely associated with adipose tissue, which animals begin using during NEB; thus, that the acid content increases. During the first week of lactation, PH and MH milk had by far the highest TVA content, while the lowest content was found in MK (Figure 1).

The study also confirmed a higher CLA content in healthy animals than sick animals during the early stage of lactation, as also indicated by results obtained by Artegoitia et al. [42] and Puppel et al. [10]. However, the studies of the above-mentioned authors did not confirm the influence of the age of cows on the formation of CLA levels with metabolic disorders. Only multiparous cows were included in Puppel et al.'s [10] study. In ruminants, CLA9 is formed in two ways. It can be obtained as an intermediate product during the biohydrogenation of linoleic acid into stearic acid using a bacterial isomerase. During this process, vaccenic acid is first formed, which is then hydrogenated to stearic acid. The second way is the endogenous synthesis of CLA9 from vaccenic acid originating from the rumen, which takes place using the enzyme Δ-9-desaturase [43]. It is estimated that more than 91% of the CLA cis9, trans11 secreted into milk comes from endogenous synthesis (i.e., cows fed on pasture) [44]. This observation is due to the fact that during lactation in dairy cows, the epithelial cells of the udder have high Δ-9-desaturase activity, as well as the fact that it is also found in the small intestine and adipose tissue of the ruminants. Studies have shown that the age of cows was an additional factor in determining the formation of CLA levels during the early stage of lactation (Figures 3 and 4). The CLA9 content in the milk of PH cows in the first week of lactation was 32.75% higher than in to PK milk, while in MH milk, it was 67.7% higher than in MK milk.

As a result, several bacterial strains—Megasphaera eldenii, Bifidobacterium, Propionibacterium, Lactococcus, Lactobacillus—produce CLA trans10,cis12 [45]. In addition, rumen pH has a significant role in keeping a viable rumen environment appropriate for the aforementioned bacteria [46]. BHBA significantly decreased the diversity of microbiota community and increased the abundance of some pathogenic bacteria, implying that ketone bodies might influence the function of mucosal barriers [47]. MH milk had the highest CLA10 content over the course of the experiment; however, a reduction of 15% was observed in the second week, along with another reduction of 19% in the third week of lactation, compared to those of each preceding week. The CLA10 content in these animals, when compared to the healthy groups, was 319% lower for primiparous cows, as well as 1.018% lower for multiparous cows. In the second week of lactation, there was still a

significant difference in CLA10 content between healthy cows and those diagnosed with ketosis, though these values were already lower in the healthy animals, amounting to 161% for primiparous cows and 233% for multiparous cows; in contrast, its value increased in animals with ketosis. This study compared primiparous and multiparous cows and shows differences between the groups, which are of great importance. As Mirzaej- Alamouti et al. [48] pointed out, this observation may be influenced by the less adapted nature of rumen primiparous cows to the fed ration. Additionally, Civiero et al. [49] reported a lower extent of negative energy balance in primiparous cows and demonstrated its association with lower BHBA serum concentrations than those recorded in multiparous cows.

5. Conclusions

Overall, it is possible to conclude that when ketosis occurs, high levels of ketone bodies inhibit the activity of acetyl-CoA, thus decreasing the transport of acetyl-CoA, which may result in a decrease in CLA9 and CLA10 synthesis. Studies have shown that the age of the cows was an additional factor in determining the formation of CLA isomer levels during the early stage of lactation. High levels of BHBA inhibit the formation of CLA9 and CLA10 during the first two weeks of lactation in multiparous cows who are in their second lactation. Further investigations of ketogenesis in primiparous cows should be carried out. Thus, we conclude that different reference limits in CLA9 and CLA10 content should be considered to take parity to account.

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Institutional Review Board Statement: The Second Ethics Committee for Animal Experimentation in Warsaw of the Ministry of Science and Higher Education (Poland) reviewed and approved all procedures [permission no. 10/2011]. All cows were handled in accordance with the regulations of the Polish Council on Animal Care, and the Warsaw University of Life Sciences Care Committee reviewed and approved the experiment and all procedures carried out in the study.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analysed during the study are included within the article.

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Conflicts of Interest: The authors declare no conflict of interest.

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Mój indywidualny udział w jej powstaniu polegał na: opracowaniu koncepcji badań i założeń metodycznych, kolekcjonowaniu materiału biologicznego, analizach laboratoryjnych, redagowaniu manuskryptu.

Indywidualny wkład pracy w publikację wynosi: 70%

Podpis

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Mój indywidualny udział w jej powstaniu polegał na: opracowaniu koncepcji oraz założeń metodycznych badań, korekcie manuskryptu, odpowiedzi na recenzje.

Indywidualny wkład pracy w publikację wynosi: 20%

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Original scientific paper

UDK: 637.112

A comparison between Polish Holstein-Friesian and F_1 hybrid Polish Holstein Friesian × Swedish Red cows in terms of milk yield traits

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Abstract

The intensive breeding work of Holstein-Friesian cattle has led to the decrease in the diversity within the population and to inbreeding depression, which may impair its functional traits. In addition, as shown by the research, production traits are negatively correlated with functional traits such as reproduction, health, and longevity, which have a very strong impact on the profit of dairy farms. The aim of this study was to compare milk yield traits of hybrids obtained by crossbreeding of Polish Holstein Friesian (PHF) cows and Swedish Red (SRB) bulls with values obtained for pure PHF cows. For the study, 100 primiparous cows were selected and divided into two groups. The experimental group consisted of 50 crossbreds (PHF×SRB), while the control group consisted of 50 purebred PHF cows. The study showed a higher content of milk components (fat by 11.78 %, protein by 9.06 %, dry matter by 5.75 %) in PHF×SRB, as compared to PHF. A lower level of SCC (by 38.94 %) has also been shown in hybrids, which indicates their higher resistance to udder diseases. The experiment demonstrated a highly significant impact of heterosis on performance parameters and technological quality of milk in F1 generation obtained as a result of crossbreeding between PHF cows and SRB bulls.

Key words: inbreeding; inbreeding depression; crossbreeding; heterosis; milk

Introduction

According to the commonly accepted definition, heterosis is a phenomenon consisting in increasing the phenotypic value of quantitative traits of the first generation of hybrids with respect to homozygous parents. The effects of heterosis are opposite to the effects of inbreeding depression, if the breeds are properly selected for breeding. Therefore, any crossbreeding program should start from an appropriate selection of breeds, which should be complementary.

Dairy farming around the world is based on the most efficient Holstein Friesian (HF) breed of two color varieties: black and white (HO), and red and white (RW). In Poland, the local variety is Polish Holstein Friesian (PHF) breed, which was created as a result of crossing the Polish Black - White and Polish Red - White cows with HF bulls (Nowicki, 2011). According to the data of the Polish Federation of Cattle Breeders and Dairy Farmers (PFCBDF), in 2019 the PHF breed accounted for almost 88.61 % of the active population (HO - 84.87 %, RW - 3.81 %). In 2018, the average life expectancy of the PHF breed of the HO variety was 5.41 years, whereas of the RW was 5.37 years, which indicates that cows have been in use for almost 3 years (HO - 2.93 years, RW - 2.79 years) (PFHBiPM, 2020), but the length of use has been shortened in relation to the results obtained in 2016 (Adamczyk et al., 2017). The earlier deficiency is associated with cow's health problems (82.84 %) (Adamczyk et al., 2017), which very often starts with high nutritional requirements, especially with regard to energy demands in the early stages of lactation, leading to problems with metabolic disorders (Puppel and Kuczyńska, 2016; Puppel et al., 2017; Puppel et al., 2019). According to Adamczyk et al., (2017), they are the direct cause of about 8 % of deficiencies in the PHF breed, which was also observed by Ghaderi-Zefrehei et al. (2017). Among metabolic diseases, ketosis is the most important and common disease affecting dairy herds (van der Drift et al., 2012). Metabolic diseases are usually only the starting point of growing expenses related to veterinary care, because very often they are accompanied by problems with udder inflammation and reproduction (Adamczyk et al., 2017; Clasen et al., 2017). However, these problems are related not only to the high energy demand and poorly balanced feed (Schaeffer et al., 2011; Puppel et al., 2018). The selection of only the best bulls in terms of production traits caused a deterioration in the results of functional traits such as health, fertility, and longevity (Smith et al., 1998; Thompson et al., 2000; Adamec et al., 2006; Bjelland et al., 2013; Pryce et al., 2014; Doekes et al., 2019; Hofmannová et al., 2019). Such a breeding model reduced the diversity of the population, leading to inbreeding depression, which reduces the level of productivity (Schaeffer et al., 2011; Smith et al., 1998; Thompson et al., 2000; Adamec et al., 2006; Bjelland et al., 2013; Pryce et al., 2014; Doekes et al., 2019; Hofmannová et al., 2019). According to Pryce et al. (2014), a 1 % increase in the inbreeding level was associated with a decrease in milk yield by 28 L/lactation in Holstein-Friesian cows, and by 12 L/lactation in Jersey cows. Additionally, the increase in inbreeding level was correlated with a decrease in milk, fat, and protein yields. Also, Smith et al. (1998) and Doekes et al. (2019) showed that an increase in the inbreeding level by 1 % in HF resulted in productivity reduction by 36.3 kg and 27 kg/lactation, respectively. In addition to the problems associated with increased homozygosity, there may also appear problems with an increased incidence of rare lethal or harmful recessive disorders, such as bovine leukocyte adhesion deficiency - BLAD (Kehrli et al., 1990) or uridine monophosphate synthase deficiency - DUMPS (Shanks et al., 1984).

The increasing level of inbreeding does not allow taking full advantage of the effect of the breeding progress. Therefore, crossing procedures become an excellent alternative because these extend the length of animal use, improve reproduction parameters, and reduce metabolic disorders (PFHBiPM, 2017; PFHBiPM, 2018; PFHBiPM, 2019; PFHBiPM, 2020; Sørensen et al., 2008). In addition, a steady increase in the inbreeding of the Holstein-Friesian population (around +0.2 % per year) indicates that crossings will be necessary for most milk producers in the future. Modern breeding should be focused on product quality as well as animal health and welfare, because they guarantee the profitability of production. The main goal of crossbreeding Holstein-Friesian cows with bulls of other dairy breeds is to improve performance traits. Thus, the aim of this study was to compare Polish Holstein-Friesian and F1 hybrid Polish Holstein Friesian × Swedish Red cows in terms of milk yield traits.

Material and methods

The experiment was carried out in an experimental dairy farm of the Warsaw University of Life Sciences (WULS, Warsaw, Poland), where about 350 cows were kept in a free stall housing system with an average yield exceeding 10,000 kg of milk in lactation. For the study, 100 primiparous cows were selected and divided into two groups. The experimental group consisted of 50 crossbreds (Polish Holstein Friesian x Swedish Red; PHF×SRB), while the control group consisted of 50 purebred PHF cows. Data from milk performance evaluation were the basis for obtaining information on milk performance (milk production in individual lactation months, fat, protein, dry matter content, somatic cell count and milk performance in the whole lactation). Samples of milk were collected from the cows for laboratory analyses at monthly intervals, at ten time points. FTIR was used to analyze the composition of the milk. The cows' feeding regime was based on the total mixed ration (TMR) diet (***ad libitum***) (Table 1).

The data obtained were analysed statistically using IBM SPSS 6.0 package. The distribution of the milk chemical composition was checked by the Shapiro-Wilk test. The ANOVA analysis was performed to establish influence of the genotype on milk chemical composition and somatic cell count (SCC). The changes in concentration of basic

Table 1. Ingredient and chemical composition of the TMR

TMR diet	
Ingredient [kg/dDM]	
Maize silage	9.23
Alfalfa silage	3.70
Corn silage	2.42
Soybean meal	2.32
Pasture ground chalk	0.20
VIT-RA BML- vitamin mix	0.20
Salt	0.05
Rapeseed meal	2.17
Magnesium oxide	0.07
Chemical composition [g/kgDM]	
Ash	5.25
Crude protein	16.05
Fat	4.94
Starch	291.11
Sugar	76.95
Acid detergent fiber	30.19
Neutral detergent fiber	41.33
Ca	0.93
P	0.63
NEL (Mcal/kg)	1.64
Total, kg of DM (offered)	21.13
Daily intake (kg)	19.22
Average milk production (kg)	33.21
UFL (unit of milk production) balance (%)	3.19
PDIN (protein digested in the small intestine when rumen-fermentable nitrogen is limiting)	2.31
PDIE (protein digested in the small intestine when rumen-fermentable energy is limiting)	-2.81

TMR - total mixed ration

chemical components in regard of genotype and lactation stage were established by the multi-variance analysis.

The following statistical model was applied:

$Y = \mu + A_i + B_j + (AxB)_{ij} + e_{ijk}$

where, μ - mean, A_i - day in lactation, B_j - genotype (PHF, PHF×SRB), AxB - interaction between day in lactation and genotype, e_{ij} - random error. Only the interactions between factors whose influence was statistically significant ($P \leq 0.01$ or $P \leq 0.05$) were considered. The Pearson's correlation was used to quantify the degree of a linear relationship between two variables (*x* and *y*).

Results and discussion

Figure 1 shows the lactation curves of PHF and PHF×SRB cows. The lactation curve is a reflection of the daily production of milk, which is a resultant of both genetic and environmental factors. The shape of both curves is similar, which may indicate that neither purebred cows or hybrids had feeding problems. Heins et al. (2006) also showed a similar trend. According to Lopez et al. (2015), the peak lactation in HF cows is between weeks 4 and 8 of lactation. However, as shown by results obtained, the peak lactation in cows from the PHF group fell on the 3rd month after calving, i.e. about the 9th week of lactation. PHF×SRB crossbreds reached their lactation peak earlier, which decreased in the second month of lactation, i.e. about 5weeks after calving. Similar tendencies were observed by Slósarz et al. (2016).

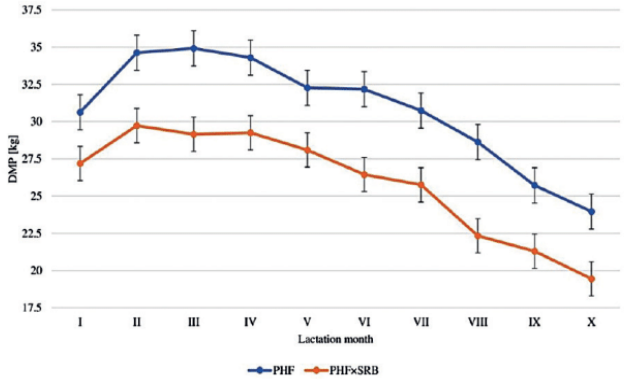

Figure 1. Lactation curve

PHF: Polish Holstein Friesian; PHF×SRB: Polish Holstein Friesian x Swedish Red; DMP: daily milk production
Samples of milk were collected from the cows for laboratory analyses at monthly intervals, at ten time points

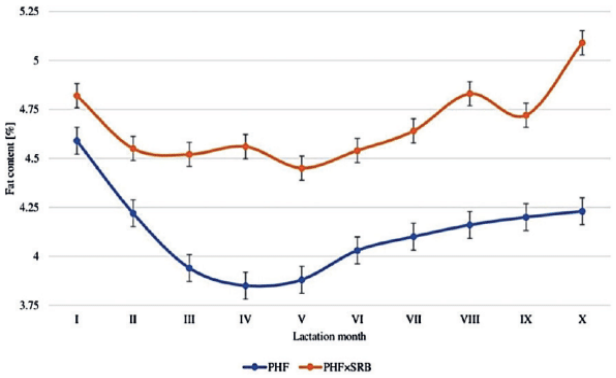

Figure 2. Changes in the content of fat depending on genotype

PHF: Polish Holstein Friesian; PHF×SRB: Polish Holstein Friesian x Swedish Red
Samples of milk were collected from the cows for laboratory analyses at monthly intervals, at ten time points

Figure 2 shows changes in fat content of milk from PHF group cows and from PHF×SRB group cows. Fat content depends on many factors including health status, food intake, performance, and physiological status. The obtained results showed significant differences in the level of this component during the lactation period. The first month of lactation was characterized by a high concentration of fat, after which it decreased to the metabolic state, and by changes in both the amount of milk produced and dietary dose. This dependence was confirmed by Heins et al. (2008) and Kuczyńska et al. (2011). The dilution effect was observed in the present study after the 1st till the 5th month of lactation, both in the purebred cows and the hybrids. However, the reduction in fat content was more tangible

in the purebred cows (-0.74 %) than in the hybrids (-0.37 %). The obtained trends confirm those reported earlier by Slósarz et al. (2016). After the 5th month of lactation, the fat content increased, due to the changing physiological and metabolic state of cows, as well as to decreasing productivity, which is confirmed in Figure 1. Changes in fat content in relation to milk production were confirmed by a negative correlation of -0.245 (Table 2).

Table 2. *Pearson correlations between individual milk components*

Variable	DMY	Fat	Protein	Dry matter	SCC
DMY	1	-0.245**	-0.583**	-0.375**	-0.049
Fat	-0.245**	1	0.397**	0.945**	0.000
Protein	-0.583**	0.397**	1	0.647**	0.860
Dry matter	-0.375**	0.945**	0.647**	1	0.110
SCC	-0.049	0.000	0.860	0.110	1

***Correlation significant at a 0.01 level (two-sided). DMY - daily milk yield; SCC - somatic cell count*

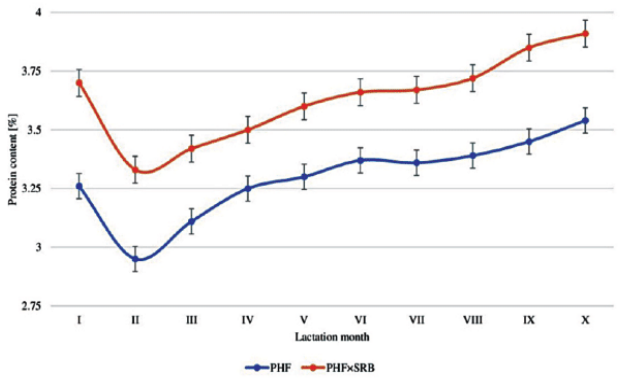

Figure 3. *Changes in the content of protein depending on genotype*

PHF: Polish Holstein Friesian; PHF×SRB: Polish Holstein Friesian x Swedish Red
Samples of milk were collected from the cows for laboratory analyses at monthly intervals, at ten time points

Figure 3 shows changes in the protein content of milk. The high technological quality of milk is associated to the concentration of total proteins; in particular to the casein content, which is responsible for both, the rate of formation of the curd and its compactness, making it one of the most important components of the raw material. This ingredient is of a great interest not only to dairies, but also to farmers, who perceive it as a factor affecting the higher price of raw material (Król et al., 2011). Protein is one of the milk constituents that is difficult to modify, since its content can mainly be obtained by working in this direction selection of genetics. This component is a highly inherited trait (h2 = 0.3-0.5) (Toghiani, 2012; Hofmannová et al., 2019), hence the measures undertaken to increase its content in milk are not difficult to control, as in the case of low-hereditary traits. The obtained results show that the protein content was similar in the whole lactation period in both the purebred and crossbred cows, which indicates, first of all, consistent breeding work of the whole dairy herd in which the experiment was conducted. The difference visible from the very beginning is related to the higher content of this component in hybrids, which is connected with the use of SRB breed, which according to PFCBDF data is characterized by a significantly higher average protein content in comparison with PHF cows (SRB 3.55 %, PHF HO 3.34 %, respectively) (PFHBiPM, 2020). However, Ezra et al. (2016) did not show any significant differences in protein content between HF and HF×NRF. In the present study, at the beginning of lactation, the protein content was at the highest level, which was associated to the physiology and colostrum secretion, i.e. colostrum is rich in immunoglobulins and whey proteins. The concentration of this component then decreased to the lowest level in the second month of lactation, which was probably associated with a high energy demand (Figure 3). Additionally, studies have shown that the protein content of milk is negatively correlated with milk production, which is confirmed by result -0.583 (Table 2). Moreover, Heins et al. (2006) confirmed a higher protein content in the milk of the crossbreds, while Hazel et al. (2017) demonstrated higher fat and dry matter contents and higher milk yields.

Changes in the dry matter content during lactation in purebreds and hybrids are presented in Figure 4. Dry matter is a very important parameter from the technological point of view, it is a resultant of components such as: protein, fat, lactose and other components defined as ash (Jaworski and Kuncewicz, 2007). Our research showed differences in the dry matter content, which may be indicative of a higher technological usefulness of hybrid milk. Studies have shown that the appearance of the curve is similar to that obtained in Figures 2 and 3, confirming the dilution effect, and a significant correlation between fat (0.945), protein (0.647), and dry matter (Table 2). A similar relationship was established by Jaworski and Kuncewicz (2007). The obtained results demonstrate a significantly higher content of particular milk components (fat by 11.78 %, protein by 9.06 %, dry matter by 5.75 %) in PHF×SRB in comparison to the milk of PHF cows. Similar trend was observed by Swalve (2007), Malchiodi et al. (2011), and Malchiodi et al. (2014).

Figure 5 shows changes in the cytological quality (SCC) during lactation. The number of somatic cells in milk is an indicator of udder health and technological quality of milk. According to Malinowski (2001), environmental factors such as cow's age, lactation phase, and calving period have a small influence on the somatic cell count in milk. Piepers et al. (2009) and De Vliegher et al. (2012) have shown the udder inflammation in heifers to be a common problem affecting milk production during the first lactation. Archer et al. (2013a, 2013b, 2014c) demonstrated that a higher content of somatic cells between the 5th and 30th day after calving reduced milk production not only during the first lactation, but also affected the possibility of udder inflammation in later lactations, life expectancy of cows, and length of their use. According to Juozaitiene and Juozaitis (2005), the SCC up to 100,000/mL in milk

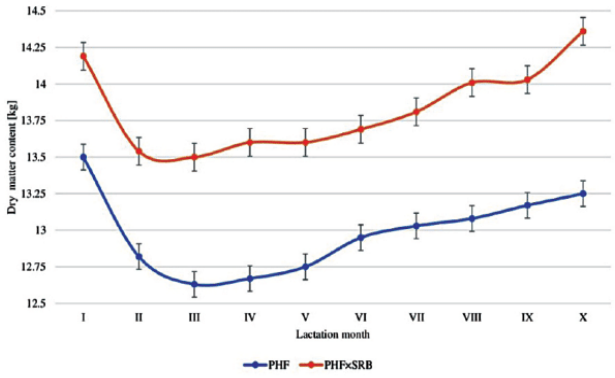

Figure 4. Changes in the content of dry matter depending on genotype

PHF: Polish Holstein Friesian; PHF×SRB: Polish Holstein Friesian x Swedish Red
Samples of milk were collected from the cows for laboratory analyses at monthly intervals, at ten time points

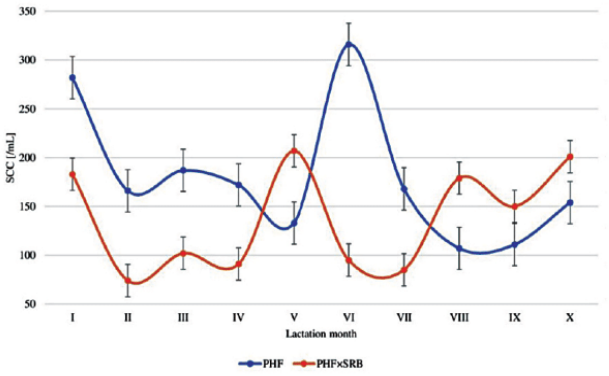

Figure 5. Changes in the content of SCC depending on genotype

PHF: Polish Holstein Friesian; PHF×SRB: Polish Holstein Friesian x Swedish Red; SCC: somatic cell count
Samples of milk were collected from the cows for laboratory analyses at monthly intervals, at ten time points

indicates healthy udder, while that above 200,000 /mL in milk indicates the occurrence of subclinical mastitis. In the present study, in the first month of lactation of purebred cows and hybrids we found a higher number of somatic cells in milk than in the following months, which is due to the beginning of lactation and changes related to the production and secretion of milk with lower efficiency. It is worth noting that the content of somatic cells in the purebred cows exceeded 200,000 /mL, which according to Juozaitiene and Juozaitis (2005) proves the occurrence of subclinical mastitis. In hybrids, however, the content of somatic cells did not exceed this value, which may indicate their higher resistance to mastitis. In the final stage of lactation, the number of somatic cells increased in both analysed groups, which was caused by the lower milk production and ongoing lactation. A similar trend, in which at the beginning and in the end of lactation the content of somatic cells in milk is higher, and at the peak of lactation a lower number of somatic cells is maintained, was shown by Jakiel et al. (2011). In our study, crossbreds were characterized by a lower level of somatic cells (38.94 %) in comparison to PHF, which indicates their greater resistance to udder disease, which was also confirmed by Heins and Hansen (2012a, 2012b). In the studies by Swalve (2007), Malchiodi et al. (2011), and Hazel et al. (2017), the crosses of HF×SRB and HF×SR were characterized by a higher SCC than the purebred HF.

Table 3 presents the average production values for purebred PHF and crossbreds. Daily milk production in the first lactation was significantly higher by 14.61 % in the purebreds than in the crossbreds. A similar relationship was found in the studies by Heins et al. (2006), Malchiodi et al. (2011), Malchiodi et al. (2014), Piccardi et al. (2014), and Saha et al. (2017). However, Puppel et al. (2018) demonstrated that PHF×MO cows were characterized by the highest milk yield, reaching 27.97 kg, while the lowest daily performance has been demonstrated for PHF×NO cows -18.93 kg. These results do not confirm the reports of other authors (Neja et al., 2010; Brodziak et al., 2012; Litwińczuk et al., 2014) concerning the daily milk production in PHF breed, which according to these researchers is higher in comparison with cows of other breeds.

On the basis of the results obtained for the fat-to-protein ratio (FPR), it can be concluded that the feeding dose for both groups of animals was composed correctly and the animals did not show any metabolic disorders; due to the average value of this parameter (1.26 for PHF and 1.29 for PHF×SRB). According to Haas and Hofirek (2004), the appropriate FPR in milk should be from 1.2 to 1.4. A ratio below 1.1 indicates the occurrence of a metabolic disorder such as acidosis. However, the ratio above 1.5 may indicates the occurrence of ketosis (Riehardt, 2004; Puppel and Kuczyńska, 2016), which was also confirmed by Ranaraja et al. (2018).

Table 3. Daily milk production [kg] and chemical composition of milk [%]

Variable		Breeds		P-value
		PHF	PHF×SR	
DMY [kg]	LSM	30.36	26.49	0.000
	SEM	7.221	6.788	
Fat [%]	LSM	4.16	4.65	0.000
	SEM	0.763	0.864	
Protein [%]	LSM	3.31	3.61	0.000
	SEM	0.311	0.367	
Dry matter [%]	LSM	13.04	13.79	0.000
	SEM	0.917	1.023	
SCC [10^3/mL]	LSM	181.47	130.61	0.064
	SEM	322.678	253.478	
Fat/Protein	LSM	1.26	1.29	0.489
	SEM	0.226	0.227	

LSM - Least square of mean; SEM - Standard error of LSM.
PHF: Polish Holstein Friesian; PHF×SRB: Polish Holstein Friesian x Swedish Red; DMY: daily milk yield; SCC: somatic cell count

Table 4. Milk performance of Polish Holstein Friesian × Swedish Red and pure Polish Holstein Friesian cows

Milk performance			Breeds		P-value
			PHF	**PHF×SRB**	
Days in Milk		LSM	297.71	273.50	0.001
		SEM	17.738	42.100	
Milk [kg]		LSM	9038.48	7245.02	0.000
		SEM	674.169	1389.856	
Fat	[%]	LSM	4.13	4.65	0.000
		SEM	0.472	0.607	
	[kg]	LSM	376	336.89	0.069
		SEM	41.854	76.728	
Protein	[%]	LSM	3.31	3.61	0.000
		SEM	0.177	0.223	
	[kg]	LSM	299.17	261.55	0.001
		SEM	17.938	42.126	
Dry matter	[%]	LSM	13.04	13.79	0.000
		SEM	0.568	0.752	
	[kg]	LSM	1178.62	999.09	0.000
		SEM	74.787	186.546	

LSM - Least square of mean; SEM - Standard error of LSM.
PHF: Polish Holstein Friesian; PHF×SRB: Polish Holstein Friesian x Swedish Red.

