LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

Mingaudas Urbutis

THE IMPACT OF NEGATIVE ENERGY BALANCE DURING DRY PERIOD AND ITS PREVENTION ON HEALTH PARAMETERS OF COWS AND CALVES

Doctoral Dissertation
Agricultural Sciences,
Veterinary (A 002)

Kaunas, 2025

Dissertation has been prepared at the Department of Large Animal Clinic of the Veterinary Faculty of the Lithuanian University of Health Sciences during the period of 2020–2025.

Scientific Supervisor:

Prof. Dr. Ramūnas Antanaitis (Lithuanian University of Health Sciences, Agricultural sciences, Veterinary – A 002).

Dissertation is defended at the Veterinary Research Council of the Lithuanian University of Health Sciences:

Chairperson

Prof. Dr. Vita Riškevičienė (Lithuanian University of Health Sciences, Agricultural Sciences, Veterinary – A 002).

Members:

Prof. Dr. Vytuolis Žilaitis (Lithuanian University of Health Sciences, Agricultural Sciences, Veterinary – A 002);
Prof. Dr. Rasa Želvytė (Lithuanian University of Health Sciences, Agricultural Sciences, Veterinary – A 002);
Assoc. Prof. Dr. Dalia Mickevičienė (Lithuanian Sports University, Natural Sciences, Biology – N 010);
Prof. Dr. Przemysław Sobiech (University of Warmia and Mazury in Olsztyn (Poland), Agricultural Sciences, Veterinary – A 002).

Dissertation will be defended at the open session of the Veterinary Research Council of the Lithuanian University of Health Sciences at 9 a.m. on the 29th of August of 2025, in the Dr. S. Jankauskas auditorium of the Veterinary Academy of the Lithuanian University of Health Sciences.
Address: Tilžės 18, LT-47181 Kaunas, Lithuania.

LIETUVOS SVEIKATOS MOKSLŲ UNIVERSITETAS

Mingaudas Urbutis

NEIGIAMO ENERGIJOS BALANSO IR JO PROFILAKTIKOS UŽTRŪKINIMO LAIKOTARPIU POVEIKIS KARVIŲ IR JŲ VERŠELIŲ SVEIKATINGUMO RODIKLIAMS

Daktaro disertacija
Žemės ūkio mokslai,
veterinarija (A 002)

Kaunas, 2025

Disertacija rengta 2020–2025 metais Lietuvos sveikatos mokslų universiteto Veterinarijos fakulteto Stambiųjų gyvūnų klinikoje.

Mokslinis vadovas

prof. dr. Ramūnas Antanaitis (Lietuvos sveikatos mokslų universitetas, žemės ūkio mokslai, veterinarija – A 002).

Disertacija ginama Lietuvos sveikatos mokslų universiteto Veterinarijos mokslo krypties taryboje:

Pirmininkas

prof. dr. Vita Riškevičienė (Lietuvos sveikatos mokslų universitetas, žemės ūkio mokslai, veterinarija – A 002).

Nariai:

prof. dr. Vytuolis Žilaitis (Lietuvos sveikatos mokslų universitetas, žemės ūkio mokslai, veterinarija – A 002);
prof. dr. Rasa Želvytė (Lietuvos sveikatos mokslų universitetas, žemės ūkio mokslai, veterinarija – A 002);
doc. dr. Dalia Mickevičienė (Lietuvos sporto universitetas, gamtos mokslai, biologija – N 010);
prof. dr. Przemysław Sobiech (Varmijos Mozūrų universitetas (Lenkija), žemės ūkio mokslai, veterinarija – A 002).

Disertacija bus ginama viešajame Veterinarijos mokslo krypties tarybos posėdyje 2025 m. rugpjūčio 29 d. 9 val. Lietuvos sveikatos mokslų universiteto Veterinarijos akademijos Dr. S. Jankausko auditorijoje.
Disertacijos gynimo vietos adresas: Tilžės g. 18, LT-47181 Kaunas, Lietuva.

CONTENTS

LIST OF ABBREVIATIONS

BCS	–	body condition score
BHB	–	β-hydroxy-butyrate
BRD	–	bovine respiratory disease
C/EBPα	–	cytosine-cytosine-adenosine-adenosine-thymidine-enhancer-binding protein alpha
CpG	–	cytosine-phosphate-guanine
DHA	–	docosahexaenoic acid
DNA	–	deoxyribonucleic acid
ELISA	–	enzyme-linked immunosorbent assay
EPA	–	eicosapentaenoic acid
FATP4	–	long-chain fatty acid transport protein 4 gene
FTPI	–	failure of transfer of passive immunity
GLU	–	glucose
GLUT1	–	glucose transporter 1 gene
$GR1_{10}$	–	hepatic glucocorticoid gene 1
HCS	–	high calf score
IgG	–	immunoglobulin G
IL-1β	–	interleukin-1 beta
IL-6	–	interleukin-6
IR	–	insulin resistance
LCS	–	low calf score
LDH	–	lactate dehydrogenase
LPS	–	lipopolysaccharide
LSD	–	least significant difference
MCRC	–	a monensin controlled-release capsule
MCS	–	medium calf score
MF	–	milk fat content
MF:P	–	milk fat and protein ratio
ML	–	milk lactose concentration
MP	–	milk protein content
mRNA	–	messenger ribonucleic acid
MY	–	milk yield
NADPH	–	nicotinamide adenine dinucleotide phosphate
NCD	–	neonatal calf diarrhea
NEB	–	negative energy balance
NEFA	–	non-esterified fatty acid
NFkB	–	nuclear factor kB
NOS2	–	nitric oxide synthase 2

NRC	–	nutrient requirements of dairy cattle
O_2	–	oxygen
OS	–	oxidative stress
PPAR	–	peroxisome proliferator-activated receptors
PPARɣ	–	peroxisome proliferator activated receptor gamma
RNA	–	ribonucleic acid
RT	–	rumination time
STP	–	serum total protein
TLR4	–	toll-like receptor 4
TMR	–	total mixed ration
TNF-α	–	tumor necrosis factor alpha
Zfp423	–	zinc finger protein 423 gene

INTRODUCTION

The dairy industry of the European Union continues to remain one of the main providers of food to the world even when facing various challenges. The European Union produced and delivered to dairies 144.9 thousand tons of raw milk in the year of 2023 and similar results were achieved in 2024 – 144.5 thousand tons, and cheese has remained the main product of exports from the Union [1, 2]. Milk yield per cow per year averaged 7 791 kg (highest milk yields were recorded in Estonia – 10 728 kg, and lowest in Romania – 3 425 kg). In the meantime, Lithuania had an average milk yield of 6 724 kg per cow per year [3]. The number of cows in Lithuania has decreased by 19% (from 2013 to 2023), in the European Union this decrease is estimated around 9.3%, while milk yield per cow increased by 20.3% – a tendency registered around the world, where cow productivity has grown exponentially [4, 5]. Even though milk production has been increasing, it is estimated that milk consumption should grow by 21.5% from 2024 till 2033, while milk production should grow only by 18.5% in the same period [3]. Such tendencies only fortify the need for the industry to further increase production per cow. As their numbers decrease, each cow becomes more costly and their cost is further increased by outside factors, such as increasing fuel and fertilizer costs (influenced by geopolitical instability and changing climate); environmental taxes and legislations [6 ,7]. The changing climate and globalization has recently brought on infectious diseases (such as Foot and mouth disease and Bluetongue disease) further decreasing the number of dairy cattle [8, 9]. As cows are bred for higher milk yields, the percentage of older cows culled from the herd has seen an increase in the last decade. Older cows are not able to balance the nutrient and energy requirements between high milk yields and stable metabolism or proper reproductive function, therefore they are removed from the herd. Therefore, the dairy industry focuses on optimizing each lactation and maintaining health status of their stock [10]. These challenges more than ever before are pushing the dairy industry to reevaluate their management strategies [6].

In order to remain profitable and relevant, the dairy industry is forced to focus on the survival of replacement heifers and maximizing their milk yield. One way of maximizing the potential for dam's productivity and health of its offspring is by ensuring a stable prepartum period [11]. During this period, the physiology of the cow is undergoing a series of changes – preparing the mammary gland for future lactation, managing colostrogenesis, providing the growing fetus with nutrients, reserving and storing lipids as energy source and adapting to a changing diet and nutrient requirements [12–15]. The fetus experiences a growth spurt during the last trimester and fully develops its organ systems in preparation for the calving [14]. Therefore veterinarians working with dairy and industry professionals are putting a lot of effort in managing prepartum cows by modifying feed rations, ensuring appropriate body condition score and energy state in order to avoid health risks for the upcoming lactation and to improve the health status of the newborn calf [16–18].

Managing the prepartum to benefit the upcoming lactation has been studied extensively, but recently more work has been published on the effects of prepartum period for the offspring health and productivity [19]. A variety of factors experienced by the dam during prepartum, from environmental to internal, have shown evidence of having a long lasting effect on the calf and even its future productivity [20]. One of the internal factors, parity of the animal, has shown evidence to negatively influence the health status of its offspring, since available nutrient has to be divided between maturing dam and a developing fetus [21]. During prepartum onset of negative energy balance state determined by initiated lipid mobilization, expressed by higher concentration of non-esterified fatty acids, is a physiological norm in highly productive individuals. As cows enter insulin resistance state in order to divide sparce energy sources, blood glucose concentrations remain low and BHB levels rise, acting as an energy alternative for tissues [22]. Similar state is also characteristic in newborn calves, as lactose levels in colostrum are insufficient to provide enough energy for calf demands, therefore lipolysis is also registered. In turn lower concentration of blood glucose and higher concentrations of BHB can be found [23]. Seeing as NEFA concentration defines lipid mobilization, blood BHB and glucose concentrations are better indicators of active metabolic activity and energy state [24]. Prophylaxis against NEB in the prepartum by feed supplementation has shown good capabilities in controlling the negative effects of this state in cows [25]. These findings point to a unique relationship between dam and fetus and an effort is being made by the scientific community to understand the internal mechanisms governing this relationship. By identifying the factors modifying fetal development and programming calf health and productivity, we could provide better care for the neonate and allow it to reach its production potential and ensure longevity [26].

Scientific novelty

The prepartum period, or it as a part of the transition period, has been studied extensively focusing on its effects on the upcoming lactation of the cow. Importance of it's proper management for the cow's health is undeniable and scientific research in this field has impacted the way dry cows are being managed in the dairy industry. Recently, a new focus has been set on to the prepartum period but through the lens of it's effect on the health of the fetus. More specifically, how environmental and internal factors of the dam can affect the fetus and in turn – the neonate. Some studies have investigated the relationship of complex blood parameters and markers between cow and her fetus together with how external and internal factors can influence them during prepartum [27, 28].

One of the main processes initiated during prepartum is the onset of negative energy balance state. The negative impact of this state can be mitigated by implementing prophylactic supplementation before parturition. A modification was done in these studies for the evaluation of the effect of prophylaxis against the negative energy balance state by monitoring herd health management system parameters and udder health indicators. Evidence of a close relationship between cow energy state and udder health parameters has been registered.

A relationship forming between prepartum cows and their calves has been confirmed by monitoring their metabolic blood and health evaluation parameters. Complementing calf health evaluation system parameters with herd health management system parameters of lactating cows helped identifying animals at a higher risk of disease susceptibility and confirmed the impact of negative energy balance state on the declining health status. Cows exhibiting specific changes in the herd health management system parameters and milk quality gave birth to calves more prone to disease.

Evaluation methods of cow and calf health status described in the study can be used by researchers, on farm veterinary practitioners and farm managers to evaluate herd health status and to show evidence of the relationship between prepartum cows and their calves. Having this data can help in making informed decisions about the management of prepartum cows and calves, improving housing conditions, feeding ration supplementation and the effectiveness of implemented prophylactic measures.

