

The role of ketosis on the occurrence of mastitis and their mutual influence on milk yield in one lactation period in cows

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Abstract

Janevski, D., Radinović, M. & Krivokapić, J. (2026). The role of ketosis on the occurrence of mastitis and their mutual influence on milk yield in one lactation period in cows. *Bulg. J. Agric. Sci.*, 32(3), 723–727

Ketosis, as a primary metabolic disorder that occurs postpartum in cows, is a risk factor for the occurrence of mastitis as a secondary disease.

To prove the objectives of the conducted study, two groups of East Friesian cows were examined.

The first group included 104 cows with ketosis, of which 60 cows in correlation with ketosis developed mastitis. Further studies were conducted only on these 60 cows. Ketosis was proven by the method of enzymatic catalysis by determining beta-hydroxybutyric acid in blood serum. Mastitis was proven by determining the number of somatic cells and by identifying the type of microorganisms, which was carried out by applying the MALDI-TOF/SARAMIS™ platform using structural ribosomal proteins that are specific for each type of microorganism.

The second group was a control group of cows, which also consisted of 60 completely healthy cows for which milk yield data was regularly collected throughout the entire lactation period.

By isolating microorganisms from mastitic milk, the most common causative agent was *Staphylococcus aureus*, which was isolated in 40% of the samples, followed by *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Both diseases have a negative impact on milk yield, where a statistically significant difference is observed using analysis of variance ($p < 0.05$).

The analysis of the data in the conducted research showed a decline in milk yield, with a decrease in milk yield of 8.56 l/day, which is one quarter less than in a healthy population of cows for one lactation period.

Keywords: bacteria; ketosis; cows; mastitis; *Staphylococcus aureus*

Introduction

The peripartum period is the most critical period in cows, in which physiological adaptations to the newly emerged situation take place. These changes are particularly expressed during early lactation when providing for increased energy needs. Physiologically, cows have a reduced appetite on the one hand, while milk production initially amounts to 40 to 60% of the total amount of milk during the lactation period. These changes lead to the appearance of a negative energy

balance due to the increased utilization of glucose from the blood and low blood glucose values. For this period, it is estimated that one third of the energy used by cows for milk production is used from body reserves (Bellato et al., 2023).

When a negative energy balance occurs, the lipomobilization process intensifies with an insufficient amount of glycogenoplastic substances, whereby the newly formed relationship in intermediary metabolism results in the accumulation of acetyl-CoA, and intensification of the ketogenesis process. Oxaloacetate plays a key role in carbohydrate

metabolism because it condenses into acetyl-CoA during the citric acid cycle. If a large amount of acetyl-CoA is produced during the breakdown of fatty acids and the amount of oxaloacetate is reduced, the citric acid cycle stops and the resulting acetyl-CoA accumulates in the mitochondria of hepatocytes and is converted into ketone bodies (acetone, acetoacetate, and beta-hydroxybutyric acid) during the ketogenesis process. The resulting ketone bodies enter the blood and supply the heart, brain, skeletal muscles, and other organs with energy, where they also enter milk, resulting in ketosis (Berge and Vertenten, 2014).

Mastitis is an inflammation of the mammary gland characterized by pathological changes in the glandular tissue and the appearance of physical, chemical and bacteriological changes in the milk (Abebe et al., 2016).

The diagnosis of mastitis is initially made by determining the number of somatic cells, while the most reliable diagnosis is made by microbiological isolation and identification of the causative agent from mastitic milk. Bacteria are isolated in the largest percentage as the causative agents of mastitis, while fungi, yeasts, algae, molds and viruses are isolated in a smaller number of mastitic glands (Dingwell et al., 2003).

The aim of the conducted research is to prove the role and mutual influence of ketosis and mastitis on milk production in cows during a lactation period, as well as to determine the prevalence of pathogenic types of microorganisms responsible for the occurrence of mastitis.

Materials and Methods

This research was conducted on two groups of cows from one farm, which were raised under the same conditions and with the same diet. The cows are dairy cows of the East Friesian breed of cattle. The first group included $n = 104$ cows with ketosis, of which $n = 60$ cows subsequently developed mastitis. In the cows in which mastitis developed (60) in correlation with ketosis, clinical and laboratory examinations were performed during the lactation period, which referred to proving ketosis, proving mastitis, isolating the microbiological cause of mastitis, and daily collection of data on the milk yield of these cows. The second group of cows $n = 60$ were completely healthy cows, for which daily data on milk yield was collected for the duration of one lactation period. In the second part of the research, comparative analyses were performed for the two groups of cows in terms of milk yield, and by statistical processing of the data, the differences in milk yield between the studied groups of cows were determined.

