

CATHL2 polymorphism and milk production and reproductive traits in dairy cattle

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Cathelicidins are a group of host defense proteins that exhibit antibacterial, antiviral and immunomodulating properties, while they also promote wound healing and participate in cell proliferation and angiogenesis. Cathelicidins have been detected in many animal species, including cattle, in which 8 genes coding for these proteins were identified. Moreover, in cows these proteins are involved in the response to mastitis. The aim of this study was to estimate associations between polymorphism in the *CATHL2* gene in the 807 *G>A* position and some milk production traits and reproductive traits. The study included 539 Polish Holstein-Friesian Black-and-White cows. The ACRS and PCR-RFLP method was used to identify genotypes. The *CATHL2/MseI* frequencies were as follows: *GG* – 0.644, *GA* – 0.311 and *AA* – 0.045. In this study, no statistically significant associations between *CATHL2* genotypes and the analyzed traits were found; however, a tendency to maintain a relationship of genotypes with milk production traits and reproductive indicators is shown.

KEY WORDS: cathelicidin / dairy cattle / *CATHL2* / polymorphism /
milk production / reproductive traits

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Cathelicidins are a major class of short cationic proteins, which are part of the immune system [Cristelo *et al.* 2021, Li *et al.* 2021, van Harten *et al.* 2022]. Together with defensins they constitute a group of host defense peptides (HDP) [Young-Speirs *et al.* 2018]. Inactive precursors are stored in neutrophilic granules and mast cells, with cathelicidines being degranulated and released under the influence of pathogens, e.g. after detection of lipopolysaccharide (LPS). During the activation of these peptides the protein structure is shortened and its final form has 12-80 amino acid residues [Petkovic *et al.* 2021, Young-Speirs *et al.* 2018]. They have antibacterial [van Harten *et al.* 2018], antiviral, antifungal and antiparasitic properties [Petkovic *et al.* 2021, Young-Speirs *et al.* 2018]. However, among their functions these proteins play an important role in angiogenesis, cell proliferation, wound healing, chemotactic ability and regulation of the immune response [Mylonas *et al.* 2021, Petkovic *et al.* 2021]. Cathelicidins have been detected in many species of mammals, birds, reptiles, and amphibians [van Harten *et al.* 2018]. Moreover, 10 cathelicidin genes have been detected in cows, while only one in humans and mice, hence these proteins are considered to be crucial for the immune response of cows [Li *et al.* 2021]. In cattle there are at least eight cathelicidin genes located at the *CATHL* locus on the long arm of chromosome 22q24, with each gene consisting of four exons and three introns. The expression of precursor cathelicidines in cattle takes place mainly in the bone marrow cells, lymphoid tissue, and the mammary gland, but also in the small intestine [Young-Speirs *et al.* 2018]. Cathelicidins are involved in the defense response during mastitis in cows [Li *et al.* 2021].

Cattle performance traits include, among other things, milk yield, fat and protein contents. Milk production and its high quality are key features of cows influencing the profitability of dairy herds. Milk yield traits are related to quantitative traits, which are controlled by environmental and genetic factors [Strzałkowska *et al.* 2009, Jóźwik *et al.* 2010ab, Liu *et al.* 2020]. Therefore, suitable candidate genes are currently being searched for. The dairy industry invested enormous resources to identify polymorphisms and select QTLs that would increase the efficiency and limit losses of dairy cattle and milk production [Liu *et al.* 2020].

In high-yielding dairy cows, during the early lactation energy absorption capabilities are reduced [Cardoso Consentini *et al.* 2021]. The consequence is a negative energy balance, that is compensated by the absorption of energy from fat tissues [Schmitz *et al.* 2018]. In order to prevent a negative energy balance, cows mobilize fat reserves from adipose tissue. This causes an increase in the content of non-esterified fatty acids in the blood and leads to an increase in the amount of ketone bodies causing ketosis [Bekuma and Galmessa 2019]. Moreover, early lactating cows develop insulin resistance, which aims to maintain high milk yield and increases the availability of glucose in the mammary gland. This reduces the availability of glucose for the muscles. In the early stage of lactation subacute rumen acidosis (SARA) may occur due to disturbance of the nutritional balance, which leads to a decrease in rumen pH [Schmitz *et al.* 2018]. The energy balance has a direct impact on metabolic

changes as well as pathways related to immune functions. This affects susceptibility to the disease, including mastitis [Wrzecińska *et al.* 2021].