Practical value

As stated above, in order to monitor the health status and metabolic parameters of cows and calves, we utilized tools that an average farm would have. On site and handheld blood parameter tests of glucose and BHB concentration are simple and quick to use. Veterinarians working in the dairy industry are already accustomed to using it in their everyday work and are able to make quick and accurate decisions about herd management and treatment plan. A calf scoring system has shown its benefit in determining sick calves and helping to diagnose and monitor them further. A unified system helps to remove biased opinions about the health status of the animal therefore a more correct decision can be made. Automatic herd management system parameters are being used for quite some time in the dairy industry and have given good results in diagnosing the animals as early as possible and monitoring changes in their health status. Combining these methods and systems allows to correctly evaluate the energy status of the animals and prevent metabolic diseases, which influence the development of other disease such as mastitis, metritis or calf diarrhea and pneumonia.

The above-mentioned tools and parameters can also be used by the dairy farm personnel in order to monitor the relationship between cow and calf and to make appropriate management decisions for the prepartum period. With the data analyzed, dairy farm managers and veterinarians can improve environmental conditions for the cows or decide on a beneficial supplementation of the feed ration – ensuring a good health condition for the lactating cow and her calf. Knowing whether parity of the cow will influence the health status and metabolism of its neonate, could lead to a necessity for more care in the prepartum period. By doing so, we can decrease the number of sick days for the calves, that way ensuring a normal development of their rumen and other organ systems. A healthy neonate has a better chance to reach puberty, become inseminated and then reach their milk yield potential. Well managed prepartum period for the cow also guarantees a better start in the lactation and lower chances of ketosis, mastitis and reproductive problems.

The layout of the dissertation

The dissertation is prepared on the basis of a set of two scientific articles. The volume of the PhD thesis is 94 pages. The thesis contains following chapters: List of abbreviations, Introduction, Literature review, Materials and methods, Results and discussion, Conclusions, Recommendation, Summary in Lithuanian, References, List of publications, Curriculum vitae, Acknowledgements. Literature review related to the topic of the research is presented at the beginning of dissertation. Paper I describes the effect of prophylaxis during prepartum on the negative energy balance state of cows and investigates its benefits to the health status of animals evaluated by the use of herd health management system parameters.

Paper II describes the metabolic state and relationship between cow and its calf and how it affects the health status of the participants evaluated via calf health evaluation schematic and herd health management system parameters.

Hypothesis

Prepartum period of the cow has an influence on the energy and health status of not only the cow, but her calf as well, therefore prophylactic supplementation against negative energy balance state during prepartum should have a positive effect on health and energy levels postpartum.

The aim of the study

Aim of the study was to evaluate the impact of negative energy balance state and it's prophylaxis during prepartum period on health and metabolic parameters of cows and their calves after parturition.

Objectives of the study

1. To evaluate the effectiveness of prepartum prophylaxis against negative energy balance state and concurrent udder inflammation by using herd management system parameters postpartum: body condition score, milk yield, milk β-hydroxybutyrate concentration and milk lactate-dehydrogenase activity;
2. To evaluate cow lactation number influence on cow and their calves metabolic blood parameters of β-hydroxybutyrate (BHB) and glucose (Glu) concentrations and health status;
3. To evaluate the metabolic relationship between prepartum cows and their calves by analyzing blood BHB and Glu concentrations postpartum and calf health monitoring system parameters;
4. To evaluate the impact of prepartum cow negative energy balance on calves health status by analyzing cow herd health system and calf health monitoring scheme parameters postpartum.

1. LITERATURE REVIEW

1.1. Prepartum period influences cow health status in the upcoming lactation

The prepartum period places a significant stress on the pregnant cow's internal processes and in turn leads to certain health risk in this period and the upcoming lactation [29]. One of the negative effects at this stage is metabolic stress which is characterized by the onset of lipid mobilization, diminished immune response and oxidative stress (OS) [30]. The immune system of the cow is compromised and it becomes more susceptible to disease at this stage. One of the reasons for a weaker immune response is the oxidetive stress that cows face [31, 32]. Nevertheless, in a study of Abuelo et al. [33], cows with more significant signs of OS tended to have a lower sensitivity of insulin in their peripheral tissues, pointing towards a negative relationship between OS and insulin resistance (IR). Immune response in prepartum cows is also impeded due to active lipolysis and excess circulating non-esterified fatty acids (NEFA). A higher concentration of these metabolites causes an increased expression of TNF-α, which reduces the production of acute phase proteins therefore weakening the immune response [34]. A surge of glucocorticoids and steroids prepartum have a negative impact on the host immune response [35]. Even though the prepartum cow faces major overhaul in hormonal regulation that may affect the immune system, but still the main reason for a diminished immune response is the negative energy balance (NEB] and the fat mobilization that follows. This was evident in the research of Kimura et al. [36] and Nonecke et al. [37]. In their studies they performed mastectomies for prepartum cows and compared the immune response and the metabolic status of these cows with lactating ones. Mastectomized cows still exhibited typical hormonal changes around calving, mostly steroid hormonal changes, but their immune system was compromised for a shorter duration in comparison to lactating cows post calving [36, 37]. The onset of lipid mobilization before calving, indicated by higher concentrations of blood NEFA, was also noted as having a negative effect on the immune system of dry cows [34]. Before parturition, the composition of NEFA in the blood changes in a way that impedes the activity of immune cells due to lack of essential fatty acids. The lipid membrane fluidity and lipid raft formation are influenced by the relative formation of fatty acids in the membrane phosphor-lipids. Nonetheless, fatty acids have a role to play in regulating intracellular signaling and are important in receptor binding [38]. During intense fat mobilization, a decrease of inflammatory responses to Gram-negative bacteria, for example, are initiated when TLR4 (toll-like receptor 4) pathogen recognition receptors on host cells interact with the lipopolysaccharide (LPS) component on the outer membrane of the pathogen. A major signaling pathway downstream of TLR4 activation is nuclear factor kB (NFkB), which plays a central role in regulating inflammatory gene responses. Certain fatty acids including lauric, palmitic and oleic acids can activate NFkB signaling through interaction with TLR4. In addition, both EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) can inhibit LPS induced NFkB activation by directly targeting TLR4 or its associated molecules. Certain saturated and PUFA also can bind a family of nuclear receptors called peroxisome proliferator-activated receptors (PPAR). Both α-linolenic acid and DHA are omega-3 fatty acids that can serve as potent ligands for PPAR and function to downregulate inflammatory reactions in many cell types, including leukocytes and endothelial cells. In contrast, palmitate and stearate can enhance pro-inflammatory signaling pathways through the activation of NFkB [38, 39].

The contents of the fatty acids in the blood due to lipomobilization can influence the immune response of the cow. One of the mechanisms for this is the oxylipids – mediators for immune cell signaling derived from phospholipids and NEFA [40]. Oxylipids originating from oxidation of omega-6 fatty acids (thromboxanes, leukotrienes and prostaglandins) have a tendency in promoting inflammation, whereas oxylipids stemming from omega-3 fatty acids (resolvins and protectins) tend to resolve the inflammation process [41]. A hypothesis was formed that by modifying the quantity and ratio of omega-6 and omega-3 fatty acids in the feed for prepartum cows, their acute phase response and neutrophil function might be improved [42]. More direct data on this relationship was observed in the work of Contreras et al. [43], where bovine endothelial cells were supplemented with omega-3 fatty acids were more stable when challenged with high NEFA concentrations. The authors note that the reduced inflammatory response was influenced by oxylipids (resolvins, lipoxins and protectins) from the omega-3 acid supplementation [43].

There is evidence of a relationship between inflammatory response and the metabolic state of the cow. In a study of Bertoni et al. [44], it was noted that preparturient cows showed a more severe immune activity even though no traces of bacterial infection or other pathologies were found in the study group. Cows more prone to obesity and fatty liver syndrome have a higher blood serum concentration of pro-inflammatory cytokine TNF-α [45]. Nevertheless, an adverse reaction was also reported in a study of Bradford et al. [46], where late lactation cows were directly infused with TNF-α and an induced insulins resistance followed together with higher concentration of triglycerides in the liver. The onset of lactation also had a negative impact on the population of peripheral blood leukocytes [36]. The diminished availability of glucose for macrophages and neutrophils during the transition period partly explains the compromised immune response [47, 48]. Not only a lack of glucose impairs the immune function, but also the increased concentrations of BHB, most often registered during NEB, have a detrimental effect on the antimicrobial mechanism of leukocytes and neutrophils [49, 50]. In another study done by Farney et al. [51], inflammation for cows in early lactation was controlled by sodium salicylate which in turn also caused hypoglycemia.

1.2. Prepartum risk factors and their effect on calf health

Calves are most at risk of succumbing to disease during the first month of life, therefore an adequate start in life should be provided for them by the dam during the dry period [52].

Protein restriction during mid and late gestation periods has a tendency to negatively affect the glucocorticoid metabolism by suppressing the expression of hepatic glucocorticoid gene $GR1_{10}$ expression [53]. In the work of Kwon et al. [54], sheep that for a short period of time received a diet lacking in amino acids, showed a change in amino acid concentrations, foetal and maternal amino acid profiles and ratios. Even after amino acid concentrations and supply returned to normal, these adaptations persisted [54]. One of these changes can be seen in the work of Houin et al., where they registered a drop in foetal glutamate concentration when the mother was underfed [55]. Glutamate is very important in foetal development since it is used in oxidation and NADPH (nicotinamide adenine dinucleotide phosphate) synthesis, which is required for further steroidogenesis, nucleoside production and lipogenesis as well [56]. Since the fetus needs to keep a certain glucose concentration ratio to maternal glucose, it synthesizes glutamate in its liver and sends it towards the placenta for further oxidation as an energy source. This allows for maternal glucose to be easily absorbed by the fetus following the glucose gradient as it is kept low in the fetus. However, during maternal hypoglycemia, fetal liver switches from glutamate synthesis to glucose, to maintain homeostasis [57]. Such changes in liver activity and glucose metabolism can lead to insulin resistance in postnatal life and have been registered in human children with diabetes [58]. The effects of undernutrition have been studied more in detail in the work of Ma et al. [59]. In their study, pregnant malnourished and normally fed ewes had samples of their cotyledonary tissues taken. These samples were examined for messenger RNA (ribonucleic acid) levels of GLUT1 (glucose transporter 1), FATP4 (long-chain fatty acid transport protein 4) genes and registered higher levels in malnourished group ($p < 0.05$, by 55% and 45% respectively). The activation of these genes shows an adaptation of the placenta to maintain glucose and fatty acid transportation in order to uphold homeostasis [59]. Through activation of these genes, off-spring born to underfed ewes in comparison to normally fed, had a tendency for more adiposity, better weight gain but suffered from insulin resistance and pancreatic failure. It is important to note that the underfed group was feed restricted only during mid-gestation and later received the appropriate diet until lambing. This indicates that even a short period of restricted metabolism can have a permanent effect on the offspring's gene expression and pheno-type [60].

Overnutrition during the mid and late gestation periods influences the expression of intramuscular adipogenesis of the fetus. In a study of Duarte et al. [61], calves of overfed cows presented with more expressed Zfp423 (zinc finger protein 423), PPARɣ (peroxisome proliferator activated receptor gamma) and C/EBPα (cytosine-cytosine-adenosine-adenosine-thymidine-enhancer-binding protein alpha) mRNA (messenger ribonucleic acid), involved in intramuscular adipocyte proliferation and differentiation. Cows exposed to metabolic stress in the prepartum, expressed by higher concentrations of NEFA and higher oxidative stress status gave birth to calves that were of lower body weight and had a weaker immune response when challenged [30].