The demonstration of ketosis was done by the method of enzymatic catalysis by determining beta-hydroxybutyric

acid in the blood serum. The examination was done using biochemical analyzers. Blood samples were taken from the sixth to the eighth day after calving, while the blood was taken by puncture of the v. coccygea. The cut-off value for determining subclinical ketosis is ≥ 1.2 mmol/L (Bellato et al., 2023).

Milk samples for mastitis testing were collected twice over a period of 60 to 90 hours, at the quarter level. Sampling for testing was done by taking milk in a sterilized tube in an amount of 40 ml, marked with the internal number and location of the quarter, the sample is taken from the entire milked and mixed milk suspected of mastitis (Gezehagn et al., 2020). Milk samples were not taken from cows that had been treated with certain types of antibiotics for various pathological conditions. For the examination, samples were taken from cows that had suffered from ketosis after calving and then had a secondary occurrence of mastitis (Almaw et al., 2008). The milk samples for examination were taken according to the recommendations set by the National Medical Commission. Disinfection of the udder buds was done using 70% Ethanol, for the samples that were used for microbiological examination (Abrahmsén et al., 2013). Then the samples were stored in a refrigerator at $+4^{\circ}\text{C}$ and transported to the microbiological laboratory, where bacteriological isolation was done by inoculating nutrient media (blood agar), with 0.02 ml of each sample inoculated onto plates that were then incubated at $+37^{\circ}\text{C}$, under aerobic conditions for 24 to 48 hours (Saidi et al., 2013).

For each sample, the location of the quarter and the serial number were marked. After incubation, each sample was individually counted and morphologically compared with the colonies and classified (Langhorne et al., 2023).

The identification of microorganisms was carried out using the MALDI-TOF/ SARAMIS™ platform, MALDI-TOF MS, which uses the structural ribosomal proteins that are specific for each M/O species, and SARAMIS™, which is a database that automatically identifies microorganisms based on their spectrum obtained from MALDI-TOF MS. Each microorganism has a unique spectrum with specific signals for the species, genus and family, and on the basis of which its identification is carried out (Sangiao et al., 2021).

The determination of the number of somatic cells in milk was carried out by first heating the milk to 40°C for 15 minutes and analyzing it with Fossomatic 6000 (Foss Electric, Denmark), while the counting of somatic cells was done according to an accredited method (Miller et al., 1986; Hisira et al., 2023).

The research and the provision of blood and milk samples from animals were approved by the ethics committee of the Ministry of Agriculture, Forestry, Waters and Veteri-

nary Administration of the Republic of Serbia, Belgrade, on 11.04. 2024, with number 001328298 2024 14841 002 001 323 022.

Results and Discussion

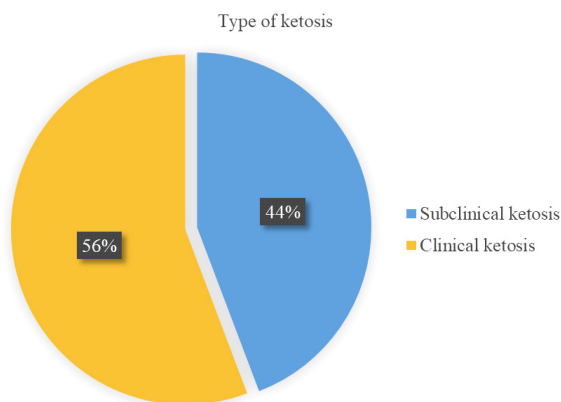


Fig. 1. Percentage of cows diagnosed with clinical and subclinical ketosis

Source: Authors' own elaboration

From Figure 1, it can be concluded that 56% or 58 cows developed clinical ketosis, while 44% or 46 cows were diagnosed with subclinical ketosis.