Table 4 presents the results concerning milk performance results for full lactation of purebred PHF and PHF×SRB cows. The longer lactation (by 63.47 days) was shown in the PHF cows in comparison to the crossbreds; which may indicate problems with reproduction of the purebred cows. During the prolonged lactation, milk production by purebred cows is significantly higher (by 2919.4 kg), and the milk is characterised by lower contents of individual components (fat -0.50 %, protein -0.28 %, dry matter -0.74 %) that determine its technological quality, in comparison to milk from the hybrids. However, the amount of raw material obtained, despite the lower concentration of the above mentioned components, results in a higher milk yield from the PHF cows (fat +76.11 kg, protein +73.7 kg, dry weight +313.97 kg). Heins et al. (2006) reported a lower milk yield by hybrids with Scandinavian Red breeds (SR) during lactation, but the difference was not as big as in the present research. Whereas Petraškiene et al. (2011) and Petraškiene et al. (2013) stated that the milk yield of HF×SRB hybrids was similar to that of the HF cows. Slósarz et al. (2016) also achieved similar results.

Conclusion

Crossbreeding between the PHF and SRB breeds has a positive effect on the content of fat, protein and dry matter in milk, as well as on udder health. Additionally, the crossbreds were characterized by shorter lactation than the purebred PHF, which may be indicative of their smaller reproductive problems. However, more study is needed on the effects of crossing between breeds on dairy cattle.

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Usporedba proizvodnih osobina poljskih holstein-friesian krava s F[1] križankama poljskog holstein-friesian × švedskog crvenog goveda

Sažetak

Intenzivan uzgojno-selekcijski rad u holstein-friesian populaciji doveo je do smanjenja genetske raznolikosti unutar populacije i povećanja razine uzgoja u srodstvu, što može oslabiti proizvodne karakteristike. Uz to, dosadašnja istraživanja upućuju na to da su proizvodne karakteristike u negativnoj korelaciji s funkcionalnim karakteristikama, kao što su reprodukcija, zdravlje i duljina života, što ima vrlo visok utjecaj na profit mliječnih farmi. Cilj ovog istraživanja je usporediti proizvodne osobine križanki poljskog holstein-friesian (PHF) × švedskog crvenog goveda (SRB), s proizvodnim osobinama jedinki čistokrvnog poljskog holstein-friesian goveda. Istraživanje je provedeno na 100 prvotelki podijeljenih u dvije skupine, 50 križanki (PHF × SRB) i 50 čistokrvnih PHF prvotelki. Utvrđen je veći sadržaj komponenti mlijeka (mliječne masti za 11,78 %, mliječnih proteina za 9,06 %, suhe tvari za 5,75 %) kod križanki (PHF × SRB) u odnosu na čistokrvne PHF prvotelke. Niža razina SCC-a (za 38,94 %) utvrđena je kod križanki, što ukazuje na njihovu veću otpornost na oboljenja vimena. Istraživanje je ukazalo na značajan učinak heterozisa na sadržaj mliječne masti, mliječnog proteina i broja somatskih stanica u mlijeku.

Ključne riječi: holstein-friesian; heterozis; proizvodnja mlijeka, broj somatskih stanica

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Podpis

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The Influence of Crossbreeding on the Composition of Protein and Fat Fractions in Milk: A Comparison Between Purebred Polish Holstein Friesian and Polish Holstein Friesian × Swedish Red Cows

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Abstract: Background/Objectives: In this study, the differences in protein and fat bioactive components between the milk from purebred Polish Holstein Friesian (PHF) cows and PHF cows crossbred with Swedish Red (SRB) were investigated. The objective was to assess the impact of genetic variation on the nutritional quality of their milk. Methods: This study was conducted at the Warsaw University of Life Sciences' (WULS) experimental dairy farm in Warsaw, Poland, and involved 60 primiparous cows divided into two groups: 30 PHF×SRB crossbred cows and 30 purebred PHF cows. All cows were housed in a free-stall system with an average lactation yield exceeding 10,000 kg/lactation. The milk composition analyses included total protein, casein, whey protein, fatty acid profiles, and vitamin content. Results: Milk from the PHF×SRB hybrids showed a significantly greater total protein content (3.53%) compared to that from the purebred PHF cows (3.28%). The casein content was higher in the hybrids' milk (2.90%) than the purebreds' milk (2.78%), while the whey protein levels were lower in the purebred milk (0.50%) than in the hybrid milk (0.63%). The hybrids exhibited higher concentrations of certain saturated fatty acids in their milk, while the purebreds' milk contained greater amounts of beneficial unsaturated fatty acids and fat-soluble vitamins—E, D, and K. Conclusions: These results indicate that genetic selection through crossbreeding can enhance the nutritional quality of milk. The differences observed in protein, fatty-acid, and vitamin content underscore the role of the genotype in milk composition, suggesting that breeding strategies can optimize dairy products' health benefits.

Keywords: milk; protein; fat; vitamins; purebred; crossbreed

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1. Introduction

Milk is recognized as an excellent food and a source of energy and high-quality protein, vitamins, and minerals in a digestible form available to humans, especially during the growing period [1]. Cow's milk is the most widely used in human nutrition and accounts for about 81% of world production [2].

The protein in cow's milk has high biological value and, importantly, has high digestibility [3]. Protein undergoes hydrolysis in the gastrointestinal tract, resulting in the formation of peptides that exhibit bioactive properties [4]. Milk protein is composed of two main fractions, namely, casein (C) (approximately 80%) and whey protein (WP) (approximately 20%) [3,5]. Although the WP content of milk is relatively low, WP has the highest biological value among the proteins consumed by humans [6].

The casein fraction is mainly responsible for the capacity to process milk in dairy production and, after consumption, is involved in regulating metabolism and activating antioxidant enzymes, as well as being a source of trace elements [7].

The main WP in ruminant milk is β-lactoglobulin (BLG), which is synthesized in the mammary glands. BLG binds to and transfers hydrophobic compounds such as retinol, vitamin D2, cholesterol, and fatty acids (FAs), thus improving their bioavailability. BLG is involved in passive immunity in newborns and in the regulation of phosphorus metabolism in the mammary glands [8–10]. BLG binds with free fatty acids (FFAs), altering the digestion of milk fat by increasing lipase activity [11,12]. It has the ability to adhere to microorganisms, preventing pathogen colonization, inhibits viral replication, and has anti-cancer effects [13,14]. The second WP in milk is α-lactalbumin (ALA), which is produced in the epithelial cells of the mammary glands and is involved in the biosynthesis of lactose and milk [15]. ALA is recognized as a major source of essential amino acids (EAAs) [16]. ALA has the ability to bind and transport ions: calcium, magnesium, sodium, potassium, zinc, and manganese [17,18]. It has anti-cancer properties, aids in mineral absorption, has immunomodulatory effects, and prevents damage to the gastric mucosa [18,19]. BLG and ALA are considered milk allergens [20].

Another WP is lactoferrin (Lf), which is responsible for chelating iron, suppressing the inflammatory response, and inhibiting oxidative stress. The action of Lf is dependent on iron saturation [21]. It has antimicrobial, antifungal, antiviral, anti-inflammatory, and anti-cancer properties; these are mainly related to its ability to alter the permeability of cell membranes, thus contributing to the lysis and death of cells considered harmful [22–24]. Yet another WP is bovine serum albumin (BSA). This is a protein derived from blood serum and enters the milk via secretory cells. BSA has a high content of EAAs; it transports long-chain fatty acids and steroid hormones and binds metal ions and aromatic compounds [18]. Lactoperoxidase (Lp) is a WP secreted into the secretory cells of the mammary gland [25]. Lp is responsible for producing an effective immune response to inflammation [26], fights against microorganisms, and is used in the protection of the mammary glands and the digestive tract of newborns [27–29]. It has anti-cancer and antioxidant properties and is also used to preserve food products [30–32]. Lysozyme (Lz) is a WP with the following properties: antibacterial, antiviral, antifungal, anti-inflammatory, with antihistamine and anti-tumor activities. Additionally, it demonstrates immunostimulant properties [33,34]. Studies have shown that Lz also has the potential to act synergistically with antibiotics [35].

The fat fraction of milk also plays an important role in the human diet. It is commonly believed that the consumption of C12:0, C14:0, and C16:0 acids has an adverse effect on the human body and is related to an increased number of low-density lipoproteins (LDLs) and the occurrence of atherosclerosis and coronary artery disease; however, the other saturated fatty acids (SFAs) found in milk have a neutralizing effect on these acids by increasing the concentration of high-density lipoproteins (HDLs) [36–38]. In addition, studies on the effects of C12:0, C14:0, and C16:0 acids indicate that, due to the presence of calcium, peptides, and phosphorus, these acids are modified in a manner that restricts their harmful properties [39,40]. From a consumer's perspective, butyric acid (C4:0–4.4%), which belongs to the SFA group (of which milk is almost the only source), plays an important role in that it has been shown to have anti-cancer properties [41] and is an essential source of energy for the intestines, maintaining homeostasis, preserving the function of the mucous membrane, and ensuring defense against pathogens [38]. The second fraction of fatty acids is unsaturated fatty acids. In this group, the highest content found is that of oleic acid (C18:1 c9–24–35%), the effect of which is related to C18:0. Both of these acids show anti-carbohydrate properties and have positive effects on human health [38]. The third fraction is polyunsaturated fatty acids (PUFAs). A particular role is played here by conjugated linoleic acid (CLA) and its isomers, formed by microbial biohydrogenation in the digestive tract of ruminants. CLA exhibits a cardiovascular immune function and has anti-cancer hypolipidemic, anti-atherosclerosis, and anti-diabetic properties [38,42,43]. The main source of CLA in the human diet is milk, but CLA is also present in ruminant meat [44,45].

Milk is also a source of vitamins. The content of fat-soluble vitamins depends on the content of fat in the milk. Vitamin A is particularly important during both human and animal growth periods as it is responsible for the body's normal development and

immune activity by supporting epithelial barrier function. It is considered an important vitamin for maintaining normal vision and is involved in inhibiting the spread of cancer through its antioxidant properties [46,47]. Vitamin D is responsible for the control of calcium homeostasis in the body and influences the inhibition of cell proliferation and apoptosis, which can enhance immunity. It also has an inhibitory effect on the growth of tumors, especially skin tumors [47,48]. Vitamin E is primarily responsible for inhibiting the clumping of platelets, and it induces the vasodilation of blood vessels. It exhibits antioxidant properties through trapping and inhibiting the production of reactive oxygen species. The antioxidant function depends on the presence of other vitamins (C and B) as well as selenium and glutathione. Vitamin D is also responsible for preventing the skin aging process and the formation of scars by protecting against oxidative stress in the skin [47,49]. Vitamin K is responsible for blood clotting, normal bone development, and the inhibition of osteoporosis and cardiovascular disease. Due to its effect on blood coagulation, vitamin K accelerates the wound-healing process and reduces reactive oxygen species due to its antioxidant properties [47,50].

The aim of the present study was to determine whether crossbreeding influences the levels of bioactive compounds and antioxidant potential, both of which play a crucial role in the nutritional quality and oxidative stability of milk. The study hypothesis was that crossbreeding PHF×SRB cows enhances milk composition, particularly by increasing its antioxidant potential, leading to improved oxidative stability and overall nutritional quality.

2. Materials and Methods

The research was conducted following the ethical guidelines of the Second Ethics Committee for Animal Experimentation in Warsaw, Ministry of Science and Higher Education (Warsaw, Poland), which approved all procedures (permission no. 10/2011). All cows involved in the study were handled according to the regulations of the Polish Council on Animal Care. The experimental design and procedures were also approved by the Warsaw University of Life Sciences Care Committee. This oversight ensured compliance with ethical standards in animal research.

2.1. Animals and Sampling

The experimental study was conducted at a dairy farm situated on the premises of Warsaw University of Life Sciences (WULS) in Warsaw, Poland. This facility houses approximately 350 cows in a free-stall housing system and boasts an average lactation yield that exceeds 10,000 kg of milk. Within the framework of this investigation, a meticulously selected group of 60 primiparous cows underwent a thorough selection process, leading to their categorization into two distinct groups. The experimental group comprised 30 crossbred cows, identified as Polish Holstein Friesian × Swedish Red (PHF×SRB), while the control group encompassed 30 purebred Polish Holstein Friesian (PHF) cows.

The dietary regimen was formulated based on the recommendations provided by the INRA system. Administered ad libitum, the diet consisted of a total mixed ration (TMR) composed of various components, including maize silage (12.10 kg/d DM), alfalfa silage (4.80 kg/d DM), corn silage (2.00 kg/d DM), soybean meal (2.50 kg/d DM), pasture ground chalk (0.20 kg/d DM), salt (0.05 kg/d DM), rapeseed meal (1.50 kg/d DM), and magnesium oxide (0.06 kg/d DM). Notable nutritional parameters pertaining to the TMR included total kilograms of dry matter (23.10) and daily intake (19.90 kg).

Milk sampling was carried out 10 times at 30-day intervals between 10 and 280 ± 5 days postpartum. Individual milk samples, each measuring 250 mL, were obtained during both morning and evening milking sessions. Subsequently, these samples were diligently preserved in sterile containers and expeditiously transported to WULS's Milk Testing Laboratory for in-depth compositional analysis. All analyses were performed in duplicate five times.

Strict criteria were implemented to select cows for the study, ensuring that only animals free from hoof issues, such as sole ulcers, and other health conditions that may have influenced the data were included. All cows were under veterinary care. There were no metabolic disorders, as indicated by the measurements of non-esterified fatty acids (NEFAs),

β-hydroxybutyric acid (BHBA), glucose, and F/P (fat/protein) ratio for the cows all having the correct values. Additionally, somatic cell count (SCC) levels did not indicate mastitis.

2.2. Chemical Analyses

The assessment of the basic milk parameters, specifically the fat, protein, lactose, and casein contents, was conducted by employing an automated infrared analysis methodology facilitated by a MilkoScan FT 120 analyzer (Foss Electric, Hillerød, Denmark).

The trans-esterification method outlined in EN ISO 12966-2:2017 [51] was employed for the methylation of fatty acids. Identification of individual fatty acids within the crude fat samples was undertaken using an Agilent 7890A GC system (Agilent, Waldbronn, Germany), following the methodology established by Puppel et al. [52]. The identification process was substantiated using pure methyl ester standards, including FAME Mix RM–6 (Lot LB 68242), Supelco 37 Comp. The FAME Mix (Lot LB 68887), methyl linoleate (Lot 094K1497), and conjugated CLA (9Z, 11E) (Lot BCBV3726) were all sourced from Supelco (Bellefonte, PA, USA).

The concentrations of α-lactalbumin, β-lactoglobulin, lysozyme, lactoferrin, bovine serum albumin, and lactoperoxidase were determined using an Agilent 1100 Series RP-HPLC device (Agilent Technologies, Waldbronn, Germany) according to the methodology described by Puppel et al. [53]. All samples were analyzed in duplicate. The total run time was 44 min, the flow rate was 1.2 mL min^{-1}, and the detection wavelength was 220 nm. The injection volume of the final solution was 25 µL. The identification of the peaks corresponding to α-lactalbumin, β-lactoglobulin, lysozyme, lactoferrin, bovine serum albumin, and lactoperoxidase was confirmed via comparison with standards (Sigma–Aldrich, St. Louis, MO, USA).

The total antioxidant status in blood plasma was measured using a NanoQuant Infinite M200 Pro analyzer (Tecan Austria GmbH, Grödig, Austria) with ELISA kits from Randox Laboratories (Crumlin, UK), specifically the Total Antioxidant Status kit (Cat. No. NX2331).

The concentrations of α-tocopherol (vitamin E), α-retinol (vitamin A), vitamin D, vitamin K, and β-carotene were determined via reversed-phase high-performance liquid chromatography (RP-HPLC) using an Agilent 1100 Series system (Agilent Technologies, Waldbronn, Germany) according to the method of Solarczyk et al. [44]. Chromatographic separations were performed at ambient temperature on a ZORBAX Eclipse XDB column (Agilent Technologies, Waldbronn, Germany) under solvent gradient conditions. The mobile phase consisted of methanol (Merck, Darmstadt, Germany) and water (Sigma–Aldrich, St. Louis, MO, USA) in a 950:50 (v/v) ratio, with a flow rate of 1.0 mL/min. Detection was carried out at a wavelength of 280 nm, with an injection volume of 25 µL. All samples were analyzed in duplicate, and peak identification was verified via comparison with standards from Sigma–Aldrich (St. Louis, MO, USA).

2.3. Statistical Analysis

The data underwent a comprehensive statistical compilation employing an analysis of variance (ANOVA) through the least-squares method facilitated by PS IMAGO PRO 10.0 software [54]. Significant differences among group means were determined using the F-statistic. The distribution characteristics of the whey protein, fatty acid, and vitamin composition were examined through the application of the Shapiro–Wilk test.

3. Results

The milk analyzed in this experiment was from cows that had no mammary gland inflammation problems or metabolic diseases, as evidenced by the results in Table 1, where the SCC for the purebred PHF cows is 116 × 10^3/mL, and that of the PHF×SRB hybrids is 123 × 10^3/mL; the EU standard for this parameter is 400 × 10^3/mL [55]. In addition, the cows were under constant veterinary care—lameness, diarrhea, and fever were absent.

Table 1. Parameters for cows participating in the experiment.

	PHF (n = 30)		PHF×SRB (n = 30)		p-Value
	LSM	SEM	LSM	SEM	
DMP [kg]	30.08	0.146	27.45	0.149	0.000
Lactose [%]	5.06	0.009	4.79	0.009	0.000
SCC [10^3/mL]	116	9.396	124	9.553	0.570
F/P	1.18	0.015	1.14	0.015	0.098
BHBA [mmol/L]	0.79	0.015	0.673	0.015	0.000
NEFA [mmol/L]	0.39	0.016	0.20	0.016	0.000
Glucose [mg/dL]	64.56	0.315	63.63	0.320	0.039

PHF—Polish Holstein Friesian cows, PHF×SRB—Polish Holstein Friesian and Swedish Red hybrid cows, DMP—daily milk production, SCC—somatic cell count, F/P—fat/protein ratio, BHBA—β-hydroxybutyric acid, NEFA—non-esterified fatty acids, LSM—least-squares mean, SEM—standard error of LSM.

3.1. Protein Fraction in the Milk

Table 2 illustrates the influence of the cow genotype on the composition of casein and whey proteins in the milk. The total protein content in the milk from the PHF×SRB crossbred cows was approximately 7.62% higher than that of the purebred PHF cows. In the purebred PHF milk, C constituted 84.76% of the total protein, whereas in the crossbred cows, it accounted for 82.15%, indicating that the C content was about 4.32% greater in the crossbred cows' milk. In contrast, WP concentrations were approximately 26% lower in the purebred PHF milk compared to those in crossbred cows' milk. This difference can be attributed to the genetic predisposition of the HF breed, recognized for its high milk production resulting from a dilution effect, along with the genetic contributions of the Swedish Red breed to the overall milk composition. Furthermore, Lz concentrations were approximately 17.19% lower in the crossbred cows' milk than in purebred PHF milk. Lf levels showed a more pronounced reduction, being about 55.26% lower in the crossbred cows' milk. Conversely, the BSA content was approximately 27.78% higher in the crossbred cows' milk compared to the purebred PHF milk. Additionally, the levels of BLG in the crossbred cows' milk were approximately 45% higher than those in the purebred PHF cows. Finally, Lp activity was about 14.71% higher in the crossbred cows' milk compared to purebred PHF milk.

Table 2. The influence of the cow's genotype on the contents of individual proteins.

	PHF		PHF×SRB		p-Value
	LSM	SEM	LSM	SEM	
Protein [%]	3.28	0.016	3.53	0.016	0.000
Casein [%]	2.78	0.011	2.90	0.011	0.000
Whey protein [%]	0.50	0.030	0.63	0.042	0.000
Lz [µg/L]	20.18	0.510	16.59	0.518	0.000
Lf [µg/L]	0.38	0.023	0.17	0.024	0.000
ALA [g/L]	1.68	0.022	1.72	0.022	0.228
BSA [g/L]	0.18	0.005	0.23	0.005	0.000
BLG [g/L]	2.40	0.046	3.48	0.047	0.000
Lp [mg/L]	0.34	0.010	0.39	0.010	0.003

PHF—Polish Holstein Friesian cows, PHF×SRB—Polish Holstein Friesian and Swedish Red hybrid cows, Lz—lysozyme, Lf—lactoferrin, ALA—α-lactalbumin, BSA—bovine serum albumin, BLG—β-lactoglobulin, Lp—lactoperoxidase, LSM—least-squares mean, SEM—standard error of LSM.

3.2. Fat Fraction in the Milk

3.2.1. Fatty Acids

The analysis of selected SFAs (Table 3) in the milk of purebred PHF cows and PHF×SRB crossbred cows revealed genotype-dependent variations. The crossbred hybrids demonstrated higher concentrations of short- and medium-chain SFAs, including butyric acid (C4:0), capric acid (C10:0), lauric acid (C12:0), and myristic acid (C14:0), when compared to the purebred PHF cows. These differences were statistically significant, indicating a genotype-driven improvement in the synthesis of these specific SFAs. In contrast, the

PHF cows had a higher content of stearic acid (C18:0), suggesting that divergent metabolic pathways affect long-chain fatty acid synthesis. The observed disparities suggest that crossbreeding selectively influences the milk's FA profile, enhancing certain FAs associated with different nutritional and functional properties.

Table 3. The influence of the cows' genotypes on selected fatty acids.

	PHF		PHF×SRB		*p*-Value
	LSM	**SEM**	**LSM**	**SEM**	
Fat [%]	3.84	0.046	3.97	0.047	0.045
Selected fatty acid [g/100 g fat]					
SFA	64.50	0.193	64.12	0.196	0.161
C4:0	2.60	0.026	2.68	0.026	0.026
C6:0	1.53	0.017	1.47	0.018	0.017
C8:0	1.02	0.014	1.01	0.014	0.370
C10:0	2.05	0.034	2.39	0.034	0.000
C12:0	2.51	0.035	2.75	0.035	0.000
C14:0	8.99	0.068	9.27	0.069	0.003
C16:0	30.71	0.150	30.48	0.152	0.277
C18:0	12.19	0.089	11.16	0.091	0.000
C16:1 c9	1.63	0.015	1.71	0.016	0.001
C18:1 t11	2.69	0.043	2.76	0.044	0.279
C18:1 c9	25.25	0.168	23.77	0.171	0.000
C18:1 c11	1.24	0.009	1.17	0.009	0.000
PUFA	3.94	0.018	3.77	0.018	0.000
C18:2 c9,c12 *n*-6	2.16	0.014	2.04	0.014	0.000
C18:3 *n*-6	0.04	0.002	0.06	0.002	0.000
C18:3 *n*-3	0.34	0.003	0.32	0.003	0.000
C18:2 c9,t11	0.53	0.005	0.51	0.005	0.000
C18:2 t10,c12	0.03	0.001	0.02	0.001	0.000
C18:2 c9,t13	0.21	0.003	0.16	0.003	0.000
C20:2 *n*-6	0.02	0.001	0.04	0.001	0.000
C20:4 *n*-6	0.15	0.002	0.16	0.002	0.001
C20:5 *n*-3	0.10	0.002	0.08	0.002	0.000
C22:5 *n*-3	0.07	0.001	0.07	0.001	0.392
C22:6 *n*-3	0.01	0.001	0.02	0.001	0.000

PHF—Polish Holstein Friesian cows, PHF×SRB—Polish Holstein Friesian and Swedish Red hybrids, SFA—saturated fatty acid, PUFA—polyunsaturated fatty acid, LSM—least-squares mean, SEM—standard error of LSM.

The comparison between the unsaturated fatty acid (UFA) content in the purebred PHF cows and that in the PHF×SRB crossbred cows revealed notable genotype-related differences. The crossbred hybrids exhibited a higher concentration of palmitoleic acid (C16:1), while the PHF cows showed significantly higher levels of C18:1 c9 and vaccenic acid (C18:1 t11). Furthermore, the PUFA content was greater in the PHF milk, with higher levels of linoleic acid (C18:2 c9,c12 *n*-6), α-linolenic acid (C18:3 *n*-3), and CLA, suggesting a more favorable UFA profile for the purebred cows. Conversely, the crossbred hybrids displayed elevated levels of γ-linolenic acid (C18:3 *n*-6) and certain long-chain *n*-6 fatty acids, indicating a differential FA metabolism between the two genotypes. While differences in trans FAs were not statistically significant, the overall findings suggest that the genotype significantly influenced the composition of UFA, with potential implications for the nutritional and functional properties of milk.

3.2.2. TAS and Lipophilic Vitamins

Table 4 presents the effect of genotypes on the total antioxidant status (TAS) and the lipophilic vitamin content in the milk. The TAS was significantly higher in the milk of the purebred PHF cows compared to that of the PHF×SRB crossbred cows, reflecting an approximately 14.0% greater antioxidant capacity in the purebred PHF milk. The concentration of vitamin E was substantially greater in PHF cows than in the crossbred cows, with a difference of approximately 28.3% in favor of the PHF cows. A similar trend was observed for vitamins D and K; specifically, the vitamin D content was about 15.3% higher in PHF cows, while vitamin K levels were approximately 10.1% higher. In contrast, no

significant differences were noted in the concentrations of β-carotene or vitamin A between the PHF cows and the crossbred cows, suggesting that these specific vitamins may not be influenced by the genetic factors associated with crossbreeding. These findings emphasize a significant genetic influence on the milk's antioxidant status and vitamin profile.

Table 4. The influence of the cows' genotypes on TAS and lipophilic vitamins.

	PHF		PHF×SRB		*p*-Value
	LSM	SEM	LSM	SEM	
TAS [mmol/L]	1.70	0.037	1.49	0.037	0.000
β-carotene [mg/L]	0.25	0.006	0.24	0.006	0.292
A [mg/L]	0.73	0.013	0.75	0.013	0.233
E [mg/L]	0.97	0.024	0.71	0.025	0.000
D [µg/L]	5.04	0.145	4.37	0.147	0.001
K [µg/L]	8.04	0.133	7.30	0.135	0.000

PHF—Polish Holstein Friesian cows, PHF×SRB—Polish Holstein Friesian and Swedish Red hybrids, TAS—total antioxidant status, LSM—least-squares mean, SEM—standard error of LSM.

4. Discussion

4.1. Protein Fraction in the Milk

The WP fraction is the main source of the so-called EAAs that are responsible for maintaining adequate homeostasis in the body [56]. These amino acids include sulfur-containing amino acids (SAAs), which are responsible for specific immune responses, reducing oxidative stress, and protecting against cancer [57]. They also include branched-chain amino acids (BCAAs), which are involved in blood glucose metabolism and homeostasis, as well as fat metabolism and regulating the synthesis of skeletal muscle tissue protein [58–60]. WPs are also involved in antioxidant activity, which is determined primarily according to the histidine and hydrophobic amino-acid content [5]. The role of WPs in the antioxidant process is to scavenge free radicals, chelate metals, and recover the thiol–SH group in proteins [7]. The results of this study reveal significant differences in the protein composition and bioactive components of the milk from purebred PHF cows compared to the PHF×SRB crossbred cows. These findings are crucial for understanding how crossbreeding influences milk quality and have direct implications for dairy production. The demand for milk and dairy products, which is driven by their nutritional value, is highlighted by Górska-Warsewicz et al. [61], who noted that milk proteins constitute a substantial portion of daily nutrient supply. The observed differences in individual protein fractions between PHF cows and PHF×SRB crossbred hybrids stem from complex interactions involving genetic and physiological factors. The total protein content was notably higher in the crossbred cows compared to the purebreds, which is advantageous for dairy production, as elevated protein levels enhance the nutritional quality of milk, rendering it more suitable for cheese production. The C content also exhibited significantly higher levels in the crossbred cows relative to the purebreds. As a primary component of milk, C is essential to cheese-making processes. Interestingly, Lindmark-Mansson et al. [62] reported shifts in the ratios of C to WP in Swedish cows, observing a decrease in the C content alongside increased WP, which they attributed to dairy cow breeding programs. Similarly, Puppel et al. [63] noted a trend toward lower casein levels in hybrid cows of Scandinavian Red descent. Furthermore, Gustavsson et al. [64] indicated that the SRB breed is characterized by a higher overall protein content in its milk compared to HF, suggesting that the genetic influence of the SRB breed may contribute to the enhanced protein profile observed in the PHF×SRB hybrid cows in this study.