A particularly important aspect in large-scale dairy cattle breeding is the assessment and monitoring of reproductive indicators [Pérez-Méndez *et al.* 2020]. Cow reproduction is a pivotal factor influencing the profitability of dairy farming and is important for the economic efficiency of dairy, as it affects continuity of milk production and the length of calving intervals, while also facilitating genetic progress [Lonergan and Sánchez 2020]. To achieve sustainable livestock production, the reproductive potential should be increased without disturbing animal welfare. This potential can be exploited by the implementation of reproductive biotechnology techniques, such as oestrus synchronization, semen analysis, or artificial insemination [Hufana-Duran and Duran 2020, Kaya *et al.* 2018]. Reproductive disturbances can lead to the lowering of milk production, culling of cows and an overall decline in farm profitability [Gussmann *et al.* 2019, Lonergan and Sánchez 2020]. Reproductive traits are influenced by both environmental factors [Eetvelde *et al.* 2017] and genetic predisposition.

It is important to constantly monitor the reproductive indicators of cattle, which are closely related to lactation and milk production. Analysis using molecular techniques in the field of genetics facilitates the search for dependence between the genetic variant of a cow and performance traits.

The aim of this research was to conduct preliminary studies upon the analysis of polymorphism in the *CATHL2* gene in the *G>A* 807 position with reference to the characteristics of milk and reproductive traits of the Polish Holstein-Friesian breed.

Material and methods

Animal source and sample collection

The research included 539 Polish Holstein-Friesian Black-and-White cows. Animals were kept in one herd located in western Poland. All subjects were kept under similar environmental conditions. Cows were fed as standard, and in spring and summer they were kept in pastures. Milking was carried out twice a day using a mechanical milking machine. The herd performance was assessed using the A4 – method. Data on the milk performance traits and reproductive indicators of cows originated from breeding documentation conducted as part of the milk performance assessment. The following traits were recorded: daily milk yield, protein yield and content, fat yield and content, calving interval, open days, pregnancy, dry period, postpartum resting period, period of insemination service and insemination index.

Peripheral blood collected from each individual from the zygomatic vein to vacuum tubes containing the anticoagulant agent K3EDTA was used to isolate DNA. Genomic DNA was isolated using a commercial DNA isolation kit (Genomic Mini Purification Kit, A&A Biotechnology) according to the manufacturer's instructions.

Amplification, genotyping, and statistical analyses

The studied polymorphism is located in exon 1 of the *CATHL2* gene at position 807 (*rs110278258*).

Genotyping was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR primers were designed based on the *CATHL2* gene sequence available in GenBank (EU380692) including amplification created restriction site (ACRS) modification in the forward primer sequence at position 805 C→T. Table 1 shows the used primer sequences, PCR conditions, PCR product size, restriction enzyme and size of fragments after restriction endonuclease digestion.

Table 1. Primer sequences, PCR conditions, PCR product size, restriction enzyme and size of fragments after restriction endonuclease digestion

Primers (mismatched nucleotide underlined)	F: GAG CTA GAC CCT ACA CCC <u>AT</u> R: AGG CTC ACC CCA TTC TCC TTG AA
PCR conditions	
initial denaturation	5 minutes at 95 °C
denaturation	30 cycles : 30 seconds at 94 °C
annealing	30 cycles : 45 seconds at 51 °C
extension	30 cycles : 30 seconds at 72 °C
final extension	8 minutes at 72 °C
Product size	253 bp
Restriction enzyme	<i>MseI</i>
Size of fragments after digestion	GG: 253 bp GA: 253, 233, 20 bp AA: 233, 20 bp

After incubation the fragments were separated in a 3% agarose gel. Based on the molecular results, the frequencies of genotypes and alleles were determined.

Means and standard deviations were calculated.

The following linear model was used for the studied association of genotypes with recorded traits:

$$y_{ijkl} = \mu + s_i + L_j + P_k + e_{ijkl}$$

where:

s_i – the fixed effect of *i*-th calving season;

L_j – the fixed effect of *j*-th lactation $j = 1, 2, 3, 4$;

P_k – the fixed effect of *k*-th genotype;

e_{ijkl} – a random error connected with *ijkl*-th observation.

Statistical inferences were based on the Fisher-Snedecor (analysis of variance) and the Duncan multiple range test. These computations were performed using the Statistica 12 PL software [Statsoft INC., 2013].