Health of the dam influences the quality of colostrum and has direct effects on the mortality of newborn calves. The importance of colostrum for the calf and a successful passive transfer was investigated in a study done by Windeyer et al. [52]. Out of 2 874 heifer calves involved in the study, 11% had failure of transfer of passive immunity (FTPI) when the cut-off point for serum total protein (STP) concentration was set at 5.2 g/dL and 32% when the cut-off point was 5.7 g/dL. The fore mentioned cut-off points were inclu-ded in the regression analysis as risk factors for mortality and susceptibility to neonatal calf diarrhea (NCD) and bovine respiratory diseases (BRD). 5.7 g/dL STP was a good indicator of BRD in the first 5 weeks of life (sensiti-vity = 40%, specificity = 69%, positive predictive value = 18%, negative pre-dictive value = 87%). 5.2 g/dL STP was a good predictor for death in the first 5 weeks (sensitivity = 27%, specificity = 89%, positive predictive value = 5% and negative predictive value = 98%). On the other hand, STP did not have good predictive value when it came to NCD. The data indicates that calves with FTPI were more at risk of disease and the chance of them dying during the first 5 weeks was increased. Average daily gain of these calves was also lower in comparison to non-immune-suppressed calves [52].

Heat stress is one of the factors influencing the health of the dam and the quality of her colostrum. In a study of do Amaral et al. [62] they investigated and compared colostrum quality of prepartum cooled and heat stressed cows. Results showed that prepartum cooled cow colostrum had higher concentrations of immunoglobulin G (IgG), which pays an important role in developing the immune system of the calf [62–64]. On the other hand, the studies carried out by Laporta et al. [65] compared colostrum IgG concentrations between cows cooled and not cooled during prepartum and found, that non-cooled cows had a higher concentration, whereas colleagues Tao et al. [66] and Monteiro et al. [67], found no significant difference in IgG concentration. However, on both occasions the calves of non-cooled cows reported increased incidence of neonatal disease, calling further study on the effects of heat stress on colostrum quality and immune system strength of calves [66–68].

Monteiro et al. [67] further investigated the relationship between prepartum heat stress, colostrum quality and calf immune system in another study. Colostrum from cooled and non-cooled cows was provided for calves born during summer, the same colostrum was frozen and provided for calves born in winter season. Results show that while there was a difference in IgG absorption during summer compared between calves of cooled and non-cooled calves, in the winter time the absorption was similar–meaning the heat stress experienced by the fetus modifies it's ability to absorb IgG and plays a more important role than colostrum IgG contents [67].

Heat stress during the third trimester has detrimental effects to the development of the fetus and will impact the health status of the neonate [69]. One of the mechanisms for this negative relationship is due to a restriction in blood flow through the placenta [70]. In a thermoneutral environment during the last trimester, the uterine blood flow of cattle increases by 4 times (from 2.9–13.2 $l\ min^{-1}$), in order to keep up with the nutritional and oxygen demands of the rapidly growing fetus, oxygen uptake by the fetus increases 16 times and glucose demand from 4 to 19% [14, 71–73]. The increase in blood flow is directly involved in the transportation of nutrients through the placenta as a difference in gradient is required for successful transfer [74]. However, during heat stress, the blood flow to the uterus and the fetus is restricted. In a study from Regnault et al. [75], pregnant ewes exposed to heat stress environment had significant changes to their umbilical and uterine blood flow in comparison to thermoneutral pregnant ewes. While uterine blood flow was significantly increased in the heat stress group, umbilical blood flow was reduced, this in turn changed the uterine/umbilical blood flow ratio from 2.28 (± 0.45) in thermoneutral group to 3.94 (± 0.35) in heat stressed ($p < 0.03$). For reference, uterine/umbilical blood flow ratio in a non-compromised pregnancy should be around 2.0. Also, while uterine vein O_2

saturation was increased in heat stressed cows compared to thermoneutral group and was still similar in the maternal artery, the fetal artery O_2 saturation was significantly lower in the heat stressed fetus (44.19% *vs* 25.73%, $p < 0.01$). The restricted blood flow and nutrient transfer experienced by the heat stressed group resulted in a 50% lower placental weight and 40% lower fetal weight [75, 76].

A number of studies have shown that heat stress, when not managed during prepartum, can lead to a significant decrease in neonate weight, ranging from a loss of 0.6 kg to 13 kg ($p < 0.001$) [69, 77]. This is a challenge for the dairy industry as calves with a lower birthweight are more at risk of death in the preweaning period, due to an insufficient development of essential organs and weaker immune response. Maldeveloped small intestines and lungs, oftentimes accompanied by smaller spleen and thymus sizes, results in more abundant cases of neonatal diarrhea and pneumonia. Underdeveloped small intestines have a retarded ability to absorb nutrients and immunoglobulins from colostrum and milk due to a higher percentage of jejunal enterocyte apoptosis; lung tissue has trouble performing blood-gas exchanges and fighting of viral infections; and smaller spleen and thymus limits the production of antibodies and other immune cells [78–80].

Research indicates that the fetus adapts to prioritize glucose as an energy source and therefore BHB and NEFA are relatively untouched, whereas with calves that were subject to heats stress abatement, BHB and NEFA were used as energy, most likely due to their rapid growth and higher energy requirement. Non-cooled calves also showed a slower insulin clearance after an insulin challenge, but a similar glucose response compared to cooled calves, suggesting a weaker insulin response in muscle and adipose tissues. While calves of non-cooled cows had a greater clearance of glucose, the pancreatic insulin response was similar to cooled calves, therefore it is theorized that prepartum heat stress modifies the calves' ability for glucose uptake by non-insulin dependent tissues [69]. Similarly, results of lactating cow plasma glucose concentration also point to this adaptation – prioritizing glucose over NEFA and BHB, therefore it adds more basis to the fact, that the fetus is also programmed for it during prepartum [81]. The negative effects during the prepartum not only affect the cow lactation and the health of the neonate, but also results in loss of production in the future for the calf. Growth up till weaning is suppressed for such calves and future lactations suffer because of heat stress in prepartum. Some evidence indicates that the genetics could be affected even for the future generations as well [65, 82].

1.3. Possible mechanism for fetal programming

Environmental and internal factors influence the development of the fetus through processes of DNA (deoxyribonucleic acid) methylation of cytosines at cytosine-guanosine pairs and by modifying the histones of the DNA molecule. Affected parts of the DNA molecule determine if the genes are available for transcription or not, thus determining the phenotype of the offspring [83]. The work of Skibiel et al. [84] delves further into fetal programming. The team have found that heat stress experienced in the prepartum period affects around 400 differently-methylated-genes and differently-methylated-cytosines that are responsible for processes like development of cells, innate immune response, transcription, translation and cell signaling. The forementioned genes were altered by having their cytosine-phosphate-guanine (CpG) sites methylated. In the same study, morphological differences between liver and mammary gland tissues between heat stressed and cooled groups were registered, giving reason to believe that heat stress caused changes in gene expression and the morphology of cells and tissues. However, the authors also state that more solid research should be performed, and more clear data should be gathered, as they did not find a strong correlation between gene methylation and gene expression. They also state that there are conflicting results between studies, where on one hand promoters rich in CpG sites are often times found methylated, and their gene expression inhibited. Whereas on the other hand, CpG poor promoters are methylated but the transcription is not affected. The authors also discuss that DNA methylation remains rather stable throughout life, but changes in gene expression are variable and monitoring them provides us with the view of changes occurring only at that exact moment. They recommend that more effort should be put into examining other mechanisms of epigenetics – histone modifications, microRNA activity and chromatin remodeling [84–90].

1.4. Difficulties in monitoring prepartum period with innovative systems

Monitoring cow health status during prepartum is challenging. Since most novel herd management system algorithms depend on milking and milk quality parameters, the physiology of a dry cow prevents dairy producers from accessing this data. Therefore, other parameters and dry cow behaviour must be utilized. Majority of monitored parameters during prepartum are connected to calving prediction and do not provide a lot of information about cow metabolism or health status [91]. Nonetheless, some systems are able to provide data that could be used for monitoring health status while their main purpose is to predict an upcoming calving. Braun et al. [92] used MSR noseband sensor (MSR Electronics, Seuzach, Switzerland) that is able to monitor cow eating and rumination behaviour and established that rumination time was a better predictor compared to eating time, as the latter tends to have a steep decline on the day of calving, while rumination time starts to decline around 10 days in advance. Rumination and eating time together with cow activity, monitored with Afimilk Silent Herdsman (Afimilk Ltd., Israel) and tail mounted Axivity systems (AX3 3-Axis logging accelerometer; Axivity, Newcastle upon Tyne, UK) respectfully, were also used for calving prediction in the study of Miller et al [93]. Feeding behaviour and cow activity are well known parameters used for metabolic disease diagnosis, reproduction management and lameness prediction [94–96]. Even if these monitoring technologies are being used in the prepartum period by the scientific community to determine animal health status, these products are not always used in the dairy industry or are only used for calving prediction. More effort should be put into the research of these products on how to better use them in industry and what benefits farm managers could get from doing so.

1.5. Herd health system parameters indicating negative energy balance

During the early lactation period cows are most at risk of diseases therefore herd health management systems are used to monitor their health status [97]. An increase in health risk arises from greater demand to maintain productivity, which causes negative energy balance [98]. Following the metabolic changes, a cascade of bodily functions and systems start to fail which develops into clinical diseases such as ketosis, abomasal displacement, metritis and udder inflammation. In order to diagnose these diseases a variety of herd health management systems are used in dairy farms that monitor a wide array of parameters through the help of innovative sensors [99]. One of the main and most often used parameter is rumination time. A decrease in rumination activity is a sure signal that there is a disturbance in the bodily functions of a cow. Though quite sensitive, rumination time is not very specific as it’s decrease can be registered with most of the diseases [100]. Similarly, a decrease in milk yield is also a sensitive indicator with most of the diseases in early lactation period, yet it also lacks in specificity [101].

Recently milk biochemistry and milk quality have been used to diagnose negative energy balance state and other diseases [102]. Increase in blood BHB concentration is a golden standard for negative energy balance and ketosis diagnosis, it is rather labor and time consuming to perform [103]. However milk BHB concentration is a very good alternative and it does not interfere with the welfare of animals [104]. Another metabolite extracted from milk and used in diagnosis is lactate-dehydrogenase enzyme activity. Higher concentration of this metabolite in the milk indicates of cell degradation and is closely related to the udder health status [105]. A simpler method and used even before advances in milk biochemistry is milk quality parameter assessment. Quality parameters as milk fat, protein and lactose concentrations and especially their dynamics together are good indicators of the metabolic state of cows. An increase in milk fat to protein ratio is widely used to diagnose ketosis or acidosis not only on a herd level, but with each individual animal as well [106]. Together with a decrease in milk lactose concentration it is a sure signal that the energy levels in cows has been compromised [107, 108].

1.6. Prepartum prophylaxis with monensin against negative energy balance and necessity for alternatives

Monensin supplementation is an example of how supplementation during prepartum can have a positive effect for the cows upcoming lactation and gives an incentive to explore further supplementation options in order to not help just the cow, but the calf as well. This ionophore has been used in the dairy industry for some time in order to diminish the negative aspects of subclinical and clinical ketosis. By inhibiting Gram-positive bacteria and at the same time not affecting the colonies of Gram-negative bacteria, it allowed to lower the synthesis and concentration of butyrate fatty acids in the rumen. This in turn allowed propionic acid producing bacteria to thrive and provide the liver with necessary components for gluconeogenesis, ensuring adequate levels of glucose for cell metabolism and diminishing the development of negative energy balance [25, 109].

Supplementation with monensin affects multiple bodily systems. Some research indicates that cows receiving this supplement were better at maintaining or even increasing their body weight and BCS compared to non supplemented groups [109]. Although other studies were not able to reproduce the same results, therefore the mechanism remains inconclusive [110]. One theory is that by ensuring propionate delivery for gluconeogenesis, monensin provides relief for the liver and minimizes the risk for lipid accumulation. Therefore, resulting in lower weight of the animal [25]. A healthy liver is also able to continue all of the necessary biosynthesis, gluconeogenesis and detoxification of metabolites [109]. Monensin in the rumen reduced the rate of protein degradation and slowed down ammonia accumulation. This ensured that more protein passed into the small intestines and were absorbed in the form of amino acids [111].