The results indicate that both Lz and Lf levels were significantly lower in the crossbred cows compared to the purebred PHF cows. Lz is a glycoprotein found in milk that has antibacterial properties. It directly contributes to the innate immune defense of the milk by breaking down the peptidoglycan layer of bacterial cell walls. This action helps maintain the microbial stability of the milk [65]. Lf, another important glycoprotein, exhibits antibacterial activity and plays a crucial role in regulating iron homeostasis and modulating

the immune response [66]. The observed lower Lz and Lf levels in the crossbred cows may be surprising given the expectation of heterosis associated with crossbreeding, which often leads to enhanced performance traits and increased resilience; however, the lower levels of these proteins may indicate that the specific genetic combinations in the crossbred population do not favor the expression of these immune-related proteins. This suggests that the crossbreeding strategy might influence not only milk production traits but also the immunological profile of the milk. The diminished presence of lysozyme and lactoferrin in the milk of the crossbred cows may reflect a complex interaction between the genetic backgrounds of the breeds involved and their respective immune response mechanisms [63]. While heterosis can enhance various traits, the findings in this study indicate that it does not uniformly translate into improved levels for all immune components.

Unlike the above proteins, the levels of ALA did not exhibit significant differences between the purebred and crossbred groups, suggesting that the genetic background of the cows did not influence the expression of this protein. ALA, which is primarily involved in lactose synthesis, plays a critical role in the milk's composition, but the stable levels across both groups indicate consistent production mechanisms. BSA concentrations were significantly higher in the crossbred cows compared to the purebred cows. The higher BSA level is particularly noteworthy, as BSA functions as a carrier protein, facilitating the transport of fatty acids, hormones, and other bioactive compounds, thereby enhancing the nutritional profile of the milk. Increased BSA levels may contribute to improved health outcomes for consumers due to the potential role of this protein in binding and transporting bioactive substances. Similarly, BLG levels were significantly elevated in the crossbred cows. The greater concentration of BLG, a predominant whey protein, is associated with enhanced functional properties, including emulsification, foaming, and gelation. These characteristics are essential for the processing and texture of various dairy products, which may be advantageous for both producers and consumers. Research, including findings by Gustavsson et al. [64], suggests that a higher BLG content could be linked to specific genetic variants, implying that breeding strategies could be designed to select for these beneficial traits. Lp levels were also significantly higher in the crossbred cows. Lp is known for its antimicrobial activity, which contributes to the preservation of milk by inhibiting the growth of pathogenic bacteria [67]. The elevated levels of Lp in the crossbred cows' milk may enhance the microbial quality and extend the shelf life of dairy products.

The increased concentrations of key whey proteins (BSA, BLG, and Lp) in the milk from the crossbred hybrids may be indicative of a more robust immune response in these animals. Enhanced immune activity could lead to better protection of the mammary gland against pathogenic intrusions, which would correlate with the observed lower somatic cell count in the milk of the crossbred hybrids. A lower SCC is typically associated with improved udder health and reduced mastitis incidence, further substantiating the role of genetic factors in influencing both milk composition and quality [68]. Furthermore, elevated levels of these whey proteins not only enhance cow health but also augment the biological activity of the milk. The proteins possess various bioactive properties that may confer health benefits upon consumers, establishing their significance in functional food applications. Notably, the exceptional heat stability of these milk proteins during thermal processing allows them to retain their functional properties, thereby improving the quality of the final dairy product [69]. Interestingly, the findings of Maurmayr et al. [70] indicate that while their hybrids displayed higher ALA concentrations, they exhibited lower BLG levels. This variability underscores the complexity of genetic factors influencing protein expression across different crossbreeding scenarios, highlighting the necessity for further investigation into the specific genetic determinants that affect whey protein profiles in dairy cattle.

4.2. Fat Fraction in the Milk

4.2.1. Fatty Acids

Milk fat is a crucial component in dairy products, significantly influencing their nutritional quality, sensory properties (taste and aroma), and functional applications in

food processing [71]. The fat in milk is predominantly composed of triacylglycerols (TAGs). The remaining components include diacylglycerols, cholesterol, phospholipids, and FFAs. The complexity of TAGs arises from their diverse composition, which includes fatty acids, each exhibiting unique physicochemical and bioactive properties. The specific composition of these FAs is influenced by various factors, such as dietary intake, microbial fermentation in the rumen, de novo synthesis in the mammary gland, and the physiological state and age of the animal [37,52,53,72–75]. This study reveals significant differences between the fatty acid profiles of the purebred PHF cows and the PHF×SRB crossbred cows. These variations can be attributed to genetic differences resulting in distinct metabolic pathways and FA synthesis mechanisms.

A critical factor influencing milk fat production is the cow's energy balance. The energy necessary for milk synthesis is derived from dietary sources, while energy deficits are compensated for by mobilizing adipose tissue [76]. The PHF breed is known for its high productivity and elevated energy requirements, which may not always be met through feed intake alone. Consequently, PHF cows often mobilize body reserves more extensively than SRB cows, which have been selectively bred for improved feed efficiency and better metabolic regulation [77]. In this study, it was observed that the PHF cows exhibited higher concentrations of certain FAs, such as stearic acid (C18:0) and C18:1 c9. Elevated levels of these FAs can indicate a state of negative energy balance, highlighting the physiological implications of milk production under conditions of varying energy availability.

The current analysis indicates that PHF×SRB crossbred hybrids had higher concentrations of short- and medium-chain fatty acids compared to the purebred PHF cows. Conversely, the PHF cows exhibited significantly higher levels of beneficial long-chain fatty acids. These findings are consistent with the existing literature, which suggests that the FA composition of milk is influenced by genetic selection and metabolic efficiency. Higher levels of specific FAs in hybrids may be linked to their metabolic pathways, which differ from those of PHF cows. While certain FAs are often associated with less favorable health outcomes, the presence of beneficial long-chain fatty acids in PHF cows suggests potential health-promoting properties. The role of *n*-3 PUFAs is particularly noteworthy due to their health benefits, including anti-inflammatory properties and cardiovascular protection [78]. Essential FAs, such as linoleic acid and α-linolenic acid (ALA) are vital for human health as they cannot be synthesized endogenously [78]. This study indicates that CLA levels were higher in milk from the PHF cows, correlating with elevated ALA content. This suggests that selective breeding strategies could enhance the presence of these beneficial FAs in milk.

The observed differences in FA profiles between PHF and crossbred cows have significant implications for dairy production and consumer health. Regarding fat content, the crossbred hybrids had higher levels of SFAs, specifically C4:0, C10:0, C12:0, and C14:0, compared to the purebred PHF cows. In contrast, the purebred PHF cows exhibited higher concentrations of beneficial UFAs, such as C18:0 and C18:1 c9. These findings indicate that the genotype significantly affects the FA profile of milk. The higher levels of beneficial long-chain fatty acids in milk from the PHF cows indicate that this breed may contribute positively to the nutritional quality of dairy products. Additionally, the increased concentrations of short- and medium-chain fatty acids in hybrids could influence their utility in various dairy applications, such as cheese production and other dairy products. These findings emphasize the importance of breeding programs focused on optimizing milk FA profiles. By selecting for desirable FA characteristics, dairy producers can enhance the nutritional value of milk, thereby meeting consumer demand for healthier dairy options.

4.2.2. TAS and Lipophilic Vitamins

The results of this study demonstrate significant differences in vitamin content between the milk of the purebred PHF cows and the PHF×SRB cows. Specifically, milk from the PHF cows exhibited higher concentrations of vitamins E, D, and K compared to that of the hybrids. These disparities can be attributed to genetic factors and the metabolic pathways involved in vitamin synthesis and accumulation. Genes influence lipid metabolism significantly in dairy cows. This metabolism is crucial for the synthesis of fat-soluble vita-

mins. Enzymes, such as lipases, which are responsible for fat breakdown, can be genetically regulated, affecting the body's ability to process lipids [79].

Vitamin E levels were markedly higher in the milk of the PHF cows compared to the hybrids. This difference may reflect the genetic capacity of PHF cows to synthesize or retain higher levels of this essential antioxidant, which plays a crucial role in maintaining cellular integrity and reducing oxidative stress [80]. The presence of vitamin E in milk is vital for both animal health and human nutrition, as it contributes to immune function and may have protective effects against chronic diseases [81].

A similar trend was observed with vitamin D, of which the PHF cows had higher concentrations compared to the hybrid cows. Vitamin D is critical for calcium metabolism and bone health, and its increased concentration in PHF milk may provide additional nutritional benefits. The genetic differences between the breeds may influence the cows' ability to mobilize vitamin D and its subsequent accumulation in milk.

The findings showed that the PHF cows had a higher content of vitamin K than the hybrids. Vitamin K is essential for blood clotting and bone health, and the presence of greater amounts in the milk from PHF cows could enhance its functional properties. The observed differences in vitamin K content are likely influenced by genetic factors that affect the metabolism and storage of the vitamin within the mammary gland. Research indicates that the rumen's fermentation process likely modulates the synthesis of the K and B vitamins, but comprehensive data on this topic remain scarce. Furthermore, studies have shown that the concentrations of vitamin K in milk often do not correlate well with cows' dietary intakes. Haug et al. [82] suggest that this discrepancy may arise from the simultaneous processes of degradation and synthesis that occur in the rumen, which can affect the overall availability of these vitamins for absorption by the milk.

The observed difference in total antioxidant status between the purebred PHF cows and the PHF×SRB crossbred hybrids indicates a significant disparity in antioxidant capacity. The 14.09% greater TAS levels for the purebred PHF cows reflect their enhanced ability to combat oxidative stress, a critical factor in preserving milk quality. Higher TAS values are indicative of greater stability against oxidative degradation, thereby delaying lipid peroxidation and preserving the nutritional integrity of the milk. As noted by Puppel et al. [83], an elevated antioxidant potential is crucial for prolonging the delayed phase of protein oxidation and mitigating the formation of detrimental compounds such as dityrosine. The capacity to resist oxidative damage not only safeguards beneficial milk constituents but also extends the overall shelf life and stability of dairy products. This is particularly relevant in dairy production, where the preservation of sensory attributes and nutritional quality is essential for consumer acceptance.

Despite the fact that the same management practices were applied to both groups, the differences in vitamin content highlight the potential influence of genetics on vitamin synthesis and accumulation. Genetic factors may determine how efficiently each breed metabolizes vitamins, thus affecting their final concentrations in milk. This suggests that selective breeding for specific traits may improve the nutritional profile of milk produced by dairy cows.

5. Conclusions

This study reveals that there are significant differences between the bioactive components in milk from purebred Polish Holstein Friesian cows and that from Polish Holstein Friesian and Swedish Red crossbred cows. While the crossbred hybrids exhibited enhanced total protein content, the concentrations of beneficial unsaturated fatty acids, particularly CLA, and fat-soluble vitamins (E, D, and K) were markedly higher in the purebred PHF cows. These findings indicate that crossbreeding may improve specific traits without universally enhancing all aspects of milk quality. Moreover, the TAS values were significantly higher in the purebred PHF cows (1.70 mmol/L) compared to the hybrids (1.49 mmol/L). The elevated TAS values suggest a superior antioxidant capacity in PHF cows' milk, which is crucial for delaying lipid peroxidation and maintaining nutritional integrity. The findings highlight the fact that genetic factors play a pivotal role in the synthesis and retention of

vital nutrients and antioxidants in milk. In summary, the results underscore the necessity for targeted genetic selection in dairy cattle breeding programs, focusing on optimizing not only protein yields but also the nutritional quality of milk, including beneficial FAs, vitamins, and antioxidant capacity. These insights can guide breeding strategies in order to enhance the health benefits of dairy products, thereby aligning with increasing consumer demand for nutritionally superior milk.

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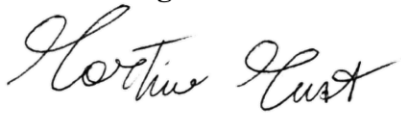

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Podpis

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dr hab. Kamila Puppel, prof. SGGW

metabolites

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Article

Effect of Age at First Calving on the Reproduction Parameters, Metabolic Profile, and Fatty Acid Composition of Polish Holstein Friesian (PHF) and Crossbreds PHF × Swedish Red (SRB) Cattle

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Abstract: Background: The high dairy production of Polish Holstein Friesian (PHF) cows determines high energy requirements in the early stages of lactation. Unfortunately, it is very often difficult to meet this demand through feedstuffs; therefore, homeostasis may be disturbed and metabolic diseases may occur, causing a majority of cows' health problems. Breeders are, therefore, looking for alternatives to the PHF breed using crossbreeding. Methods: This experiment involved 30 PHF cows and 30 PHF × Swedish Red (SRB) crossbred hybrid cows, divided into two age groups, <2 years and >2 years, at first calving. Milk and blood samples were collected at 35 ± 5 days postpartum for analysis. Data on reproductive performance were also analyzed. Results: This study revealed lower milk production for the crossbreds hybrid (27.44 kg compared to 32.08 kg), with a higher basic composition content than PHF cows (fat: 3.97% compared to 3.83%, protein: 3.53% compared to 3.27%). The heifers of the crossbreds hybrid reached sexual maturity earlier but did not affect the lower age at first calving. Dividing the cows into age categories provided a more detailed perspective of the impact of genotypic differences on reproductive and metabolic profiles in PHF and PHF × SRB cattle. The findings highlight the importance of considering age-specific effects when assessing the performance and health of dairy cattle with diverse genotypes. Conclusions: The choice between PHF and PHF × SRB should depend on the specific goals and priorities of the cattle farming operation. Factors such as overall milk yield requirements, market demands, reproductive management strategies, and health considerations should be carefully evaluated to determine the most suitable breed for a given farming context.

Keywords: crossbreeding; reproductive performance; metabolic profile

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1. Introduction

High animal performance is often a factor contributing to stress, leading to health problems. The most challenging period for dairy cows is undoubtedly the transitional period, which includes the final phase of pregnancy, calving, the onset of lactation, and the increase in productivity up to the peak of lactation, occurring at around the 100th day post-calving. During this time, dynamic changes in the body's homeostasis can lead to the development of inflammatory conditions and a decrease in immunosuppressive abilities with hormonal origins, increasing the likelihood of metabolic, mammary gland,

and reproductive system disorders [1–5]. During the transition period, metabolic disorders are by far the most important group of diseases that occur [3].

During this period, animals have the highest nutrient demand and require a properly balanced ration. Due to the sudden increase in the demand for energy and nutrients related to the initiation of colostrum and milk synthesis (fat, protein, and fatty acids), as well as the stress arising at this time, dairy cows are unable to take in an adequate energy supply with the normal ration. This results in a negative energy balance (NEB). Therefore, animals use the fat reserves accumulated in their body, turning them into energy and ketone bodies, causing the most common metabolic disorder: ketosis [2,5–8]. According to Gordon et al. [9], ketosis can occur in as many as 80% of all cows in individual herds. As reported by Duffield [10], clinical ketosis is diagnosed in 2 to 15% of all cows in the first two months of lactation, while McArt et al. [11] indicate that in the first week of lactation, 75% of high-yielding cows are diagnosed with subclinical ketosis, suggesting that this problem is a significant challenge for farmers. Among the changes observed by breeders occurring during the onset of ketosis in females are weight loss and a reduced condition. However, according to Horst et al. [4], weight loss immediately after parturition is a normal phenomenon in female mammals.

The loss of weight is related to the mobilization of adipose tissue to stimulate lactation, which is why many farmers do not pay much attention to the changes in the body weight of cows after parturition. Nevertheless, the prolongation of this state or rapid weight loss leads to the formation of stress in the body and, as a result, inflammation, which can cause the onset of ketosis and, subsequently, the onset of other diseases. Hence, an important aspect in the prevention of metabolic disease control is a timely diagnosis [12,13]. To detect their occurrence, a test is used to determine the level of ketone bodies, which are the intermediate metabolites of mobilized fat. Ketone bodies are formed during ketogenesis occurring in the liver, where the mitochondrial β-oxidation of long-chain fatty acids (FAs) occurs [6,14]. Ketone bodies include non-esterified fatty acids (NEFAs), β-hydroxybutyric acid (BHBA) acetone, and acetoacetic acid (in the form of acetoacetate anion) [15]. The levels of NEFA and BHBA increase during the course of the disease, while the levels of cholesterol and glucose, which play a key role in milk synthesis, decrease [14,16].

The diagnosis of metabolic disorders uses the determination of the concentration of ketone bodies in blood, urine, or milk. In the blood, the content of parameters such as NEFA, BHBA, or glucose is usually determined [17]. According to Serrehno et al. [17], analyses of urine and milk indicate the onset of a condition with a two-day delay compared to a blood test. In addition to ketone bodies, the determination of selected FAs, as well as whey proteins of milk, can be used in diagnosis [2,5–8].

Unfortunately, the occurrence of a negative energy balance during the transition period is very often the beginning of the problems farmers encounter with dairy cows. With the onset of NEB in cows, there are reproductive problems such as short, silent, or missing estrus, making it difficult to choose the right time for cows to mate. In addition, the pregnancy often does not develop even if fertilization occurs or there is a miscarriage at an early stage of embryonic development. All of this affects the extension of the postpartum downtime, increasing the number of semen straws used for successful insemination, and extending the service, the inter-pregnancy, and the inter-calving period, which will automatically decrease the milk yield [1,18–20].

Crossbred hybrids with an increased value of functional traits may prove to be an alternative to keeping high-yielding Holstein Friesian (HF) cows [21–24]. Crossbreeding is a well-known, appreciated, and often-used method of pairing individuals of two breeds into a parental pair, which is widely used in poultry production [25], pig breeding [26,27], and beef cattle [28]. The main advantage of this method is that it affects the improvement of less-heritable traits due to the possibility of the heterosis effect, defined as the exuberance of hybrids [27]. Crossbreeding between breeds positively influences many traits, encouraging the vigor of newborn offspring, health, body development, growth rate, feed utilization, productivity, and fertility [29]. According to Solarczyk et al. [30], crossbreeding dairy cattle

with beef cattle has a beneficial effect on the bioactive molecule content of meat. There are also reports in the literature on crossbreeding dairy cattle in which it has been shown that functional traits such as fertility, health, and longevity are improved [29,31–33], as well as the content of bioactive milk fractions [34]. As reported by Sørensen et al. [31], due to low reproduction rates, there is growing interest in this method in many developed countries due to potential economic gain and possible consumer interest in products from hybrids.

To reduce the high cost of animal maintenance, studies have also been undertaken to determine the optimal age at the first calving (AFC) of dairy cows because the rearing of heifers for herd renovation has a significant influence on production costs. It is widely believed that ideally, the AFC should fall between 23 and 24 months of age [35–37], as a result of which cows achieve optimal results in milk and breeding performance.

The objective of this study was to assess the influence of genetic factors on key physiological traits in PHF cattle and PHF × SRB crossbreds. Particular attention was given to the effects of age at first calving, with cows categorized into two groups: those calving before 24 months and those calving at or after 24 months of age. The analysis focused on evaluating milk production, reproductive parameters, metabolic profiles, and fatty acid composition in relation to genotype. The results provide valuable insights into the genetic determinants of these traits and offer implications for optimizing breeding programs and herd management practices in dairy production.

2. Materials and Methods

2.1. Animals and Sampling

This study was conducted at the experimental dairy farm situated on the premises of the Warsaw University of Life Sciences (WULS) in Warsaw, Poland. This facility housed approximately 350 cows in a free-stall housing system, boasting an average lactation yield exceeding 10,000 kg of milk. Within the framework of this investigation, a meticulously selected group of 60 primiparous cows underwent thorough analysis, leading to their categorization into two distinct groups. The experimental group comprised 30 crossbreds identified as Polish Holstein Friesian × Swedish Red (PHF × SRB) cows, while the control group encompassed 30 purebred Polish Holstein Friesian (PHF) cows. The primiparous cows selected for the experiment were required to be in optimal health. Inclusion criteria specified the absence of locomotor disorders, with a particular emphasis on excluding any cases of hoof inflammation. Additionally, cows with a history of mastitis or related complications were excluded from the study. This study aimed to provide a detailed analysis of the age at first calving in PHF and PHF × SRB crossbreds cattle by categorizing the cows into two age groups: those less than 2 years old (<2 years; PHF—23.3 months, PHF × SRB—23.2 months) and those 2 years or older (>2 years; PHF—25.9 months, PHF × SRB—24.8 months). The sample comprised 15 PHF cows in the <2 years category, 15 PHF cows in the >2 years category, 15 crossbred cows in the <2 years category, and 15 crossbred cows in the >2 years category, ensuring equal representation of both breeds within each age class.

The dietary regimen was formulated based on the recommendations provided by the INRA system [38]. Administered ad libitum, the diet consisted of a total mixed ration (TMR) including maize silage (12.00 kg/d DM), alfalfa silage (4.20 kg/d DM), corn silage (2.10 kg/d DM), soybean meal (2.80 kg/d DM), pasture ground chalk (0.20 kg/d DM), salt (0.05 kg/d DM), rapeseed meal (1.80 kg/d DM), and magnesium oxide (0.06 kg/d DM). Notable nutritional parameters pertaining to the TMR included total kg of DM (23.10), daily intake (19.90 kg), net energy lactation (1.75 Mcal/kg), average milk production (37.02 kg), unit of milk production balance (3.45%), protein digested in the small intestine when rumen-fermentable nitrogen was limiting (2.51), and protein digested in the small intestine when rumen-fermentable energy was limiting (2.23).

Milk and blood were sampled at 35 ± 5 days postpartum. Milk and blood were collected from all 60 cows taking part in the experiment. Individual milk samples, each measuring 250 mL, were obtained during both morning and evening milking sessions. Subsequently, these samples were diligently preserved in sterile containers and expeditiously

transported to WULS's Milk Testing Laboratory for in-depth compositional analysis. In parallel, blood samples of 10 mL each were drawn from the jugular vein using specialized tubes (Vacuette, Essen, Germany). Post-collection, the blood samples underwent centrifugation at 1800× *g* and 4 °C for a duration of 15 min. The ensuing supernatant was promptly conveyed to WULS's Veterinary Centre for a comprehensive suite of analyses.

Reproductive data were obtained from breeding records and included the following information: date of birth, date of first insemination, date of successful insemination, date of first calving, date of first service post-calving, date of successful service post-calving, the number of semen doses required for successful conception, and the date of subsequent calvings. Based on these records, the following reproductive parameters were calculated: AFI (age at first insemination), PI (pregnancy index), SP (service period), GL (gestation length), AFC (age at first calving), PPD (postpartum downtime), IP (inter-pregnancy interval), and PBC (calving interval). The service period (SP) was calculated as the interval between the first insemination and successful conception, with a value of 1 assigned in cases where conception occurred at the first estrus, in accordance with Kuczaj's methodology [32]. The pregnancy index (PI) was calculated based on the number of semen straws used to achieve successful conception.

2.2. Chemical Analyses

The assessment of basic milk parameters, specifically fat and protein content, was conducted employing an automated infrared analysis methodology facilitated by a Milkoscan FT 120 analyzer (Foss Electric, Hillerød, Denmark).

The trans-esterification method outlined in EN ISO 12966-2:2017 [39] was employed for the methylation of FAs. The identification of individual FAs within crude fat samples was undertaken using an Agilent 7890A GC system (Agilent, Waldbronn, Germany), following the methodology established by Puppel et al. [40] The identification process was substantiated through the utilization of pure methyl ester standards, including FAME Mix RM–6 (Lot LB 68242), Supelco 37 Comp. FAME Mix (Lot LB 68887), Methyl linoleate (Lot 094K1497), and CLA Conjugated (9Z, 11E) (Lot BCBV3726), all sourced from Supelco (Bellefonte, PA, USA).

Quantitative glucose, protein, creatine, and GGTP analyses were conducted utilizing a BS800M biochemical analyzer (PZ Cormay, Warsaw, Poland) positioned within the Veterinary Centre of WULS. The reagents used for analysis with A-800 GLUCOSE (PZ Cormay, Warsaw, Poland) include enzymatic reagents such as glucose-6-phosphate dehydrogenase and NADP+. For A-800 GGT (PZ Cormay, Warsaw, Poland), gamma-glutamyl substrate, specifically gamma-glutamyl-p-nitroanilide, and a buffer were utilized. In the case of A-800 CREA ENZYMATIC (PZ Cormay, Warsaw, Poland), enzymes including creatinase and a buffer were employed. For A-800 TOTAL PROTEIN (PZ Cormay, Warsaw, Poland), copper was used with the biuret method, along with a buffer to stabilize pH.

2.3. Statistical Analysis

For the statistical analysis, an ANOVA was employed using the least-squares method to compare group means facilitated by the PS IMAGO PRO 7.0 software [41]. Prior to conducting an ANOVA, key assumptions were tested:

1. Normality: The Shapiro–Wilk test was applied to assess whether the data followed a normal distribution. All variables yielded *p*-values greater than 0.05, confirming that normality was not violated.
2. Homogeneity of Variance: Levene's test for homogeneity of variances was conducted, ensuring that variances across groups were equal ($p > 0.05$). This indicates that the data met the assumption of homoscedasticity, essential for a valid ANOVA analysis.
3. Independence of Observations: Data collection procedures ensured that individual measurements were independent, as no repeat measures were taken from the same animals.

Given that all assumptions were satisfied, an ANOVA was deemed appropriate for comparing the differences between groups. Significant differences were identified using the F-statistic and post hoc comparisons were conducted where necessary to explore specific group differences.

These comprehensive checks ensured the validity and reliability of the statistical findings, providing confidence in the reported differences in reproductive parameters, metabolic profiles, and milk composition between the Polish Holstein Friesian (PHF) and PHF × Swedish Red (SRB) crossbred groups.

3. Results

3.1. Basic Composition of Milk

Table 1 presents the influence of cow genotype on the modulation of performance parameters pertaining to milk production as well as milk yield. Milk yield exhibited a significant 14.39% reduction in the PHF × SRB crossbreds relative to the PHF (p-value < 0.001). This divergence suggests nuanced genetic orchestration governing milk yield. Complementing this, the fat percentage subtly ascended by 3.47% in the PHF × SRB crossbreds compared to the PHF group (p-value = 0.045), revealing intricate lipid metabolism under genetic influences. Of paramount significance, the protein percentage escalated remarkably, by 7.83% within the PHF × SRB crossbreds group compared to the PHF (p-value < 0.001), signifying genetic modulation of protein synthesis. Casein content demonstrated a substantial elevation of 4.44% within the PHF × SRB crossbreds versus the PHF group (p-value < 0.001), denoting the genetic sway on specific milk protein fractions. Conversely, the fat-to-protein (F/P) ratio experienced a marginal decline of 2.88% in the PHF × SRB crossbreds relative to the PHF group (p-value = 0.098). Although statistically nonsignificant, this minor modulation underscores the intricate equilibrium between the lipid and protein pathways guided by genetic and environmental inputs (Table 1).

Table 1. Influence of cow genotype on the formation of performance parameters of milk and milk yields.

	PHF (n = 30)		**PHF × SRB (n = 30)**		***p*-Value**
	LSM	**SEM**	**LSM**	**SEM**	
Milk yield [kg]	32.08	0.292	27.44	0.297	<0.001
Fat [%]	3.83	0.046	3.97	0.047	0.045
Protein [%]	3.27	0.016	3.53	0.016	<0.001
Casein [%]	2.77	0.011	2.89	0.011	<0.001
F/P	1.17	0.015	1.14	0.015	0.098

PHF—Polish Holstein Fresian; PHF × SRB—Polish Holstein Fresian × Swedish Red; LSM—least-square means; SEM—standard error of LSM; F/P—fat/protein.