Results and discussion

In this study, where the fragment of the *CATHL2* gene was analyzed, three genotypes were found: *GG* (253 bp), *GA* (253, 233, 20 bp) and *AA* (233, 20 bp). Frequencies of genotypes and alleles are presented in Table 2.

The effect of nucleotide substitution on selected milk yields of cows was analyzed. Table 3 presents the milk yield of cows in subsequent 305-day lactations depending on the genetic variant of *CATHL2*. There were no statistically significant differences; however, some trends were observed between the genotype and the analyzed traits.

Table 2. Genotype and allele frequencies of *CATHL2/MseI* polymorphism

Numbers of cows	Genotype frequencies		Allele frequencies	
347	<i>GG</i>	0.644	<i>G</i>	0.800
168	<i>GA</i>	0.311	<i>A</i>	0.200
24	<i>AA</i>	0.045		

Table 3. Means and standard deviations of studied traits in references to genotypes

Genotype	Number of cows	Milk yield (kg)	Fat yield (kg)	Fat content (%)	Protein yield (kg)	Protein content (%)
			1st lactation			
<i>GG</i>	347	9342 (1373)	358.80 (57.30)	3.86 (0.45)	320.10 (59.90)	3.44 (0.48)
<i>GA</i>	168	8973 (1632)	345.52 (62.750)	3.88 (0.45)	306.98 (66.91)	3.43 (0.51)
<i>AA</i>	24	9210 (1515)	355.08 (53.90)	3.89 (0.48)	322.62 (51.90)	3.51 (0.21)
			2nd lactation			
<i>GG</i>	346	10730 (1921)	419.90 (78.30)	3.94 (0.46)	371.50 (62.40)	3.48 (0.23)
<i>GA</i>	168	10759 (1845)	422.40 (74.20)	3.95 (0.48)	371.50 (58.20)	3.47 (0.24)
<i>AA</i>	24	10258 (1325)	400.20 (73.70)	3.91 (0.59)	365.60 (43.00)	3.58 (0.22)
			3rd lactation			
<i>GG</i>	302	11419 (7137)	453.50 (276.00)	3.99 (0.47)	391.10 (248.70)	3.44 (0.26)
<i>GA</i>	150	11165 (1677)	441.60 (83.40)	3.97 (0.58)	379.20 (59.80)	3.41 (0.36)
<i>AA</i>	22	10931 (1641)	440.30 (83.00)	4.03 (0.49)	375.60 (52.10)	3.45 (0.21)
			4th lactation			
<i>GG</i>	206	10893 (1796)	430.90 (80.30)	3.97 (0.47)	370.50 (54.70)	3.42 (0.23)
<i>GA</i>	103	10839 (1936)	426.10 (86.90)	3.95 (0.49)	369.70 (61.10)	3.43 (0.25)
<i>AA</i>	16	10779 (1548)	428.50 (69.50)	3.99 (0.50)	367.30 (48.90)	3.42 (0.21)

p \geq 0.05 – differences between mean values were statistically non-significant.

Table 4. Averages and standard deviations of selected reproductive indicators in the tested cow population, taking into account *CATHL2/MseI* polymorphism

Traits	<i>CATHL2/MseI</i>		
	<i>GG</i>	<i>GA</i>	<i>AA</i>
Intercalving period (days)	496.00 (191.76)	487.00 (182.36)	496.00 (192.21)
Interpregnancy period (days)	124.00 (87.70)	123.00 (88.00)	133.00 (77.39)
Pregnancy (days)	279.89 (23.07)	279.16 (16.17)	277.40 (12.79)
Dry period (days)	73.94 (36.63)	72.57 (28.25)	77.33 (30.87)
Postpartum resting period (days)	58.63 (18.94)	60.24 (19.00)	57.25 (19.02)
Period of insemination service (days)	63.02 (79.79)	60.92 (71.65)	75.93 (84.43)
Insemination index	3.03 (2.44)	2.91 (2.13)	3.15 (2.23)

p \geq 0.05 – differences between mean values were statistically non-significant.

The potential relationships between *CATHL2/MseI* polymorphism and recorded reproductive indicators in the studied herd were also analyzed (Tab. 4).

