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Concentration of blood acetone during prepartum and the whole lactation in primiparous cows

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Summary

The aim of this study was to analyse the acetone concentration in the blood of cows in the period immediately preceding the first calving and during the whole first lactation. The study was conducted on 124 clinically healthy primiparous cows of Polish Holstein-Friesian breed born, reared and managed on the same farm (Agricultural Experimental Farm Dłoń) at least up to the second calving. Blood samples were collected from the animals according to the following schedule: 7-8 and 1-2 weeks before the expected calving, as well as 2, 5, 30 (± 3), 60 (± 3), 90 (± 3), 120 (± 3), 200 (± 3), and 280 (± 3) days after calving. The concentration of acetone was determined in the blood serum by the gas chromatography method. The mean acetone concentration in the blood of cows examined was 0.41 mmol/L (SD = 0.18). The highest acetone concentration, exceeding the physiological level (0.4 mmol/L), was noted 7-8 and 1-2 weeks before calving, and at 5, 200 and 280 days of lactation. The threshold corresponding to symptoms of clinical ketosis (above 2 mmol/L) was not exceeded in any of the samples. It was found that the blood acetone level is a feature of low repeatability (ρ up to 0.07). Significant positive correlations ($p \leq 0.01$) were observed between acetone concentrations determined 30 to 120 days after calving (r from 0.26 to 0.39). Significant positive correlations ($p \leq 0.05$) were also found between the acetone concentration before calving and in the first 60 days of lactation (r from 0.19 to 0.22), whereas no correlation was observed between concentrations determined before calving. The results obtained in this study prove that the blood acetone concentration in dairy cows is of special significance during late pregnancy because of a relatively high level of this ketone body and because of its impact on the blood acetone concentration in the initial phase of lactation.

Keywords: dairy cows, blood acetone concentration, prepartum period, lactation

Acetone is numbered among ketone bodies, which also include acetoacetic acid and beta-hydroxybutyric acid (BHB). In dairy cows, acetone constitutes approximately 19% and 60% of all ketone bodies in blood and milk, respectively (6, 7). This disproportion results from the fact that acetone passes from blood to milk much more easily than the other two ketone bodies. It has been observed that the difference in the concentration of this metabolite in the two liquids does not exceed 5%, whereas in the case of the other ketone bodies, especially BHB, it is considerably higher (1, 7). This makes acetone a very attractive diagnostics material in dairy cows during lactation, and its quantitative analyses may be very helpful in the assessment of carbohydrate and lipid metabolism.

An increased level of ketone bodies – a state called ketosis – is harmful. Depending on the intensity of the disorder, one may distinguish subclinical and clinical ketosis. With regard to the concentration of acetone in the blood and milk, the threshold level for subclinical ketosis was established as 0.4 mmol/L (2, 23), whereas the threshold level corresponding to clinical symptoms of the disease was established as 2 mmol/L (2, 14). The symptoms of clinical ketosis include an aversion to the intake of concentrates, decreased milk production and body condition, aberrant appetite, abnormal rumen motility, firm faeces, sight disorders, unsteady gait, and nervous symptoms like alternant apathy and excitement (1, 9).

The literature sources describe several types of ketosis. The classical distinction (3, 17, 29) is made between primary ketosis (which occurs as a result of a shortage or insufficient consumption of acceptable food at the initial period of lactation, and whose symptoms appear 3-6 weeks after calving) and secondary ketosis (the consequence of other diseases occurring especially in the partum period; symptoms during the first days of lactation). An alternative systematics of the types of ketosis

occurring at the first stage of lactation was presented by Holtenius and Holtenius (13), who distinguished type I ketosis (hypoglycemic – hypoinsulinemic; symptoms at 3-4 week of lactation; occurs as a result of a negative energy balance) and type II ketosis (hyperglycemic – hyperinsulinemic; symptoms during the first days of lactation; most of cases are caused by diseases of the peripartum period). The type I ketosis corresponds to primary ketosis, and type II ketosis corresponds approximately to secondary ketosis. Since an increase in insulin resistance is being observed in high-yielding cows (4), type II (secondary) ketosis is likely to become increasingly common. To determine with absolute certainty the type and severity of ketosis, it is necessary to analyze at least the levels of all three ketone bodies, free fatty acids and glucose (4, 12, 13, 15).