3.2. Reproductive Parameters

Significant differences emerged in the age at the first insemination. The PHF × SRB crossbreds exhibited an average age of 434.9 days, manifesting a substantial 4.32% reduction versus the PHF purebreds at 454.5 days (p-value < 0.001). This marked divergence underscores the genetic influence on the timeline of reproductive maturation. Conversely, the pregnancy index displayed a marginal 2.41% elevation in the PHF × SRB crossbreds compared to the PHF; however, this was insufficient for statistical significance (p-value = 0.465). This nuanced variation hints at the potential role of crossbreeding in reproductive success, although it does not conclusively establish a causal connection. Considering the service period, a pivotal metric of reproductive efficiency, PHF × SRB crossbreds exhibited a slight 4.31% extension (14.0 days) relative to the PHF group (13.4 days), albeit without statistical significance (p-value = 0.656). This marginal alteration suggests that while crossbreeding introduced genetic diversity, it may not substantially influence the temporal facet of reproductive cycles. The gestation length showcased a 0.21% elevation in PHF × SRB crossbreds (280.9 pregnancies per year) compared to the PHF (280.3 pregnancies per year). However, statistical insignificance (p-value = 0.200) implies that the effect of crossbreeding on gestation length remained subdued (Table 2).

Table 2. The influence of cow genotype on the formation of reproductive parameters.

	PHF (n = 30)		PHF × SRB (n = 30)		*p*-Value
	LSM	SEM	LSM	SEM	
AFI [days]	452.5	1.40	434.9	1.42	<0.001
PI [in units]	1.44	0.033	1.48	0.034	0.465
SP [days]	13.4	0.95	14.00	0.96	0.656
GL [days]	280.3	0.32	280.9	0.32	0.200

PHF—Polish Holstein Fresian; PHF × SRB—Polish Holstein Fresian × Swedish Red; LSM—least-square means; SEM—standard error of LSM; AFI—the age of the first insemination; PI—pregnancy index; SP—service period; GL—gestation length.

During the postpartum downtime, both PHF × SRB (<2: 78.5 days, >2: 86.2 days) exhibited reductions versus the PHF (<2: 97.5 days, >2: 87.7 days) (p-value $\leq$ 0.001). This reduction underscores genetic influences on postpartum recovery, likely attributable to intricate genetic interactions shaping recovery rates. The pregnancy index, a pivotal determinant of reproductive efficiency, had PHF × SRB (<2: 2.27, >2: 1.54) demonstrating significantly lower indices relative to the PHF counterparts (<2: 2.09, >2: 2.43) (p-value $\leq$ 0.001), indicative of the intricate genetics governing reproductive prowess. The service period, a critical parameter of reproductive efficiency, exhibited similarity within age categories across both genetic groups (<2: 59.8 days, >2: 62.9 days for PHF; <2: 37.1 days, >2: 28.9 days for PHF × SRB) (p-value = 0.224), hinting at comparable temporal dynamics irrespective of genetic backgrounds. Likewise, the inter-pregnancy period, a hallmark of reproductive intervals, remained akin across age categories and genetic groups (<2: 157.3 days, >2: 150.6 days for PHF; <2: 115. 5 days, >2: 115.1 days for PHF × SRB) (p-value = 0.556), suggesting resilient temporal dynamics under diverse genetic contexts. Calving frequency, encapsulated within the period between calving, echoed consistency across all groups (p-value = 0.878), implying minimal influence of age categories or genetic backgrounds. Notably, the pregnancy period, mirroring gestational duration, had a significant reduction within crossbred animals (PHF × SRB) (<2: 283.3 days, >2: 278.2 days) versus their PHF counterparts (<2: 280.5 days, >2: 280.0 days) (p-value $\leq$ 0.001). This discrepancy implies that crossbreeding potentially alters gestational length through intricate genetic interplay, heralding new implications for herd management strategies (Table 3).

Table 3. Influence of cow genotype and age of first calving on the formation of reproductive parameters.

AFC	PHF				PHF × SRB				*p*-Value
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	
	<2 (n = 15)		>2 (n = 15)		<2 (n = 15)		>2 (n = 15)		
AFC [days]	708.3	1.67	787.1	1.48	705.0	1.67	754.4	1.54	<0.001
PPD [days]	97.5	2.23	87.7	1.97	78.5	2.23	86.2	2.05	<0.001
PI [in units]	2.09	0.082	2.43	0.073	2.27	0.082	1.54	0.076	<0.001
SP [days]	59.8	4.87	62.9	4.31	37.1	4.87	28.9	4.48	0.224
IP [days]	157.3	5.49	150.6	4.87	115.5	5.49	115.1	5.05	0.556
PBC [days]	437.7	5.38	430.6	4.77	398.8	5.38	393.3	4.95	0.878
GL [days]	280.5	0.47	280.0	0.41	283.3	0.47	278.2	0.43	<0.001

PHF—Polish Holstein Fresian; PHF × SRB—Polish Holstein Fresian × Swedish Red; LSM—least-square means; SEM—standard error of LSM, AFC—the age of the first calving; PPD—period of postpartum downtime; PI—pregnancy index; SP—service period; IP—inter-pregnancy period; PBC—the period between calving; GL—gestation length.

3.3. Metabolic Profile

Table 4 reports the influence of cow genotype and age first calving on the selected metabolic profile parameters. The NEFA is an important metabolite formed during the mobilization of adipose tissue for energy used to diagnose the occurrence of metabolic disorders. The examination of NEFA levels in PHF groups and PHF × SRB hybrids highlighted their clear differences. The interbreed hybrids (PHF × SRB) showed significantly

lower NEFA levels compared to the corresponding PHF age groups. The NEFA value is approximately 70% higher in PHF cows than in hybrids.

Table 4. The influence of cow genotype and age of first calving on the selected metabolic profile parameters.

Age	PHF				PHF × SRB				*p*-Value
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	
	<2 (n = 15)		>2 (n = 15)		<2 (n = 15)		>2 (n = 15)		
NEFA [mmol/L]	0.405	0.042	0.420	0.104	0.281	0.042	0.282	0.038	<0.001
BHBA [mmol/L]	0.725	0.038	1.111	0.034	0.695	0.038	0.711	0.035	<0.001
Glucose [mg/dL]	65.234	0.817	61.812	0.724	64.061	0.817	60.559	0.751	<0.001
Protein [g/L]	70.488	1.204	66.640	1.067	66.725	1.204	64.690	1.107	0.016
Albumins [g/L]	35.999	0.583	35.356	0.517	38.453	0.583	40.306	0.537	0.008
Creatynine [mg/dL]	0.970	0.022	0.939	0.019	1.016	0.022	1.198	0.020	<0.001
GGTP [U/L]	19.572	2.652	24.506	1.215	16.427	2.652	15.853	1.638	0.015

PHF—Polish Holstein Fresian; PHF × SRB—Polish Holstein Fresian × Swedish Red; LSM—least-square means; SEM—standard error of LSM; NEFA—non-esterified fatty acids; BHBA—β-hydroxybutyric acid; GGTP—gamma-glutamyl transferase.

BHBA is the most important indicator used to diagnose ketosis to study BHBA levels in the PHF and PHF × SRB hybrid groups. The hybrids showed lower BHBA levels in each group, while PHF cows that calved at over 2 years of age had very high BHBA levels, which may indicate a higher possibility of ketosis. Glucose, a vital metabolite reflecting metabolic equilibrium and cattle well-being, revealed breed-specific differences across distinct cattle groups. An examination of glucose levels within PHF and PHF × SRB groups highlighted notable distinctions. Crossbreds (PHF × SRB) consistently manifested significantly lower glucose levels compared to both age categories of PHF. The observed percentage differences of −1.81% for cattle with an age of first calving <2 years and a more pronounced −6.99% for those with an age of first calving >2 years accentuated the extent of these differences. The statistical significance of these findings ($p < 0.001$) underscored the role of genetic interactions and breed-specific factors in modulating metabolic processes, significantly influencing glucose homeostasis within the assessed cattle groups.

On transitioning to protein levels, pivotal indicators of physiological status, a comparison between the PHF and PHF × SRB groups revealed significant differences. Crossbreds cattle (PHF × SRB) consistently exhibited markedly lower protein levels across both age categories. The calculated percentage differences of −5.65% for cattle with an age of first calving <2 years and −2.97% for those with an age of first calving >2 years underscored the extent of these differences and the statistical significance ($p = 0.016$) highlighted the possible effects of genetic and breed-specific elements on protein metabolism, with potential implications for nutrient utilization within the studied cattle groups.

Albumins, crucial plasma proteins governing osmotic balance and molecular transport, revealed potential differences in nutrient utilization and overall health dynamics upon comparing the distinct cattle groups. A detailed analysis of albumin levels underscored significant differences between the PHF and PHF × SRB groups. Crossbreds cattle (PHF × SRB) consistently exhibited significantly higher albumin levels in both age categories than PHF cattle. The observed percentage differences of +6.42% for cattle with an age of first calving <2 years and a notable +13.75% for those with an age of first calving >2 years accentuated the potential influence of genetic and breed-specific components on nutritional metabolism, thereby affecting albumin dynamics and potential health implications within the studied cattle groups.

The analysis of creatinine levels, again, revealed notable differences between the PHF and PHF × SRB groups. Crossbreds cattle (PHF × SRB) consistently presented significantly higher creatinine levels within both age categories compared to PHF cattle. The observed percentage differences of +4.23% for cattle with an age of first calving <2 years and a notable +27.93% for those with an age of first calving >2 years underscored the potential influence of genetic and breed-specific factors on renal dynamics. The statistical significance of these

differences ($p < 0.001$) pointed to the potential impact on creatinine levels and broader health implications within the studied cattle groups.

Gamma-glutamyl transferase (GGTP), a critical hepatic and biliary enzyme associated with liver health, exhibited significant differences within the PHF and PHF × SRB groups. Remarkably, crossbreds cattle (PHF × SRB) consistently displayed significantly lower GGTP levels in both age categories than PHF cattle. The observed percentage differences of −20.94% for cattle with an age of first calving <2 years and an even more substantial −35.47% for those with an age of first calving >2 years emphasized the significant nature of these differences. The statistical significance of these findings ($p = 0.015$) highlights the potential influence of genetic and breed-specific factors on hepatic function, potentially impacting GGTP levels and broader health implications within the studied cattle groups.

3.4. Fatty Acid Profile

Table 5 reports the influence of cow genotype and the age of first calving on the formation of selected FAs. PHF cattle calving at less than 2 years exhibited higher levels of C6:0 (1.868 g/100 g) in comparison to both the PHF × SRB groups (1.548 g/100 g for <2 and 1.600 g/100 g for >2). This difference was statistically significant ($p = 0.001$). Moving on to C10:0, there was a significant difference in levels between the PHF and PHF × SRB groups calving at 2 years or more, where the latter group had higher levels (2.745 g/100 g vs. 2.167 g/100 g), with a significant *p*-value of 0.024. A similar trend was observed in the case of C12:0, with PHF × SRB cattle calving at 2 years or more displaying higher levels (3.058 g/100 g) compared to PHF cattle (2.800 g/100 g), and this distinction was statistically significant ($p = 0.001$). Notably, while displaying higher levels in PHF × SRB cattle calving at 2 years or more (32.148 g/100 g) compared to PHF cattle (32.195 g/100 g), C16:0 did not exhibit statistical significance ($p = 0.107$), suggesting a more subtle difference. Moving to C18:0, no significant differences were observed between the PHF and PHF × SRB groups for either age category, with $p = 0.587$. However, C20:0 showed a substantial distinction, especially in cattle calving at less than 2 years. The PHF × SRB cattle in this category displayed significantly lower levels (0.072 g/100 g) compared to PHF cattle (0.243 g/100 g), as indicated by a *p*-value of <0.001. Two distinct conjugated linoleic acid isomers, CLA c9, tr11, and CLA tr10, c12, also exhibited significant differences. Both of these isomers were lower in the PHF × SRB groups compared to PHF cattle, irrespective of the age category, with *p*-values of <0.001 and 0.059, respectively. Lastly, C22:0, a metabolite with a relatively low presence, was significantly different between the groups, particularly in cattle calving at 2 years or more ($p = 0.006$).

Table 5. The influence of cow genotype and age of first calving on the formation of selected fatty acids.

Age	PHF				PHF × SRB				*p*-Value
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	
	<2 (n = 15)		>2 (n = 15)		<2 (n = 15)		>2 (n = 15)		
C6:0 [g/100g fat]	1.868	0.045	1.457	0.040	1.548	0.045	1.600	0.042	< 0.001
C10:0 [g/100g fat]	1.983	0.087	2.167	0.077	2.929	0.087	2.745	0.080	0.024
C12:0 [g/100g fat]	2.247	0.090	2.800	0.080	2.909	0.090	3.058	0.083	0.001
C16:0 [g/100g fat]	27.655	0.388	32.195	0.344	29.238	0.388	32.148	0.357	0.107
C18:0 [g/100g fat]	13.102	0.231	11.983	0.205	10.236	0.231	9.802	0.213	0.587
C20:0 [g/100g fat]	0.243	0.010	0.153	0.009	0.072	0.010	0.103	0.009	< 0.001
CLA9 [g/100g fat]	0.480	0.012	0.437	0.011	0.518	0.012	0.469	0.011	< 0.001
CLA10 [g/100g fat]	0.038	0.003	0.030	0.003	0.034	0.03	0.018	0.003	0.059
C22:0 [g/100g fat]	0.011	0.006	0.063	0.005	0.020	0.006	0.048	0.005	0.006

PHF—Polish Holstein Fresian; PHF × SRB—Polish Holstein Fresian × Swedish Red; LSM—least-square means; SEM—standard error of LSM; CLA9—CLA *cis-9*, *trans 11*; CLA10—CLA *trans-10*, *cis-12*.

4. Discussion

Adopting crossbreeding strategies in livestock production has sparked analytical interest due to the perceived advantages of crossbred animals over their parental breeds. Notably, research by Mäki-Tanila [42] has highlighted the improved robustness and economic efficiency associated with crossbreeding. This notion is further reinforced by the endorsements of Hansen [43] and Kalm [44], adding weight to the viability of crossbreeding as a pragmatic approach in livestock management. In Poland, the crossbreeding of dairy cattle has been quite popular for several years, and hybrids account for about 7% of the active population of animals [45].

4.1. Basic Composition of Milk

Milk production and the basic composition of milk are important aspects of cattle breeding, which, for many years, has been the primary breeding objective affecting the profit of dairy farming. Interesting information was provided by a study by Heins et al. [46] in which it was reported that HF cows have the highest productivity, followed by Holstein Friesian × Scandinavian Red (HF × SR) hybrids, then Holstein Friesian × Montbeliarde (HF × MO) hybrids, and the lowest in Holstein Friesian × Normande (HF × NO) hybrids. Most of the available studies provide information on milk yield for a full 305-day lactation, with the majority showing that the milk yield of HF × SR hybrids is lower than that of purebred HF cows [32,47–50], while Heins et al. [48] and Hazel et al. [51], in their studies, indicated a higher lifetime yield for HF × SR hybrids than purebred HF cows. Curiously enough, Benak et al. [52] confirmed this in the context of specific hybrids from the SR group of breeds, which are HF × NRF hybrids, and similar observations were made by Pytlewski et al. [53], whereas Ezra et al. [54] indicated that Holstein Friesian × Norwegian Red (HF × NRF) hybrids also have lower lactation performance. The confirmation of milk yield can be found in daily milk production. Our study showed a 14.39% reduction in milk yield within PHF × SRB crossbreds compared to PHF, indicating the genetic modulation of milk yield in HF and SRB breeds [55]. Similar lower milk yields with HF × SR were confirmed by Malchiodi et al. [56], Piccardi et al. [57], Saha et al. [58], Solarczyk et al. [59], Saha et al. [60], and Piazza et al. [61] in their studies. Interestingly, as in the case of higher productivity, higher daily milk production is observed in HF × NRF hybrids than in purebred HF cows, as indicated not only by the previously cited items but also in the study by Puppel et al. [34]. In the same study, the results of Holstein Friesian × Danish Red (HF × RDM) hybrids were also analyzed, where their productivity was lower than that of purebred HF cows. This is quite an interesting observation considering that in the improvement of the SR breed group, all the breeds included the Danish Red, Finnish Ayrshire, Norwegian Red, and Swedish Red breeds [62].

The study by Heins et al. [46] provided valuable insights into the variations in fat plus protein production among crossbred cows, showcasing distinct trends across different crossbred combinations. The HO × NO hybrids exhibited a significant 8.6% reduction in fat plus protein production compared to pure Holsteins, while the HF × MO crossbreds displayed a 3.8% decrease. In contrast, the HF × SR crossbreds showed a minor 2.2% reduction without statistical significance. Similarly, a 3.47% increase in fat percentage within PHF × SRB crossbreds signifies genetic nuances in lipid metabolism. Importantly, a remarkable 7.83% elevation in protein percentage within PHF × SRB crossbreds highlights genetic control over protein synthesis pathways, and a 4.44% rise in casein content (p-value ≤ 0.001) underscores genetic influence on specific milk proteins. The higher production of fat and protein in the milk of hybrids is confirmed by the available studies, which is certainly related to genetic determinants and lower milk production, which has a lower milk dilution effect [32,34,49,58,59,63]. Interesting results were obtained by Benak et al. [52], where the content of both fat and protein in the milk of HF × NRF hybrids was higher despite higher daily production than for the HF breed, while in the case of the results of Pytlewski et al. [53], the hybrids had a lower fat and protein content, which may confirm the dilution effect of the milk components. The higher casein content in the milk of

HF × SR hybrids was similarly confirmed in our own study by Maurmayr et al. [64] and Puppel et al. [34].

4.2. Reproductive Parameters

The emphasis on increased production in cows frequently aligns with the detrimental effects on their health, fertility, and lifespan. Extensive investigation into the complex interplay of genetics governing production and functional traits like fertility and vulnerability to health concerns has resulted in diverse outcomes and incongruities across studies and reviews [65,66]. This inherent trade-off highlights the intricate complexities of simultaneously enhancing a range of traits in cattle breeding endeavors. Significantly, dairy cows with lower yields demonstrate a distinct advantage over their high-yielding counterparts, particularly concerning disease resistance. This distinction is particularly evident in aspects like udder health, fertility, longevity, and metabolic disorders. By comprehending these genetic antagonisms and trade-offs stemming from the selection for intense production, we acquired critical insights into the multifaceted task of enhancing livestock performance across diverse agricultural contexts [66,67]. These insights hold implications for refining breeding methodologies and management strategies with the aim of bolstering the sustainability and well-being of dairy cattle populations [66,68,69]. A significant reduction of 4.32% in the age of first insemination was demonstrated in PHF × SRB crossbreds compared to PHF purebreds. Similar values were obtained by Malchiodi et al. [70] in their studies with HF and HR × SR hybrids. Underlining the genetic influence on reproductive maturation timelines, according to Hutchison et al. [64], is a positive response to breeding programs and herd management because it increases profits. Due to the achievement of early sexual maturity and similar efficiency of the insemination procedure in PHF × SRB hybrids with purebred PHF heifers, the age at first calving (AFC) in the hybrids was also lower, at 729.9 days against 747.7 for the PHF breed. Taylor et al. [71] indicated that cows calving at <2 years of age exhibit longer lives and higher lifetime production. As indicated by Hutchison et al. [72], an earlier age of first insemination and consequently earlier AFC is related to better growth and fertility characteristics. As Berglund [73] points out, a huge influence on fertility is linked to breed and breeding goals. A decrease in the value of reproductive traits is mainly seen in the HF breed, even in animals from Scandinavia, where reproductive traits have been on the breeding program for 50 years, while in the Scandinavian Red breed group, the value of reproductive traits has remained constant for many years. Better fertility and a shorter parturition interval in purebred SRB cows compared to HF cows were also shown by Buckley et al. [74], Clasen et al. [75], Andree O'Hara et al. [55], and Bieber et al. [76] in their studies, confirming the higher value of reproductive traits in the SRB breed than the HF breed. During postpartum downtime, both age categories of PHF × SRB exhibited reductions in comparison to PHF, illustrating genetic influences on postpartum recovery. Similar results were obtained by Pipino et al. [77]. This underscores the intricate genetic and physiological interactions shaping recovery rates. As Hazel et al. [78] point out, metabolic disorders are diagnosed less frequently in HF × SR hybrids, and the costs related to treating these animals are lower than those related to treating HF cows. The pregnancy index, a vital determinant of reproductive efficiency, was significantly lower in both age categories of PHF × SRB compared to their PHF counterparts. Similar observations were obtained by Malchiodi et al. [70], Hazel et al. [51], and Pipino et al. [77]. Remarkably, the pregnancy period, reflecting gestational duration, exhibited a significant reduction within crossbred animals (PHF × SRB) compared to their PHF counterparts. This suggests that crossbreeding potentially alters gestational length through intricate genetic interactions, which has implications for herd management strategies. According to Pereira et al. [79], GL should be determined by the breed of the calf. In the case of this experiment, primiparous PHF × SRB hybrids were covered with MO breed bull semen, and the pregnancy of females calved for the first time at less than two years of age lasted 283.3 days; this was consistent with previous reports on the effect of the MO breed on gestation length [49,79].

4.3. Metabolic Profile

Differences in metabolic parameters between purebred HF cows and their hybrids with SR primarily arise from distinct metabolic pathways influencing energy mobilization and fat metabolism during lactation. The process of lipid mobilization in dairy cows is critical during both the transition period and early lactation. In purebred HF cows, rapid fat mobilization can lead to increased levels of NEFA and BHBA, indicative of a higher risk of ketosis [80]. In contrast, SRB hybrids may exhibit more regulated lipid mobilization, potentially due to genetic differences that enhance FA oxidation. This may involve more active pathways mediated by peroxisome proliferator-activated receptors (PPARs), resulting in lower NEFA and BHBA concentrations during early lactation [81]. Our research confirms that there is a greater mobilization of fats at the beginning of lactation in PHF cows than in PHF × SRB hybrids, as indicated by higher levels of ketone bodies in PHF cows. The BHBA levels in calving cows over 2 years of age were particularly worrying: BHBA PHF <2; 0.725, >2; 1.111 to PHF × SRB <2; 0.695, >2; 0.711 mmol/L. According to Hazel et al. [78], HF × SR hybrids do not show metabolic problems as often as HF cows, which is related to the SR breed's influence on these animals' metabolism. Differences in metabolism between HF and SRB cows were proven by Ntallaris et al. [82], where a lower NEFA value was shown in SRB cows than in HF cows. The metabolism of body fat is also influenced by the amount of fat stored, according to Ospina et al. [83]. Cows that enter the reproductive period later tend to accumulate more fat, which may, at the same time, result in a greater mobilization of spare matter in the postpartum period.

Glucose metabolism is responsible for the formation of energy and hormones; being highly dependent on insulin is another key factor differentiating these breeds. Insulin sensitivity may vary between HF and SRB cows, with SRB cows likely exhibiting improved insulin responsiveness. This enhanced sensitivity may facilitate greater glucose uptake and utilization during lactation. The process of gluconeogenesis, particularly from propionate derived from fiber digestion, may be more efficient in SRB cows, leading to elevated blood glucose levels compared to HF cows. The regulation of gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase could be more effective in SRB hybrids, promoting stable glucose concentrations. In our own research, low glucose levels were observed in cows calving at >2 years of age in both PHF breeds and PHF × SRB hybrids; however, as reported by Mohammed et al. [84], the accepted values are at an appropriate level. According to Ntallaris et al. [82], blood glucose content in HF cows is lower than in SRB cows, which could account for the influence of breed on this parameter; however, based on our research, we can conclude that fat mobilization and the formation of ketone bodies affect gluconeogenesis quite strongly, during which glucose is formed [80]. The higher blood glucose content in cows calving at less than 2 years of age may also indicate a lower demand for energy due to a smaller body frame [85].

Additionally, variations in blood protein levels, specifically total protein and albumin, may indicate differences in immune responses and inflammation. Elevated protein levels in HF cows often correlate with inflammatory states during early lactation. This inflammation is mediated by pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), influencing protein metabolism. In contrast, SRB hybrids may demonstrate a more robust immune response, potentially due to genetic traits that enhance immune function and reduce inflammatory responses [86].

Creatinine levels, as a marker of muscle metabolism, may also differ between these breeds [87]. In HF cows, the increased mobilization of protein reserves to meet energy demands may lead to lower creatinine concentrations, reflecting greater protein catabolism. Conversely, SRB hybrids may maintain muscle mass more effectively, resulting in higher creatinine levels.

Liver enzyme activity such as gamma-glutamyltransferase (GGTP) is indicative of hepatic health and metabolic stress. HF cows often exhibit elevated GGTP levels, particularly those calving at older ages, which may signify liver stress and fat accumulation. Excessive fat mobilization during early lactation can overload the liver's capacity for fat oxidation,

resulting in fatty liver syndrome and increased GGTP [8,88]. In contrast, SRB hybrids may demonstrate superior hepatic function and lipid metabolism, potentially linked to an enhanced expression of genes involved in fatty acid oxidation and liver regeneration. In conclusion, the observed differences between purebred HF cows and SRB hybrids stem from a complex interplay of genetic, biochemical, and physiological factors influencing lipid and glucose metabolism, protein catabolism, immune function, and hepatic health.

4.4. Fatty Acid Profile

The observed differences in milk FA profiles between PHF cows and PHF × SRB hybrids can be understood through the complex interaction of genetic, metabolic, and physiological factors that influence lipid metabolism. These differences can be attributed to the distinct metabolic pathways involved in de novo FA synthesis in the mammary gland and the mobilization of FAs from adipose tissue, alongside the regulation of these processes by genetic and metabolic factors such as breed-specific characteristics, metabolic health (e.g., ketosis), and the stage of lactation [89].

Breed-related genetic differences, particularly between PHF and SRB cows, contribute significantly to the variation in milk FA composition. Genetic selection in dairy breeds like HF has historically focused on high milk yield, which can indirectly influence lipid metabolism. In contrast, breeds like SRB are typically selected for their robustness, including greater resistance to metabolic disorders. This divergence in breeding objectives leads to differences in the regulation of key enzymes involved in FA synthesis pathways such as acetyl-CoA carboxylase (ACC) and FA synthase (FAS), which are essential for the de novo synthesis of short- and medium-chain FAs in the mammary gland [90].

The de novo synthesis of FAs (up to C16:0) occurs primarily in the mammary gland from precursors such as acetate and BHBA, which are produced during rumen fermentation. The differences in the levels of caproic acid, capric acid, and lauric acid in our study could reflect breed-specific differences in the activity of these metabolic pathways. For example, Poulsen et al. [91] demonstrated that SRB cows tend to have lower levels of certain medium-chain FAs compared to Holstein Friesians. This may be linked to lower the activity of enzymes like FAS in SRB cows, resulting in a reduced synthesis of C6:0 and C10:0, as observed in our study. Conversely, higher levels of de novo FAs in PHF cows can be associated with greater FAS and ACC activity, possibly due to genetic selection for high milk yield.

The metabolic state of the cow, particularly during early lactation when cows are in a negative energy balance, significantly impacts the mobilization of FAs from adipose tissue. In this phase, cows often exhibit elevated levels of NEFAs in the bloodstream due to the mobilization of stored triglycerides, which are then transported to the liver for β-oxidation or directed to the mammary gland for incorporation into milk fat. The mobilization of long-chain FAs such as stearic acid and arachidic acid primarily originates from adipose tissue rather than de novo synthesis [6].

In cows experiencing a negative energy balance or metabolic stress such as ketosis, the excessive mobilization of adipose reserves leads to increased concentrations of long-chain FAs in milk. This can explain the higher levels of C18:0 and C20:4 in PHF cows, which are often more prone to metabolic disorders like ketosis due to their high milk production demands. As suggested by Puppel et al. [6], higher concentrations of BHBA in cows with subclinical ketosis are associated with an altered FA metabolism, potentially leading to increased levels of mobilized FAs, including C18:0. Our findings that PHF cows had higher levels of these long-chain FAs support this notion, indicating that HF may be more susceptible to mobilizing body fat during early lactation.

