UDC 619:612.015.3:636.2 doi: https://doi.org/10.15407/ubj96.05.096

SHORT-TERM REDUCTION IN FEED INTAKE BY DAIRY COWS IN THE POSTPARTUM PERIOD LEADS TO SUBCLINICAL KETOSIS DEVELOPMENT

V. VLIZLO[1,2]✉, O. STASIV[1], H. SEDILO[1],
N. FEDAK[1], M. PETRYSHYN[1], R. VOLOSHYN[1]

[1]Institute of Agriculture of Carpathian Region the National Academy of Agrarian Sciences of Ukraine, Obroshyne Lviv district of Lviv region, Ukraine;
[2]Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Lviv, Ukraine;
✉e-mail: vasyl.vlizlo@lvet.edu.ua

Received: 22 May 2024; **Revised:** 08 August 2024; **Accepted:** 07 October 2024

Subclinical ketosis is widespread in highly productive dairy cows after calving and often remains undiagnosed, leading to reduced productivity. Physiologically controlled feeding in the first weeks after calving and during the intensive lactation period can reduce the incidence of ketosis. The study aimed to determine how a short-term reduction of feed after calving affects the formation of ketone bodies in blood, urine and milk of dairy cows. The group of ten Ukrainian black-spotted dairy breed cows after calving aged from 4 to 6 years was involved in the experiment lasted for 72 h. In 24 h the amount of compound feed, haylage, and silage received by cows was gradually reduced until complete exclusion in the diet. Blood, urine, and milk samples were collected three times a day. The content of ketone bodies, β-hydroxybutyrate and glucose was estimated with the corresponding sets of indicator strips. The content of total bilirubin, cholesterol, albumin and enzymes activity in the blood serum were determined on biochemical analyzer. It was found that in 24 h after the beginning of feed reduction the level of blood glucose decreased, persistent hypoglycemia within 48 and 72 h was developed, the concentration of β-hydroxybutyrate in blood and milk and of ketone bodies in urine was elevated. The increase in total bilirubin concentration and liver enzymes activity in the blood serum with a simultaneous decrease in albumin level and delayed sodium propionate conversion into glucose were observed. Thus, short-term reduction in feed intake by dairy cows after calving causes ketosis development and violation of liver functions.

K e y w o r d s: ketosis, dairy cows, feeding level, β-hydroxybutyrate, glucose, liver damage.

A critical factor in reducing the duration of productive use of dairy cows is the mismatch between the intake of energy and nutrients in the body with feed and costs to ensure high milk productivity and the body's own needs, which is especially relevant in the first weeks after calving [1, 2]. When energy deficiency occurs in cows after calving, gluconeogenesis is enhanced with reserve fats and proteins, thus leading to the formation of ketone bodies [3, 4]. However, the cow should not lose much weight. Excessive weight loss of animals during intensive lactation indicates the activation of lipomobilization. This is especially common in high-yielding, fat, and well-fed cows [5, 6]. Uncontrolled negative energy balance may be accompanied by active accumulation of ketone bodies in the body, metabolic disorders, and the occurrence of ketosis [7]. The impetus for the development of ketosis can be short-term malnutrition or starvation, along with a lack of energy and increased lipomobilization [8, 9]. This is observed in cases of poor-quality feed and postpartum disorders with a decrease in appetite or anorexia [10].

Ketosis, particularly its subclinical course, is widespread during the first weeks after calving in farms that keep highly productive dairy cows [11, 12]. On average, subclinical ketosis is diagnosed in more than 40% of cows in different countries, including Ukraine [13-15]. Subclinical ketosis often remains undiagnosed and untreated, leading to a decrease in the efficiency of dairy farming due to reduced productivity, impaired reproductive capacity, and the emergence of various comorbidities [16-18]. Therefore, providing physiologically controlled feeding and maintaining moderate fat mobilization in the first weeks after calving and during the intensive lactation period can reduce the incidence of ketosis.

The study aimed to determine how a short-term reduction of feed for dairy cows from two to four weeks after calving affects the time of energy deficiency in the body, the accumulation of ketone bodies in blood, urine, and milk, and to determine the correlation between blood parameters of ketosis and the functional state and structure of the liver.