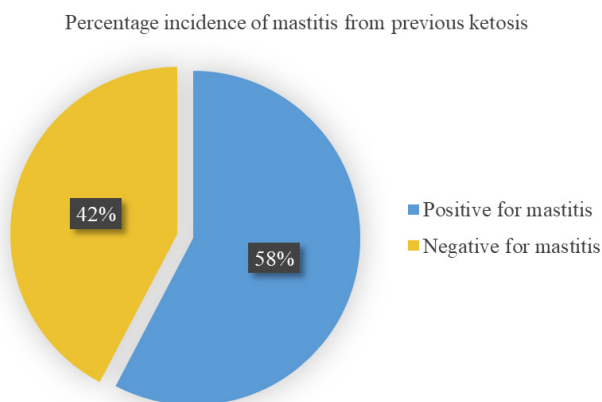


Fig. 2. Percentage of cows with mastitis out of the total number of cows with ketosis

Source: Authors' own elaboration

From Figure 2 it can be seen that out of 104 cows with ketosis, 58% or 60 cows developed secondary mastitis.

From Figure 3 it can be seen that 36 cows or 60% developed clinical mastitis, while 24 cows or 40% developed subclinical mastitis.

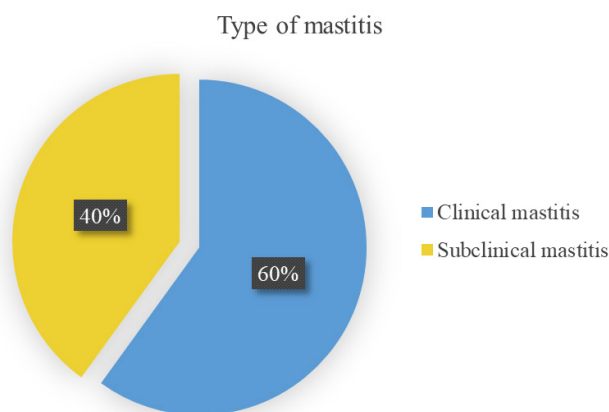


Fig. 3. Percentage of mastitis type in cows

Source: Authors' own elaboration

From Figure 4 it can be seen that in 40% or 24 cows, *Staphylococcus aureus* was isolated as the most common cause of mastitis, in 18% or 11 cows, *Streptococcus agalactiae* was isolated, *Streptococcus dysgalactiae* was isolated in 9 cows and accounted for 15% of the total percentage, *Streptococcus uberis* was represented by 12% and appeared in 7 cows, in 10% or 6 cows, the cause of mastitis was *Escherichia coli*, and *Pseudomonas aeruginosa* was represented by 5% or 3 cows, causing mastitis.

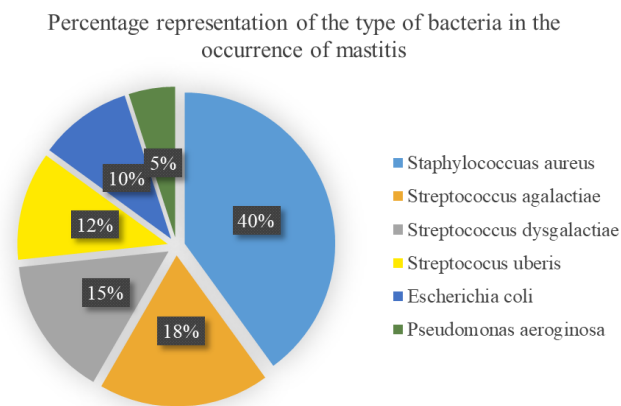


Fig. 4. Percentage of the type of bacteria that caused mastitis

Source: Authors' own elaboration

Table 1 shows the milk yield values of the group of cows with mastitis and the control group of healthy cows. The average milk yield in the control group was 30.45 l/day, while in the group of cows with mastitis it was 21.89 l/day, the minimum milk yield in the cows with mastitis was 8 l/day.

Table 1. Statistical presentation of the average milk yield during the lactation period

Group of cows	Average	Min	Max	Standard deviation	Coefficient of variation (%)
Control group	30.45	19.34	41.23	5.25	11.85
Cows with mastitis	21.89	8.00	29.00	5.32	23.17

Source: Authors' own elaboration

day, and the highest yield was 29 l/day, while in the control group the lowest daily yield was 19.34 l/day, while the highest daily milk yield was 41.23 l/day.

According to the values of the standard deviations and coefficients of variation, the variability of milk yield is significantly higher in the group of cows with ketosis and mastitis and amounts to 23.17% compared to the control group with healthy cows which amounts to 11.85%.