Proper dosage of this supplement is important for the productivity benefits and to avoid toxicity. Some studies indicate that a dose of 23 ppm daily provided a sought-after effect of increased milk yield, but further increasing the dose caused the milk yield to drop. Similarly, raising the dose always had a negative effect on the dry matter intake of animals. Interestingly, higher than normal dose of monensin managed to always increase the milk protein concentration [110].

However, monensin in higher doses is toxic for cattle and needs to be supplemented with extreme care in order to avoid significant economic loses [112]. Special products for use in the dairy industry have been developed that guarantee adequate dosage and release of required amount of the substance into the rumen [113]. However, due to a lack of quality control and increasing number of cases, where the faulty product was in reach of non-intended animals and caused toxicosis, together with rising concerns of possible antimicrobial resistance, the product was banned from European Union on the 15th of May 2024 [114, 115]. Therefore a need for alternatives arises to manage the negative energy balance state during prepartum and to avoid clinical diseases in the upcoming lactation. Supplementation with carbohydrates, fatty acids, amino acids and other substances are being researched as possible cases [42, 56, 116].

Considering the data from scientific literature, we set out to investigate the relationship between pregnant dam and its calf concerning their energy status and its effect on health status postpartum and neonatal life. One of the factors, influencing the energy levels and metabolism in cows is parity, therefore its effect on neonate vitality and health status needs to be studied. Supplementation during prepartum to mitigate the harmful effects of negative energy balance has been studied and results indicate a lower risk of ketosis in supplemented cows. However, more data is needed for its effects on the development of other diseases in cows, especially udder inflammation. Moreover, the herd health system parameters indicating of udder inflammation and their relationship with negative energy balance indicators need more investigation. Combining parameters of herd health systems and calf management schemes with prophylaxis against negative energy status during prepartum can improve health status and productivity in dairy farms.

2. MATERIALS AND METHODS

2.1. Studying effects of prepartum prophylaxis on negative energy balance state and health status of cows

2.1.1. Farm and animal selection

This study was carried out during 2021.03.01–2021.09.30 at one Lithuanian dairy farm with 550 dairy cows. All cows were multiparous, in their 2nd and 3rd lactation since they are more at risk of negative energy balance compared to primiparous cows. The cows were fed total mixed ration (TMR) at 5 A.M. and 5 P.M. (twice per day) and housed in a loose housing system. The feed ration was balanced to fit the energy and nutrient requirements calculated based on the NRC [117] for Holstein cows producing, on average, 40 kg/day of milk and weighing 550 kg on average. The cows were milked twice per day (at 5 A.M. and 5 P.M.) using a parlor system. The average energy corrected milk yield (4.15% fat, 3.6% protein) in 2020 was 10 500 kg per cow and year.

During prepartum period, all of the cows enrolled in the study were kept in a separate freestall barn with straw bedding. They were provided a total mixed ration that consisted of 26% crude protein, 32% ashes, 11% acid detergent fiber, 21% neutral detergent fiber and 4% crude fat. The feed also contained dietary minerals containing 20% calcium, 5% phosphorus, 8% magnesium, 0.62% potassium, 0.7% sulphur, 1.83% sodium, 0.3% chloride and traces of iron, manganese, zinc and copper.

2.1.2. Study and control group determination

As a prepartum prophylactic measure against negative energy balance state, the monensin controlled-release capsules (Kexxtone Elanco GmbH, Bad Homburg, Germany) were used for the study. The capsules emitted monensin at daily doses of 335 mg, when administered once with an oral balling gun in accordance with the manufacturer's instructions. Two experimental groups were formed as follows: 1. GK – cow group supplemented with monensin as a prophylactic measure against negative energy balance state (n = 42) and 2. GO – cow group that were administered a capsule containing no monensin (n = 42) (Fig. 2.1.2.1). Treatment began 21 days before calving as per instruction from the product manual. Both groups were housed in the same environment until calving. After calving they were transferred to a group of other lactating cows and were milked in a robotic milking system. They were monitored for 30 days.

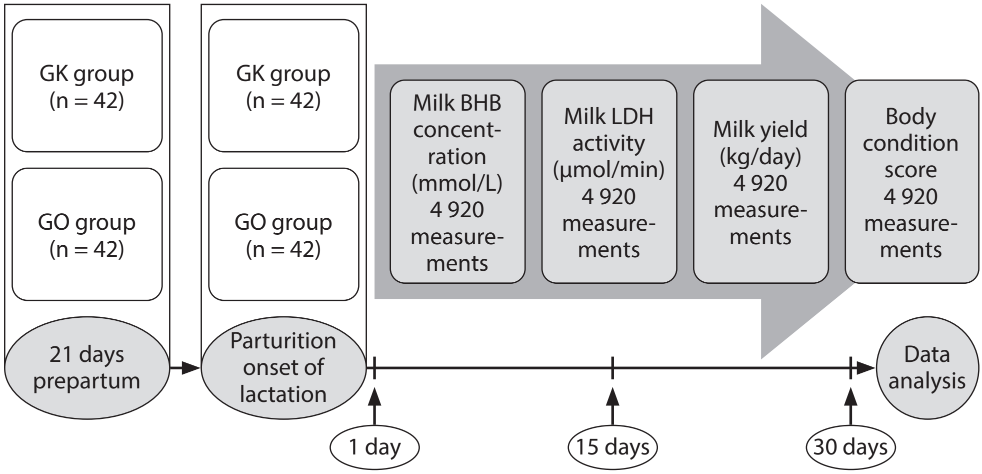


Fig. 2.1.2.1. *Schematic presentation of first study*

GK group – Cow group that received prophylaxis of monensin slow-release capsules in the prepartum; GO group – Cow group that received an empty capsule without monensin; n – number of animals in group; BHB – β-hydroxybutyrate; LDH – lactate-dehydrogenase; Measurements – number of parameter values registered throughout study period.

2.1.3. Herd health management system data analysis

With the help of the Delaval milking robot (DeLaval Inc., Tumba, Sweden) and Herd Navigator analyzer (Lattec I/S, Hillerød, Denmark), values of milk yield (kg/day), milk β-hydroxybutyrate (BHB) concentration (mmol/L) and lactate dehydrogenase (LDH) activity (µmol/min) were registered during milking. 4920 measurements for each parameter were recorded during the study period between cows from both groups. A representative milk sample of several milliliters from each cow was taken by an inline automatic sampler during the robotic milking process. Optical milk meter measured and determined the milk yield from each milking. Milk BHB concentration was determined by dry-stick method integrated into the analyzer. Normal levels of milk BHB are referenced in the study of De Jong et al. [104]. LDH activity was calculated and determined, dividing it from the milk yield of the latest milking session. Normal LDH activity threshold is referenced in the work of Larsen Torben [118].

Before entering the milking robot, each cow's body condition score (BCS) was recorded by a 3D BCS camera (DeLaval International AB, Sweden). Animals were recorded from above, and the rear part of the back (from the short ribs to the tail end) and assigned a score. Every time a cow walks under the camera, the system identifies the specific movement and captures images of the cow; it then chooses the best image of the cow in the video recording. The 3D-camera uses light coding technology, which projects a pattern of infrared ray dots on the back of the cow. Following this, the distances between these certain dots are measured; according to the manufacturer, a 3D-image of the back is modeled, and an algorithm converts that image information into a body condition score. As a golden standard, the scale used to develop the algorithm was based on visual scoring using a 1–5-point scale system [119]. In this scale, the spinous to transverse processes are assessed and given a specific score, where one corresponds to the lowest and five to the highest condition score. A normal BCS range of 3–3.5 for cows in early lactation period was also established in a study of Roche et al. and was used in this study to compare results [119].

2.2. Studying the metabolic relationship between cow and calf with its impact to health status

2.2.1. Farm and animal selection

In order to perform this study, an approval under the number G2-227 was received from the State Food and Veterinary Service's Department of Animal Welfare. All of the procedures were performed according to Lithuanian Law on Animal Welfare and Protection.

To study the relationship between cow and calf, in 2022 we chose a dairy farm of up to 1200 milking Holstein-Friesian cows that was situated at 54°58'34.9" N 23°46'04.2" E coordinates in Lithuania. The farm had a free-stall barn system where the milking process was carried out by Lely Astronaut 3 milking robots (Lely, Maassluis, The Netherlands). Cows were fed a total mixed ration daily at 5 A.M. and 5 P.M. all year round. Primiparous and multiparous cows received the same feed ration which consisted of 50% grain slurry, 16% grass silage, 24% corn silage, 5% grass hay and 5% mineral mixture. 48% of the ration consisted of dry matter, of which acid detergent fiber accounted for 20%, 39% consisted of non-fiber carbohydrates, 16% crude protein and 28% made up of neutral detergent fiber. According to the National Research Council (NRC), such a feed provided enough nutrients for a 550 kg Holstein-Friesian cow with an average milk yield of 40 kg/day [117]. Around 12 000 kg of milk per cow per year was produced during 2021.

To compare the effect of parity to the relationship between cow and calf, 20 calves from primiparous (2 years of age) and 20 calves from multiparous (only 2nd and 3rd lactation, 3 and 4 years of age, respectively) cows were selected (Fig. 2.2.1.1).

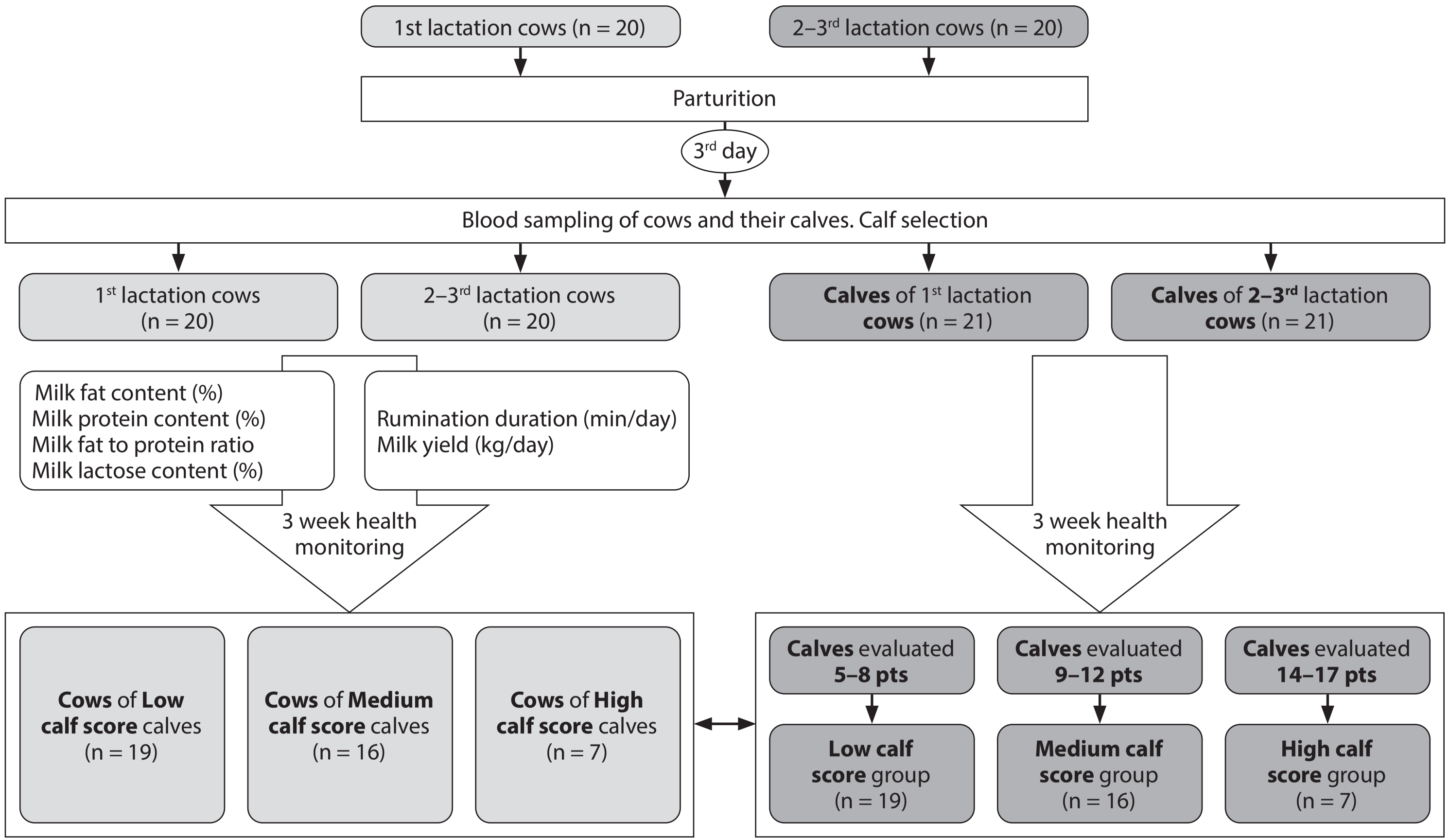


Fig. 2.2.1.1. *Schematic of the second study*

n – number of animals in group; pts – point values from a calf health monitoring scheme assigned to a calf, according to it’s clinical symptoms.