Materials and Methods

Animal selection. To conduct the experiment, a group of Ukrainian black-spotted dairy breed cows was formed in 10 heads, aged from 4 to 6 years, and in the period from 2 to 4 weeks after calving. Dairy productivity was 26-32 kg of milk per day. The structure of the diet of experimental cows consisted of 12.1% of roughage, 45.2% − succulent, and 42.7% − concentrates of total nutrients. The dry part of the diet contained 248.86 MJ of metabolic energy and 2301.99 g of protein. The metabolic energy in the diet from concentrated compound feed was 97.8 MJ, or 39.4%, haylage, and silage − 32.0%. The compound feed was given five times a day via an automatic dispenser.

Ethical committee approval. The Ethical Committee approved animal experiments and schemes of the Institute of Agriculture of the Carpathian Region of NAAS of Ukraine (15.01.2023, №1).

The scheme of the experiment. For the experiment with reducing the amount of feed or reducing the energy-protein level of nutrition, ten cows were allocated to a separate part of the room. In 24 h, the amount of compound feed, haylage, and silage received by cows was gradually reduced by ¼, ½, ¾ until its complete exclusion from their diet. The animals were on a reduced diet for the next two days. During the experiment (three days), the cows had free access to hay and water around the clock. Clinical examination of cows, their urine, and milk was performed three times a day. Blood was taken from the jugular vein before the start of feed reduction and in 24, 48, and 72 h after the start of feed reduction.

Laboratory tests of the blood, urine, and milk. Ketone bodies in urine were examined with universal indicator strips Combur9 Test® (Germany) to establish a qualitative reaction (from − to +++), β-hydroxybutyrate in milk using universal indicator strips KetoLac BHB company Horchst, Germany (qualitative reaction from − to ++). Glucose and β-hydroxybutyrate levels were determined in whole blood immediately after sampling by CareSens Dual meter (i-SENS, Inc.) using test strips CareSens PRO for glucose and KetoSens for ketone (Republic of Korea). The contents of total bilirubin, cholesterol, and albumin, the activity of aspartate aminotransferase (AST, EC 2.6.1.1), γ-glutamyl transpeptydase (GGT, EC 2.3.2.2.), glutamate dehydrogenase (GLDH, EC 1.1.1.27) in the blood serum were determined. Blood serum studies were performed on a biochemical analyzer (Evolution 3000, Italia) using sets of reagents from the company "SpineLab" (Ukraine).

The gluconeogenesis state was determined by the method Y. Gröhn [19], but we used sodium propionate *per os* instead of intravenous administration. It was set inside from a special rubber bottle and pre-dissolved in water at the rate of 40 g per 100 kg of body weight. Sodium propionate was administered to 10 experimental cows before the start of the experiment on feed reducing (0 h) and at the end of the experiment after 72 h of energy-protein deficiency. The drug's assimilation rate was determined by examining blood glucose before sodium propionate and then 10, 30, 60, and 180 min after its administration.

All procedures were carried out in a way that minimizes animal suffering and in compliance with the guidelines of the European Convention "For the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and "Common Ethical Principles for Animal Experiments" (Kyiv, 2001). The experiments were carried out following the principles of humanity set out in the European Union Directive (DIRECTIVE 2010/63/EU).

Statistical analysis. Data were expressed as mean ± m (arithmetic mean error). The means were compared using Student's *t*-distribution and the values of $P < 0.05{-}0.001$ were considered statistically significant. The correlation analyses were based on a two-tailed test to calculate the Pearson correlation coefficient.

Results and Discussion

Clinical studies of cows did not show changes in their general condition at the beginning of the experiment. The content of β-hydroxybutyrate in the

T a b l e 1. Changes in glucose and ketone bodies in cows with reduced feeding

Study time after reduced feeding, h	Glucose, in the blood, mmol/l	β-hydroxybutyrate in the blood, mmol/l	β-hydroxybutyrate in milk, qualitative reaction (-) - (++)	Ketone bodies in urine, qualitative reaction (-) - (+++)
0	2.7 ± 0.1	0.69 ± 0.10	–	–
24	2.2 ± 0.1[b]	0.70 ± 0.17	(–) – (+)	(+) – (++)
48	2.0 ± 0.1[c]	1.32 ± 0.31[a]	(+) – (++)	(+) – (+++)
72	1.8 ± 0.1[c]	1.64 ± 0.33[a]	++	(++) – (+++)

Note. Data are presented as mean ± m. Values with different alphabet superscript in a column were significantly different ($^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$). Qualitative reaction in urine: – negative, + weakly positive, ++ positive, +++ strongly positive; qualitative reaction in milk: – negative, + positive, ++ strongly positive.

blood was 0.69 ± 0.10 (0.1–0.9) mmol/l. Reactions to the content of β-hydroxybutyrate in milk and ketone bodies in urine were adverse.