Metabolic disorders such as ketosis significantly affect lipid metabolism and consequently the FA composition of milk. In cases of ketosis, elevated levels of BHBA, a product of incomplete FA oxidation in the liver, indicate an impaired energy metabolism. During ketosis, cows are unable to meet their energy requirements through dietary intake alone, resulting in the excessive breakdown of adipose tissue triglycerides and increased NEFA

levels in circulation. These NEFAs are then incorporated into milk fat, altering the fatty acid profile, and increasing the levels of C18:0 and other long-chain FAs [2] in particular.

Additionally, ketosis is associated with shifts in the synthesis of conjugated linoleic acid isomers in milk. The CLA is primarily formed in the rumen through the biohydrogenation of linoleic acid, and its synthesis is modulated by rumen microbial activity and the metabolic state of the cow. CLA9 and CLA10, two bioactive isomers, have been identified as potential biomarkers for metabolic health [5]. In our study, the higher levels of CLA9 in PHF × SRB hybrids calving before 2 years of age and lower levels of CLA10 in hybrids calving after 2 years suggest that these isomers may be reflective of the metabolic status and possibly the incidence of ketosis. As noted by Puppel et al. [6], higher CLA concentrations, particularly CLA10, have been associated with cows in better metabolic health, while lower levels may indicate subclinical metabolic disturbances.

The variation in palmitic acid levels observed in our study, with higher levels in cows calving after 2 years of age, reflects this shift. Palmitic acid, synthesized both de novo and through mobilized lipids, is one of the most abundant FAs in milk. As lactation advances, healthy cows typically exhibit higher C16:0 concentrations, as noted by Puppel et al. [6], which is indicative of improved metabolic stability and efficient fat synthesis. The higher levels of C16:0 in older cows in our study may reflect better energy management and metabolic health in these animals, as younger cows, particularly those calving before 2 years of age, often face greater metabolic stress during lactation.

The differences in milk FA composition between PHF cows and PHF × SRB hybrids arise from the interplay of breed-specific genetic factors, metabolic health, and lactation dynamics. These factors influence key metabolic pathways, including the de novo synthesis of FAs in the mammary gland and the mobilization of adipose-derived FAs. Genetic differences between PHF and SRB cows affect the activity of enzymes such as ACC and FAS, leading to variations in medium-chain FAs, while metabolic disorders like ketosis alter lipid mobilization, increasing the concentration of long-chain FAs. The stage of lactation further modulates these processes, with de novo synthesis predominating as cows return to a positive energy balance. These findings underscore the importance of understanding the metabolic and genetic factors influencing milk composition, as they have implications for both dairy production efficiency and animal health management.

5. Conclusions

In summary, the crossbred PHF × SRB cows demonstrated notable advantages in milk composition, reproductive efficiency, and postpartum recovery time. While PHF cows exhibited a higher total milk yield compared to PHF × SRB hybrids, the latter group displayed enhanced levels of fat, protein, and casein in their milk, which may better align with specific market demands and processing requirements. The reproductive performance of the PHF × SRB hybrids was characterized by earlier sexual maturation compared to purebred PHF cows, indicating an improvement in reproductive efficiency. Although the pregnancy index was marginally elevated in PHF × SRB hybrids, the service period for PHF cows was longer; however, this extension did not reach statistical significance.

In addition to reproductive metrics, the metabolic profiles of the two breeds provided further insight into their physiological adaptations. Distinct differences in metabolic parameters—such as glucose, protein, albumin, creatinine, and GGTP levels—were observed, reflecting the unique physiological dynamics of each breed. For instance, crossbreds exhibited consistently lower glucose and protein levels compared to PHF cows, which may indicate different energy mobilization and metabolic efficiencies. The lower glucose levels in PHF × SRB hybrids could suggest a more efficient utilization of available energy, particularly in younger cows, while variations in albumin and creatinine levels may reflect differing health statuses and muscle condition between the breeds.

An analysis of the age at first calving, categorized into those calving at less than 2 years and those at 2 years or older, revealed that PHF × SRB hybrids experienced a significant reduction in milk yield relative to PHF across both age categories. Nevertheless, there was a

marked increase in fat, protein, and casein content in the milk of the crossbreds, indicating a genetic influence on these components. The genotypic impact was further highlighted by the timing of first insemination, with PHF × SRB hybrids reaching sexual maturity earlier, particularly among those calving before 2 years of age. Although the pregnancy index showed a slight increase in crossbreds, the service period was also extended, although this change was not statistically significant in either age group.

The division into age categories facilitated a comprehensive understanding of how genotypic differences affect first-calving age, milk production, reproductive metrics, and metabolic traits in both PHF and PHF × SRB cattle. These findings underscore the necessity of considering age-specific effects when evaluating the performance and health of dairy cattle with diverse genetic backgrounds. Ultimately, the choice between PHF and PHF × SRB should be guided by the specific objectives and priorities of the cattle farming operation. A careful assessment of factors such as overall milk yield, market demands, reproductive management strategies, and health considerations is essential to determine the most suitable breed for a given agricultural context.

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Mój indywidualny udział w jej powstaniu polegał na: opracowaniu koncepcji badań i założeń metodycznych, kolekcjonowaniu materiału biologicznego, analizach laboratoryjnych, redagowaniu manuskryptu.

Indywidualny wkład pracy w publikację wynosi: 60%

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Effect of Breed on the Level of the Nutritional and Health-Promoting Quality of Semimembranosus Muscle in Purebred and Crossbred Bulls

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Simple Summary: Beef is an important natural source of nutrients as well as of bioactive ingredients that improve our health. It is an excellent source of alanine, creatine, carnosine, anserine, and polyunsaturated fatty acid (PUFA), which plays an important preventative role in carcinogenic processes. The strategy for rearing and feeding cattle for slaughter should be directed at reducing saturated fatty acid (SFA) in beef fat and/or increasing PUFA, especially n-3. Therefore, the aim of the study was to determine the influence of breed types on the nutritional and pro-health quality of beef. The experiment was conducted in Poland, on 62 bulls from three breeds: Limousin, Polish Holstein-Friesian, and Polish Holstein-Friesian × Limousin. Bulls were slaughtered at 21–23 months of age, and samples of semimembranosus muscle (300 g) were cut parallel to the muscle axis at 24 h postmortem. It can be concluded that commodity crossbreeding significantly improved the quality of beef, resulting in similar or even better results than purebred cattle.

Abstract: Meat from commercial breed cattle are very often used to crossbreed with dairy breeds. The effect of heterosis is most evident when crossbreeds are genetically different from each other. Therefore, the aim of the study was to determine the influence of breed types on the nutritional and pro-health quality of beef. The experiment was conducted on 62 bulls from three breeds: Limousin, Polish Holstein-Friesian, and Polish Holstein-Friesian (PHF) × Limousin. During the fattening period, the animals were fed ad libitum using the same diet. Bulls were slaughtered at 21–23 months of age. The meat of PHF × Limousin hybrids was characterized by the lowest level of SFA and the highest content of n-3 PUFA fatty acids, carnosine, and α-tocopherol compared to the values obtained for the Polish Holstein-Friesian and Limousin breeds. In the case of PHF × Limousin hybrids, there was a 6% increase in n-3 PUFA, 21% in carnosine, and 66% in α-tocopherol compared to the Polish Holstein-Friesian breed. Commodity crossbreeding significantly improved the quality of beef analyzed in this study, resulting in similar or even better results than purebred cattle. This meant that beef from the hybrids with PHF was of the best nutritional and health-promoting quality.

Keywords: breed; meat; quality; crossbreeding

1. Introduction

Breed, gender, and the feeding system are factors that determine, to a large extent, the level of bioactive muscle tissue components, because their contribution is closely correlated with the degree of fat cover, and thus is a consequence of diet and genotype [1,2]. Differences between many breeds of cattle have been reported for Red Angus and Simmental steers [3], Aberdeen Angus, Belgian Blue,

and Limousine bulls [4], and for different double muscle genotype bulls [1]. Various feeding strategies are often used to increase the content of polyunsaturated fatty acid (PUFA) n-3 fatty acid and to improve beef intramuscular PUFA n-6/PUFA n-3 ratio [5–9]. Beef is an important natural source of alanine, creatine, carnosine, anserine, and polyunsaturated fatty acid, which plays an important preventative role in carcinogenic processes [3,4]. It should be noted that both carnosine and anserine block the production of advanced end-products of glycation, which lead to diseases such as arteriosclerosis, diabetes, cataracts, and Alzheimer's disease [10,11]. Another important function of these compounds is the formation of complexes with the ions of certain metals, supporting biological activity, which can be exemplified by the inhibition of growth and development of *Helicobacter pylori* [12]. Beef is a source of vitamins soluble in fat. Vitamin A takes part in the processes of growth; it mainly influences the differentiation of the epithelial cells of the oral mucosa, digestive, urinary, and respiratory tracts, and sight organs. Vitamin A catalyzes the oxidation of unsaturated fatty acids, and its presence is essential for the biosynthesis of fat from sugars or from fatty acids and glycerol [13]. The effect of vitamin E is mainly due to its antioxidant properties, thanks to which it protects the body against degenerative diseases [14]. A periodic increase in demand for tocopherols is observed during increased exposure to heavy metals, exposure to free radicals, with a lower supply of other antioxidants, increased physical activity, and in old age [13,15]. Vitamin E prevents the rapid dissipation of ion gradients within muscle reducing rates of tenderization [16,17]. Additionally, Rowe et al. [18] concluded that the use of antioxidants in meat could improve tenderness. Omega-3 and omega-6 fatty acids evoke antibacterial, antiviral, antifungal, antioxidant, and antiparasitic effects [19]. Das [20] reported that the ability of eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) to suppress the production of pro-inflammatory cytokines and induce their anti-inflammatory effects results indirectly from their ability to increase mRNA Peroxisome proliferator-activated receptor gamma and protein activity. Additionally, Cheah et al. [21] reported that diets rich in monounsaturated fatty acids (MUFAs) have been shown to have favorable anti-inflammatory and cardiovascular benefits.

Beef is characterized by a moderate and, unlike protein, quite varied fat content, representing 0.7–10% of tissue mass. The composition of intramuscular fat varies from breed to breed, and the proportions between the different types of fiber are different. Late maturing breeds (Belgian Blue, Limousine, and Blonde d'Aquitaine) are characterized by better musculature and lower fat content compared to the parameters achieved by early maturing breeds, e.g., Angus [22]. In addition, Chambaz et al. [23] reported that the Angus breed showed a growth rate similar to Simmental and Charolais while Limousin grew slower, became oldest, and provided the heaviest carcasses and best conformation. The feeding system also influences the fat content of the carcass. The highest content is obtained from intensively fattened animals and the lowest from the extensive fattening method [24,25].

One way to improve low-hereditary functional characteristics is by crossbreeding [26]. This is why there has been a growing interest in this method of animal refinement for several years [27,28]. This method has been recognized as possibly causing heterosis, which is conditioned by the presence of a favorable gene combination. The effect of heterosis is most evident when crossbreeds are genetically different from each other. According to Hansen et al. [28], the effect of heterosis on production traits may amount to as much as 6.5%, while for fertility, health, and survival it may amount to 10%. Meat from commercial breed cattle are very often used to crossbreed with dairy breeds. Carcasses of such hybrids should have a higher proportion of meat and a lower fat content. The beef from these animals is more tender but less marbled than purebred beef cattle. The most commonly used bulls for mating are from breeds such as Limousin, Charolais, Simmental, and Piedmontese, and Hereford, Angus, and Salers are rarely used for this purpose [29]. The offspring of the Limousin and Piedmontese breeds grow rapidly and are well-muscled, and their meat is low in fat and of high quality [30,31].

According to Statistics Poland [32] data, approximately 93% of the cattle population in Poland are dairy cows, and only 1% are cattle of beef breeds. The most popular meat breed in Poland is the Limousin, which is approximately 70% of the population of purebred cows, followed by Charolaise and Hereford. The main criterion for choosing the method of creating a beef herd is economic considerations.

Many farmers who decide to create a breeding herd in a short period of time face the problem of high costs associated with the purchase of breeding material. It is possible to reduce such high expenditures by using other methods which, however, involve many years of work. One solution is to create a herd by means of crossbreeding. It is a long-term process, allowing for the gradual elimination of undesired genes and obtaining animals of as similar phenotype and genotype as possible to the paternal breed. Genetic variability in beef quality has been linked to differences between lines or breeds, variations due to the crossing of breeds, and variations between animals [2]. The research hypothesis assumes that commodity crossbreeding Polish Holstein-Friesian (PHF) × Limousin will significantly improve the quality of beef, resulting in similar or even better results than purebred PHF cattle. What is important is that beef production in Poland is mostly related to this dairy breed. Therefore, the aim of the study was to determine the influence of breed (purebred vs. crossbred cattle) on the nutritional and pro-health quality of beef.

2. Material and Methods

The experiment was conducted on 62 bulls from three breeds: Limousin (n = 25), Polish Holstein-Friesian (PHF, n = 12), and Polish Holstein-Friesian × Limousin (n = 25). The detailed characteristics of the bulls are presented in Table 1.

Table 1. Bull characteristics on the day of slaughter.

Bull Characteristics	Breed					
	Limousin		Polish Holstein-Friesian		PHF × Limousin	
	Mean	SD	Mean	SD	Mean	SD
Number (n)	25		12		25	
Age (d)	608	12.54	602	11.25	602	11.89
Live weight (kg)	695	35.23	534	24.47	668	34.25
Carcass weight (kg)	412	29.25	317	26.15	382	28.65

The bulls were kept on one farm located at Warmia and Masuria. Cattle were harvested at 21–23 months of age, and hot carcass weight was recorded. Then carcasses were chilled at 2–4 °C and samples of semimembranosus muscle (300 g) were cut parallel to the muscle axis at 24 h postmortem.

Slaughter and postslaughter processing were carried out in accordance with Council Regulation (EC) No. 1099/2009 of 24 September 2009 [33].

The animals were kept in alcoves in a free-standing system, in accordance with the minimum standards of cattle maintenance. During the fattening period, the animals were fed the same diet, ad libitum, with a total mixed ration (TMR) (Table 2).

Table 2. Ingredients and chemical composition of feed (as fed).

Ingredients	Content
Corn silage (%)	67.80
Barley (%)	29.20
Supplement * (%)	3.00
Diet composition:	
Dry matter (%)	54.24
Crude protein (g/kg)	128.40
NEm (Mcal/kg)	1.77
NEg (Mcal/kg)	1.15
NDF (g/kg)	343.23
ADF (g/kg)	193.56
Crude fat (g/kg)	19.20

* The supplement was composed of: 56.5% barley, 10% rape meal, 2% urea, 25% limestone, 3% salt, 0.066% vitamin E 500, 1% premix, 0.05% flavor, and 2.5% molasses, which provided 5% of diet in Dry matter (DM), and supplemented 1 kg diet (in DM) with additional 14.67 mg copper, 58.32 mg zinc, 26.73 mg manganese, 0.66 mg iodine, 0.23 mg cobalt, 0.29 mg selenium, 4.825 IU vitamin A, 478 IU vitamin D, and 32 IU vitamin E.

2.1. Chemical Analysis

Beef samples were chopped, then placed in a blender and ground until a homogeneous mass was obtained, which was analyzed later using a near infrared spectrophotometer. The basic chemical composition of meat was determined with a Food Scan™ analyzer (Foss Electric, Hillerød, Denmark).

Meat fat extraction was carried out according to the Folch method [34]. Fatty acid methylation was performed according to the trans-esterification method EN ISO 5509 [35]. Individual fatty acids were identified in crude fat using an Agilent 7890A GC (Agilent, Waldbronn, Germany) with HP Chem Station software (Agilent, Waldbronn, Germany), a flame ionization detector (FID), and a Varian Select FAME column (100 m length, 0.25 mm diameter, 0.25 µm film thickness; Varian/Agilent Technologies, Waldbronn, Germany) according to the methodology by Batorska et al. [36]. The analysis involved a programmed run with temperature ramps under the following conditions and temperatures: the injector was held at 240 °C fitted with siltek deactivated split/splitless liner packed with glass wool (Agilent, Waldbronn, Germany). The column had a total flow rate of 25 cm/s. One microliter of sample was injected with a split ratio of 50:1. The oven method was as follows: 130 °C held for one minute, increased to a temperature of 170 °C at the rate of 6.5 °C/min, then increased to a temperature of 215 °C at the rate of 2.5 °C/min held for 12 min. Then, it was increased to a temperature of 230 °C at the rate of 20 °C/min, held for three minutes. Helium was used as the carrier gas. The FID was operated at 300 °C. All samples were analyzed in duplicate. Each peak was identified using pure methyl ester standards (Supelco, Bellefonte, PA, USA).

The determination of α-tocopherol (vitamin E), α-retinol (vitamin A), and β-carotene were established using an Agilent 1100 Series reverse phase high performance liquid chromatograph (Agilent Technologies, Waldbronn, Germany) according to the methodology by Puppel [37]. Separations were performed at ambient temperature using solvent gradient on a ZORBAX Eclipse XDB column (Agilent Technologies, Waldbronn, Germany). The chromatographic conditions were as follows: Solvent A was MeOH (Merck, Darmstadt, Germany) and water (Sigma-Aldrich, St. Louis, MO, USA) in a ratio of 950:50 (v/v). The flow rate was 1.0 mL/min and the detection wavelength was 280 nm. The injection volume of the final solution was 25 µL. All samples were analyzed in duplicate. The identification of peaks was confirmed by a comparison with the standards (Sigma-Aldrich, St. Louis, MO, USA).

The determination of anserine, carnosine, taurine, coenzyme Q10, creatinine, and creatine was established using Agilent 1100 reversed-phase high performance liquid chromatography (RP-HPLC). Separations were performed at ambient temperature using solvent gradient on the Jupiter column C18 300A (Phenomenex, Torrance, CA, USA) according to the methodology by Łukasiewicz et al. [11]. The chromatographic conditions were as follows: Solvent A was acetonitrile (Merck, Darmstadt, Germany), water (Sigma-Aldrich), and trifluoroacetic acid (Sigma-Aldrich, St. Louis, MO, USA) in a ratio of 30:970:1 (v/v/v). Solvent B was acetonitrile, water, and trifluoroacetic acid in a ratio of 970:30:1 (v/v/v). The flow rate was 1.4 mL/min and the detection wavelength was 214 nm. The injection volume of the final solution was 25 µL. All samples were analyzed in duplicate. The identification of peaks was confirmed by a comparison with the standards (Sigma-Aldrich, St. Louis, MO, USA).

2.2. Statistical Analysis

The data obtained were subjected to analysis of variance using ANOVA analysis, with breed as fixed factors. Significant means were separated using Duncan (at $p < 0.05$). The distribution of bioactive components was checked using the Shapiro–Wilk test. All tests were processed using the IBM SPSS 23 package [38]. Data were presented as least squares means (LSM) with standard error of the mean.

The following statistical model was used:

$$Yijk = \mu + Ai + eij \quad (1)$$

where: *Yijk* = value of the tested trait; μ = mean; A*i* = effect of the *i* (the breed) (*I* = 1–3); *eij* = standard error.

3. Results and Discussion

Many genetic factors influence the quality of beef; the main one is the cattle breed [22,39,40]. They differ in their content and composition of intramuscular fat, the properties of connective tissue, especially collagen, and the proportion between the types of muscle fibers [22]. Table 3 shows a comparison of the protein, fat, and collagen contents in Limousin, Polish Holstein-Friesian, and PHF × Limousin hybrids.

Table 3. The influence of breed on the formation of the basic chemical composition of muscle tissue.

Component	Breed			SEM	*p*-Value
	Limousin	Polish Holstein-Friesian	PHF × Limousin		
Protein (g/100 g)	21.31 ^{AB}	19.4 ^{AC}	22.41 ^{BC}	0.241	0.000
Fat (g/100 g)	1.85 ^{A}	2.95 ^{AB}	1.89 ^{B}	0.035	0.010
Collagen (mg/100 g)	511.68 ^{AB}	592.24 ^{AC}	492.24 ^{BC}	1.231	0.042

A–C: Means in the same row marked with the same letters differ significantly at: capitals, $p \leq 0.01$. SEM, standard error of least square means (LSM).

The biological and nutritional value of meat protein is determined by the content of intramuscular connective tissue. Greater protein content in muscle tissue was found in the PHF × Limousin hybrids, at 22.41 g/100 g of meat, and the lowest in Polish Holstein-Friesian at 19.4 g/100 g. In the study by Malczyk et al. [41], the protein content was 21.61 g/100 g in the semitendinosus muscle and 21.51 g/100 g in the longest lumbar muscle in Lowland Black and White × Limousin hybrids. It should be noted that Holsteins may require concentrate-rich instead of silage-rich diets to express their full growth potential [42]. Additionally, purebred Holsteins have a low carcass weight compared to beef breeds or crossbreds [43], which was confirmed by the results obtained in the experiment (Table 1). Studies have shown that the concentration of protein (*p*-value 0.000) was significantly influenced by the breed.

Breeders usually choose animals that are high caliber and late maturing, which achieve very good fattening results. Late maturing breeds such as Limousin, Belgian White, and Blue have less fat and better musculature than early maturing breeds, but less marbling. The Italian and French cattle breeds are characterized by a low proportion of fat and bone and high muscle content. They also achieve satisfactory slaughter yields [30]. European beef cattle, Limousin, and Charolais have, among other features, better slaughter performance, less fat with higher musculature, but less marbling than British breeds such as Hereford or Angus [44]. Table 3 shows that muscle tissue in Limousin cattle was characterized by the lowest level of fat (1.85 g/100 g), and the highest level was found in Polish Holstein-Friesian at 2.95 g/100 g of meat. Studies by Zając [45] showed that the highest fat contents, of 5.30% and 5.15%, respectively, were found in the musculus serratus ventralis and the comb muscle. For comparison, the lowest fat content was in the semimembranosus muscle (2.38%) and semiendodermis muscle (2.77%). In the research conducted by Malczyk et al. [41], the level of fat in Black and White cattle was 2.07% in the heel muscle and 5.03% in the lumbar muscle. Additionally, Nogalski et al. [46] found that Sida silage can improve carcass and meat quality characteristics of Polish Holstein-Friesian bulls. The morphology, composition, and amount of intramuscular connective tissue vary depending on the muscle type, breed, and age of the animal [47].

Another important ingredient in meat is collagen. Table 3 shows that the highest content was found in Polish Holstein-Friesians (592.94 mg/100 g) and the lowest in PHF × Limousins (492.24 mg/100 g). The meat from dairy breeds is characterized by relatively high total collagen content compared with meat obtained from beef breeds [48]. In research by Dąbrowska et al. [49], the following collagen levels were found in meat from Black and White × Limousin crossbred bulls, depending on the muscle tissue analyzed: finger rectifier muscle (musculus extensor digitorum) 3032.2 mg, medium gluteus

muscle (musculus gluteus medius) 1506.5 mg, large lateral muscle (musculus vastus lateralis) 878.9 mg, semispongiform muscle (musculus semitendinosus) 525.6 mg, and large lumbar muscle (musculus psoas major) 300.4 mg. Studies have shown that the concentration of collagen was significantly influenced by the breed [50].

The health-promoting quality of meat depends on the saturated fatty acid (SFA) content. A high saturated fatty acid diet has long been implicated with an increased risk of cardiovascular disease [21]. Studies have shown that C12:0, C14:0, and C16:0 have atherogenic properties, while C14:0, C16:0, and C18:0 have thrombogenic properties [22]. Table 4 shows the share of SFAs in Polish Holstein-Friesian, Limousin, and PHF × Limousin hybrids in muscle tissue.

Table 4. The influence of breed on the formation of the saturated fatty acid composition of muscle tissue.

Component (g/100 g of Fat)	Breed			SEM	*p*-Value
	Limousin	Polish Holstein-Friesian	PHF × Limousin		
C12:0	0.06	0.09	0.05	0.011	0.991
C14:0	2.26 ^{AB}	1.45 ^{AC}	1.92 ^{BC}	0.132	0.004
C16:0	25.57 ^{Ab}	27.47 ^{AC}	24.22 ^{bC}	0.317	0.025
C18:0	18.40 ^{aB}	19.26 ^{aC}	16.87 ^{BC}	0.153	0.000
SFA	48.30 ^{aB}	49.74 ^{aC}	43.99 ^{BC}	0.752	0.000

aa, AA, etc.: Means in the same row marked with the same letters differ significantly at: small letters, $p \leq 0.05$; capitals, $p \leq 0.01$. SEM, standard error of LSM. SFA, saturated fatty acid.

The highest level of SFA, amounting to 49.74 g/100 g, was found in the muscle tissue of Polish Holstein-Friesian cattle. It should be emphasized that this breed exhibited the highest content of all fatty acids studied: lauric acid (C12:0; 0.09 g/100 g), palmitic acid (C16:0; 27.47 g/100 g), and stearic acid (C18:0; 19.26 g/100 g). The levels of the above-mentioned fatty acids in Limousin meat and their hybrids with Polish Holstein-Friesian were similar, especially for C12:0, C14:0, and C16:0. A significant difference was found in C18:0 acid, where in purebred cattle the level of stearic was 18.40 g/100 g, and in hybrids, 16.87 g/100 g. The lowest total content of SFA (43.99 g/100 g) was observed in the PHF × Limousin hybrids. Studies have shown that the concentration of SFA and C18:0 were significantly influenced by the type of breed (*p*-value 0.000). Almeida et al. [51] showed that the formation of a fatty acid profile is also linked to muscle type. Studies have shown that SFA concentration in the biceps femoris is more than three times higher than in the semimembranosus.

Other fatty acids that influence the nutritional and health-promoting quality of beef are monounsaturated fatty acids (MUFAs) and PUFAs.. The main n-3 PUFA in beef are α-linolenic (18:3 n-3), eicosapentaenoic (20:5 n-3), and docosahexaenoic (22:6 n-3) acids [45]. Table 5 presents the content of selected fatty acids in the muscle tissue of Polish Holstein-Friesian, Limousin, and PHF × Limousin hybrids.

Table 5. The influence of breed on MUFA and PUFA levels in muscle tissue.

Component (g/100 g of Fat)	Breed			SEM	*p*-Value
	Limousin	Polish Holstein-Friesian	PHF × Limousin		
C18:1 trans-11 (TVA)	1.13 ^{ab}	0.83 ^{aC}	1.31 ^{bC}	0.071	0.048
C18:2 n-6 (LA)	8.70 ^{Ab}	6.24 ^{Ac}	7.97 ^{bc}	1.041	0.021
C18:2 cis-9, trans-11 (CLA)	3.26 ^{Ab}	2.59 ^{AC}	3.59 ^{bC}	0.147	0.033
C18:3 n-3 (LNA)	0.59 ^{ab}	0.49 ^{ac}	0.71 ^{bc}	0.087	0.042
C20:5 n-3 (EPA)	0.58 ^{a}	0.42 ^{ab}	0.65 ^{b}	0.064	0.049
C22:6 n-3 (DHA)	0.14 ^{A}	0.07 ^{AB}	0.16 ^{B}	0.045	0.714
n-6 PUFA	11.01 ^{A}	8.54 ^{AB}	10.44 ^{B}	0.121	0.036
n-3 PUFA	2.42 ^{Ab}	2.06 ^{AC}	2.81 ^{bC}	0.175	0.006

aa, AA, etc.: Means in the same row marked with the same letters differ significantly at: small letters, $p \leq 0.05$; capitals, $p \leq 0.01$. SEM, standard error of LSM; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid.

Cheah et al. [21] reported that MUFAs may be an ideal substitute for SFA, obviating the adverse effects of SFAs; therefore, the aim should be to increase their share in the diet. The first of the lipid components analyzed was vaccenic acid (C18:1 trans-11; TVA), responsible for the efficiency of tissues and organs. Its highest level was found in PHF × Limousin hybrids (1.31 g/100 g), and its lowest in Polish Holstein-Friesians (0.83 g/100 g).