Associations between the genotypes and milk production traits were not statistically significant. Milk, protein, and fat yield (kg) were compared in each lactation where no effect of the animal genotype was found. Protein and fat contents (%) was similar for three genotypes: *AA*, *AG* and *GG* in each lactation.

There were no statistically significant differences; however, some trends were observed. Duration of the parturition interval was the shortest in heterozygous cows, whereas in homozygous *GG* and *AA* individuals there was the same number of days. The parturition interval was similar in the case of cows with genotypes *GG* and *GA*, while in animals with genotype *AA* it was slightly prolonged. The pregnancy length was almost the same for genotypes *GG* and *GA*, while in cows with genotype *AA* the length was slightly shortened. It was observed that in the case of the drying period length the animals with the *AA* genotype were characterized by a prolonged time in relation to animals of genotypes *GG* and *GA*, in which the drying time was similar. *GA*-genotype animals had a slightly extended postnatal resting time relative to the other genotypes analyzed. In the case of insemination period a significant extension was observed in the case of animals with genotype *AA* in relation to cows of the genotype *GG* and *GA*, which had the shortest insemination period. Results related to the insemination index are the same, because the highest value was recorded in cows with genotype *AA*, while the lowest in cows with genotype *GA*.

To link the dependence between polymorphism and milk performance traits, a number of studies on genes encoding proteins of the so-called somatotropic axis were undertaken [Habel and Sundrum 2020, Haubold *et al.* 2020]. Among them, insulin-like growth factor type 1 (IGF-1) can be distinguished [Habel and Sundrum 2020]. In the case of IGF-1 there are some dependencies in connection with cathelicidins. IGF-1 is a factor that induces the production of cathelicidins [Chieosilapatham *et al.* 2018]. It can therefore be assumed that cathelicidins are also indirectly related to the milk yield of cows [Seo *et al.* 2016]. IGF-1 is one of the main regulators of growth and metabolism after birth. Its key role can be observed in the development of the mammary gland, control of lactation and reproduction in cattle [D'Occhio *et al.* 2019, Habel and Sundrum 2020, Sharmin *et al.* 2021]. IGF-1 influences the proliferation of mammary gland cells [Cohick 2022, Sharmin *et al.* 2021] and may increase the proliferation of epithelial cells lining the mammary gland [Cohick 2022]. Cathelicidins also have properties associated with stimulation of cell proliferation [Cristelo *et al.* 2021].

During the analysis no statistically significant differences were found. However, it can be seen that in the 2nd lactation the highest values of milk yield, fat yield and fat content were observed for cows with genotype *GA*. In turn, the *AA* genotype affected the highest fat content (1st, 3rd and 4th lactation) and the highest protein content (1st, 2nd and 3rd lactation).

In the case of reproductive indices the following traits were analyzed: calving interval, parturition interval, length of pregnancy, duration of the drying period, period of insemination service and insemination index.

The calving interval should be between 360 and 400 days [Dalcq *et al.* 2018]. In this study the average calving interval was 493 days, which is a considerable deviation from the assumed norm. In the case of high-yield cows a fertility problem can be seen. They are a consequence of high efficiency in the last weeks of lactation, which results in problems with drying [Mezzetti *et al.* 2020]. Drying period should last about 40-60 days [Pascottini *et al.* 2020]. In this study an average drying period of nearly 75 days has been obtained on the basis of analysis. Extending the drying period can lead to the exposure to mastitis [Khalil and Hussein 2019].