After calving, the high milk productivity of cows requires a significant amount of energy. The body compensates for the lack of energy from its reserves [20, 21]. Initially, compensation occurs through the breakdown of liver and muscle glycogen. Decreased energy safety of diets causes a rapid decrease in blood glucose [22, 23]. However, glycogenolysis is rapidly extinguished. Therefore, the process of reduction of compound feed, haylage, and silage feeding led to a rapid decline in dietary energy, leading to hypoglycemia and hyperketonemia, ketonuria, and ketonolactia. Thus, during the first 24 h after the feed reduction, blood glucose concentration was decreased by 19 % (Table 1). Subsequently, the lack of compound feed, haylage, and silage led to an even more significant decrease in blood glucose and caused an increase of β-hydroxybutyrate in the blood plasma and milk and ketone bodies increase in the urine (Table 1). The glucose concentration in the blood plasma of experimental cows decreased by 26% after 48 h and by 33% after 72 h, also, all cows had hypoglycemia.

A decrease in blood glucose in dairy cows to 2.2 mmol/l and below indicates an energy deficit and the possible development of ketosis [24]. Having large fat reserves in the depot, the body tries to eliminate energy loss through lipolysis, causing the mobilization of fat from the depot of the body [11]. However, excessive and prolonged intake of lipids causes insufficient conversion in the tricarboxylic acid cycle with increased ketone bodies formation and the development of ketosis [25]. The accumulation of ketone bodies is mainly due to β-hydroxybutyrate, so it is crucial to determine its level in the blood of cows for the diagnosis of ketosis [26, 27] as its increase over 1.2 mmol/l can be an indicator of subclinical ketosis [28, 29]. We found that after a decrease in energy feeding, the concentration of ketone bodies in some cows increased after the first day, and all studied cows were diagnosed with hyperketonemia, ketonuria, and ketonolactia after two days (Table 1). The first signs of a negative energy balance were characterized by a negative correlation between a decrease in glucose and an increase in β-hydroxybutyrate in the blood (r = -0.9), which was established by other researchers [30].

During enhanced lipomobilization, fats are transferred to the liver, where the mitochondria of hepatocytes are mainly involved in their breakdown,

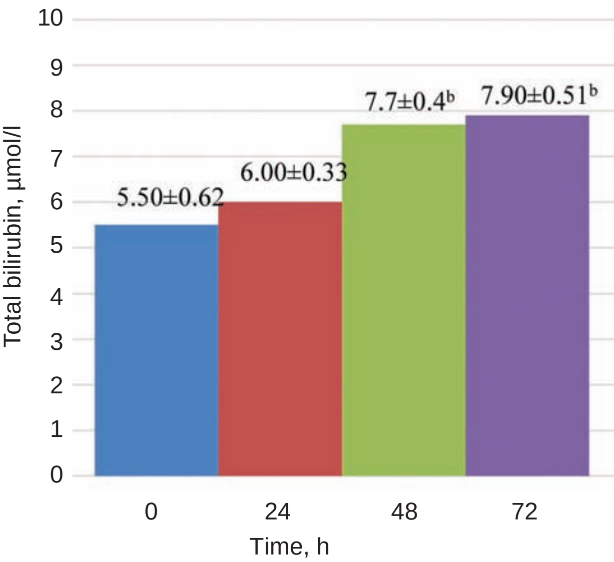

Fig. 1. The concentration of total bilirubin in the blood serum of cows. Values with different alphabet superscript in a column were significantly different ($^bP < 0.01$)

oxidation, and assimilation. Active mobilization of fat from the depot to the liver causes disruption of its essential functions and the development of liver obesity [31, 32]. Therefore, a decrease in feed intake and energy and protein supply of cows leads to an increase in total bilirubin concentration in the blood serum [33, 34], which we have established throughout the experiment (Fig. 1), and total bilirubin concentration is closely positively correlated with an increase of β-hydroxybutyrate in the blood plasma (Table 2).