Table 2 shows the results of the application of the one-factor analysis of variance. A statistically significant difference ($p < 0.05$) was observed in milk yield in the two examined groups of cows.

From the statistical processing of the results, we determined a high degree of correlation between ketosis and mastitis, whereby in the conducted research, out of a total of 104 cows with ketosis, 60 cows or 58% developed secondary mastitis, which is confirmed by the data from (Bellato et al., 2023).

The percentage of mastitis in terms of its type was as follows. 36 cows or 60% were diagnosed with clinical mastitis, while 24 cows or 40% with subclinical mastitis. In terms of the microbiological cause, 6 types of bacteria were isolated from the mastitic glands as the causes of mastitis. The most common was *Staphylococcus aureus*, which was isolated from 24 cows and accounted for 40% of the total number of mastitis cases, which is fully consistent with the research conducted by Saidi et al. (2013), Vanhoudt et al. (2018) and Abebe et al. (2016), followed by *Streptococcus agalactiae* with 18%, which was isolated from 11 cows, while *Streptococcus dysgalactiae* was isolated from 9 cows and accounted for 15% of the total percentage, *Streptococcus uberis* was isolated from 7 cows with a participation of 12%, which partially coincides with the research conducted by (Langhorne et al., 2023), while *Escherichia coli* was isolated from 6 cows with a participation of 10%, *Pseudomonas aeruginosa* was represented by 5%, i.e. in 3 cows it caused mastitis, the percentage ratio of microbial causes partially

coincides with the results from (Dingwell et al., 2003). The research determined a high degree of impact on ketosis and mastitis on milk production in cows. The difference in average milk yield between the two groups indicates the large economic losses that occur with the occurrence of ketosis and the impact of mastitis is particularly pronounced, which also affects the further health status of the animal.

The average milk yield in the control group was 30.45 l/day, while in the group of cows with ketosis and mastitis it was 21.89 l/day, indicating a significant decrease in milk yield, which in terms of comparative research is most similar to the results of research by (Hagnestam et al., 2007).

The average milk yield in the control group was 30.45 l/day, while in the group of cows with mastitis it was 21.89 l/day, which indicates the impact of mastitis on milk yield with a decrease of almost a quarter in the average milk yield, which is confirmed by the values of the standard deviations and the coefficients of variation, with the variability of milk yield being significantly higher in the group of cows with ketosis and mastitis and it is 23.17% compared to the control group with healthy cows which is 11.85%.

By applying the one-factor analysis of variance, a statistically significant difference ($p < 0.05$) is observed in the milk yield of the two examined groups of cows, which is confirmed by the research conducted by (Dohoo and Martin, 1983).

Conclusion

In the study conducted, from the processing of the obtained results, we prove a high rate of association of postpartum ketosis with the occurrence of mastitis, which indicates the direct impact of immunosuppression that occurs during ketosis, which creates optimal conditions for the growth and development of infectious agents of mastitis.

From the results obtained when isolating microorganisms from mastitic milk, we confirmed the dominant role of

Table 2. ANOVA test for average daily milk yield

Effect	SS	df	MS	F	p-value
Intercept	100341.2	1	100301.4	6192.102	0.000
Tretman	1109.1	1	592.7	34.341	0.000

Source: Authors' own elaboration

Staphylococcus aureus as one of the most common factors in the occurrence of mastitis, which we isolated from 40%, and *Streptococcus agalactiae* which was isolated from 11 cows and represented 18% of the total number of mastitic cows, together with 58%.

Microbiological agents from the environment surrounding the animals should not be neglected as etiological agents, i.e. opportunistic pathogens that, under favorable conditions of growth and development, can cause mastitis in individuals. In the conducted research, (*Streptococcus dysgalactiae*, *Streptococcus uberis* *Echerichia coli*, *Pseudomonas aeruginosa*) were isolated, which together participated in the occurrence of mastitis in 25 cows or 42% of the total number of mastitic cows.

Through the control of metabolic disorders in the postpartum period, the rational use of antimicrobial drugs, and the application of measures to prevent the isolation and eradication of pathogenic microorganisms, the occurrence of mastitis would be largely prevented.

Based on the analysis of the results related to milk yield, the mutual influence of ketosis and mastitis on milk yield was determined with statistically significant differences ($p < 0.05$), whereby in mastitic cows there was a decrease in milk yield by 8.56 l/day, which is one quarter less than in the healthy population of cows.