Calves born from 25th of May 2022 to 25th of April 2022 were selected in order to minimize the seasonal and climatic effects on the animals. In order for the calf to be included in the study and to maintain similar conditions for the participants, a calf needed to fit certain criteria: 1. Calving was easy and no assistance from a veterinary professional was required; 2. Cow was healthy prior calving; 3. Calf received good quality colostrum from its dam. Colostrum quality was evaluated with MS Colostro Balls (MS Schippers, Kerken, Germany) system – color coded tablets of different density. It was done at a required temperature of 20–30 °C as per manual. Trained staff members provided 4 L of colostrum for the calves in the first hour after birth and repeated after 12 h. If all of the tablets float, this indicates that the colostrum density has reached at least 1 075 g/dm^3 indicated as "very good" [120, 121]. Colostral density unit threshold of 1 075 g/dm^3 converted to specific gravity would amount to 1.075. Good quality colostrum is indicated by a specific gravity of > 1.047 and is confirmed to be used for IgG concentration evaluation, since it correlates well with IgG concentration evaluation performed by radial immunodiffusion (r = 0.77) and ELISA (enzyme-linked immunosorbent assay) (r = 0.79) methods [122–124].

After receiving their first colostrum, calves were separated into single pens. Their feeding consisted of milk replacer (Sprayfo Yellow, Trouwnutrition, Putten, The Netherlands) and concentrated feed. Composition of milk replacer: whey powder, 10% skimmed milk powder, palm and coconut oil, hydrolyzed wheat proteins supplemented with vitamins and minerals. This amounted to 21.5% of crude protein, 17.5% crude oils and fats together with 9% crude ash. There was no crude fiber in the milk replacer. Each kilogram of milk replacer also contained 25 000 IU of vitamin A, 5 000 IU of vitamin D_3, 90 mg of iron, 10 mg of copper, 300 mg of vitamin E and 0.3 mg of selenium. The solution was mixed with water at 45–55 °C at a ratio of 130–140 g per liter. A total of 4 L of replacer was fed to the calves twice a day every 12 h. The temperature at the time of feeding was 40 °C.

2.2.2. Calf study group forming and health status evaluation

On the 3rd day after calving, a full clinical examination was carried out for each calf (n = 42, note that the number of calves differs from the number of cows in the study, since two cows had twin births, and the calves were enrolled into the study). A trained veterinary professional with 5 years of work experience performed the clinical examination and continued the evaluation of calf health throughout the study period. Clinical examination and calf health evaluation methodology was based on McGuirck [125]. Nasal discharge, ear position, eye discharge, rectal temperature, diarrhoea and presence of disease were evaluated. In this scheme (Fig. 2.2.2.1), a score of 0 represents a lack of clinical symptoms and indicates a healthy calf, while presence of other symptoms and their severity indicate a possible disease. We did not record clinical diagnoses such as pneumonia and / or diarrhoea, as the scoring system is not designed that way. A higher score in multiple examined symptoms is a good indicator of disease.

School of Veterinary Medicine
UNIVERSITY OF WISCONSIN–MADISON

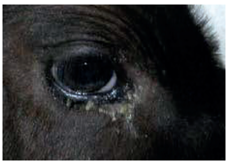
Calf Health Scoring Criteria			
0	**1**	**2**	**3**
Rectal temperature			
100–100.9	101–101.9	102–102.9	≥ 103
Cought			
None	Induce single cough	Induced repeated coughs or occasional spontaneous cough	Repeated spontaneous coughs
Nasal discharge			
Normal serous discharge	Small amount of unilateral cloudy discharge	Bilateral, cloudy or excessive mucus discharge	Copious bilateral mucopurulent discharge
			
Eye scores			
Normal	Small amount of ocular discharge	Moderate amount of bilateral discharge	Heavy ocular discharge
			
Ear scores			
Normal	Ear flick or head shake	Slight unilateral droop	Head tilt or bilateral droop
			

Fig. 2.2.2.1. *Calf scoring chart for health evaluation*

Source: McGuirk SM, Peek SF. Timely diagnosis of dairy calf respiratory disease using a standardized scoring system. *Animal Health Research Reviews*. 2014;15(2):145–147.

Calves that fit all of the required criteria were included in the study and a clinical examination was performed every 2–3 days for a period of 3 weeks. After each clinical examination calves were evaluated according to the severity of clinical symptoms and received a score for that day. At the end of each calves' 3 week examination period, every score of that calf was summed up and a final calf score was determined. After all of the calves were evaluated and had their final score, a class interval of "4" was used to assign calves into distinct groups. A similar method for group division has been described in the work of Maier et al. and has been modified for this study [126]. Calves with a score of 5–8 were assigned to the Low calf score group (LCS), calves that showed the least symptoms and were at a low risk of disease (n = 19). Since no calf had a score of "13", the Medium calf score group (MCS) consisted of calves that reached a score of 9–12 (n = 16) and were considered at a medium risk of disease. The High calf score group (HCS) consisted of calves that scored 14–17 points (n = 7) and were considered calves at a high risk of disease.

2.2.3. Cow and calf blood sampling and evaluation

On the third day after parturition, a blood sample via jugular venipuncture was taken from calves into a blood biochemistry tube (BD Vacutainer Red, Mississauga, Canada) without any conservatives. A drop of blood was drawn from the tube and used in a hand-held blood glucose (Glu) and β-hydroxybutyrate (BHB) meter (CentriVet GK, Acon, San Diego, USA). The blood samples were transported in 4 °C to the laboratory for further analysis within an hour from sampling. In the laboratory, the samples were centrifuged at 1 200 RPM for 8 min.

The centrifuged serum was then analyzed to determine the serum protein concentration and to evaluate the presence of failed passive transfer. The sample serum was evaluated with a hand-held refractometer (RHC200, YHequipment, Shenzhen, China). According to Renaud et al. [127], the threshold for failed passive transfer is < 5.2 g/dL. No calves were removed from the study and had an adequate passive immunity as all calves had an average serum protein concentration of 7.6 g/dL.

As sampling was done for the calves, on the same day, meaning on the 3^{rd} day of lactation, blood samples were taken from their dams. A blood sample from the coccygeal vein was taken into a red biochemistry tube (BD Vacutainer, Mississauga, Canada); a drop of blood was used to determine the concentration of Glu (mmol/L) and BHB (mmol/l) by using a hand-held device (CentriVet GK, Acon, San Diego, USA). The samplings for both cows and calves were performed once on the third day of life and lactation. By measuring blood BHB of cows we also intended to identify any cows with increased levels of this metabolite, indicating health issues and early onset of NEB and/or clinical ketosis. Such cows would have been removed from the study. We chose to monitor NEB state, as the main study point of the thesis, with the use of herd health monitoring system parameters that provided us with more data points and continuous surveilance of our study animals.

2.2.4. Cow study group determination and herd health management system parameter data

After 3 weeks of lactation for each cow and when the evaluation of their calf health score was performed, they were assigned into groups according to their calf health score (LCS, MCS, HCS). Then data from automated milking system Lely Astronaut A3 (Lely, Maassluis, The Netherlands) were retrieved for a period of 3 weeks. Parameters such as rumination time (RT) (duration of rumination in minutes per day, 1 200 measurements), milk yield (MY) (kilograms of milk produced per day, 2 400 measurements), milk protein content (MP) (percentage of milk protein in milk, 2 400 measurements), milk fat content (MF) (percentage of milk fat in milk, 2 400 measurements), milk lactose concentration (ML) (percentage of milk lactose in milk, 2 400 measurements) and milk fat and protein ratio (MF:P, 2 400 measurements) were monitored, analyzed and compared between groups. These parameters were registered each day for each cow and the mean value of the 3-week period was used for statistical analysis.

2.3. Statistical analysis

SPSS 26.0 (SPSS Inc., Chicago, USA) was used for statistical analysis. For the data concerning the study of prepartum prophylaxis, statistical analysis of the automatically registered body condition score and inline biomarker data was evaluated for normal distribution using the Shapiro-Wilk test. Milk LDH values were converted to logarithmic expressions ($\log_{10}$). We used the general linear repeated measures model (repeated measures with the "between subject factor" test) to compare the indicators of days (1, 15 and 30) and between GO and GK groups of cows. The results of the study were given as means and standard error (SEM) and 95% confidence interval for mean. The Bonferroni test was used to compare data by day and by group. A linear and second-order polynomial regression was used to analyze the change in the continuous variables of the automatically registered body condition score and inline biomarkers during the experiment. Milk BHB measurements were classified according to the values recorded in the study (0.04, 0.05 and

0.06 mmol/L). Fisher's exact test was used to assess the relationship between β-hydroxybutyrate levels.

On data gathered from the study concerning cows and their calves' metabolic relationship and its effect on health status, Shapiro–Wilk test was used to determine the normal distribution of blood biochemistry data, as the number of measurements was low. Cow BHB and Calf BHB data were not normally distributed. However, for normally distributed data of cow and calf glucose concentrations, a difference between parity groups was determined using One-way ANOVA. The results were presented as means and standard deviation. Correlation analysis of normally distributed parameters was performed via Pearson correlation and the strength of the correlation was set as follows: |0.1–0.3| = low, |0.3–0.5| = moderate and |0.5–1.0| = strong. Non-normally distributed data were analyzed with the Mann–Whitney U test and the distribution of calf score class between parity groups was evaluated using Pearson Chi-square.

Kolmogorov–Smirnov test was used to evaluate the normal distribution of the milk parameters and data from automatic milking system since measurement count was high. Parameters of rumination time, milk yield, milk protein concentration, milk fat concentration, milk fat and protein ratio and milk lactose concentration were normally distributed. Student's t-test was used to evaluate the difference in means of milking parameters between primiparous and multiparous cows. One-way ANOVA was used to evaluate if there was a significant variation in parameters between cows in groups according to the calf score. LSD (least significant difference) *post hoc* test was used to determine which groups of calf score differed significantly from each other. The results were presented as means with standard error. Degree of significance was set to $p < 0.05$.

3. RESULTS

3.1. Prepartum prophylaxis effects on cow negative energy balance and health status evaluated by herd health management system parameters

The difference between the mean values of the body condition score was statistically significantly higher in the GK cows compared to the GO group at the 15th (+0.24, $p = 0.003$) and 30th (+0.52, $p < 0.001$) days after calving.