Simultaneously, the development of pathology in hepatocytes causes impaired protein synthesis [35, 36], which was manifested by a decrease in blood serum albumin (Fig. 2). Albumin concentration decreases correlatively to the increase of β-hydroxybutyrate in the blood (Table 2).

The amount of cholesterol in the blood serum of cows varied a little during the reduction of energy and protein supply, but the content of cholesterol in the blood serum was negatively correlated with the amount of β-hydroxybutyrate in the blood plasma (Table 2).

An increase in the activity of enzymes indicative of the liver was found during the time of energy-protein deficiency in the blood serum of most experimental cows (Fig. 3). There is a possibility that cows developed cytolysis and impaired permeability of hepatocyte membranes and their organelles, which led to the elimination of enzymes into the blood [33, 37]. While characterizing changes in the activity of indicator enzymes, it should be noted that increased lipomobilization causes, above all, damage to the mitochondria of hepatocytes, which are mainly involved in fat utilization, thus, fatty infiltration of the liver, which occurs during ketosis, leads to increased activity of mitochondrial GLDH, as well as AST (Fig. 3). Therefore, these enzymes have a

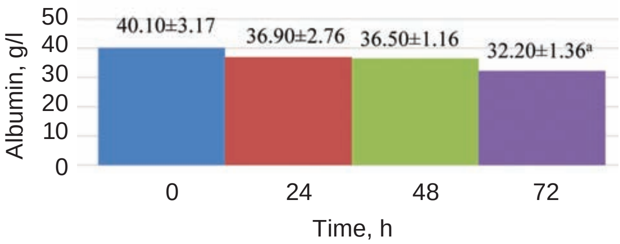

Fig. 2. The amount of albumin in the blood serum of cows. Values with different alphabet superscripts in a column were significantly different ([a]P < 0.05)

Table 2. Correlation coefficient (r) between the content of β-hydroxybutyrate in the blood and indicators of the functional and structural state of the liver

Blood indicators	β-hydroxybutyrate in blood
Total bilirubin	+ 0.73
Albumin	− 0.26
Cholesterol	− 0.20
Glutamate dehydrogenase	+ 0.25
Aspartate aminotransferase	+ 0.31
γ-glutamyl transpeptydase	+ 0.74

positive correlation with β-hydroxybutyrate in the blood (Table 2).

The activity of GGT in the blood serum of cows during the experiment did not increase significantly (Fig. 3). It should also be noted a high positive correlation between the content of β-hydroxybutyrate in the blood and the activity of GGT (Table 2), which indicates the inclusion in the pathological process of the biliary system and the possible destruction of cells that form the bile ducts. Because γ-glutamyl transferase activity was positively correlated (r = +0.41) with total bilirubin concentration, this can be seen as the development of parenchymatous jaundice in cows.

In gluconeogenesis, propionic acid is converted to glucose [19]. In the case of giving sodium propionate before the experiment, the concentration of glucose in the blood plasma of cows increased within 30 min after administration of sodium propionate, then there was a gradual decrease in its content, and in three hours, it was almost the same as before. Administration of sodium propionate to cows showed that in experimental cows that developed severe hyperketonemia, ketonuria and ketonolactia, the time of conversion of propionate to glucose was longer, compared with the indicators before the reduction of feeding (Fig. 4). Thus, in cows with the β-hydroxybutyrate content in the blood plasma 1.64 ± 0.33 mmol/l, and positive qualitative tests for the β-hydroxybutyrate content in milk and the ketone bodies content in urine, the assimilation of sodium propionate began only after thirty minutes and lasted for three hours. Thus, the development of ketosis disrupts gluconeogenesis - one of the essential functions of the liver, due to which high-yielding