While the consequences of mastitis that occur in the physiological function of the mammary gland are only one of the reasons to take a series of prophylactic measures to prevent and treat mastitis.

Conflict of interest

The authors declare that they have no conflict of interests.

References

Abebe, R., Hatiya, H., Abera, M., Megresa, B. & Asmare, K. (2016). Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Veterinary Research*, 12(270), 1 – 11.

Abrahamsén, M., Persson, Y., Kanyima, B. M. & Båge, R. (2013). Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda. *Tropical Animal Health and Production*, 46(1), 99 – 105.

Almaw, G., Zerihun, A. & Asfaw, Y. (2008). Bovine mastitis and its association with selected risk factors in smallholder dairy farms in and around Bahir Dar, Ethiopia. *Tropical Animal Health and Production*, 40(6), 427 – 432.

Bellato, A., Tondo A., Dellepiane, L., Dondo, A., Mannelli, A. & Bergagna, S. (2023). Estimates of dairy herd health indicators of mastitis, ketosis, inter-calving interval, and fresh cow replacement in the Piedmont region, Italy. *Preventive Veterinary Medicine*, 2112(1), 1 – 3.

Berge, A. C. & Vertenten, G. (2014). A field study to determine the prevalence, dairy herd management systems, and fresh cow clinical conditions associated with ketosis in western European dairy herds. *Journal of Dairy Science*, 97(4), 2145 – 2154.

Dohoo, I. R., Martin, S. W., Meek, A. H. & Sandals, W. C. D. (1983). Disease, production and culling in Holstein-Friesian cows I. The data. *Preventive Veterinary Medicine*, 1(4), 321 – 334.

Dingwell, R. T., Kelton, D. F. & Leslie, K. E. (2003). Management of the dry cow in control of peripartum disease and mastitis. *Veterinary Clinics of North America: Food Animal Practice*, 19(1), 235 – 265.

Gezehagn, K., Betelihem, T. & Belege, T. (2020). Isolation and Identification of Major Pathogenic Bacteria from Clinical Mastitic Cows in Asella Town. *Veterinary medicine international*, 13(1), 1 – 6.

Hagnestam, C., Emanuelson, U. & Berglund, B. (2007). Yield Losses Associated with Clinical Mastitis Occurring in Different Weeks of Lactation. *Journal of Dairy Science*, 90(5), 2260 – 2270.

Hisira, V., Zigo, F., Kadaši, M., Klein, R., Farkašová, Z., Vargová, M., & Mudroň, P. (2023). Comparative analysis of methods for somatic cell counting in cow's milk and relationship between somatic cell count and occurrence of intramammary bacteria. *Veterinary Sciences*, 10(7), 468.

Langhorne, C., Gupta, S. D., Horsman, S., Wood, C., Wood, B. J., Barker, L., Deutscher, A., Price, R., McGowma, N. M. R., Humphris, M., Ranjbar, S., Henning, J. & Gibson, J. S. (2023). Bacterial culture and antimicrobial susceptibility results from bovine milk samples submitted to four veterinary diagnostic laboratories in Australia from 2015 to 2019. *Veterinary Epidemiology and Economics*, 10(1), 1 – 7.

Miller, R. H., Paape, M. J., & Fulton, L. A. (1986). Comparison of milk somatic cell counts by Coulter and Fossomatic counters. *Journal of Dairy Science*, 69(7), 1942–1946.

Saidi, R., Khelef, D. & Kaidi, R. (2013) Bovine Mastitis: Prevalence of Bacterial Pathogens and Evaluation of Early Screening Test. *African Journal of Microbiology Research*, 7(9), 777 – 782.

Sangiao, E. T., Rodriguez, C. L. & Riestra, C. G. (2021). Application and Perspectives of MALDI – TOF Mass Spectrometry in Clinical Microbiology Laboratories. *Journal of Dairy Science*, 20(9), 1539 – 1543.

Vanhoudt, A., Hees – Huijps, K., Knegsel, A. T. M., Sampinon, O. C., Vernooij, J. C. M., Nielen, M. & Werven, T. (2018). Effects of reduced intramammary antimicrobial use during the dry period on udder health in Dutch dairy herds. *Journal of Dairy Science*, 101(4), 3248 – 3260.