PUFAs, as essential fatty acids, are only available through dietary consumption [21]. The highest content of LA, 8.70 g/100 g, was found in meat obtained from the Limousins. Slightly lower levels were found in crossbreeds (7.97 g/100 g), and the lowest in Polish Holstein-Friesians (6.24 g/100 g). C18:2 cis-9, trans-11 (CLA) has many functions, which are mainly antioxidant, antiatherosclerotic, and anticancer [52]. The muscle tissue of purebred Limousin and their hybrids with the Polish Holstein-Friesian breed were recorded as 3.26 g/100 g and 3.59 g/100 g, respectively, i.e., more than the 2.59 g/100 g from the Polish Holstein-Friesians. Studies have shown that the concentration of CLA was significantly influenced by the breed.

α-linoleic acid (C18:3 n-3; LNA) plays a key role in hepatic glycolysis, de novo lipogenesis, and fatty acid regulation [53]. The highest level was found in PHF × Limousin hybrids and amounted to 0.71 g/100 g, whereas the lowest was in Polish Holstein-Friesians at 0.49 g/100 g. Other acids analyzed were eicosapentaenoic and docosahexaenoic, which support the functioning of the nervous system. Siscovick et al. [54] reported that the American Heart Association recommends 1 g/day of combined EPA/DHA intake or 2–4 g/day for those with hypertriglyceridemia. Both EPA and DHA were found to be the most abundant in crossbred meat (0.65 g/100 g and 0.16 g/100 g) and the least in Polish Holstein-Friesian meat (0.42 g/100 g and 0.07 g/100 g) (Table 5).

Bioactive dipeptides, including carnosine and anserine, are important components of beef that influence its pro-health value. Table 6 shows the content of bioactive protein fraction components in the muscle tissue of Polish Holstein-Friesians, Limousins, and PHF × Limousin hybrids.

Table 6. The influence of breed on bioactive protein fraction component levels in muscle tissue.

Component (mg/100 g)	Breed			SEM	*p*-Value
	Limousin	Polish Holstein-Friesian	PHF × Limousin		
Anserine	83.64 ^{Ab}	61.22 ^{AC}	81.46 ^{bC}	1.170	0.000
Carnosine	462.48 ^{AB}	387.3 ^{AC}	492.36 ^{BC}	8.226	0.000
Taurine	42.13 ^{AB}	34.28 ^{AC}	48.99 ^{BC}	0.551	0.005
Coenzyme Q10	2.35 ^{Ab}	1.87 ^{AC}	2.67 ^{bC}	0.125	0.044
Creatinine	5.48 ^{AB}	4.12 ^{AC}	6.36 ^{BC}	0.054	0.037
Creatine	448.22 ^{AB}	396.96 ^{AC}	481.25 ^{BC}	7.012	0.000

aa, AA, etc.: Means in the same row marked with the same letters differ significantly at: small letters, $p \leq 0.05$; capitals, $p \leq 0.01$. SEM, standard error of LSM.

Anserine (β-alanyl-L-(N-methyl) histidine) is a methyl carnosine derivative. It is a dipeptide consisting of β-alanine and L-(N-methyl) histidine. It occurs mainly in skeletal muscles and the brain, and in mammalian organisms it acts as an antioxidant. The concentration of carnosine in raw beef is 300–500 mg/100 g of tissue. For comparison, the average carnosine content of pork is 211–419 mg/100 g tissue. Differences in the concentration of carnosine in muscle tissues result primarily from different levels of muscle oxidation. Carnosine concentration is lower in muscles with high proportions of oxidative muscle fibers [11]. Breed, age, gender, and the rearing system also influence this concentration. The highest level of anserine (83.64 mg/100 g) was found in Limousine meat, and the lowest in Polish Holstein-Friesian meat (61.22 mg/100 g). The carnosine content in crossbreeds was the highest at 492.36 mg/100 g and the lowest in Polish Holstein-Friesians at 387.3 mg/100 g. Mateescu et al. [55] conducted research on the content of bioactive dipeptides in the longest back muscle of the Angus (by sex). The highest carnosine level was found in steers at 370 mg/100 g, in heifers at 367 mg/100 g, and in bulls at 366 mg/100 g. The content of anserine was almost the same in all categories of cattle and ranged from 66 to 67 mg/100 g. Carnosine levels were also determined in muscles and selected organs

in a study by Purchas and Busboom [56]. Its content in the longest dorsal muscle was 432.6 mg/100 g, in the semitendinous muscle, 452.6 mg/100 g, and in the musculus triceps brachii, 299.1 mg/100 g.

Taurine, full chemical name 2-aminoethylsulfonic acid, belongs to the amino acids and is commonly found in animal tissues. A lack of taurine in the diet results in a decrease in the number of leukocytes, and in the ability of neutrophils to oxygen burst and phagocytosis. The administration of taurine before an inflammatory reaction or after exposure to oxidative stress prevents or reduces the intensity of pro-inflammatory changes [57]. The highest level of taurine, 48.99 mg/100 g, was found in PHF × Limousin hybrids, and the lowest in Polish Holstein-Friesian meat, 34.28 mg/100 g. Purchas et al. [58] reported significant differences between cheek muscle and semitendinosus muscle.

Coenzyme Q10, also called ubiquinone, is a compound found in every cell of the body and plays a key role in it. Lowering the level of coenzyme Q10 favors the development of diseases arising from, among others, as a result of a reactive oxygen species, e.g., cardiovascular disease or cancer [59]. It is known that as a result of a deficiency of coenzyme Q10, the respiratory chain does not function properly and, as a result, there is insufficient production of high energy compounds [60], which in consequence may reduce the efficiency of the cell, tissue, and the whole organism. It was also found that clinical symptoms associated with deficiency of coenzyme Q10 can be eliminated or reduced by replenishing its quantity in the body with pharmaceutical preparations or dietary supplements [61]. The highest level of coenzyme Q10, 2.67 mg/100 g, was found in PHF × Limousin hybrids, and the lowest in Polish Holstein-Friesian meat, 1.87 mg/100 g. Mattila et al. [62] reported 1.6 mg/100 g for an unspecified beef muscle.

Table 7 shows the content of vitamins soluble in fat in the muscle tissue of Polish Holstein-Friesians, Limousins, and PHF × Limousin hybrids. β-carotene is a precursor of retinol, which is necessary for cell division and differentiation and reproduction [63]. Retinol also participates in the regulation of immune function by supporting the production of white blood cells [64]. Studies have shown that the concentrations of β-carotene and α-retinol were significantly influenced by the types of breed. The study showed almost double the level of β-carotene in the PHF × Limousin group than those in the Polish Holstein-Friesian group. Darwish et al. [65] reported that grass-fed steers had significantly higher amounts (five- to seven-fold) of β-carotene, compared to grain-fed animals, due to the high β-carotene content of fresh grasses as compared to cereal grains.

Table 7. The influence of breed on vitamins soluble in fat in muscle tissue.

Component (µg/g)	Breed			SEM	*p*-Value
	Limousin	Polish Holstein-Friesian	PHF × Limousin		
β-carotene	0.29 ^{AB}	0.20 ^{AC}	0.45 ^{BC}	0.003	0.000
α-retinol	0.75 ^{AB}	0.66 ^{AC}	0.92 ^{BC}	0.008	0.018
α-tocopherol	3.14 ^{AB}	1.61 ^{AC}	4.76 ^{BC}	0.098	0.024

aa, AA, etc.: Means in the same row marked with the same letters differ significantly at: capitals, $p \leq 0.01$. SEM, standard error of LSM.

The highest level of α-tocopherol, 4.76 µg/g, was found in PHF × Limousin hybrids, and the lowest in Polish Holstein-Friesian meat, 1.61 µg/g. Liu et al. [66] reported that a minimum of 3 to 3.5 µg/g meat of α-tocopherol is necessary to prevent discoloration. Additionally, Clausen et al. [67] reported that raw meat with approximately 2 µg/g meat of α-tocopherol showed a high degree of lipid oxidation. Therefore, it can be concluded that increased tissue levels of α-tocopherol protected against retail discoloration and lipid oxidation. It should be emphasized that the quality of muscle tissue varies, with this variability being significantly influenced by type of breed.

4. Conclusions

Commodity crossbreeding significantly improved the quality of beef analyzed in this study, resulting in similar or even better results than purebred cattle. The meat of PHF × Limousin hybrids

was characterized by the lowest level of SFA and the highest content of omega-3 fatty acids (LNA, EPA, DHA), carnosine, taurine, coenzyme Q10, and vitamins soluble in fat. This meant that beef from the hybrids with PHF was of the best nutritional and health-promoting quality. It should be emphasized that the scheme of crossbreeding Polish Holstein-Friesian or Limousin cattle with other breeds, or in beef herds, should be based on the expectations of the breeder. There are many possibilities from using one or two breeds, or through suppressing crossbreeding. In order for these measures to be profitable, the breeder must consistently follow his breeding strategy. The crossbreeding of beef cattle has two main advantages: hybrid animals show heterosis, and crossbred animals combine the forces of different breeds used to form the cross.

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Podpis

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Article

The Impact of Calf Rearing with Foster Cows on Calf Health, Welfare, and Veal Quality in Dairy Farms

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Abstract: This study assessed the impact of different calf rearing systems on calf health, behavior, meat quality, and oxidative stability. The study involved two groups of bull calves: conventionally penned calves (control, fed with use of automatic feeders) and calves reared alongside foster cows (experimental). The presence of foster cows was found to have a significant positive influence on calf health. Calves raised with foster cows experienced lower rates of diarrhea, delayed instances of coughing, and a reduced occurrence of rhinitis compared to conventionally reared calves. Behavioral observations revealed differences in sucking and licking behaviors between the two groups. Calves with foster cows displayed more consistent patterns of these behaviors, while conventionally reared calves exhibited greater variability. Additionally, the experimental group consistently achieved higher daily weight gains, suggesting the potential for larger and more valuable carcasses at slaughter. Importantly, there were no significant differences in the quality of veal between the two rearing groups. This included fatty acid composition, color attributes, and myoglobin levels, indicating consistent meat quality. In summary, this research highlights the advantages of rearing systems that prioritize calf health and behavior, emphasizing maternal care and natural behaviors. Such systems hold promise for improving calf welfare and enhancing the sustainability of the meat production industry. The integration of foster cows into dairy farming practices emerges as a practical and effective approach, particularly for the rearing of bull calves.

Keywords: calves; health; behavior; veal; oxidative stress; fatty acids

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1. Introduction

The development of biotech reproduction techniques, including semen preservation and artificial insemination, has increased the reproductive capacity of males [1]. Additionally, sexed semen is used to increase the likelihood of desired female calves being born in dairy farming [2–4]. However, this has led to intensive selection among males, reducing genetic diversity and increasing the risk of genetic diseases, as well as diminishing the value of production traits [5–11]. Furthermore, this selection model negatively impacts low-inbred functional traits like health, reproduction, and longevity [6,8,12], particularly noticeable on organic farms where feed differs significantly [13]. Despite the possibility of using sexed semen, it is rarely used due to the occurrence of reproductive problems in dairy cows associated with high milk yield [12,14,15] and poor herd management (mainly heat detection) [15,16]. As indicated by Frijters et al. [17], the causes of reduced fertility in cows with which sexed semen is used are due to lower sperm counts and sperm damage in the sexed semen Diskin et al. [18] and O'Callaghan et al. [19] pointed out that all focus only on the reproductive problems of cows and neglect the contribution of males to the fertilization process, and further remarked the need for male selection to improve ejaculate quality and

semen preservation capabilities. According to Seidel and DeJarnette [4], the use of sexed semen in the USA approaches 30%. In a study by Januś et al. [20], it was indicated that the use of sexed semen is more effective in heifers and primiparous females, while conventional semen is most often used with multiparous females, due to the percentage of inseminations; which is why bulls are born in addition to heifers desired by breeders (as the typical sex distribution in newborn calves is 1:1) [21,22]. Due to the predominant share of dairy cows in Poland (more than 90% of females are dairy cows), male off are sent to be fattened to high weight values for beef production [23]. According to Solarczyk et al. [24] and Sakowski et al. [25], beef from Holstein–Friesian bulls has a significantly inferior nutritional value to dairy–meat hybrids and purebred individuals from meat breeds, therefore, an alternative to using Holstein–Friesian bulls may be to obtain veal [3].

As noted by Ngapo and Gariepy [26], the definition of veal varies widely and depends on the author and the author's country of origin. According to Resano et al. [27] and Domaradzki et al. [28], veal is the meat derived from the butchering of calf carcasses, and has specific qualities such as low fat content, a light color, tenderness, and a delicate taste due to the young age of the animals being slaughtered. According to Regulation 1254/99 of the Council of the European Union, in the European Union, a calf is considered to be an animal up to 300 kg in weight, with no permanent teeth [29]. Europeans regard veal as a delicacy and a dietary product, which is why its consumption is not popular everywhere. In Poland, about 20 t of veal was produced in 2010 and the average per capita consumption of this meat was 335 g [30]. According to Sans and de Fontguyon [31], veal accounts for about 20% of the meat from cattle in the EU, with over 33% of this total coming from dairy herds, where the majority, around 75%, is from male calves. Traditional rearing is conducted by leaving calves with their mothers after birth so that the calf takes colostrum straight from the cow's teat, and that will also stay with the mother during rearing and take milk directly from the mother ad libitum at least twice a day. This model is mainly used in beef cattle herds [31]. On the other hand, in modern calf rearing on dairy farms, the calves are almost immediately separated from their mothers after birth [22] so as not to cause either calves or cows the unnecessary stress associated with separation [32]. Calves in this type of system are fed from the very beginning using bottles and buckets fitted with teats, and, on large farms, using special vending machines for feeding colostrum and milk, or conventional milk-substitute formulae, which provide the necessary nutrients while also increasing farmers' profits, as the milk can then be sold [33]. Some veterinarians even believe that by weaning calves quickly, breeders will provide better rearing conditions for their calves [34]. As reported by Haskell [22], some of the calves destined for veal procurement are transported at about eight days of age to special rearing houses where they are housed until slaughter, i.e., 8–10 months of age. These preparations are not used on organic farms, where only whole milk is used in rearing [35]. In this system, it would therefore be most cost-effective to keep the calves with suckler cows, which is a natural system in which the calf can take milk directly from the cow, which can further reduce the labor involved in milking and feeding the calves [36]. This is seen by many consumers as the most appropriate and most important system for improving animal welfare [37–41].

The rearing of calves in dairy herds is a huge challenge, which is why various practices are used on farms to obtain the best possible results. These activities are focused primarily on the rearing of healthy females, which in the future will be used for the renovation of the herd, while any bulls that are born are quite a problem because they do not represent valuable breeding material in these herds [21,42]. Practices related to the rearing of male animals vary. Many farmers decide to euthanize males immediately after birth; some farmers decide to sell their bulls in the first week of life, after which the animals are slaughtered for hide, rennet, and meat for pet animals. Still, other farmers sell these males to special rearing facilities where the animals are destined to become veal or beef [22,42–44]. With this last choice, the length of time animals are kept, and thus the type of meat obtained, depends largely on the preferences of the consumers in a given country. In Poland, due to the rather low consumption of veal, bulls of the Holstein–Friesian breed are sent for

fattening to reach high weight values, which, as indicated by Solarczyk et al. [24] and Sakowski et al. [25], is not the best solution due to the resulting rather low quality meat. In dairy herds, regardless of sex, the most common practice is to wean calves from their mothers almost immediately [45], which can be for various reasons: from a desire to better care for the calf, including the administration of the right amount and the best quality colostrum (i.e., to ensure adequate transfer of passive immunity); to reduce the stress associated with weaning calves from their mothers; to reduce the incidence of disease entities; and—what is in truth the most important factor—to be able to reduce the rearing costs associated with administering milk replacers and the sale of whole milk from cows, as well as the comfort to take care of animal handlers [32]. In the case of male calves, very often breeders do not pay attention to the quality of colostrum or the timing of its administration, due to which their passive immunity is not at a high level [46,47]. Recently, the attention of more and more consumers has been drawn to the welfare of animals; therefore, they are very often surprised by the practices used on dairy farms imagining that calves' long-term contact with their mothers is the most natural process, and which typically allows proper behavior [48–51]. For weaning older calves, there are also different models: single-stage weaning, which involves completely separating the calves from the mothers; and two-stage weaning, which involves putting on special nose-flaps. According to Valente et al., by far the best way to wean older calves from their mothers is the single-stage weaning, which is less stressful [52].

The quality of veal is the result of a complex interplay among numerous factors, such as nutrition, age at slaughter, exercise, stress levels, rearing conditions, genetics, carcass handling, calf health, and consumer preferences [41]. Variations in rearing systems can influence these factors, ultimately shaping the characteristics and quality of veal meat. In summary, a profound scientific understanding of the natural behaviors, anatomy, and physiology of cows and calves is essential for informed decision-making in calf rearing, whether it is mother-bonded, fostered, or motherless calf rearing. This knowledge is critical for ensuring calf health, welfare, and overall well-being, regardless of the chosen rearing method. The objective of the experiment was to investigate how different rearing methods affect calf behavior, calf health, and the quality of veal produced.

2. Materials and Methods

The study was conducted on an organic dairy farm in Wyczechowo (PL), utilizing two groups of bull calves, each comprising 5 calves of the same age and origin. The experiment was replicated three times, resulting in a total of 30 calves per group. The farm's breeding practices adhered to the guidelines outlined in Regulation (EU) 2018/848 of the European Parliament and the Council, dated 30 May 2018, pertaining to organic production and the labeling of organic products [53]. It is essential to recognize that there are notable distinctions between organic animal husbandry and conventional animal husbandry. These distinctions encompass the use of allopathic medicinal products, the potential utilization of milk replacers for calf rearing, as well as the approaches employed for managing pain-inducing procedures. Of significance is the observation that organic systems typically provide animals with increased space within livestock buildings, constituting a noteworthy difference when compared to conventional systems. The control group was reared conventionally using automatic feeders, with each calf receiving approximately 1000 kg of milk over a 6-month period, in addition to access to hay. The milk was provided twice a day and came from cows kept on the farm. The experimental group, on the other hand, had permanent access to two foster cows and hay. The calf's diet was meticulously calculated to comprise 15–20% of their body weight in whole milk. This parameter was rigorously assessed and maintained consistently across both experimental groups for precise nutritional monitoring.

The selection of foster cows involved an assessment of specific criteria to ensure an optimal calf rearing environment. Priority was given to cows with established strong maternal instincts and a successful history of mothering. Additionally, the health status

of the chosen cows was a paramount consideration, with a requirement for them to be in good health and free from contagious diseases. The study's results did not reveal any significant differences in crucial milk performance parameters or the cytological quality of milk between foster cows and conventionally milked cows on the farm. Specifically, the milk sourced from both categories consistently displayed bacterial levels below the threshold of 100,000 CFU and somatic cell counts below 200,000 cells per milliliter.

Calf weighing was conducted using the CalmScale system (Jantar Sp. z o.o., Bielsko-Biała, Polska), which is designed to minimize stress during the process. The system is placed in the cattle's watering area and employs RFID tags and antennas for precise identification. Weight data, along with other relevant information, is recorded and made available for analysis after processing. Average daily gain (ADG) was calculated by taking the weight difference from day 0 (pre-treatment weight) and dividing it by the number of days since day 0, providing a crucial metric for assessing calf growth.

After the calves reached the appropriate age (6 months), they were slaughtered, and their carcasses were cooled for 24 h at 2–4 °C to facilitate proper meat preservation. Following the cooling period, 300 g samples of semimembranosus muscle were collected from each calf parallel to the muscle axis.

The daily health assessment of the animals was conducted by a veterinarian, who documented all incidents, encompassing episodes of diarrhea, occurrences of coughing, and instances of rhinitis. In tandem with these observations, standard preventive measures targeting contagious viral diseases and parasitic infections were implemented. In conclusion, the paramount strategy for curtailing the transmission of Cryptosporidia and Coccidia in calves revolves around the unwavering commitment to hygiene protocols, which includes the routine disinfection of all surfaces that have direct contact with the calves.

The behavioral data were collected as averages from 5-h observation periods conducted monthly during the first 6 months of a calf's life. In the ethogram-based analysis of calf behavior, behaviors were initially categorized into three main categories: active, resting, and abnormal (Table 1). Active behaviors involved movement, resting behaviors indicated immobility, and abnormal behaviors included actions like calves sucking or licking other calves (mouth, ears, navel, tail, and scrotal) and sucking or licking objects within their pens. These abnormal behaviors were seen as deviations from typical calf behavior patterns and could potentially indicate issues like stress or boredom. This systematic observation approach allowed for a comprehensive understanding of the behavioral patterns and interactions among the calves during their early development. Behaviors were categorized into specific points, with each behavior recorded as a total frequency. This total frequency encompassed the sum of behaviors like sucking or licking objects and the sum of behaviors involving sucking or licking other calves per hour.

Table 1. Ethogram and categorization of behaviors.

Category	Behavior	Description
Active behaviors: behaviors involving movement	Locomotion	Walking or running.
	Play (alone)	Manipulating non-food object.
	Smell	Smelling the ground or an object.
Resting behaviors: behaviors involving little to no movement	Lying down	Lying down with very little movement, whether asleep or awake.
	Defecate/Urinate	Categorized as resting because movement behavior must be paused.
	Groom	Scratching, gnawing, or licking oneself.
	Social rest	Lying while in physical contact with at least one other calf.
Abnormal Behavior	Sucking or licking	Sucking or licking other calf's (mouth, ears, navel, tail, and scrotal).
		Sucking or licking objects in pens.

2.1. Fatty Acid Analysis

Intramuscular fat was extracted from 10 g of muscle using a mixture of chloroform and methanol in a 2:1 (*v*:*v*) ratio. The fatty acids were then converted into fatty acid

methyl esters (FAME) through a base-catalyzed transesterification process using sodium methoxide in methanol. To standardize the measurements, methyl nonadecanoate (C19:0) was used as the internal reference compound. The fatty acids were separated using a gas chromatograph (model TRACE GC; Thermo Finnigan, Milan, Italy), which employed a high-polar fused silica capillary column measuring 100 m in length, with a diameter of 25 mm and a film thickness of 0.25 µm (SP. 24056; Supelco Inc., Bellefonte, PA, USA). Identification of the FAME compounds was achieved using a flame ionization detector (FID). The gas chromatography conditions and the process for identifying FAME compounds followed the protocol as outlined by Natalello et al. [54].

2.2. Meat Oxidative Stability

Oxidative stability assessment of fresh meat followed a protocol described by Natalello et al. [55]. Three 2 cm-thick meat slices were prepared from each Longissimus thoracis et lumborum (LTL) muscle sample and stored in polystyrene trays wrapped with three layers of domestic cling film. The storage took place in the dark at 4 °C for different durations: zero days (after 2 h of blooming), three days, and seven days. After each storage period, one of the three slices was used to measure color parameters. Color stability assessment utilized a Minolta CM 2022 spectrophotometer, configured in the specular components excluded (SCE) mode. Measurements were performed with illuminant A and a 10° standard observer. Three non-overlapping measurements were taken on the meat's surface, and the mean value was calculated. Color descriptors, including L* (lightness), a* (redness), b* (yellowness), C (saturation), and hab (hue angle), were recorded in the CIE L* a* b* color space.

Lipid oxidation analysis involved measuring the concentration of 2-thiobarbituric acid reactive substances (TBARS) at the conclusion of each storage period. The method was adapted from Natalello et al. [55]. Meat samples (2.5 g) were homogenized with 12.5 mL of distilled water using a Heidolph Diax 900 tissue homogenizer (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany), placed in a water/ice bath during homogenization. Subsequently, 12.5 mL of 10% (*w*/*v*) trichloroacetic acid (TCA) was added to precipitate proteins, and the samples were then filtered. The clear filtrate (4 mL) was mixed with 1 mL of 0.06 M aqueous thiobarbituric acid in Pyrex glass tubes. Tubes were incubated in a water bath at 80 °C for 90 min, and absorbance was read at 532 nm using a Shimadzu UV/vis spectrophotometer (UV-1601). Concentration of malondialdehyde (MDA) in each sample was determined using a calibration curve prepared with TEP (1,1,3,3-tetraethoxypropane) in distilled water at concentrations ranging from 5 to 65 nmol/4 mL. Results were expressed as milligrams of MDA per kilogram of meat.

2.3. Myoglobin Analysis

Myoglobin concentration was determined following a method described by Krzywicki [56], with some modifications. Briefly, 2 g of muscle were homogenized using a Heidolph Diax 900 tissue homogenizer operating at 9500 rpm with a phosphate buffer. The homogenized samples were then subjected to centrifugation at 6800× *g* at 4 °C for 15 min and subsequently filtered through Whatman 541 paper. The filtered supernatant was scanned using a UV/VIS spectrophotometer (UV-1601; Shimadzu Co., Milan, Italy), and the absorbance at 525 nm was measured. Myoglobin concentration was calculated based on these absorbance measurements and expressed as milligrams per gram (mg/g) of fresh tissue.

2.4. Statistical Model

The experimental data from the evaluation of the calves' fattening performance were analyzed using the GLM Repeated Measures Procedure by SAS, ver.9.0 and following statistical model:

$$y_{ijk} = \mu + T_i + R_j + T \times R_{ij} + e_{ijk}$$

where y_{ijk} is the investigated trait; μ is the overall mean; T_i is the fixed effect of the i-th experimental group where 1 is the calves reared in a pen; and 2 is the calves kept with suckler cows. R_j is the fixed effect of the j-th replicated group of the experiment; $G \times R_{ij}$ is the fixed effect of the interaction between group and replicated group; and e_{ijk} is the random error.

The fat, fatty acid, and myoglobin content was estimated using one-way analysis of variance and following the statistical model:

$$y_{ij} = \mu + T_i + e_{ij}$$

where y_{ij} is the investigated trait; μ is the overall mean; T_i is the effect of the treatment group (i = "in pen"; "foster cow"); and e_{ij} is the residual error.

To assess the oxidative stability data (color and lipid oxidation over time of storage) the MIXED Procedure by SAS, ver. 9.0 was used following the statistical model:

$$Y_{ijkl} = \mu + T_i + D_j + I_k\,(T) + (T \times D)_{ij} + e_{ijkl}$$

where y_{ijkl} is the observation; μ is the overall mean; T_i is the fixed effect of the treatment group (i = "in pen"; "foster cow"); D_j is the fixed effect of the day of storage (j= 0, 3, 7 days for color and 0. 7 for lipid oxidation); $I_k(T)$ is the random effect of the individual animal nested within the dietary treatment; $(T \times D)_{ij}$ is the interaction between treatment and day of storage; and e_{ijkl} is the residual error. Differences between means were assessed using Tukey's adjustment for multiple comparisons [57].

Chi-square analysis was employed to evaluate the disease status (diseased or not diseased) in relation to the group assignment (control group vs. experimental group).

3. Results

3.1. Animal Behavior

Figures 1 and 2 show the results of the calves' behavior during the experiment.

The behavioral data were obtained as averages from a 5-h observation period conducted at monthly intervals during the first 6 months of a calf's life and included recording the sum of instances in which the calves engaged in sucking/licking per hour. For the control group (calves in pen), the calves sucked or licked various objects most frequently during the fourth month of life (69 times) and least frequently during the third month (7 times). For the experimental group (calves with foster cows), they sucked or licked objects most frequently during the sixth month of the experiment (51 times) and least frequently during the second month of the experiment (9 times).

Figure 2 shows the results for the sum of sucking or licking other calves during a one-hour period. The calves in the pen group sucked or licked a total of 187 times, while the calves with the foster cows sucked or licked 113 times, which was 74 times fewer than the control group. The calves in the pen group sucked or licked other calves most frequently during the fourth month of the experiment (70 times) and least frequently during the second month of the experiment (20 times). In the third month of the experiment, no such behavior was observed in the control group. In the case of the experimental group, the calves sucked or licked another individual most frequently in the sixth month of the experiment (44 times) and least frequently in the first month of the experiment (2 times).