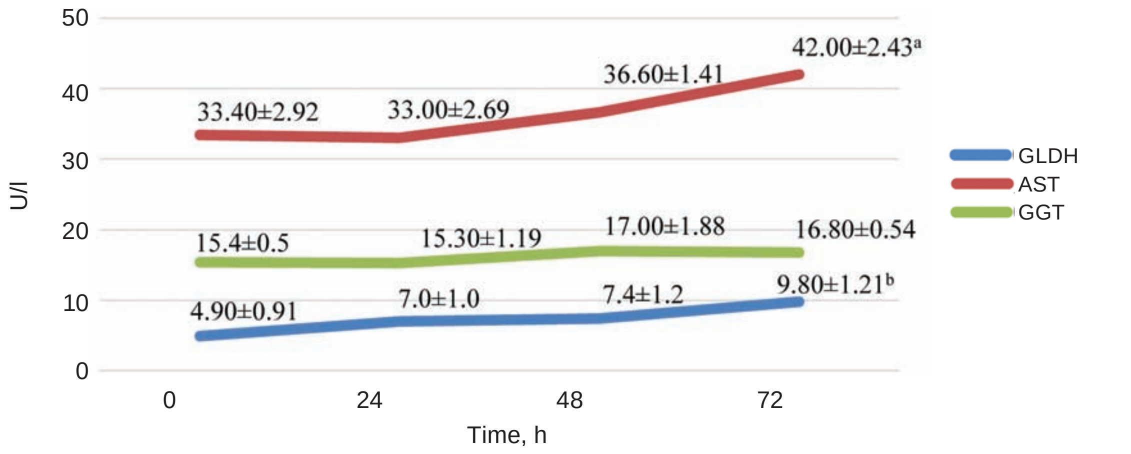

Fig. 3. Activity of enzymes in the blood serum of cows. Values with different alphabet superscripts in a column were significantly different ([a]P < 0.05, [b]P < 0.01)

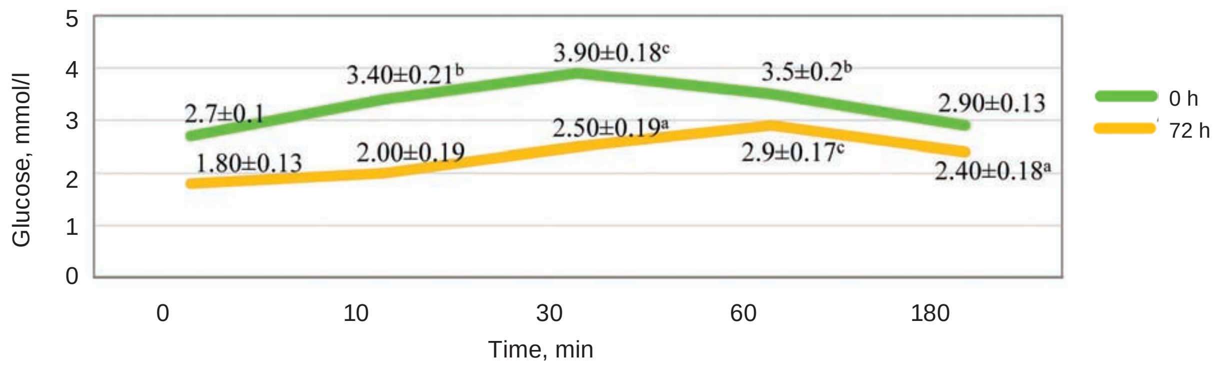

Fig. 4. Glucose concentration in the blood plasma of cows after per os administration of sodium propionate. Before reducing feeding (0 h), 72 h after reducing feeding (72 h). Values with different alphabet superscripts in a column were significantly different ([a]P < 0.05, [b]P < 0.01, [c]P < 0.001)

cows are supplied with glucose. There is a deficiency of the latter, which stimulates lipomobilization, lipolysis, ketogenesis, and fatty infiltration of the liver [35, 38].

Conclusions. A short-term reduction in the amount of feed in the diet of cows from 2 to 4 weeks after calving causes increased mobilization of fat from the body's depot to the liver; its cells are unable to cope with excessive lipids, thus leading to a rapid increase of ketogenesis and infiltration of hepatocytes, which causes the development of ketosis and disruption of the essential functions and structure of the liver.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Funding. The work was carried out at the Institute of Agriculture of the Carpathian Region of National Academy of Agrarian Sciences of Ukraine, state registration number 0123U104875.