The body condition score in the GO group decreased throughout the study period ($p < 0.001$), while in the GK group it decreased on the 15th day after calving (–1.58%, $p = 0.005$) and then increased (+0.56%, $p = 0.040$) on the 30th day after calving ($p < 0.001$). However, it is important to note that BCS in GK group remained in the physiological range throughout the study period, while in GO group it was lower than normal from the 15th day of the study and continued to decrease.

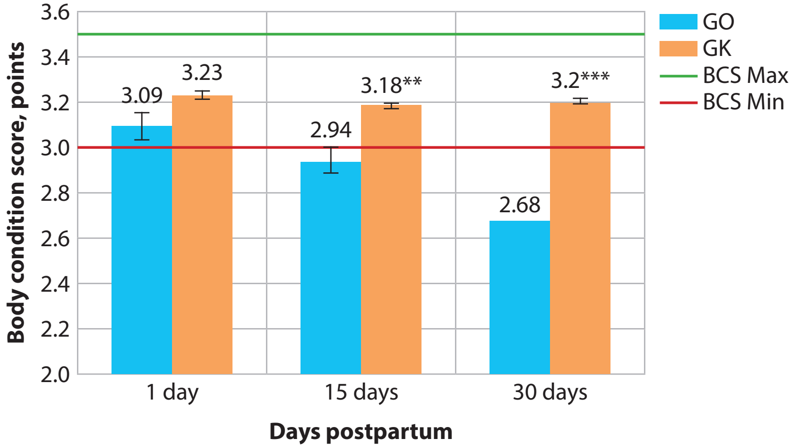


Fig. 3.1.1. Body condition score comparison and change between groups throughout study period

GO – cow group that did not receive prophylaxis; GK – cow group that received prophylaxis; BCS Max – upper threshold of normal body condition score at early lactation period; BCS Min – lower threshold of normal body condition score at early lactation period; **$p = 0.003$; ***$p < 0.001$.

The average milk yield of cows and their body condition in the GO group was significantly lower on the 15th and 30th days after calving (–7.87 kg and –9.08 kg, respectively, $p < 0.001$) compared with the GK group.

The milk yield (kg/day) of the GK group constantly increased ($p < 0.001$). The productivity of cows in this group increased by 10.25% from the 1st to the 15th day and by 22.49% from the beginning of the experiment to the 30th day ($p < 0.001$), while in the GO group there was a decrease (–10.00%, $p < 0.001$) and a subsequent increase (+10.00%, $p < 0.001$) in milk yield ($p < 0.001$).

Lactate dehydrogenase activities in the milk of both groups were almost the same on the first day after calving, but on the following days of the study they were lower in the GK group than in the GO group ($p < 0.001$).

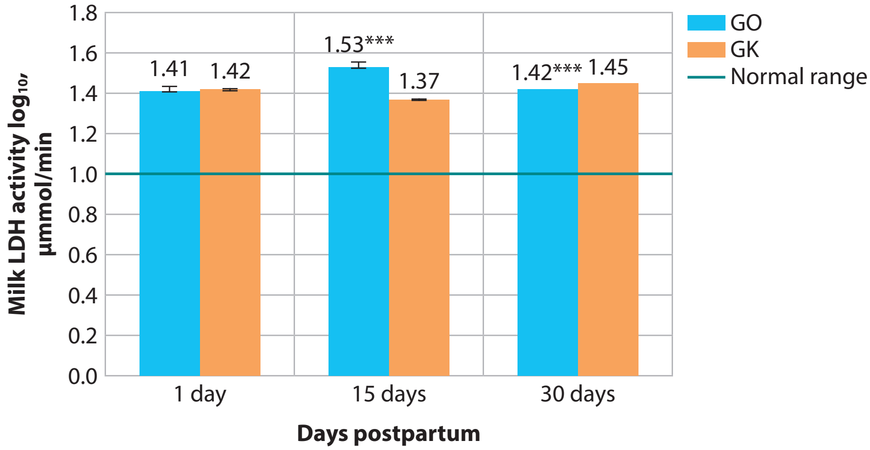


***Fig. 3.1.2.** Comparison and change of milk LDH activity between groups throughout study period*

LDH – lactate-dehydrogenase; GO – cow group without prophylaxis during prepartum; GK – cow group that received prophylaxis during prepartum; Normal range – physiological norm of LDH activity; ***$p < 0.001$.

From the beginning to the end of the study, the activity of LDH in the milk of GO cows increased by 19.15% ($p < 0.001$), while in the GK group such an increase in this indicator was stopped.

Table 3.1.1. *Comparison of milk BHB concentration (mmol/L) between groups throughout the study period*

Days after calving	Group	M	SEM	95% CI		p between groups
				Lower bound	Upper bound	
1	GO	0.060[a]	0.001	0.059	0.061	< 0.001
	GK	0.048[A]	0.001	0.047	0.049	
15	GO	0.057[b]	0.001	0.055	0.058	< 0.001
	GK	0.049[A]	0.001	0.047	0.050	
30	GO	0.054[b]	0.001	0.052	0.055	< 0.001
	GK	0.049[A]	0.001	0.047	0.050	

GK – cow group that received prophylaxis during prepartum; GO – cow group that did not receive prophylaxis during prepartum; M – mean milk BHB concentration, mmol/L ; SEM – standard error mean; 95% CI – confidence interval for mean; [a,b,c] different letters indicate that the difference between days after calving (1, 15, 30) for GO group are statistically significant at $p < 0.05$; [A,B,C] different letters indicate that the difference between days after calving for GK group were statistically significant at $p < 0.05$.

The mean BHB value of the GO group exceeded the mean value of the GK group in all study periods (from 0.005 to 0.012 mmol/L, $p < 0.001$), but the difference between the groups decreased as the number of days after calving increased (Table 3.1.1). The average value of BHB in the GK group did not change during the study, and in the GO group it decreased (from 0.060 to 0.054 mmol/L; $p < 0.001$).

The analysis showed that, in the GK group, the number of cows with a BHB value of 0.06 mmol/L decreased from the beginning of the experiment to 15–30 days by 4.70% (from 19.00% to 14.30%).

3.2. Cow and it's calf metabolic and health status relationship

When comparing glucose concentrations of cows between different parity groups, a significant difference was registered (Table 3.2.1). Primiparous cows had a 16% higher blood glucose concentration (3.03 mmol/L, Std. error ± 0.093) compared with multiparous cows (2.61 mmol/L, Std. error ± 0.102) ($p < 0.01$). However, blood glucose concentrations between groups of calves according to their cow's parity group did not differ statistically significantly ($p > 0.05$).

Table 3.2.1. *Comparison of serum glucose concentration (mmol/L) in cows and calves according to cow parity groups*

Indicator	Cow parity group	n	Mean	Std. deviation	Std. error	95% CI		Min	Max	p
						Lower bound	Upper bound			
Glucose concentration in cows	Primiparous	20	3.03	0.417	0.093	2.83	3.22	2.20	3.90	0.004
	Multiparous	20	2.61	0.456	0.102	2.39	2.82	1.80	3.40	
Glucose concentration in calves	Primiparous	21	6.86	1.513	0.33	6.17	7.55	4.20	11.60	0.605
	Multiparous	21	6.61	1.620	0.35	5.87	7.34	3.70	9.30	

n – number of measurements and animals; 95% CI – confidence interval for mean; Min – minimum; Max – maximum; p – probability.

No significant difference in BHB concentration between parity groups of cows was recorded (Table 3.2.2). Primiparous cows had a mean rank of 18.65 and multiparous cows had a mean rank of 22.35 ($U = 163$, $n = 40$, $p > 0.05$). After comparing calf BHB between groups, no significant difference was registered. The primiparous group had a mean rank of 21.57 and that of the multiparous group was 21.43 ($U = 219$, $n = 42$, $p > 0.05$).

Table 3.2.2. *Comparison of blood serum BHB concentrations of cows and calves according to cow parity groups*

BHB	Cow parity group	n	Mean rank	Sum of ranks	p
Cows	Primiparous	20	18.65	373	0.311
	Multiparous	20	22.35	447	
Calves	Primiparous	21	21.57	453	0.967
	Multiparous	21	21.43	450	

BHB – β-hydroxybutyrate; n – number of animals and measurements; p – probability.

When evaluating the distribution of calves' score class between parity groups, no significant difference was also recorded ($\chi^2(2) = 1.588$, $p > 0.05$) (Table 3.2.3). There was no significant difference between the Low calf score class and the Medium ($p > 0.05$) and High score classes ($p > 0.05$), as well as between the Medium and High score classes ($p > 0.05$).

Table 3.2.3. Distribution of calf score groups among parity groups of cows

Cow parity group	Statistic	Calves' score groups		
		Low	Medium	High
Primiparous	n	10	9	2
	%	47.6	42.9	9.5
Multiparous	n	9	7	5
	%	42.9	33.3	23.8

$\chi 2 = 1.588$, df = 2, $p > 0.05$; Low – Low calf score group that scored 5–8 on the health evaluation chart throughout the study period and is considered least susceptible to disease; Medium – Medium calf score group that scored 9–12 on the health evaluation chart throughout the study period and is considered more susceptible to disease; High – High calf score group that scored 14–17 on the health evaluation chart throughout the study period and is considered most susceptible to disease; n – number of calves in the calf score group from its respectable cow parity group; Significant results are considered when $p < 0.05$.

A significant moderate negative correlation was calculated between cow BHB concentration and cow glucose concentration ($r = -0.353$, $p < 0.05$) (Table 3.2.4). Between cow BHB and calf BHB concentrations, a significant moderate negative correlation ($r = -0.476$, $p < 0.01$) was also determined.

Table 3.2.4. Correlation coefficients between cow and calf blood parameters

Parameters	Cow BHB	Cow Glu	Calf BHB	Calf Glu	Calf score
	Correlation coefficient				
Cow BHB		−0.353*	−0.476**	−0.147	−0.05
Cow Glu	−0.353*		0.2	0.183	−0.164
Calf BHB	−0.476**	0.2		0.096	−0.207
Calf Glu	−0.147	0.183	0.096		0.111
Calf score	−0.05	−0.164	−0.207	0.111	

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed); Cow BHB – β-hydroxybutyrate concentration of cow blood serum; Cow Glu – cow blood serum glucose concentration; Calf BHB – β-hydroxybutyrate concentration of calf blood serum; Calf Glu – calf blood serum glucose concentration; Calf score – a score assigned according to the calf disease symptom severity.

Student's t-test revealed significant differences in herd health and milk quality parameters between parity groups (Table 3.2.5). Primiparous cows had a 6% lower rumination time (min/day) compared with multiparous cows (Std. error ± 6.086, $p < 0.001$). Multiparous cows had a significantly larger milk yield (kg/day) compared with primiparous cows, a difference of 32% (Std. error ± 0.49, $p < 0.001$). Milk protein concentration (%) also differed between groups. Multiparous cows had 3.81%, while primiparous protein concentration was 3.77% (Std. error ± 0.016, $p < 0.01$). Multiparous cows had a higher milk fat percentage compared with primiparous cows, which amounted to a difference of 3% (Std. error ± 0.032, $p < 0.001$). This can also be seen in the difference in the milk fat to protein ratio. Multiparous cows had a ratio of 1.15, whereas primiparous cows had a lower ratio of 1.12, a difference of 3% (Std. error ± 0.008, $p < 0.001$). Milk lactose concentration (%) was also higher in multiparous cows with 4.67% compared with 4.61% in primiparous cows. It was higher by 2% (Std. error ± 0.004, $p < 0.001$).