Ketosis is one of the most frequently observed production diseases, affecting usually 20-30% of cows in the herd (15). However, cases of its considerably more frequent occurrence, even in all animals, are known (14, 15). The most extensive studies in Poland have been conducted by Filar (8) and Zieliński (33), who observed that ketosis afflicted 20-55% and 16.6% of cows in the Lublin province and the former Leszno province, respectively. Wolańczyk et al. (31) found ketosis in 50-80% of cows kept in large cowsheds in the Gdańsk coast area.

Ketosis causes significant direct losses in productivity, fertility and animal health status (15, 26). Indirect losses caused by ketosis may also be considerable since that disease is significantly associated with the occurrence of all other production diseases, such as fatty liver, dystocia, milk fever, retained placenta, ovarian cysts, abomasum displacement, metabolic acidosis, mastitis, metritis, and hoof diseases (10, 11, 15, 18).

Despite great improvement in the general knowledge of ketosis, the literature supplies very scarce information on the levels of ketone bodies in body fluids in the prepartum period, especially in heifers, as well as in the middle and final parts of lactation.

The aim of the present study was therefore to analyse acetone concentration in the blood serum of healthy primiparous cows in the period preceding the first calving and during the whole first lactation.

Material and methods

The study was conducted on 124 primiparous cows of the Polish Holstein-Friesian breed born, reared and managed in uniform conditions on the same farm, that is the Agricultural Experimental Farm Dłoń. The initial number of animals (7-8 weeks before the expected date of the first calving) was nearly 200, and only those of them that remained in the herd at least up to the second calving and were clinically healthy during this period were examined. During the first 305-day lactation they produced on average 6664 kg of milk, and the average length of their first calving interval was 384.1 days.

High-pregnant heifers and cows on the farm were maintained in a tied system, in stalls of medium length littered with straw. Irrespective of the season of the year, the animals used the extensive yard for at least 2 hours every day. They were fed in a traditional system, with two meals per day: in the morning (7.00) and in the afternoon (16.00). The rations were standardised according to the IZ PIB-INRA system (16). The basic ration for primiparous cows in lactation met the maintenance and production demands of animals with body mass of 550 kg and the average daily milk yield of 19 kg containing 4.0% of fat and 3.1% of proteins. The morning ration constituted 40% of the total daily intake, and the afternoon ration the remaining 60%. Leftovers were removed every day before the morning feeding. Lactating cows were additionally given a special complementary feed mixture, and its amount was strictly determined according to their level of milk production and lactation stage. The complementary mixture was fed with a premix with high-protein fodder material. The basic ration for primiparous cows in lactation was introduced for high-pregnant heifers three weeks before the expected calving. Basic roughages (maize silage and alfalfa silage) and components of a complementary feed mixture (ground maize, triticale, barley and wheat grains, and extracted rape meal) were fed at constant amounts throughout the period of the experiment.

Blood samples were collected from the external jugular vein (*v. jugularis externa*) according to the following schedule: 7-8 and 1-2 weeks before the expected calving (ap), as well as 2, 5, 30 (± 3), 60 (± 3), 90 (± 3), 120 (± 3), 200 (± 3), and 280 (± 3) days after calving (pp). The samples were collected between 11.00 and 12.00 a.m. Within one hour, the serum was obtained by a two-fold centrifugation (3 min., 3000 rpm). In order to prevent acetone volatilisation, test tubes with serum were filled whole and tightly closed with rubber plugs. Then the serum was cooled to the temperature of about +4°C, and on the second day after collection the concentration of acetone was determined by the gas chromatography method. In total, 1240 samples were assayed.

The data was analyzed statistically with the SAS software (25). The following analyses were conducted (the names of SAS package procedures in parentheses): frequency (FREQ), arithmetic means (MEANS), repeatability (MIXED) and Pearson's correlations (CORR). In order to estimate repeatability coefficients (ρ), a model containing the following effects was applied: sire, cow within the sire, series of blood sample collection, season at calving (October-March, April-September), interaction sire × season at calving and random residual effect. The coefficients were estimated by dividing the variance for the cow within the sire by the variance for the residual effect.