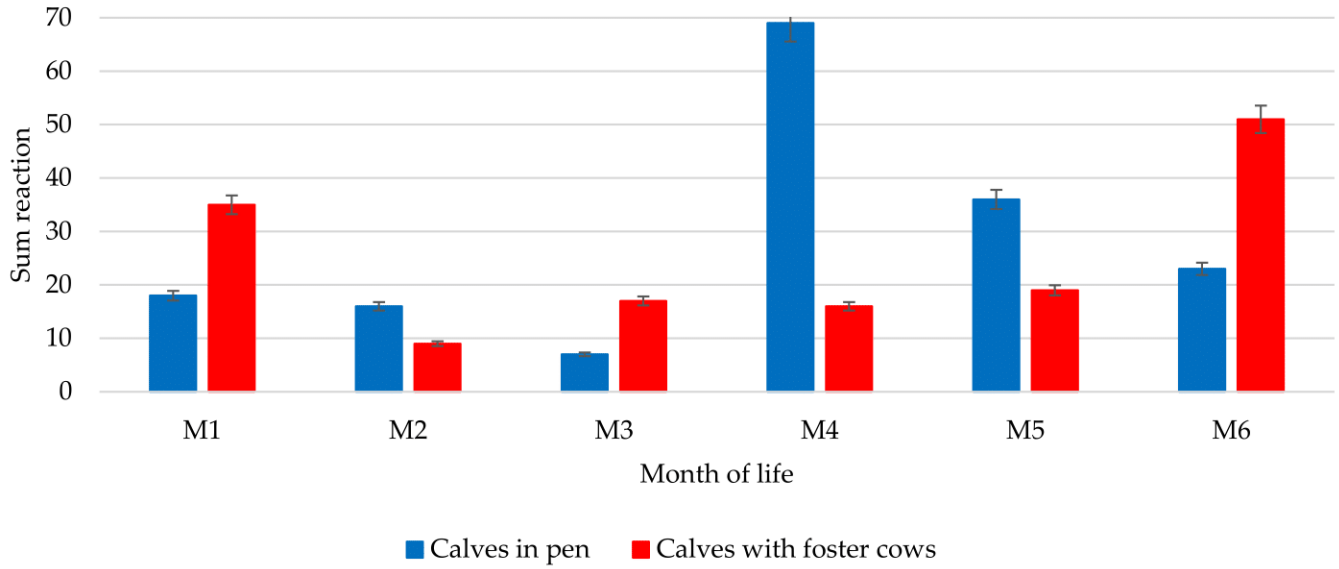

Figure 1. Behavioral observations, conducted at monthly intervals during the first 6 months of a calf's life, included recording the sum of instances in which the calves engaged in sucking/licking things per hour.

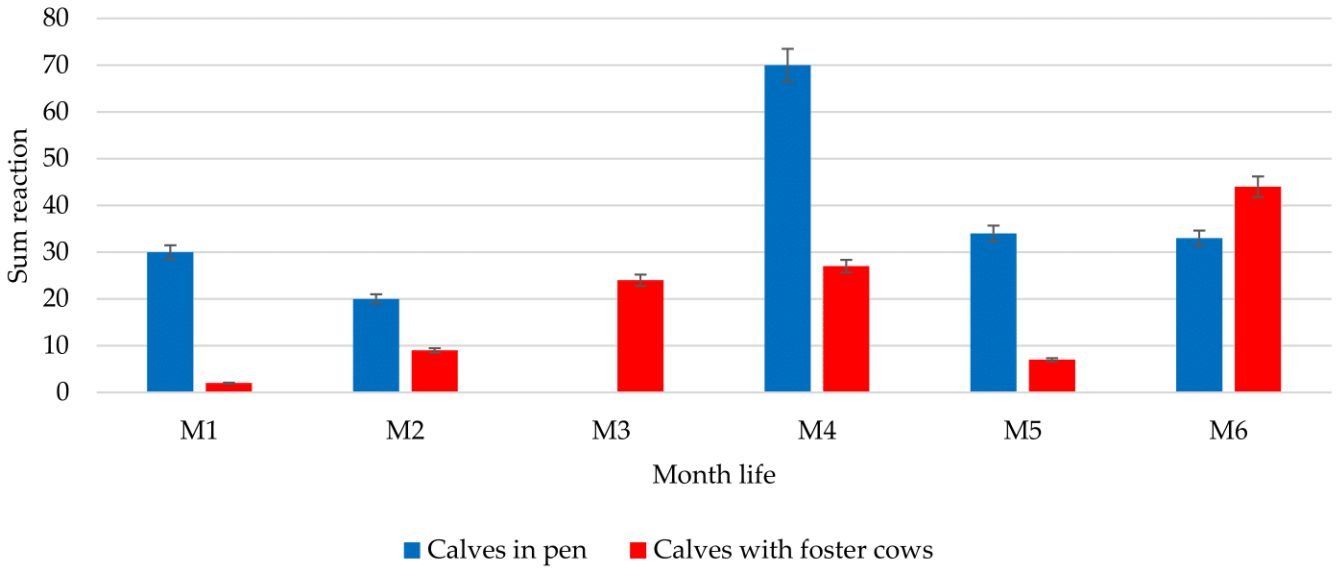

Figure 2. Behavioral observations, conducted at monthly intervals during the first 6 months of a calf's life, included recording the sum of instances in which the calves engaged in sucking/licking another calf per hour.

3.2. Animal Health

Figures 3–5 show the results for animal health.

Figure 3 shows the results for the occurrence of diarrhea. Diarrhea occurred in calves during the first four months of the experiment. In the calves in pen group, diarrhea occurred in six animals during the first month of the experiment, while in the calves with foster cows group it occurred in only one calf, and this was the only occurrence of diarrhea in this group. In the case of the control group, diarrhea was still occurring in the second month of the experiment in four individuals, in the third month of the experiment in two individuals, and in the fourth month of the experiment in one individual.

Figure 4 shows the incidence of coughing in the experimental calves. According to the collected information, coughing occurred in both the control and experimental groups. In the calves in pen group, it occurred in the first month of the experiment in two calves, in

the third month in three calves, and in the fifth month also in three calves. Coughing in the calves with foster cows group did not occur until the sixth month of the experiment and only affected one individual.

Figure 5 shows the results for the incidence of rhinitis. The observations showed that rhinitis only occurred in the calves in pen group during the first month in one individual, during the second month in three individuals, during the fourth month also in three individuals, and during the sixth month in one individual. No such observation was recorded in the calves with foster cows group.

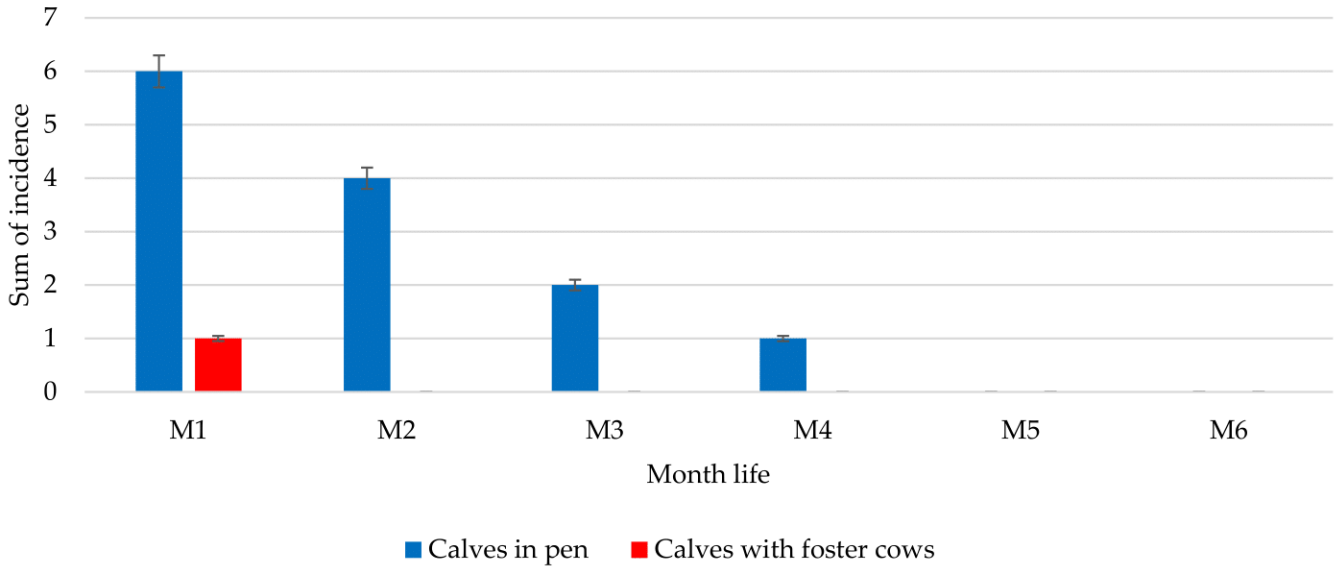

Figure 3. Incidence of diarrhea in calves.

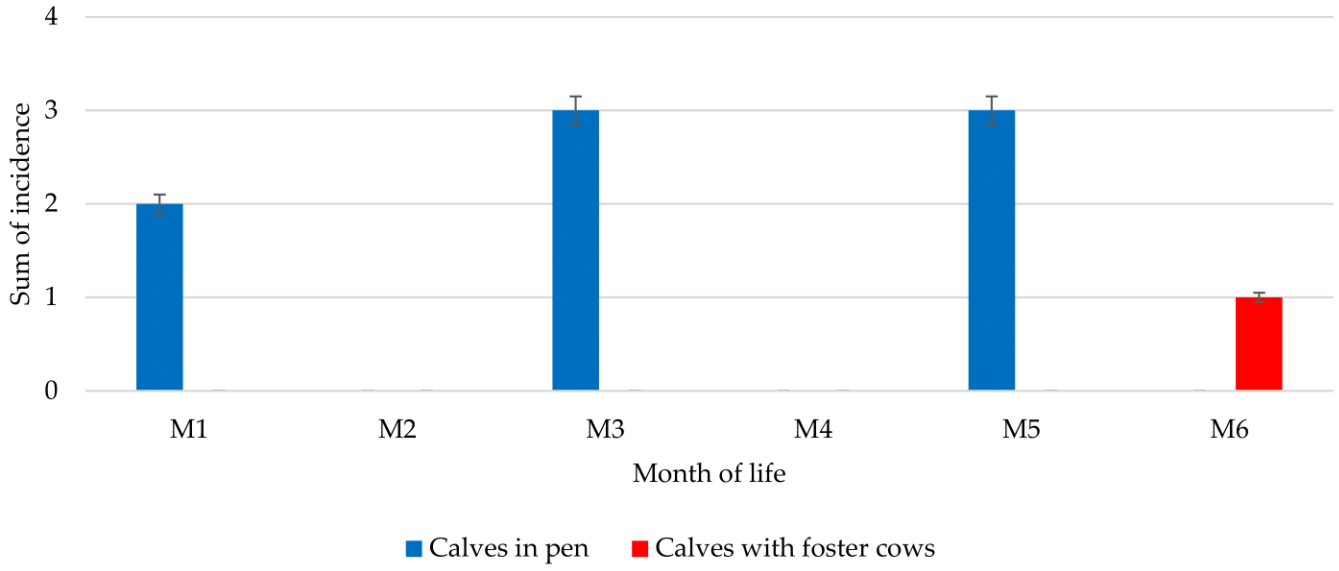

Figure 4. Incidence of coughing in calves.

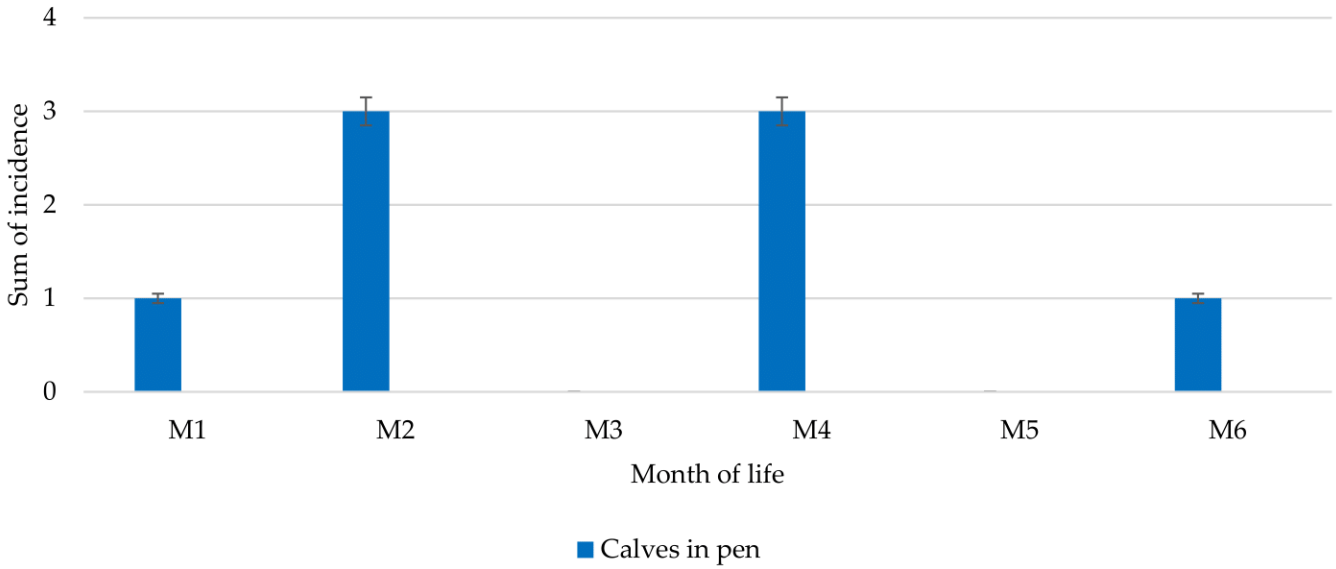

Figure 5. Occurrence of rhinitis.

3.3. Animal Body Weight

Figures 6 and 7 show the result body weight.

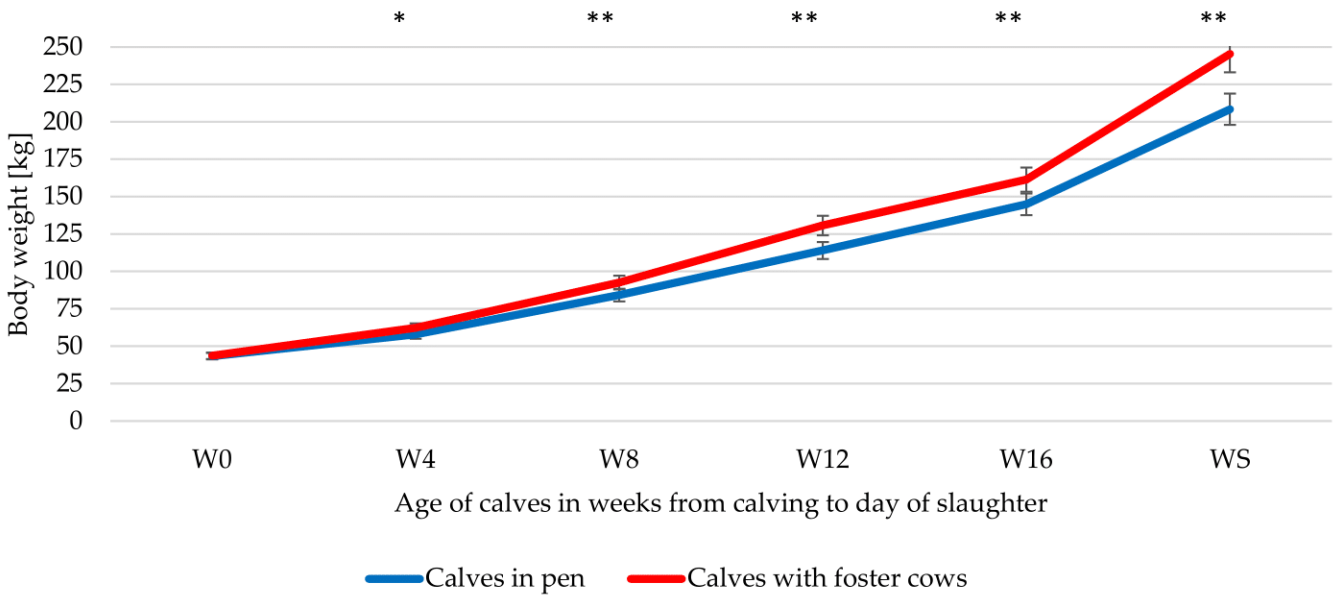

Figure 6. Changes in body weight. * $p \leq 0.05$, ** $p \leq 0.01$.

The body weight results are shown in Figure 6. The body weight measurements were taken six times during the experiment at four-week intervals from the day of birth until the day the calves were slaughtered.

The average body weight of the calves on the day of birth was similar for both groups and was 43.36 kg for the control group, in which the calves were fed using a calf feeder. In the experimental group, where the calves were fed by sucklers, the weight of the calves was 43.64 kg. The difference between the groups was −0.28 kg (control–experimental group). The second calf weight measurement was taken at week four of calf life. The average body weight of the calves in the calves in pen group was 58.76 kg an increase of 15.4 kg, while in the calves with foster cows group it was 62.21 kg, an increase of 18.58 kg: in percentage terms. This was an increase of 33.44% for the calves in pen group and 42.58% for the calves with foster cows group, compared to birth weight. The next average body

weight measurement was taken at week eight of the experiment. The animals in the calves in pen group had reached a weight of 84.21 kg, an increase of 40.85 kg (94.21%) compared to the initial weight. In the calves with foster cows group, the weight of the animals for the third measurement was 92.57 kg, an increase of 48.93 kg compared to the initial weight, indicating an increase of 112.12%. The difference between the weight of the calves in the calves in pen group to that of the calves in the calves with foster cows group was −8.36 kg ($p \leq 0.05$). The fourth weight measurement was taken at 12 weeks of calf age. The average body weight in the calves in pen group was 114.00 kg an increase of 70.64 kg (162.92%) compared to the initial weight. The calves in the calves with foster cows group had reached a weight of 130.71 kg an increase of 87.07 kg (199.52%). The difference in the weight of the control compared to the calves with foster cows group was −16.71 kg ($p \leq 0.01$). The next weight measurement was taken at week 16 of the experiment; the calves in the calves in pen group had reached a weight of 144.86 kg, an increase of 101.5 kg (234.09%). The weight of the calves in the calves with foster cows group was 161.36 kg, an increase of 117.72 kg (269.75%). The difference in the average body weight of the control group compared to the experimental group was -16.5 kg for this measurement ($p \leq 0.01$). The last weight measurement for the calves was taken on the day they were slaughtered. The calves in the calves in pen group had reached a weight of 208.43 kg, and the weight of these animals had increased by 165.07 kg (380.43%). Calves in the calves with foster cows group had reached a weight of 245.36 kg, and their weight increased by 201.72 kg (462.24%) compared to the calves' birth weight. On the day of slaughter, the weight of the calves in the control group was 36.93 kg ($p \leq 0.01$) lower than that of the calves in the experimental group. On the basis of the obtained body weight results, it was possible to estimate the daily gains, which are shown in Figure 7.

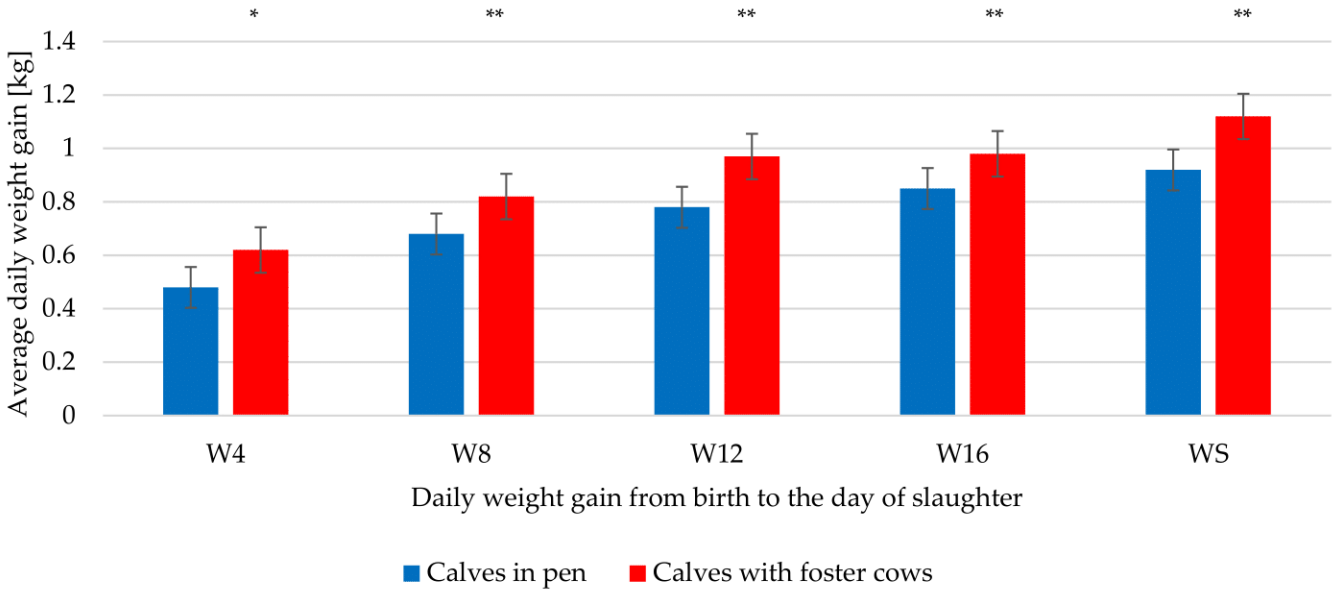

Figure 7. Daily weight gain from birth to the day of slaughter. * $p \leq 0.05$, ** $p \leq 0.01$.

In Figure 7, the daily gains were determined using the changes in body weight between individual weight measurements, with the first weight growth being determined in week four of the experiment. From the very beginning of the experiment there was an evident difference between the daily gains of the calves from each group, with the calves with foster cows group's weights being higher. In week 4, the daily difference was −0.14 kg ($p \leq 0.05$), in week 8 the daily difference was the same −0.14 kg ($p \leq 0.01$), in week 12 it was −0.19 kg ($p \leq 0.01$), in week 16 it was −0.13 kg ($p \leq 0.01$), while on the day of slaughter the daily difference was −0.20 kg ($p \leq 0.01$). The results confirm the faster growth rate of calves kept with sucklers and the possibility of obtaining higher animal weights on the day of slaughter.

3.4. Analysis of Veal

3.4.1. Quality of Veal

Basic composition is of great importance in assessing the nutritional value of the meat, the results of which are shown in Table 2. In this experiment, using muscle tissue from the calves in the control group, the protein content was found to be 31.24 g, and was 2.16 g lower ($p \leq 0.01$) than that of the animals in the experimental group, where it was 33.4 g.

Table 2. The basic composition of the veal.

	Calves in Pen		Calves with Foster Cows		*p*-Value
	LSM	SEM	LSM	SEM	
Protein	31.24	0.127	33.4	0.225	0.01
Fat	2.01	0.042	1.88	0.099	0.05
IMF	1.84	0.741	1.47	0.740	0.01

LSM—least square means. SEM—standard error of LSM. IMF—intramuscular fat.

The second parameter assessed was the fat content, which was 0.13 g lower in the muscle tissue of the animals in the calves with foster cows group (compared to the calves in pen group) and amounted to 1.88 g, while in the calves in pen group it was 2.01 g ($p \leq 0.05$). Concerning the adipose fraction, we can distinguish between perimuscular fat and intramuscular fat. In the results obtained from the muscle tissue, the intramuscular fat content was also higher in calves from the control group, the difference being 0.37 g ($p \leq 0.01$).

The next analysis concerned the determination of the fatty acid profiles. Table 3 summarizes the results for saturated fatty acids, Table 4 for monounsaturated fatty acids, and Table 5 for polyunsaturated fatty acids.

Table 3. Profile of saturated fatty acids.

Component (g/100 g of Fat)	Calves in Pen		Calves with Foster Cows		*p*-Value
	LSM	SEM	LSM	SEM	
C10:0	0.04	0.010	0.05	0.014	0.000
C12:0	0.13	0.040	0.20	0.067	0.000
C13:0	0.03	0.013	0.04	0.014	0.009
C14:0 iso	0.04	0.010	0.06	0.018	0.000
C14:0	3.26	0.725	3.96	1.131	0.001
C15:0 iso	0.11	0.023	0.15	0.043	0.000
C15:0 anteiso	0.16	0.059	0.23	0.062	0.000
C15:0	0.41	0.125	0.61	0.142	0.000
C16:0 iso	0.18	0.035	0.20	0.046	0.010
C16:0	21.86	1.779	24.66	3.129	0.000
C17:0 iso	0.29	0.049	0.34	0.051	0.000
C17:0 anteiso	0.39	0.076	0.44	0.094	0.003
C17:0	0.84	0.120	0.94	0.197	0.002
C18:0	11.27	1.748	12.58	2.242	0.001
C20:0	0.10	0.025	0.13	0.036	0.000
C22:0	0.01	0.015	0.04	0.034	0.000
C24:0	0.53	0.205	0.78	0.428	0.000
SFA	37.15	3.614	42.39	5.142	0.000

LSM—least square means. SEM—standard error of LSM. SFA—saturated fatty acids.

In this experiment, the 17 saturated fatty acids and the sum of the content of these fatty acids were determined. The values of all fatty acids obtained were higher for the calves with foster cows group than for the calves in pen group ($p \leq 0.01$). Among the saturated fatty acids determined in this experiment, C16:0 had the highest content, with a level of 21.86 g for the calves in pen group and 24.66 g for the calves with foster cows group (2.8 g

higher compared to the calves in pen group). The second most abundant acid was C18:0, whose level in the calves in pen group was 11.27 g, while in the calves with foster cows group it was 12.58 g (1.31 g higher). The next most abundant fatty acid was C14:0, whose content in the calves in pen group was 3.26 g, while in the calves with foster cows group it was 3.96 g (0.70 g higher). The value of the other fatty acids was below 1 g.

Table 4. Profile of monounsaturated fatty acids.

Component (g/100 g of Fat)	Calves in Pen		Calves with Foster Cows		*p*-Value
	LSM	SEM	LSM	SEM	
C14:1 *cis9*	0.79	0.227	0.72	0.161	0.025
C16:1 *trans9*	0.05	0.012	0.05	0.009	0.508
C16:1 *cis7*	0.26	0.042	0.27	0.065	0.617
C16:1 *cis9*	3.17	0.613	2.85	0.599	0.010
C18:1 t6+t7+t8	0.07	0.033	0.06	0.026	0.122
C18:1 *trans9*	0.20	0.036	0.19	0.036	0.030
C18:1 *trans10*	0.20	0.183	0.08	0.030	<0.001
C18:1 *trans11*	0.40	0.134	0.48	0.162	0.014
C18:1 *cis6*	0.35	0.103	0.37	0.089	0.361
C18:1 *cis9*	28.69	3.671	24.04	2.811	<0.001
C18:1 *cis12*	0.20	0.013	0.20	0.069	0.007
C18:1 *cis13*	0.16	0.039	0.16	0.041	0.057
C20:1 *cis11*	0.13	0.023	0.14	0.024	0.013
C24:1 *cis9*	0.12	0.041	0.15	0.075	0.002
MUFA	36.35	4.378	31.15	3.047	<0.001

LSM—least square means. SEM—standard error of LSM. MUFA—monounsaturated fatty acids.

Table 5. Profile of polyunsaturated fatty acids.

Component (g/100 g of Fat)	Calves in Pen		Calves with Foster Cows		*p*-Value
	LSM	SEM	LSM	SEM	
C18:2 *cis9cis12*	5.23	1.601	5.75	2.742	0.822
C18:3 *cis6.9.12*	0.03	0.018	0.04	0.0271	0.166
C18:3 *cis9,12,15*	0.72	0.162	0.91	0.298	0.269
C18:2 *cis9 trans11*	0.24	0.054	0.19	0.037	0.006
C20:2 *cis11,14*	0.05	0.025	0.06	0.037	0.006
C20:3 *n-6*	0.44	0.164	0.56	0.301	0.010
C20:4 *n-6*	2.07	0.752	2.65	1.478	0.008
C22:4 *n-6*	0.02	0.017	0.03	0.025	0.002
C22:5 *n-3*	0.16	0.082	0.17	0.093	0.512
PUFA	9.67	2.989	11.32	5.371	0.033
n-6 PUFA	8.55	2.798	10.06	5.030	0.037
n-3 PUFA	0.88	0.227	1.07	0.384	0.001
n-6/n-3	9.52	1.070	8.95	1.876	0.029

LSM—least square means. SEM—standard error of LSM. PUFA—polyunsaturated fatty acids.