The search for the relationships between mastitis and genetic predispositions were conducted by Szyda *et al.* [2019]. According to Bates and Dohoo [2016], Oprzadek *et al.* [2018] and Welderufael *et al.* [2017], there is a relationship between the Holstein-Friesian breed and susceptibility to mastitis, and the research results show that the greater the share of this breed in the genotype of animals, the greater the risk factor for mastitis susceptibility. Mastitis is an infectious disease responsible for economic losses in dairy farms. It is inflammation of the mammary gland which occurs as a result of infection with pathogenic microorganisms or trauma to the teat [Pascu *et al.* 2022]. This disease results in a reduction in milk yield and quality of milk and may also reduce the fertility of cows [Ashraf and Imran 2020]. Mastitis has an impact on embryo mortality and abortion in dairy cattle, which may be due to the immune response [Dahl *et al.* 2017, Dalanezi *et al.* 2020]. Moreover, mastitis lowers the conception rate, disrupts the development of oocyte and embryos [Dalanezi *et al.* 2020]. Many specific and non-specific mechanisms are responsible for the natural protection of the mammary gland against mastitis. Initial protection against pathogens is ensured by the continuity of the teat skin, as well as the secretion of fatty acids through the skin, which are bacteriostatic. On the other hand, closing the teats after milking by the muscles of the teats inhibits the penetration of bacteria. The presence of microflora in the teat and milk ducts of cow's udder, which protects against pathogens, is found. In turn, contact with pathogens is followed by the production of inflammatory mediators, i.e. cytokines, chemokines and β -defensin [Katsafadou *et al.* 2019]. Moreover, after contact with pathogens in the udder cells cathelicidin is synthesized, which is produced under the influence of leukocytes flowing into the mammary gland. Cathelicidin is also secreted by neutrophils, which neutralizes the bacteria in the teat [Cubeddu *et al.* 2017, Katsafadou *et al.* 2019]. In the epidermis cathelicidins are secreted, which affects cell proliferation and wound healing. However, in research conducted by the authors of this study no association of the *CATHL2* gene genotype was observed with a possible shortening of the drying period, and at the same time a reduction in susceptibility to mastitis.

In the case of the parturition interval the optimum length is 110-130 days [Miciński *et al.* 2010]; however, in high-performance cows Krzyżewski *et al.* [2004]

determined the average length of the parturition interval for 111 days, while stating that extending this period up to 160 days does not bring any adverse effects on milk yield. In the conducted studies the length of the parturition interval was determined to be on average 127 days.

Post-partum resting period in the examined herd amounted to 60 days on average. However, according to Borkowska *et al.* [2012], the rest period should be extended to min. 90 days in high-yielding cows in order to preserve the proper functioning of the reproductive system.

When analyzing the average duration of an insemination service, one can notice a significant extension for all tested genotypes in the authors' studies. This period amounted to an average of 71 days, which significantly deviates from the assumed standards; however, it was noted that the period of insemination service for high-performance cows was extended. The insemination index should be in the range of 1.7-2.5, while above 2.5 it is considered unfavorable. When analyzing the insemination index in the conducted studies it can be seen that its average value of around 3.0 is excessively high. However, the relationship between a high insemination index and high milk yield was described by researchers [Cardoso Consentini *et al.* 2021, Marumo *et al.* 2022].

Conclusion

Summing up the obtained research results, trends can be noticed and confirmed indicating deterioration in dairy cattle breeding indices at the expense of high milk yield. In the conducted analyses there were no statistically significant differences in the associations between *CATHL2* gene polymorphism and milk performance traits; however, some promising trends were observed. However, the research is preliminary in nature and knowledge related to the analysis of genetic predisposition in relation to the characteristics of milk yield and reproductive indicators should be broadened in order to find possible ways to obtain cows with high milk yield, which will not affect the reproductive capacity of cows in the future.

Ethical statement. *The study was conducted according to the Resolution No. 13/2016 of National Ethics Committee for Experiments on Animals (Poland). All animals used in this study were handled in strict accordance with good practices following EU legislation (Directive 2010/63/EU).*

Data availability. *The data from this study can be accessed from the authors upon a reasonable request.*

Author contributions. *SH and IK designed the study, conduct analysis and interpretation and wrote the paper. SH, IK, ECP and DB performed the statistical analysis. SH and MW reviewed and edited the paper.*

Competing interests. *The authors declare that they have no conflict of interest.*