Results and discussion

The mean acetone concentration in the blood of cows amounted to 0.41 mmol/L (SD = 0.18). Thus, according to standards developed by other authors (2, 23), the mean acetone level in cows was at the threshold of the physiological level and subclinical ketosis.

The concentration of acetone in the samples collected ranged from 0.05 to 1.70 mmol/L (fig. 1). Considering the maximum concentration of acetone observed in the study, it should be stated that no cases indicating clinical ketosis were found, as the threshold level of milk acetone associated with clinical symptoms of the disease was established as 2 mmol/L (2, 14). The absence of

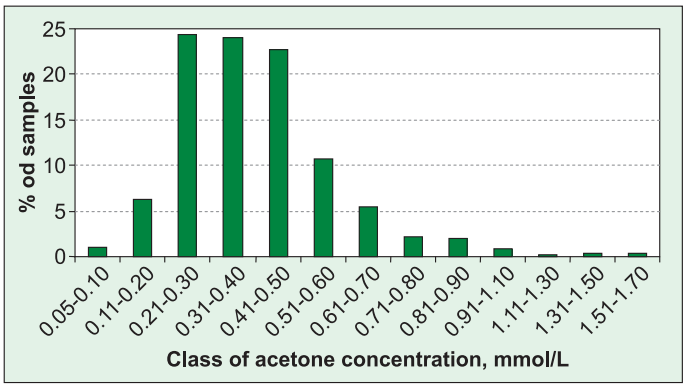

Fig. 1. Frequency distribution of blood acetone concentration in 1240 samples

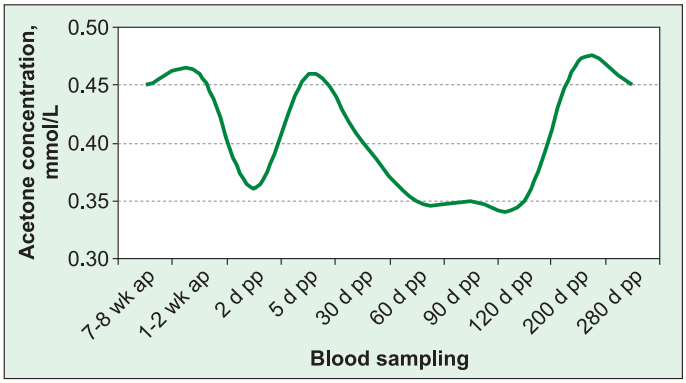

Fig. 2. Blood acetone concentrations throughout the investigated period

Explanations: wk – week, d – day, ap – before calving, pp – after calving

such cases may be explained among others by the fact that only primiparous cows were examined for it has been noted that in this age group of cows the occurrence of ketosis is the lowest (3, 32).

In the examined period, five peaks of the mean blood acetone concentration were observed (each at the level of 0.45-0.47 mmol/L): 7-8 and 1-2 wk ap, and 5, 200 and 280 d pp (fig. 2). The literature provides plentiful information on the level of ketone bodies in the blood (and milk) during the first stage of lactation and on ketosis occurring at the same time, proving unambiguously that ketosis is a problem of the first 5-6 weeks of lactation. Some of the studies on the acetone level reveal that the maximum level of this metabolite is observed 5-10 days postpartum (6, 32), whereas the authors of others observed the maximum acetone concentration at 3-4 week of lactation (1, 10, 24, 27). Referring to literature data, Goff (10) also points out a similar discrepancy, attributing it to differences in the feeding of cows examined.

The fact that in the present study the blood acetone concentration was considerably lower at 2 d pp than during the tests conducted directly before that date (1-2 wk ap) and after that date (5 d pp) is very interesting. Vazquez-Añon et al. (28) also observed that in cows in the partum period the level of BHB decreased significantly, and its lowest values were observed about 2 days before calving. Several authors claim that an increased production of ketone bodies at this time is a positive phenomenon since their metabolism makes it possible to maintain a desirable level of glucose, which serves as an additional energy source and a substrate in a glucogenesis process, as well as a substrate for the synthesis of fat contained in the colostrum and milk (12, 13, 17, 28).