Table 3.2.5. *Comparison of herd management and milk quality parameters between cow parity groups*

Parameters	Parity group	n	Mean	Mean difference	Std. error	p
Rumination time (min/day)	Primiparous	20	481.21	−32.554	6.086	< 0.001
	Multiparous	20	512.77			
Milk yield (kg/day)	Primiparous	20	32.38	−15.016	0.497	< 0.001
	Multiparous	20	47.42			
Milk protein concentration (%)	Primiparous	20	3.77	−0.038	0.016	< 0.010
	Multiparous	20	3.81			
Milk fat concentration (%)	Primiparous	20	4.24	−0.133	0.032	< 0.001
	Multiparous	20	4.38			
Milk fat to protein ratio	Primiparous	20	1.12	−0.025	0.008	< 0.001
	Multiparous	20	1.15			
Milk lactose concentration (%)	Primiparous	20	4.61	−0.059	0.004	< 0.001
	Multiparous	20	4.67			

n – number of animals in group; p – probability.

There were significant differences in automatic milking system parameter means between calf score groups (Table 3.2.6). Rumination time (min/day) was highest in the High calf score group, followed by the Medium calf score group and the Low calf score group ($F = 61.86$, $p < 0.001$). Milk yield was also the highest in the High calf score group, followed by the Low calf score group and the Medium calf score group ($F = 52.42$, $p < 0.001$). The Low calf score group had the highest milk protein concentration (%), followed by the

Medium calf score group and the High calf score group ($F = 29.17$, $p < 0.001$). Milk fat concentration (%) was lowest in the Medium calf score group, preceded by the Low calf score group, and the highest concentration was found in the High calf score group ($F = 11.45$, $p < 0.001$). The milk fat to protein ratio was also highest in the High calf score group, followed by the Medium calf score group and the Low calf score group ($F = 47.56$, $p < 0.001$). Milk lactose concentration (%) was lowest in the High calf score group. The Medium calf score group had a higher milk lactose concentration, and the highest concentration was in the Low calf score group ($F = 38.76$, $p < 0.001$). Further analysis of differences in means between groups is presented further below.

Table 3.2.6. *Comparison of herd health management system registered parameter means between cows according to their calf score groups*

Parameters	Groups	n	Mean	Std. deviation	F	p
Rumination time (min/day)	Low	119	461.94	134.80	61.86	< 0.001
	Medium	116	505.56	108.54		
	High	77	550.79	110.67		
Milk yield (kg/day)	Low	119	38.83	10.70	52.42	< 0.001
	Medium	116	36.31	11.62		
	High	77	44.80	14.84		
Milk protein concentration (%)	Low	119	3.84	0.34	29.17	< 0.001
	Medium	116	3.78	0.25		
	High	77	3.68	0.36		
Milk fat concentration (%)	Low	119	4.28	0.70	11.45	< 0.001
	Medium	116	4.25	0.61		
	High	77	4.47	0.65		
Milk fat to protein ratio	Low	119	1.11	0.16	47.56	< 0.001
	Medium	116	1.12	0.14		
	High	77	1.21	0.16		
Milk lactose concentration (%)	Low	119	4.66	0.10	38.76	< 0.001
	Medium	116	4.62	0.11		
	High	77	4.61	0.08		

Low – Low calf score group that scored 5–8 on the health evaluation chart throughout the study period and is considered least susceptible to disease; Medium – Medium calf score group that scored 9–12 on the health evaluation chart throughout the study period and is considered more susceptible to disease; High – High calf score group that scored 14–17 on the health evaluation chart throughout the study period and is considered most susceptible to disease; n – number of cows; F – F value; p – probability.

Post hoc analysis revealed significant differences in milking parameters between calf score groups (Table 3.2.7). Rumination time was longest in the HCS group and were 16% longer compared with that of the LCS ($p < 0.001$) and 8% longer compared with the MCS ($p < 0.001$). The MCS rumination time mean was statistically significantly higher compared with that of the LCS as well, by 8% ($p < 0.001$). Milk yield was also highest in the HCS group, 19% higher compared with that of the MCS ($p < 0.001$) and 13% higher than the LCS ($p < 0.001$). There was also a significant difference between the MCS and LCS groups with 6% ($p < 0.001$). Milk protein concentration was highest in the LCS group, 1.5% higher than that in the MCS ($p < 0.001$) and 4% higher than the HCS ($p < 0.001$). The difference between the MCS and LCS was also statistically significant ($p < 0.001$). The HCS group had the highest milk fat concentration. It was 4% higher compared with that of the LCS group ($p < 0.001$) and 5% higher than the MCS group ($p < 0.001$). Though the LCS group had a higher concentration compared with the MCS, this difference was not significant ($p > 0.05$). Milk fat to protein ratio was highest in the HCS group and was 7% higher than in the MCS ($p < 0.001$) and 8% higher than in the LCS ($p < 0.001$). No significant difference between the LCS and MCS groups in this parameter has been calculated ($p > 0.05$). The LCS group was determined to have the highest concentration of milk lactose. It was 1% higher compared with that of the MCS ($p < 0.001$) and 1.07% higher than the HCS group ($p < 0.001$). As with other milk quality parameters, there was no significant difference between the HCS and MCS groups ($p > 0.05$).

Table 3.2.7. *Analysis of mean differences of herd health management system parameters between cows according to their calf's score group*

Dependent variable	(I) Calf score group	(J) Calf score group	Mean difference (I–J)	Std. error	p
Rumination time (min/day)	LCS	MCS	−43.62 *	6.46	< 0.001
		HCS	−88.85 *	8.28	< 0.001
	MCS	LCS	43.62 *	6.46	< 0.001
		HCS	−45.22 *	8.40	< 0.001
	HCS	LCS	88.85 *	8.28	< 0.001
		MCS	45.22 *	8.40	< 0.001
Milk yield (kg/day)	LCS	MCS	2.51 *	0.63	< 0.001
		HCS	−5.97 *	0.81	< 0.001
	MCS	LCS	−2.51 *	0.63	< 0.001
		HCS	−8.49 *	0.82	< 0.001
	HCS	LCS	5.97 *	0.81	< 0.001
		MCS	8.49 *	0.82	< 0.001

Table 3.2.7. *Continued*

Dependent variable	(I) Calf score group	(J) Calf score group	Mean difference (I–J)	Std. error	p
Milk protein concentration (%)	LCS	MCS	0.07 *	0.01	< 0.001
		HCS	0.16 *	0.02	< 0.001
	MCS	LCS	−0.06 *	0.01	< 0.001
		HCS	0.09 *	0.02	< 0.001
	HCS	LCS	−0.16 *	0.02	< 0.001
		MCS	−0.09 *	0.02	< 0.001
Milk fat concentration (%)	LCS	MCS	0.02	0.03	0.437
		HCS	−0.18 *	0.04	< 0.001
	MCS	LCS	-0.02	0.03	0.437
		HCS	−0.21 *	0.04	< 0.001
	HCS	LCS	0.18 *	0.04	< 0.001
		MCS	0.21 *	0.04	< 0.001
Milk fat to protein ratio	LCS	MCS	-0.01	0.01	0.204
		HCS	−0.10 *	0.01	< 0.001
	MCS	LCS	0.010	0.01	0.204
		HCS	−0.09 *	0.01	< 0.001
	HCS	LCS	0.10 *	0.01	< 0.001
		MCS	0.09 *	0.01	< 0.001
Milk lactose concentration (%)	LCS	MCS	0.04 *	0.01	< 0.001
		HCS	0.05 *	0.01	< 0.001
	MCS	LCS	−0.04 *	0.01	< 0.001
		HCS	0.01	0.01	0.065
	HCS	LCS	−0.05 *	0.01	< 0.001
		MCS	−0.01	0.01	0.065

LCS – Low calf score group; MCS – Medium calf score group; HCS – High calf score group; * indicates that the difference is significant at the level of $p < 0.05$; p – probability.

4. DISCUSSION

Prepartum period is a fitting time to initiate prophylaxis against negative energy balance. As the dry matter intake decreases and the onset of lipid mobilisation is registered by higher concentrations of blood NEFA, these are the gold standard changes describing negative energy balance [128]. In the work of Hayirli et al. this change is evident as the dry matter intake decreased by 32% from 21 days prior to calving [129]. A relationship between higher concentrations of NEFA and a decrease in feeding is stated in the work of Grummer et al. [130]. One could argue that a decrease in dry matter intake is the initiating factor to the onset of lipid mobilisation expressed through higher concentrations of NEFA, but studies show NEFA concentration increase registered before the feed intake starts to diminish [131]. NEFA are transported to the liver in order to be oxidised to fulfill energy demands, therefore the liver increases the capacity of peroxisomal oxidation. Unfortunately, the product of the first step of this oxidation is hydrogen peroxide, contributing to a higher concentration of reactive oxygen substances such as lipid peroxides leading to a suppressed immune response [132, 133]. Diminishing the level of negative energy balance can be beneficial for the immune system and health of cows. Glucose is the main energy source used by immune cells like macrophages and neutrophils, but during prepartum and postpartum cows are in an insulin resistant state, in order to funnel glucose into the mammary gland, therefore immune cells have limited energy resources and their immune activities are diminished [134]. As an energy source for other tissues higher concentration of BHB is registered in blood plasma of cows experiencing negative energy balance. Level of BHB in blood and other bodily fluids of cows has been used for some time to diagnose and evaluate the severity of negative energy balance in cows [104, 106].

Appropriate energy status can be provided during prepartum by providing prophylaxis as seen in our results. Prophylaxis against negative energy balance effectiveness is monitored by registering BHB levels. Higher BHB concentration is one of the main signs of negative energy balance, which is also discussed in other studies [135]. As monensin acts by modifying the concentration of butyric acid producing bacteria and diminishes the competition for propionic acid producing bacteria. Less BHB and sufficient levels of glucose are being synthesized in the liver [136]. Therefore, we can see that supplemented group controlled the expression of negative energy balance.

During negative energy balance the milk yield of cows is decreasing due to the inability of the metabolic system to provide energy for lactogenesis [22, 135]. Glucose is a primary source for lactose synthesis and in turn milk lactose concentration is the main determinant parameter influencing milk yield by regulating osmosis of fluids into the milk gland [107]. However, in our study we received results where cows at risk of negative energy balance, expressed by higher milk fat to protein ratio, lower milk lactose concentration, had higher milk yield compared to cows with lower risk. As a loss of milk yield is one of the main symptoms of metabolic clinical ketosis, other studies claim that highly productive cows are able to maintain their high milk yield up to a point when the metabolism and lipolysis in the liver is unable to keep up with the nutrient demand for lactation. Such cows maintain their high milk yield, but other milk quality parameters as beforementioned milk fat to protein ratio and usually quite stable milk lactose concentration show signs of a negative energy balance experienced by the cow [137].

Suppression in appettite coupled with increasing energy demands for the upcoming lactation and intense growth of the fetus initiates lipolysis, therefore a decrease in BCS is noted during last trimester, followed by lower concentrations of glucose, insulin resistance and higher NEFA concentrations [138]. The loss of BCS is especially noticeable in obese animals and carries the most risk healthvise as it is parameter of fatty liver syndrome [139]. Although BCS monitoring is usually implemented in post partum cow monitoring systems, monitoring of BCS loss during prepartum is a good indicator of a higher risk of negative energy balance. In a study of Casaro et al., it was noted that obese cows (BCS > 4) had a lower dry matter intake (9.97 ± 0.21 kg/day) compared to moderate (11.15 ± 0.14 kg/day) BCS cows (3.25–3.75) and thin (11.92 ± 0.22 kg/day) cows (BCS < 3.0). Energy balance in prepartum was also lower in obese cows (−4.16 ± 0.61 Mcal/day) in comparison to moderate and thin cow groups (−1.20 ± 0.56, and 0.88 ± 0.62 Mcal/day respectively) and the same can be seen during post partum as well. BCS affected the milk yield of the forementioned groups. While obese cows had a reduced daily milk yield of 4.4 kg, moderate cows had an increase of 6.0 kg. It signifies the importance of prepartum energy and BCS monitoring as a means of affecting cow production and health parameters monitored in the prepartum [140]. In our study, the results of the control group showing a decrease in BCS score and milk yield during the study period, are also described in other studies and give a good example of changes happening due to negative energy balance and subclinical ketosis. Energy requirement for lactation is a priority after calving and it is maintained through lipolysis, hence the lower BCS score. Recorded lower milk yield in GO group is a good indicator, that homeorhesis is not balanced properly, and that energy from lipolysis is not sufficient to maintain stable milk yield as is reported by Caixeta et al [141]. GK group showed stable and increasing milk yield with higher on average BCS scores, providing evidence that monensin supplementation negated the effects of negative energy balance. Similar results are described in the work of Richards et al. [142].