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REFERENCES

1. ASHRAF A., IMRAN M., 2020 – Causes, types, etiological agents, prevalence, diagnosis, treatment, prevention, effects on human health and future aspects of bovine mastitis. *Animal Health Research Reviews* 21(1), 36-49.
2. KHALIL A. A. Y, HUSSEIN M. M., 2019 – A retrospective study on the relationship among different dry period lengths, udder health status and their possible effects on the reproductive performance of Holstein-Friesian cows. *Journal of Veterinary Medical Research* 26(2), 186-199.
3. BATES A.J., DOHOO I., 2016 – Risk factors for peri-parturient farmer diagnosed mastitis in New Zealand dairy herds: findings from a retrospective cohort study. *Preventive Veterinary Medicine* 127, 70-76.
4. BEKUMAH A., GALMESSA U. G., 2019 – Combating Negative Effect of Negative Energy Balance in Dairy Cows: Comprehensive Review. *Approaches in Poultry, Dairy & Veterinary Sciences* 6(2), 1-4.
5. BORKOWSKA D., PIĄTEK D., JANUŚ E., MUCHA J., 2012 – Fertility indices of cows in a high-yielding herd. *Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego* 8(3), 21-29.
6. CARDOSO CONSENTINI C.E., WILTBANK M.C., SARTORI R., 2021 – Factors That Optimize Reproductive Efficiency in Dairy Herds with an Emphasis on Timed Artificial Insemination Programs. *Animals* 11, 301, 1-30.
7. CHIEOSILAPATHAM P., IKEDA S., OGAWA H., NIYONSABA F., 2018 – Tissue-specific Regulation of Innate Immune Responses by Human Cathelicidin LL-37. *Current Pharmaceutical Design* 24(10), 1079-1091.
8. COHICK W.S., 2022 – The role of the IGF system in mammary physiology of ruminants. *Domestic Animal Endocrinology* 79, 106709, 1-8.
9. CRISTELO C., MACHADO A., SARMENTO B., GAMA F.M., 2021 – The roles of vitamin D and cathelicidin in type 1 diabetes susceptibility. *Endocrine Connections* 10(1), R1-R12.
10. CUBEDDU T., CACCIOTTO C., PISANU S., TEDDE V., ALBERTI A., PITTAU M., DORE S., CANNAS A., UZZAU S., ROCCA S., ADDIS M.F., 2017 – Cathelicidin production and release by mammary epithelial cells during infectious mastitis. *Veterinary Immunology and Immunopathology* 189, 66-70.
11. DAHL M.O., MAUNSELL F.P., DE VRIES A., GALVAO K.N., RISCO C.A., HERNANDEZ J.A., 2017 – Evidence that mastitis can cause pregnancy loss in dairy cows: A systematic review of observational studies. *Journal of Dairy Science* 100(10), 8322-8329.
12. DALANEZI F.M., JOAQUIM S.F., GUIMARÃES F.F., GUERRA S.T., LOPES B.C., SCHMIDT E.M.S., CERRI R.L.A., LANGONI H., 2020 – Influence of pathogens causing clinical mastitis on reproductive variables of dairy cows. *Journal of Dairy Science* 103(4), 3648-3655.
13. DALCQ A.C., BECKERS Y., MAYERES P., REDING E., WYZEN B., COLINET F., DELHEZ P., SOYEURT H., 2018 – The feeding system impacts relationships between calving interval and economic results of dairy farms. *Animal* 12(8), 1662-1671.
14. D'OCCHIO M.J., BARUSELLI P.S., CAMPANILE G., 2019 – Influence of nutrition, body condition, and metabolic status on reproduction in female beef cattle: A review. *Theriogenology* 125, 277-284.