ВПЛИВ КОРОТКОТРИВАЛОГО ЗНИЖЕННЯ СПОЖИВАННЯ КОРМУ МОЛОЧНИМИ КОРОВАМИ В ПІСЛЯРОДОВИЙ ПЕРІОД НА РОЗВИТОК СУБКЛІНІЧНОГО КЕТОЗУ

В. Влізло[1,2]✉, О. Стасів[1], Г. Седіло[1], Н. Федак[1], М. Петришин[1], Р. Волошин[1]

[1]Інститут сільського господарства карпатського регіону НААН, с. Оброшине Львівський р-н, Львівська обл., Україна;
[2]Львівський національний університет ветеринарної медицини та біотехнологій ім. С. З. Гжицького, Львів, Україна;
✉e-mail: vasyl.vlizlo@lvet.edu.ua

Субклінічний кетоз поширений серед високопродуктивних молочних корів у післяродовий період. Патологія завдає значних економічних збитків внаслідок зниження продуктивності. Фізіологічно збалансована годівля корів у перші тижні після отелення і в період інтенсивної лактації є основою зменшення захворюваності на кетоз. Метою нашої роботи було встановити як короткотривале зменшення згодовування кормів коровам у перші тижні після отелення впливає на рівень кетонових тіл у крові, сечі та молоці. Дослідження проводили на 10 коровах української чорно-рябої молочної породи, у віці від 4 до 6 років, 2−4 тижні після отелення. Протягом 24 год коровам поступово зменшували згодовування комбікорму, сінажу та силосу на ¼, ½, ¾ до повного їх виключення з раціону, залишивши вільний доступ до сіна та води. Такий рівень живлення корів тривав до 72 год. Вміст кетонових тіл у сечі, β-гідроксибутирату у молоці, β-гідроксибутирату та глюкози у крові визначали на початку та через 24, 48 і 72 год. Кількість загального білірубіну, холестеролу та альбуміну і активність ензимів у сироватці крові вимірювали на біохімічному аналізаторі. Встановлено, що через 24 год після початку зменшення згодовування кормів у крові знижувався рівень глюкози, а через 48 та 72 год розвивалася стійка гіпоглікемія і зростала концентрація β-гідроксибутирату у крові та молоці й кетонових тіл у сечі. Через 72 год у крові корів зростав вміст загального білірубіну та знижувався альбумін і зростала активність ензимів, а також сповільнювалося перетворення натрію пропіонату у глюкозу. Таким чином, короткотривале зниження споживання корму молочними коровами після отелення спричиняє розвиток кетозу і порушення функцій печінки.

Ключові слова: кетоз, дійні корови, рівень годівлі, β-гідроксибутират, глюкоза, ураження печінки.

References

1. Raja S, Palanisamy M, Prabaharan V, Rajkumar R, Kumar SS. Prevalence of subclinical ketosis in anestrum dairy cows. *J Entomol Zool Stud*. 2021; 9(1): 2044-2046.
2. Stivanin SCB, Vizzotto EF, Matiello JP, Machado FS, Campos MM, Tomich ThR, Pereira LGR, Fischer V. Behavior, feed intake and health status in Holstein, Gyr and Girolando-F1 cows during the transition period: Behavior and health of dairy cows in the transition period. *Appl Anim Behav Sci*. 2021; 242: 105403.
3. González FD, Muiño R, Pereira V, Campos R, Benedito JL. Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. *J Vet Sci*. 2011; 12(3): 251-255.
4. Xu W, Vervoort J, Saccenti E, Kemp B, van Hoeij RJ, van Knegsel ATM. Relationship between energy balance and metabolic profiles in plasma and milk of dairy cows in early lactation. *J Dairy Sci*. 2020; 103(5): 4795-4805.
5. Li S, Wei X, Song J, Zhang C, Zhang Y, Sun Y. Evaluation of Statistical Process Control Techniques in Monitoring Weekly Body Condition Scores as an Early Warning System for Predicting Subclinical Ketosis in Dry Cows. *Animals (Basel)*. 2021; 11(11): 3224.
6. Ha S, Kang S, Jeong M, Han M, Lee J, Chung H, Park J. Characteristics of Holstein cows predisposed to ketosis during the post-partum transition period. *Vet Med Sci*. 2023; 9(1): 307-314.
7. Atalay H. The effect of body condition score on nutritional diseases and milk yield in dairy cattle. *Turk J Vet Anim Sci*. 2019; 43(5): 692-697.
8. Schulz K, Frahm J, Meyer U, Kersten S, Reiche D, Rehage J, Dänicke S. Effects of prepartal body condition score and peripartal energy supply of dairy cows on postpartal lipolysis, energy balance and ketogenesis: an animal model to investigate subclinical ketosis. *J Dairy Res*. 2014; 81(3): 257-266.