The literature does not provide any data concerning the level of ketone bodies in pregnant heifers and cows and the occurrence of ketosis during the early stage of the dry period. Nor is any information available concerning the above question in relation to the earlier period of the last trimester of lactation. The data concerning the last 3-4 weeks before the expected calving derive from observations conducted on a relatively small number of animals and indicate that the concentration of ketone bodies in cows at this time may exceed the physiological level (21, 22, 28). In the present study, on both dates before calving, and especially on the second (1-2 wk ap), an increased acetone level was observed, and subclinical ketosis was noted in more than half of the cows. The literature suggests four possible causes of this phenomenon. One is the stress associated with advanced pregnancy, which may be particularly important in cows before their first calving (15, 29). In the present study, this hypothesis has to be rejected since an increased blood acetone concentration was also observed before the second calving (the end of the first lactation). The second hypothesis is that an increased level of blood acetone during the first and second late pregnancies may be related to alimentary ketosis, caused by feeding ketogenic rations that include silages containing high amounts of butyric acid, as well as beetroots, molasses and turnip (3, 17, 29). This hypothesis, however, should also be rejected, as typically ketogenic components were absent from the feeding rations, and the silages were offered during advanced pregnancy in smaller amounts than during middle lactation, when the acetone concentration was the lowest. The third, plausible cause of an increased acetone level during advanced pregnancy may be an increased activity of the rumen epithelium, which is related to intensive BHB production (3, 15). Finally, the fourth and also plausible cause may be hormones associated with the sexual cycle and pregnancy, as well as pheromones, which have a significant influence on the blood acetone level in cows (5, 30).

The coefficient of repeatability for the blood acetone concentration during the whole experimental period (from 7-8 wk ap to 280 d pp) was 0.05. Even lower repeatability coefficients were estimated in specific sub-periods: before calving (ρ = 0.01), after calving (ρ = 0.04) and from 60 to 280 d after calving (ρ = 0.02). Other authors also reported low repeatability coefficients for the level of ketone bodies or indices connected to the level of these compounds, and the values observed were within the range from 0.09 to 0.19 (19, 20). Table 1 presents the Pearson's correlation coefficients between the acetone concentrations in blood collected on

particular dates. The highest correlation coefficients (r from 0.26 to 0.39; $p \leq 0.01$) were observed for the samples collected 30 to 120 days after calving. Significant correlation coefficients ($p \leq 0.05$) were also found between the concentration of acetone 7-8 wk ap and the concentration of this compound at 2, 30 and 60 d pp (r from 0.19 to 0.22), as well as between the concentration determined 1-2 wk ap and at 60 d pp (r = 0.21). The significant positive correlations between acetone concentrations found on both dates before calving and acetone concentrations during the first two months after calving suggest a significant influence of gestational ketosis in cattle on the occurrence of ketosis in the initial stage of lactation. At the same time, the absence of a significant correlation between the blood acetone levels determined on both dates before calving is proof of a different background of ketogenesis at this time.

Tab. 1. Correlations among blood acetone concentrations

Blood sampling	7-8 wk ap	1-2 wk ap	2 d pp	5 d pp	30 d pp	60 d pp	90 d pp	120 d pp	200 d pp
1-2 wk ap	0.09								
2 d pp	0.20*	0.09							
5 d pp	–0.16	0.03	0.09						
30 d pp	0.19*	0.12	0.17	–0.02					
60 d pp	0.22*	0.21*	0.05	0.03	0.32**				
90 d pp	–0.08	0.11	0.06	0.07	–0.04	0.39**			
120 d pp	0.09	0.19	–0.01	0.09	0.10	0.26**	–0.01		
200 d pp	0.15	0.04	–0.08	0.01	–0.10	0.04	–0.02	0.19	
280 d pp	–0.05	–0.05	0.02	0.04	–0.07	0.05	0.15	–0.05	0.11

Explanations: wk – week, d – day, ap – before calving, pp – after calving; ** $p \leq 0.01$, * $p \leq 0.05$

Concluding, the blood acetone concentration in healthy dairy cows is a trait of high variability and low repeatability. The highest concentrations of this compound, which exceeded the physiological level (0.4 mmol/L), were noted 7-8 and 1-2 weeks before calving, as well as 5, 200 and 280 days after calving. The results also indicate that the blood acetone concentration is of special importance during late pregnancy because of its relatively high level and its significant correlation with the concentration of this ketone in blood during the fresh period.

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