15. EETVELDE M.V., HERAS S., LEROY J.L.M.R., SOOM A.V., OPSOMER G., 2017 – The Importance of the Periconception Period: Immediate Effects in Cattle Breeding and in Assisted Reproduction Such as Artificial Insemination and Embryo Transfer. *Periconception in Physiology and Medicine. Advances in Experimental Medicine and Biology* 1014, 41-68.
16. GUSSMANN M., DENWOOD M., KIRKEBY C., FARRE M., HALASA T., 2019 – Associations between udder health and culling in dairy cows. *Preventive Veterinary Medicine* 171, 104751, 1-7.
17. HABEL J., SUNDRUM A., 2020 - Mismatch of Glucose Allocation between Different Life Functions in the Transition Period of Dairy Cows. *Animals* 10(6), 1028, 1-21.
18. HAUBOLD S., KRÖGER-KOCH C., TUCHSCHERER A., KANITZ E., WEITZEL J.M., HOEFLICH A., STARKE A., TRÖSCHER A., SAUERWEIN H., HAMMON H.M., 2020 – Effects of a combined essential fatty acid and conjugated linoleic acid abomasal infusion on metabolic and endocrine traits, including the somatotrophic axis, in dairy cows. *Journal of Dairy Science* 103(12), 12069-12082.
19. HUFANA-DURAN D., DURAN P.G., 2020 – Animal reproduction strategies for sustainable livestock production in the tropics. IOP Conference Series: Earth and Environmental Science 492, 012065, 1-17.
20. JÓŹWIK A., BAGNICKA E., KRZYŻEWSKI J., HORBAŃCZUK K., PYZEL B., SŁONIEWSKA D., HORBAŃCZUK J.O., 2010a – The concentration of free fatty acids in goat milk as related to the stage of lactation, age and somatic cell count. *Animal Science Papers and Reports* 28, 4, 389-396.
21. JÓŹWIK A., STRZAŁKOWSKA N., BAGNICKA E., ŁAGODZIŃSKI Z., PYZEL B., CHYLIŃSKI W., CZAJKOWSKA A., GRZYBEK W., SŁONIEWSKA D., KRZYŻEWSKI J., HORBAŃCZUK J.O., 2010b – The effect of feeding linseed cake on milk yield and milk fatty acid profile in goats. *Animal Science Papers and Reports* 28, 3, 245-251.
22. KATSAFADOU A.I., POLITIS A.P., MAVROGIANNI V.S., BARBAGIANNI M.S., VASILEIOU N.G.C., FTHENAKIS G.C., FRAGKOU I.A., 2019 – Mammary Defences and Immunity against Mastitis in Sheep. *Animals* 9(10), 726, 1-17.
23. KAYA A., GÜNEŞ E., MEMİLİ E., 2018 – Application of reproductive biotechnologies for sustainable production of livestock in Turkey. *Turkish Journal of Veterinary and Animal Sciences* 42, 143-151.
24. KRZYŻEWSKI J., STRZAŁKOWSKA N., REKLEWSKI Z., DYMNIKI E., RYNIOWICZ Z., 2004 – Wpływ długości okresów międzyciążowych u krów rasy hf na wydajność, skład chemiczny mleka oraz wybrane wskaźniki reprodukcji (Influence of calving interval length in HF cows on milk yield, its composition and some reproduction traits). In Polish with English summary. *Medycyna Weterynaryjna* 60(1), 76-79.
25. LI Y., MA X., YANG J., WU X., YAN Z., HE B., 2021 – Expression Pattern of Cathelicidins in Dairy Cows During Endometritis and Role of Bovine Endometrial Epithelial Cells in Production of Cathelicidins. *Frontiers in Veterinary Science* 8, 675669, 1-11.
26. LIU L., ZHOU J., CHEN C.J., ZHANG J., WEN W., TIAN J., ZHANG Z., GU Y., 2020 – GWAS-Based Identification of New Loci for Milk Yield, Fat, and Protein in Holstein Cattle. *Animals* 10(11), 2048, 1-15.
27. LONERGAN P., SÁNCHEZ J.M., 2020 – Symposium review: Progesterone effects on early embryo development in cattle. *Journal of Dairy Science* 103(9), 8698-8707.
28. MARUMO J.L., LUSSEAU D., SPEAKMAN J.R., MACKIE M., HAMBLY C., 2022 – Influence of environmental factors and parity on milk yield dynamics in barn-housed dairy cattle. *Journal of Dairy Science* 105(2), 1225-1241.