9. Gruber S, Mansfeld R. Herd health monitoring in dairy farms - discover metabolic diseases. An overview. Tierarztl Prax Ausg G Grosstiere Nutztiere. 2019; 47(4): 246-255.
10. Seifi HA, Huzzey JM, Khan MA, Weary DM, von Keyserlingk MAG. Addition of straw to the early-lactation diet: Effects on feed intake, milk yield, and subclinical ketosis in Holstein cows. J Dairy Sci. 2021; 104(3): 3008-3017.
11. Smith GL, Friggens NC, Ashworth CJ, Chagunda MGG. Association between body energy content in the dry period and post-calving production disease status in dairy cattle. *Animal*. 2017; 11(9): 1590-1598.
12. Vudmaska I, Petrukh I, Sachko S, Vlizlo V, Kosenko Y, Kozak M, Petruk A. Using hop cones, vitamin E, methionine, choline and carnitine for treatment of subclinical ketosis in transition dairy cows. *Adv Anim Vet Sci*. 2021; 9(1): 55-62.
13. Suthar VS, Canelas-Raposo J, Deniz A, Heuwieser W. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. *J Dairy Sci*. 2013; 96(5): 2925-2938.
14. Brunner N, Groeger S, Canelas Raposo J, Bruckmaier RM, Gross JJ. Prevalence of subclinical ketosis and production diseases in dairy cows in Central and South America, Africa, Asia, Australia, New Zealand, and Eastern Europe. *Transl Anim Sci*. 2018; 3(1): 84-92.
15. Loiklung C, Sukon P, Thamrongyoswittayakul C. Global prevalence of subclinical ketosis in dairy cows: A systematic review and meta-analysis. *Res Vet Sci*. 2022; 144: 66-76.
16. Steeneveld W, Amuta P, van Soest FJS, Jorritsma R, Hogeveen H. Estimating the combined costs of clinical and subclinical ketosis in dairy cows. *PLoS One*. 2020; 15(4): e0230448.
17. Rodriguez Z, Shepley E, Endres MI, Cramer G, Caixeta LS. Assessment of milk yield and composition, early reproductive performance, and herd removal in multiparous dairy cattle based on the week of diagnosis of hyperketonemia in early lactation. *J Dairy Sci*. 2022; 105(5): 4410-4420.
18. Alemu TW, Santschi DE, Cue RI, Duggavathi R. Reproductive performance of lactating dairy cows with elevated milk β-hydroxybutyrate levels during first 6 weeks of lactation. *J Dairy Sci*. 2023; 106(7): 5165-5181.
19. Gröhn Y. Propionate loading test for liver function in spontaneously ketotic dairy cows. *Res Vet Sci*. 1985; 39(1): 24-28.
20. Ruoff J, Borchardt S, Heuwieser W. Short communication: Associations between blood glucose concentration, onset of hyperketonemia, and milk production in early lactation dairy cows. *J Dairy Sci*. 2017; 100(7): 5462-5467.
21. Sabek A, Li C, Du C, Nan L, Ni J, Elgazzar E, Ma Y, Salem AZM, Zhang S. Effects of parity and days in milk on milk composition in correlation with β-hydroxybutyrate in tropic dairy cows. *Trop Anim Health Prod*. 2021; 53(2): 270.
22. Mezzetti M, Minuti A, Piccioli-Cappelli F, Amadori M, Bionaz M, Trevisi E. The role of altered immune function during the dry period in promoting the development of subclinical ketosis in early lactation. *J Dairy Sci*. 2019; 102(10): 9241-9258.
23. Habel J, Sundrum A. Mismatch of glucose allocation between different life functions in the transition period of dairy cows. *Animals (Basel)*. 2020; 10(6): 1028.
24. Vanholder T, Papen J, Bemers R, Vertenten G, Berge AC. Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. *J Dairy Sci*. 2015; 98(2): 880-888.
25. Snedec T, Theinert K, Pietsch F, Leonhardt AS, Kadáši M, Klein R, Spilke J, Bannert E, Schären M, Köller G, Baumgartner W, Theile S, Kaiser A, Rachidi F, Starke A. Validation of a cow-side blood test for beta-hydroxybutyrate concentration in dairy cows. *Tierarztl Prax Ausg G Grosstiere Nutztiere*. 2021; 49(1): 5-11.
26. McArt JAA, Nydam DV, Oetzel GR. Epidemiology of subclinical ketosis in early lactation dairy cattle. *J Dairy Sci*. 2012; 95(9): 5056-5066.
27. Du X, Zhu Y, Peng Z, Cui Y, Zhang Q, Shi Z, Guan Y, Sha X, Shen T, Yang Y, Li X, Wang Z, Li X, Liu G. High concentrations of fatty acids and β-hydroxybutyrate impair the growth hormone-mediated hepatic JAK2-STAT5 pathway in clinically ketotic cows. *J Dairy Sci*. 2018; 101(4): 3476-3487.
28. Benedet A, Manuelian CL, Zidi A, Penasa M, De Marchi M. Invited review: β-hydroxybutyrate concentration in blood and milk and its