The total content of the SFA family of fatty acids in the calves in pen group was 37.15 g, while in the calves with foster cows group it was 42.39 g, making it 5.24 g higher than the calves in pen group ($p \leq 0.01$).

The results for the monounsaturated fatty acid content are summarized in Table 4. Among these fatty acids, the highest content was oleic acid (C18:1). The content of this acid in the calves in pen group was 28.69 g, while in the calves with foster cows group it was 24.04, with the difference being as much as −4.65 ($p \leq 0.01$). In this group, the second most abundant fatty acid was C16:1 cis 9, with a content of 3.17 g in the calves in pen group and 2.85 g in the calves with foster cows group (0.32 g lower) ($p \leq 0.01$). The content of the other acids of the MUFA family was less than 1 g.

The total content of MUFA acids for the calves in pen group was 36.35 g and was at a higher level (5.2 g ($p \leq 0.01$)) than the calves with foster cows group where it was 31.15 g.

The polyunsaturated fatty acid content is presented in Table 5. C18:2 cis9 cis12 linoleic acid was characterized by the highest content in this group of acids. The value of this fatty acid in the calves in pen group was 5.23 g, while in the calves with foster cows group it was 5.75 g, 0.52 g higher ($p = 0.822$). The second most abundant fatty acid was C20:4 n-6 arachidonic acid, whose content in the calves in pen group was 2.07 g, while in the calves with foster cows group it was 2.65 g, 0.58 g higher than in the calves in pen group ($p \leq 0.01$). The value of the other PUFA acids was less than 1 g in 100 g of fat.

The sum of PUFA acids in the calves in pen group was 9.67 g, while in the calves with foster cows group it was 11.32 g (1.65 g higher than the calves in pen group) ($p \leq 0.05$). As for the group of fatty acids included in PUFA n-6, their content in the calves in pen group was 8.55 g, while in the calves with foster cows group it was 10.06 g, higher by 1.51 g ($p \leq 0.05$). The PUFA n-3 group in the calves with foster cows group was 0.88 g, while in the calves in pen group it was 1.07 g, higher by 0.19 g ($p \leq 0.01$). The ratio of n-6 to n-3 was higher in the calves in pen group at 9.52, while in the calves with foster cows group it is 8.95, 0.57 less ($p \leq 0.05$).

3.4.2. Oxidative Stability

The collected tissues were subjected to analyses to determine oxidative stability. Figure 8 shows the myoglobin content in the *semimembranosus muscle*. Figure 9 shows the changes in the veal's MDA content. Table 6 shows the changes in meat color.

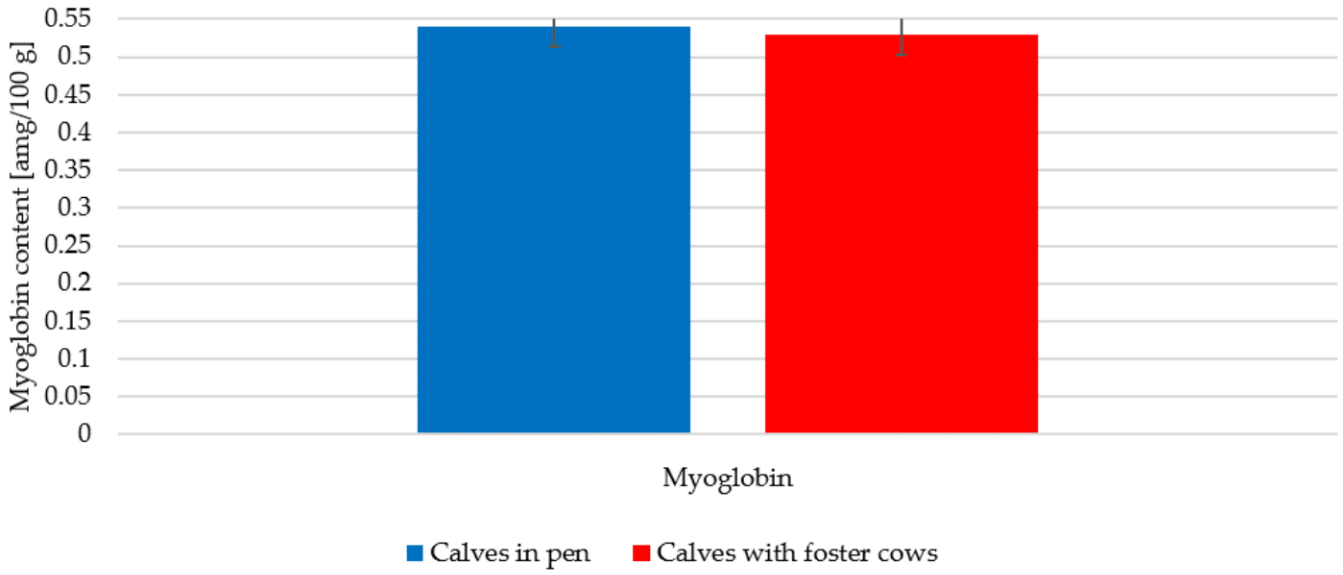

Figure 8. Myoglobin content.

The myoglobin content in the collected tissues from both groups was at a similar level: in the calves in pen group, 0.54 mg, while in the calves with foster cows group it was 0.53 mg, a difference of only 0.01 mg ($p \leq 0.725$).

Figure 9 shows the changes in MDA content during storage of the muscle tissue. The first analysis was performed 24 h after slaughter. The MDA value during the first measurement for the calves in pen group was 0.82 mg, while in the calves with foster cows group it was 0.22 mg (0.6 mg lower than in the calves in pen group) ($p \leq 0.01$). The second MDA measurement was made on day seven after slaughter; the MDA level in the calves in pen group increased by 3.73 mg to a value of 4.55 mg, an increase of 454.88%. In the calves with foster cows group it increased by 3.06 mg to a value of 3.28 mg, an increase of 1390.91%. The MDA value was higher in the calves in pen group than in the calves with foster cows group, and this difference at day seven post-slaughter, between the groups was 1.27 mg ($p \leq 0.01$).

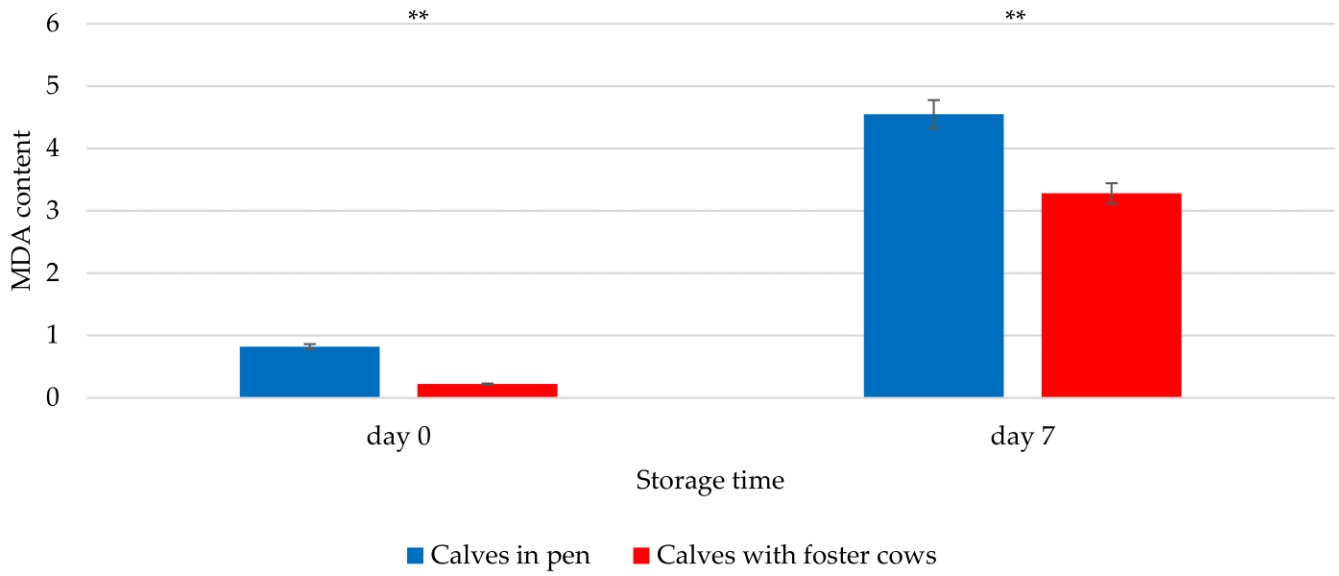

Figure 9. Changes in the MDA content of veal in relation on storage time. ** $p \leq 0.01$.

Table 6. Changes of color veal.

	Day	Calves in Pen		Calves with Foster Cows		*p*-Value
		LSM	SEM	LSM	SEM	
L*	1	46.25	4.551	46.13	5.487	0.830
	4	57.56	4.081	57.30	3.737	0.470
	7	45.87	4.900	44.46	2.246	0.080
a*	1	18.35	3.189	18.02	2.150	0.777
	4	7.41	2.969	7.82	3.750	0.293
	7	9.31	1.889	11.13	2.406	<0.001
b*	1	16.14	4.835	12.60	2.516	0.528
	4	−4.85	2.997	−4.25	5.368	0.191
	7	13.10	1.856	12.72	2.249	0.545
C*	1	24.53	5.401	23.86	3.104	0.571
	4	9.90	1.417	11.25	1.451	<0.001
	7	16.16	2.183	16.98	2.949	0.096
h°	1	40.61	4.462	40.66	2.768	0.848
	4	286.96	66.749	228.29	104.858	<0.001
	7	54.79	5.341	48.84	4.872	<0.001

LSM—least square means. SEM—standard error of LSM.

Another parameter assessed was the color of the calves' meat tissue; this was analyzed three times: the first time at 24 h after slaughter, the second time at four days after slaughter, and the third time at seven days after the slaughter of the calves.

Twenty-four hours after slaughter, the parameters for brightness (L*), redness (a*), yellowness (b*), saturation and pigment content (C*), and degree of hue deviation (h°) were at similar levels in both the calves in pen and calves with the foster cows groups.

On the fourth day after slaughter, the L*, a*, and b* parameters remained at similar levels in both groups. However, the value of saturation and pigment content was distinctly higher for the calves with foster cows group while the degree of hue deviation was lower than that of the calves in pen group. As for the changes between the individual measurements, there was an increase in the values of the L* and h° parameters, but a decrease in the other parameter values.

On the seventh day after slaughter, the values of the L* and b* parameters for both groups remained at a similar level. The C* parameter was also at a similar level, while the

value of the a* parameter was higher in the calves with foster cows group. In the case of the h° parameter, as on the fourth day after slaughter, the parameter had lower values in the calves with foster cows group. As for changes between individual measurements, there was an increase in the values of a*, b*, C*, while L* and h° decreased.

4. Discussion

4.1. Animal Behavior

Animal behavior is a complex outcome influenced by various factors, holding significance not only for the animals within a group but also for the caregivers. Proper behavior development in animals is closely tied to their natural way of life [58,59], particularly during the critical period from birth and contact with the mother. Before birth, mothers often seek isolation from the herd for calving, followed by a period of licking the newborn calf for over 30 min [60]. During this time, a bond forms between the calf and its mother, and the calf receives vital colostrum. However, calves frequently face challenges in successfully taking in colostrum due to factors like the structure of the cow's udder and the weakness of newborn calves [61–64]. Concerns about the right quantity, quality, and timing of colostrum intake are reasons why some farmers choose to separate calves from their mothers immediately after birth or shortly thereafter. Depending on the farm's size and infrastructure, various methods are used for calf feeding. This can include using buckets equipped with teats, feeding once or twice a day, or employing special vending machines that provide calves with constant access to colostrum, milk, or milk replacer, mimicking a more natural process where calves have some control over the timing of their intake [48,65]. In essence, proper behavior development in animals, particularly in the early stages, is closely linked to maternal bonding and colostrum intake, and farmers employ different strategies to ensure the health and well-being of their calves. The observations regarding calf behavior and their interactions with their mothers or the rearing environment underscore the complexity of calf rearing practices. The initial bonding and colostrum intake facilitated by maternal care are crucial for calf health and immunity. However, challenges in ensuring adequate colostrum consumption and concerns about calf well-being have led to the adoption of various rearing methods. Calves separated from their mothers and provided with controlled access to milk through buckets or vending machines can have distinct behavioral patterns. They may exhibit a higher frequency of sucking and licking behaviors as they adapt to this different feeding system (Figures 1 and 2). On the other hand, calves reared by their mothers tend to have longer suckling sessions but may eventually display more independent behaviors. According to Rosenberger et al., calves can take in as much as 12 L of milk per day [66] during 8 to 12 rest [61,67], indicating fairly small amounts of milk intake per rest period. During this time, calves have the opportunity to calm their suckling reflex. According to Appleby et al. [68], calves that stay with their mothers suckle for an average of 47 min; while, according to Hammell et al., calves drinking from a bucket provided with a pacifier suckle for only 18 min per day [69]. In an experiment in which calves that were given constant access to vending machines, but were given colostrum, milk or milk replacer twice a day, the animals appeared near the machines throughout the day [66,70,71] and showed an eagerness to take goo through sucking and licking reflexes, while animals staying with their mothers showed such behavior much less frequently [65]. A similar trend was evident in our study, where animals that were fed using an automatic feeder showed more frequent desire to lick and suck other animals as well as objects in their environment [72] compared to animals in the experimental group, where calves had permanent access to foster cows.

Calves in the experimental group consistently fulfilled their food requirements and showed little need to lick and suckle other individuals or objects. Similar observations were confirmed in a study by Margerison et al. [65]. However, as indicated by our own research and that of de Passille et al. [71], at a later age, calves that stayed with their mothers can manifest an increased number of such behaviors—this may be related to reduced milk production and not fully covering the maintenance requirement. According to Whalin

et al. [73], the actions of licking and suckling other individuals and objects is probably related not only to the desire to retrieve food and satisfy the natural need to suckle, but may also be related to the need for skin and head hair cleaning in calves, which is performed by cows, as well as the formation of social bonds between individuals. As indicated by several authors [32,33,40,41,73] these actions are not only related to the presence of their mothers but are also dependent on the age at which the calves are weaned.

Restricted feed intake only at a designated time, according to a study by Hammon et al. [33], affects not only the behavior but also the physiological state of the animals [74]. Animals fed at a set time were characterized by higher levels of cortisol in the blood, which indicates the occurrence in these animals of stress associated with feed intake restrictions and increases the reduction of immunity [75,76]. Animals that stay with their mothers take in more feed, which may, additionally, stimulate their growth. Also, calves staying longer with their mother were associated with the mimicking of her behavior and taking in solid feed [77]. However, as stressed by farmers, animals that are reared by a human are more docile and easier to handle than those animals that stayed with their mothers [50,51,67].

It is worth noting that the weaning age and feeding schedules play significant roles in shaping calf behavior. Calves weaned at different ages may exhibit variations in their social interactions and feeding-related behaviors. Moreover, the impact of rearing methods on physiological aspects, such as cortisol levels and immunity, highlights the importance of considering both behavioral and physiological indicators of calf well-being. Ultimately, the choice of rearing method should be based on a careful assessment of the welfare, health, and growth of the calves, as well as the management practices and resources available on the farm. Balancing the natural behaviors of calves with the practicalities of farm operations remains a critical consideration in calf rearing decisions.

4.2. Animal Health

Disease prevention plays a very important role in animal maintenance. According to Palczynski et al. [78] preventive measures can reduce the occurrence of diseases and thus reduce the frequency of treatment, including the administration of antibiotics, and therefore reduce costs and increase the possibility of achieving better results. There is a huge problem with calf health [78] and calf mortality [77] in dairy herds. As indicated in a study by Palczynski et al. [74], farmers very often report concerns about calves having an adequate enough transfer of passive immunity, which is associated with an inadequate intake and quality of colostrum, and the timing of colostrum intake by calves that stay with foster cows [62,79]. Therefore, more disease entities are possible; but studies have not clearly defined the etiology of the occurrence of various disease entities.

Diarrhea is a common health concern in young calves and can have significant implications for their growth and well-being. The data indicate that diarrhea was more prevalent in the control group of calves reared conventionally in pens. This could be attributed to factors such as stress associated with separation from the mother, suboptimal colostrum intake, and the feeding regimen. In contrast, the group of calves with foster cows experienced a notably lower incidence of diarrhea. The presence of foster cows may have contributed to reduced stress and better feeding practices, resulting in improved calf health (Figure 3). As Meagher et al. and Beaver et al. point out in their review [40,41], it is possible for animals that stay with their mothers to contract various disease entities that are mainly related to the presence of inflammation of the mammary gland, which causes diarrhea. Diarrhea and respiratory disorders are the most frequently mentioned disease entities occurring in calves [58–60,78]. In our study, animals in the experimental group showed significantly better health than those in the control group, and it was noted that only one individual developed diarrhea during the course of the experiment. However, when fed whole milk, diarrhea is very often mistaken for watery feces, which is a normal phenomenon [40,41].

On the other hand, when it comes to the occurrence of respiratory conditions, pneumonia is the most common problem. In our study, the occurrence of this disease was excluded through examinations performed by a veterinarian, although coughs and rhinitis were

confirmed. Coughing is often associated with respiratory issues in calves. The data show that coughing occurred in both the control and experimental groups. However, the onset of coughing in the group of calves with foster cows was delayed, with the first occurrence noted in the sixth month of the experiment and affecting only one calf. This delayed onset suggests that the rearing conditions, including the presence of foster cows, may have provided a more favorable respiratory environment for the calves. The control group, reared conventionally in pens, experienced coughing earlier and in multiple individuals. Rhinitis, or inflammation of the nasal passages, is another respiratory condition that can impact calf health. The results indicate that rhinitis occurred primarily in the control group during the early months of the experiment, affecting multiple individuals. In contrast, no cases of rhinitis were reported in the calves with foster cows. This observation suggests that the rearing system involving permanent access to foster cows may have contributed to a lower incidence of respiratory issues in the experimental group.

The elimination of the occurrence of diseases in young calves is a very important factor that affects not only the cost of production, but, above all, its efficiency, because only after eliminating the occurrence of diseases can all physiological processes run properly. In summary, the data suggest that the rearing system involving foster cows may have advantages in terms of reducing the incidence of diarrhea, delaying the onset of coughing, and preventing rhinitis in calves compared to conventional rearing in pens. These findings highlight the potential benefits of incorporating maternal care and natural behaviors into calf rearing practices, ultimately promoting better calf health and welfare. However, further research and monitoring are needed to validate these trends and assess long-term effects on calf development.

4.3. Body Weight

The proper growth and development of calves is influenced by many factors, especially environmental factors, the most important of which are nutrition and the health of the animals. Animal weight and weight gain depend on whether the animals' feed requirements are fully covered. If animals are not provided with adequate nutrition, their growth rates may be lower [63,66,80]. The daily weight gain of calves is a critical factor in determining their overall growth and development. In this study, the calves with foster cows consistently exhibited higher daily weight gains compared to the control group, reared conventionally in pens. This difference was evident from the early stages of the experiment and persisted throughout (Figure 7). The consistently higher daily weight gain in the group of calves with foster cows has important implications for their weight at slaughter. The data suggest that these calves have the potential to reach higher slaughter weights compared to those reared conventionally (Figure 7). This finding is of significance in the context of meat production, as it implies the possibility of obtaining larger and potentially more valuable carcasses.

As Hammon et al. [81] point out, the use of restricted milk intake during the rearing period is associated with the occurrence of higher blood cortisol levels compared to animals with constant access to colostrum, milk or milk replacer, which indicates the occurrence of stress in these animals, associated with the restrictions in feed intake, and may result in reduced immunity [64,75,76]. In addition, in an experiment by Hammon et al. [81], this type of rearing was associated with the presence of high levels of non-esterified fatty acids (NEFA), which may indicate the periodic occurrence of negative energy balances (NEB) in these calves [75].

In our study, the body weight of the animals in the experimental group was higher for each successive measurement compared to that of the animals in the control group. These animals also had higher daily gains. These studies confirm the results of Khan et al. [82] and Chapman et al. [83]. Animals in the experimental group, having constant access to milk, independently chose the time of day when they wanted to feed, due to which they did not feel hungry, and, as indicated by the presence of foster mother, may stimulate

calves to intake solid feed earlier. This is related to the imitation of behavior that calves observe in the foster mother.

Several factors may contribute to the enhanced growth observed in the group of calves with foster cows. The presence of foster cows may reduce stress and promote better feeding practices, leading to improved nutrient intake and growth. Additionally, the natural behavior of suckling from a mother cow may result in more efficient feeding and higher weight gain (Figure 7). As Khan et al. [82] indicate, animals permanently housed with foster mother usually begin solid feed intake earlier, but as a result do not take in too much solid feed; while animals fed limited amounts of milk or milk replacer, due to feelings of hunger, at the same time take in more solid feed [84]. However, as Johnsen et al. point out, despite the intake of a larger amount of solid feed, very often, insufficient milk intake will delay the subsequent growth of such calves [45]. In our own research, the growth rate of the calves from the control group may have been further affected by the occurrence of diarrhea, but also rhinitis and coughing, indicating the presence of lowered immunity. This leads to increased nutritional requirements to fight the disease but may, at the same time, cause a lower willingness to take food, which confirms results obtained by Stanton et al., Windeyer et al., and Renaud et al. [43,85,86].

4.4. Quality of Veal

Increasing consumer awareness of food is of great importance in shaping the highest possible quality, which, in turn, has a decisive role in the nutritional value of the raw material. Veal is considered by many to be a delicatessen meat, high in protein and fat, and rich in bioactive compounds. The content of individual fractions depends on factors such as genotype and environment, i.e., nutritional health status and stress. According to Domaradzki et al. [28], the average protein content of veal is at a level of 20.7–23.3%. In our study, the protein content was 31.24 g in the control group, and 33.4 g in the experimental group; a 31.24–33.4% protein content in meat, is a very high value, representing very high meat quality. Bittante et al. [87] obtained definitively lower values in their study on calves derived from crossbreeding dairy breeds with meat breeds, obtaining values of 20.70–22.20%. In addition to the amount of protein in the meat, intramuscular fat content and fatty acid profile play a very important role, despite their fairly low values in the meat's composition [88,89].

According to Domaradzki et al. [28], in their study, the intramuscular fat content varied between 0.4% and 2.5%; while in our study the intramuscular fat content was 1.85 g in the control group and 1.47 g in the experimental group, with a total fat content of 2.01 g in the control group and 1.88 g in the experimental group. Calves in the experimental group were characterized by lower total and, thus, lower intramuscular fat content compared to the control group. In contrast, Bittante et al. [87] obtained results of 1.97–4.32%. Intramuscular fat plays a rather important role in shaping the quality of veal. In most cases, SFAs in meat have a dominant role over MUFA and PUFA groups. As indicated by Domaradzki et al. [28], they make up from 33.99% to 52.4% of fatty acids, while MUFAs make up 29.38–51.00%, and PUFAs 5.35–30.80%. In our study, the SFA content was 37.15%, MUFA 36.35%, and PUFA 9.67%, meaning that the values are similar to previously published works.

The predominant role in veal SFAs is played by C16:0 and C18:0, while for MUFAs it is C18:1 cis9, and C6:1 cis9, and for PUFAs C18:2 n-6, C20:4 n-6, as confirmed by our own studies [81,85,90,91].

4.5. Oxidative Stability

A number of biochemical processes take place in meat after slaughter, leading to meat maturation. According to Ripoll et al. [92], producing high-quality products should be the goal of producers, so it is important that the raw materials obtained have adequate oxidative stability [93]. Among the parameters affecting oxidative stability, we can mention meat color, which directly affects its appearance, and is one of the first factors in determining the consumer's choice of meat.

Meat color depends on a number of conditions, starting with pre-slaughter factors that affect the post-slaughter factors including oxidative stress and myoglobin content. In our study, the myoglobin content in the control group was at a higher level than in the experimental group, but the differences between the groups were small—which is a desirable phenomenon. During the maturation process, the color of meat may change due to progressive oxidation processes, which can lead to meat spoilage [93]. Oxidation products are formed during the oxidation of lipids and proteins, indicating a loss of meat quality. Results on oxidative stability are very important from the point of view of the consumer, so there are many results for the most popular types of meat, such as pork, beef, and poultry meat, while for veal there are a very few reports in which this topic has been addressed.

In a study by Lušnic Polak et al. [93], the value of the L* parameter 24 h after slaughtering was at a similar level (48.36) to that in our own study (46.25–46.13), and similar to values obtained by Bittante et al. [87]. While in the case of the a* and b* parameters, the values were significantly lower (a* 11.79 and b* 2.80 [93] and a* 7.40b*–8.2 and a* 13.60b*–14.20 [87]) than in our own study (a* 18.35–18.02 and b* 16.14–12.60, respectively). On the seventh day of meat maturation, the parameters in the study by Lušnic Polak et al. [93] increased while in our own study the values decreased slightly. As Henriott et al. [94] points out, a decrease in values may favorably indicate the meat's adequate oxidative stability and a low oxidation value for myoglobin. The obtained eigenvalues differ from the results published by Florek et al. [90] and Vitale et al. [95].

Another parameter indicating the oxidative stability of meat in our study was the change in TBARS values for malondialdehyde (MDA). In the case of MDA, the values on the day of slaughter were lower in the experimental group (0.22 mg) than in the control group (0.82 mg). During the 7-day meat storage, MDA values in both groups increased—to a level of 3.73 mg in the experimental group, while in the control group to 4.55 mg. Higher TBARS values indicate higher levels of oxidation, but as Penko et al. [96] points out, this does not always change sensory characteristics. Meat with high TBARS values is not desirable, therefore, as indicated by Clausen et al. [97], the best solution is to vacuum package the meat, which protects the meat from the oxygen present in the atmosphere.

5. Conclusions

In summary, this study sheds light on the implications of rearing methods on various aspects of calf health and behavior. The presence of foster cows in the rearing environment emerged as a favorable factor, leading to improved calf health outcomes. Specifically, calves reared alongside foster cows exhibited reduced rates of diarrhea, delayed instances of coughing, and a diminished occurrence of rhinitis when compared to conventionally reared counterparts confined to pens. Behavioral observations unveiled distinctions in sucking and licking behaviors between the two groups. Calves with foster cows displayed a more consistent pattern of these behaviors, while conventionally reared calves exhibited greater variability. Moreover, this research underscores that calves reared alongside foster cows consistently attained higher daily weight gains. This finding implies the potential for larger and more valuable carcasses upon slaughter. Consequently, the rearing system involving foster cows presents notable advantages in terms of both calf health and growth. Notably, the study did not reveal significant disparities in the fatty acid composition, color attributes, or myoglobin levels of veal between the two rearing groups, implying that meat quality remained consistent. In light of these findings, this research encourages the exploration and adoption of rearing systems that prioritize calf health, behavior, and growth, underpinned by maternal care and natural behaviors. Such endeavors hold promise for enhancing the well-being of calves and the sustainability of the meat production industry. Additionally, the incorporation of foster cows into dairy farming practices may represent a pragmatic and effective approach to advancing calf rearing protocols.

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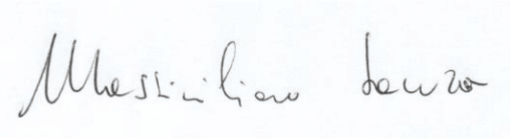

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The individual contribution to the publication was 5%

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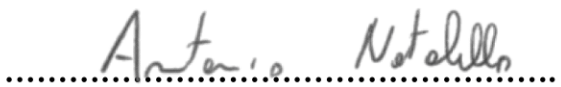

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