29. MEZZETTI M., MINUTI A., PICCIOLI-CAPPELLI F., TREVISI E., 2020 – Inflammatory status and metabolic changes at dry-off in high-yield dairy cows. *Italian Journal of Animal Science* 19(1), 51-65.
30. MICIŃSKI J., ZWIERZCHOWSKI G., BARAŃSKI W., GOŁĘBIEWSKA M., MARŚÁLEK M., 2010 – Locomotor activity and daily milk yield of dairy cows during the perioestrous period in successive lactations. *Journal of Agrobiolgy* 27(2), 111-119.
31. MYLONAS A., HAWERKAMP H., WANG Y., DEMARIA O., MELLER S., HOMEY B., DOMIZIO J.D., MAZZOLAI L., HOVNANIAN A., GILLIET M., CURDIN C., 2021 – Type I interferons link skin-associated dysbiotic commensal bacteria to pathogenic inflammation and angiogenesis in rosacea (preprint). In Review. <https://doi.org/10.21203/rs.3.rs-279140/v1>
32. OPRZĄDEK J.M., BRZOZOWSKA A.M., URTNOWSKI P., RUTKOWSKA K., LUKASZEWICZ M., 2018 – Association of BoLA-DRB3 genotype with somatic cell count in milk of Polish Holstein cattle. *Revista Brasileira Zootecnia* 47, 1-7.
33. PASCOTTINI O.B., LEROY J.L.M.R., OPSOMER G., 2020 – Metabolic Stress in the Transition Period of Dairy Cows: Focusing on the Prepartum Period. *Animals* 10(8), 1419, 1-17.
34. PASCU C., HERMAN V., IANCU I., COSTINAR L., 2022 – Etiology of Mastitis and Antimicrobial Resistance in Dairy Cattle Farms in the Western Part of Romania. *Antibiotics* 11(1), 57, 1-12.
35. PÉREZ-MÉNDEZ J.A., ROIBÁS D., WALL A., 2020 – Somatic cell counts, reproduction indicators, and technical efficiency in milk production: A stochastic frontier analysis for Spanish dairy farms. *Journal of Dairy Science* 103(8), 7141-7154.
36. PETKOVIC M., MOURITZEN M.V., MOJSOSKA B., JENSSEN H., 2021 – Immunomodulatory Properties of Host Defence Peptides in Skin Wound Healing. *Biomolecules* 11(7), 952, 1-15.
37. SCHMITZ R., SCHNABEL K., VON SOOSTEN D., MEYER U., SPIEKERS H., REHAGE J., DÄNICKE S., 2018 – The effects of energy concentration in roughage and allowance of concentrates on performance, health and energy efficiency of pluriparous dairy cows during early lactation. *Archives of Animal Nutrition* 72(2), 100-120.
38. SEO M., LEE H.-J., KIM K., CAETANO-ANOLLES K., JEONG J.Y., PARK S., OH Y.K., CHO S., KIM H., 2016 – Characterizing Milk Production Related Genes in Holstein Using RNA-seq. *Asian-Australasian Journal of Animal Sciences* 29(3), 343-351.
39. SHARMIN M.M., HAYASHI S., MIYAJI M., ISHIZAKI H., MATSUYAMA H., HAGA S., YONEKURA S., 2021 – Insulin-like growth factor-1 induces IRE1-XBP1-dependent endoplasmic reticulum biogenesis in bovine mammary epithelial cells. *Journal of Dairy Science* 104(11), 12094-12104.
40. Statsoft INC., 2013. , STATISTICA (data analysis software system). StatSoft, Inc.
41. STRZAŁKOWSKA N., JOŹWIK A., BAGNICKA E., KRZYŻEWSKI J., HORBAŃCZUK J.O., 2009 – Studies upon genetic and environmental factors affecting the cholesterol content ion cow milk. I: Relationship between the polymorphic form of beta-lactoglobulin, somatic cell count, cow age and stage of lactation and cholesterol content of milk. *Animal Science Papers and Reports*, 27, 2, 95-105.
42. SZYDA J., MIELCZAREK M., FRĄSZCZAK M., MINOZZI G., WILLIAMS J.L., WOJDAK-MAKSYMIEC K., 2019 – The genetic background of clinical mastitis in Holstein-Friesian cattle. *Animal* 13(10), 2156-2163.
43. VAN HARTEN R., VAN WOUDEBERGH E., VAN DIJK A., HAAGSMAN H., 2018 – Cathelicidins: Immunomodulatory Antimicrobials. *Vaccines* 6(3), 63, 1-23.
44. VAN HARTEN R.M., VELDHUIZEN E.J.A., HAAGSMAN H.P., SCHEENSTRA M.R., 2022 – The cathelicidin CATH-2 efficiently neutralizes LPS- and E. coli-induced activation of porcine bone marrow derived macrophages. *Veterinary Immunology and Immunopathology* 244, 110369, 1-8.

45. WELDERUFAEL B.G., JANSS L.L.G., DE KONING D.J., SØRENSEN L.P., LØVENDAHL P., FIKSE W.F., 2017 – Bivariate threshold models for genetic evaluation of susceptibility to and ability to recover from mastitis in Danish Holstein cows. *Journal of Dairy Science* 100(6), 4706-4720.
46. WRZECIŃSKA M., CZERNIAWSKA-PIĄTKOWSKA E., KOWALCZYK A., 2021 - The impact of stress and selected environmental factors on cows' reproduction. *Journal of Applied Animal Research* 49(1), 318-323.
47. YOUNG-SPEIRS M., DROUIN D., CAVALCANTE P.A., BARKEMA H.W., COBO E.R., 2018 – Host defense cathelicidins in cattle: types, production, bioactive functions and potential therapeutic and diagnostic applications. *International Journal of Antimicrobial Agents* 51(6), 813-821.