associations with cow performance. *Animal*. 2019; 13(8): 1676-1689.

29. Hubner A, Canisso IF, Peixoto PM, Coelho WM Jr, Ribeiro L, Aldridge BM, Menta P, Machado VS, Lima FS. Characterization of metabolic profile, health, milk production, and reproductive outcomes of dairy cows diagnosed with concurrent hyperketonemia and hypoglycemia. *J Dairy Sci*. 2022; 105(11): 9054-9069.
30. Antanaitis R, Džermeikaitė K, Januškevičius V, Šimonytė I, Baumgartner W. In-Line Registered Milk Fat-to-Protein Ratio for the Assessment of Metabolic Status in Dairy Cows. *Animals (Basel)*. 2023; 13(20): 3293.
31. Couperus AM, Schroeder F, Hettegger P, Huber J, Wittek T, Peham JR. Longitudinal Metabolic Biomarker Profile of Hyperketonemic Cows from Dry-Off to Peak Lactation and Identification of Prognostic Classifiers. *Animals (Basel)*. 2021; 11(5): 1353.
32. Vlizlo V, Prystupa O, Slivinska L, Gutyj B, Maksymovych I, Shcherbatyy A, Lychuk M, Partyka U, Chernushkin B, Rusyn V, Leno M, Leskiv K. Treatment of animals with fatty liver disease using a drug based on the seeds of Silybum marianum. *Regul Mech Biosyst*. 2023; 14(3): 424-431.
33. Ha S, Kang S, Han M, Lee J, Chung H, Oh SI, Kim S, Park J. Predicting ketosis during the transition period in Holstein Friesian cows using hematological and serum biochemical parameters on the calving date. *Sci Rep*. 2022; 12(1): 853.
34. Rodriguez Z, Picasso-Risso C, Gaire TN, Nakagawa K, Noyes N, Cramer G, Caixeta L. Evaluating variations in metabolic profiles during the dry period related to the time of hyperketonemia onset in dairy cows. *PLoS One*. 2023; 18(8): e0289165.
35. Simonov M, Vlizlo V. Some blood markers of the functional state of liver in dairy cows with clinical ketosis. *Bulgarian J Vet Med*. 2015; 18(1): 74-82.
36. Tóthová C, Nagy O, Nagyová V, Kováč G. Serum protein electrophoretic pattern in dairy cows during the periparturient period. *J Appl Anim Res*. 2016; 46(1): 33-38.
37. Đoković R, Šamanc H, Petrović MD, Ilić Z, Kurćubić V. Relationship among blood metabolites and lipid content in the liver in transitional dairy cows. *Biotechnol Anim Husbandry*. 2012; 28(4): 705-714.
38. Rodriguez-Jimenez S, Haerr KJ, Trevisi E, Loor JJ, Cardoso FC, Osorio JS. Prepartal standing behavior as a parameter for early detection of postpartal subclinical ketosis associated with inflammation and liver function biomarkers in peripartal dairy cows. *J Dairy Sci*. 2018; 101(9): 8224-8235.