An increase in LDH activity is a good indicator of intramammary infection as it correlates well with sommatic cell count of milk ($r = 0.8$, $p < 0.001$) and immunoglobulin G concentration ($r = 0.53$, $p <0.001$). Its sensitivity is evident in the work of Khatun et al. where it was combined with sommatic cell count and the area under the curve for Gram-positive bacterial infection was 0.984 (Sensitivity = 0.963, specificity = 0.918, $p = 0.01$) [143, 144]. There is evidence that the negative energy balance experienced by the cow has a negative effect on its immune response. Glucose, the main energy source for immune cells, is being diverted to the mammary gland for milk production, thus the immune system is compromised [145, 146]. Therefore, our results agree with other findings of higher LDH in cows experiencing negative energy balance as described in works of Klein and Ha et al [105, 147]. As supplementation with monensin helped to mitigate negative energy balance effects, the immune system was able to properly protect the mammary gland from inflammation, seen by lower concentrations of LDH in the supplemented group.

Analyzing milk quality parameters can help identifying negative energy balance in cows. In our second experiment we found that there was no significant difference in blood BHB concentration at the beginning of lactation, therefore we continued to monitor the NEB state through milk quality parameters of milk fat to protein ratio and milk lactose concentration. Such method allowed us to identify cows more at risk without having to take blood samples, once again proving the benefit of using herd health systems for diagnostics. We hypothesized that cows with more pronounced signs of negative energy balance will give birth to calves with a compromised health status. By grouping the cows according to their calves health score we then compared their milk quality parameters and herd health management system indicators and found that cows in the High calf score group have more clear signs of negative energy balance expressed by the highest milk yield, highest milk fat concentration and milk fat to protein ratio, coupled with the lowest milk protein and lactose concentrations. As the severity and conditions for negative energy balance in the upcoming lactation are set during the prepartum already, there is reason to believe, that the strain on the metabolism and other organ functions can lead to changes in the health status of a growing fetus as well [148, 149]. This study, even though carried out with a smaller sample size, shows signs of this relationship, as the calves more prone to disease were birthed by cows that suffered from negative energy balance. Milk fat to protein ratio is a good indicator of negative energy balance, subclinical and clinical ketosis in high yielding cows, indicating an increase in energy requirements [150]. Some studies indicate it being a better indicator of low energy balance compared to ketone body levels in blood and body condition score [151].

Increase in rumination time and milk yield indicate a cows increased demand for nutrients and is an early sign for negative energy balance. In our second study cows that were considered more at risk of negative energy balance, expressed by higher milk fat to protein ratio and lower milk lactose concentration, had a longer rumination duration compared to cows that were at a lesser risk of negative energy balance. While this does not fit the usual description of a cow affected by restricted energy sources, expressed by lower milk yield and lower rumination duration, if combined with changes in milk quality parameters these changes indicate a cow that is in negative energy balance state, but is still able to maintain high milk yields [106]. In order to maintain a higher milk yield such cows are able to feed properly and during rumination they ferment fatty acids that will be used for energy production in the liver [152].

Milk lactose concentration is also an approved indicator of energy status in the cow, as glucose in blood is the primary source for lactose synthesis in the mammary gland [107]. Lactose concentration is kept at a constant concentration in milk as it is responsible for the osmotic pressure of milk and ensures fluid absorption from blood to the mammary gland, increasing the milk volume [153]. Since glucose is the main source for lactose in milk, even minimal decreases in milk lactose concentration are a good indicator that the balance of glucose synthesis and uptake has been modified. Proof for a relative stability of milk lactose concentration has been discussed in the work of Andersen et al., where comparing milk lactose concentration between cows fed a high caloric diet and normocaloric, the concentration in the high caloric group increased only by 0.05 percentage points [154]. Other works also discuss the use of fat to lactose ratio as a good indicator of negative energy balance, with a calculated negative medium strong correlation of $r = -0.589$ ($p < 0.05$) [155, 156]. Therefore, lower milk lactose concentration and decreased milk fat to protein ratio are certain and sensitive indicators of negative energy balance.

Other studies state that cow parity does have an effect on calf health status – multiparous cows gave birth to heavier calves [157]. Higher birth weight is a good indicator for lower risk of early culling, since the organs are on average heavier, therefore more developed [79]. Pregnant heifers in an intense dairy production system need to partition their nutrients between their own growth and the development of their calf, therefore they are more susceptible to the negative effects of malnutrition. This in turn leads to weaker immune response of their offspring [158]. Another theory is that heifer reproductive system tissues lack efficiency and are not fully adapted to fetal development, for example smaller cotyledonary surface and weight limit nutrient transfer [21, 159]. Other studies indicate, that dry matter intake and especially it's decrease before parturition differs between parities as well. While multiparous cows showed a tendency for the feeding intensity to decrease 21 days till parturition, in heifers a drastic decrease is noted around a few days before parturition [160]. The difference in metabolism and it's effect for the IgG concentration in colostrum was noted in the work of Vasquez et al. While multiparous cows supplemented with monensin during prepartum had lower colostrum IgG concentration (60.0 ± 11.9 g/L) compared to the control group (91.4 ± 11.9 g/L, p = 0.09), in primiparous cows no effect of monensin supplementation to the IgG concentration was established (61.4 ± 8.4 *vs.* 65.5 ± 8.4 g/L monensin supplemented and control cows respectively). Authors claim that the mechanism behind this phenomenon is not broadly described in literature and one theory is that it is connected to the excretory capacity of the milk gland [161, 162]. This data coincides with the fact, that multiparous cows are under more metabolic stress in preparing for the lactation and calving. For further studies, a higher number of primiparous and multiparous cows should be studied in order to have a better comparison.

Prepartum period is integral for the upcoming lactation and the calves' life, therefore more effort should be put into managing it to achieve better results. As our work and the work of our colleagues indicate, the status of the cow during prepartum influences the phenotype and health of the fetus. This is indicated by more sick calves being birthed by cows that show clearer signs of negative energy balance by analysing their milk parameters. Environmental factors like heat stress and nutrition put internal systems under a lot of stress and calls forth certain adaptations for survivability and maintenance of the fetus [28, 62, 67, 163]. Research shows that prepartum heat stress could cause a genetic predisposition for pneumonia and neonatal diarrhea in calves [80]. Some examples of adaptations include changes in uterine and fetal blood flow during heat stress, metabolism shifts when experiencing nutrient restriction or overfeeding [159, 164].

During prepartum and postpartum, when negative energy balance state is experienced by cows, it negatively affects the immune response and calf health status. Cytokine production and regulation of inflammation is diminished as the nutrient-responsive kinases (such as protein kinase B and mechanistic target of rapamycin pathway) signalling is modified [165]. Obese periparturient animal adipose tissue cells and molecules point to a connection between metabolism and immune system. Overconditioned animals had lower levels of insulin receptor mRNA, proteins and mRNA of TNF-α with higher level of peroxisome proliferator activated receptor gamma

(PPARγ), which gives reason to believe that insulin signalling in adipose tissue was weakened [166]. PPARγ is expressed in cow adipose tissues and is essential in transcriptional control in genes responsible for encoding protein synthesis that are responsible for lipid and glucose metabolism [167, 168]. Interestingly, another study provides differing results, obese cow adipose tissues did not show more intense signals of insulin resistance [169]. Some authors state that not the fact of obesity, but more pronounced weight loss, initiated by negative energy balance, is the factor initiating insulin resistance in adipose tissues [170, 171]. In our other study the data indicates that cows showing symptoms of negative energy balance gave birth to neonates that were more at risk of disease. The registered changes in cows were increased productivity, causing negative energy balance, increase in milk fat to protein ratio and a decrease in milk lactose concentration. In future studies it would be beneficial to take into account the body weight and even body condition score change as to investigate this parameter as an indicator of negative energy balance and use it to elaborate on the relationship between cow and calf metabolism and immune response. As not only fat deposits but protein and their amino acids are used as energy source in severe negative energy balance, immune reaction is weaker as amino acids are essential for the synthesis of molecules responsible for antioxidation, inflammation signalling and immune response [172]. Amino acids play an integral role in 1-carbon metabolism which is connected with protein synthesis through the mechanistic target of rapamycin pathway, antioxidant synthesis, metabolism of energy and epigenetic regulation mechanisms [173]. Results of one scientific group point to direct effects of methionine supplementation prepartum to calf increase in neutrophil phagocitic capacity, hepatic activity and higher taurine concentrations, weaker expression of TNF-α [174–176]. Calves born to supplemented cows also had lower concentration of oxidative stress blood parameters of reactive oxygen metabolites, myeloperoxidase and ceruloplasmine [177]. Therefore we have evidence that the prepartum period of the cow can modify the bodily functions of calves.

Some studies express, that a lower BHB concentration in calf serum is a physiological trait in early life, leading us to believe, that the correlation in our study could be without causation – if the dam enters negative energy balance in early lactation, it's BHB concentration will increase, while the calves physiology will drive the BHB concentration down, resulting in a negative correlation [178–180]. However, other studies claim that according to the metabolic status of the dam and environmental factors, the fetus may adapts its metabolism to prioritize a different energy source. Calves of malnourished dams have a tendency to prioritize lipolysis, therefore their blood samples reveal insulin resistance and hypoglycemia – BHB becomes a primary energy source for cells [181]. Interestingly, overfed animals also show evidence of glucose intolerance and are programmed for lipolysis [182]. In another study where cows were supplemented with fat during the prepartum, their calves registered increased concentration of IgG by 28% ($p < 0.01$), better absorption of IgG ($p < 0.01$) and a higher average daily gain compared to calves born to nonsupplemented cows (597 and 558 g/day, $p = 0.02$, respectfully) [183]. Calves from supplemented cows also had higher concentration of cholesterol compared to control calves. This is explained by higher concentrations of cholesterol registered in fat supplemented cows, knowing cholesterol is able to pass through the placenta [183–185]. However, Garcia et al. claim that in their study fat supplementation did not have an effect on cholesterol concentration, and the researchers imply that improved liver function, expressed by more active peroxisome proliferator activated receptor α gene [186]. In our study monensin was used as a prophylaxis during prepartum against negative energy balance and a study performed by Vasquez et al. points to it having a negative effect on IgG concentration in colostrum [162]. However, in a study of Vedovatto et al. monensin supplementation had no effect on colostrum IgG concentration, but calves of supplemented cows had a higher body weight at weaning by 24 kg compared to nonsupplemented animals [187]. The mechanism of how monensin supplementation of cow can affect the metabolic parameters and colostrum quality is not readily described and in some of our unpublished data we were able to record that calves from supplemented cows had higher blood BHB concentration. When taking the effects of heat stress into consideration, more accurately, heat abatement in the prepartum, some studies indicate that calves from cooled cows have lower glucose concentrations together with lower levels of BHB and NEFA. This is explained by a much more rapid growth of the cooled calves and a need for a different energy source, when glucose has been used up [82]. Therefore, for future studies, the growth and weight gain of the calves should be registered more accurately, in order to correctly evaluate the nutrient and energy requirements and metabolism of calves.

The above described mechanisms and work of colleagues delves deeper into reactions and pathways of fetal programming or otherwise known as epigenetics. Though the mechanism of actions is still under a lot of debate in the scientific community, more and more data is being published about it affecting not only the current animal but its future generations as well [27, 188]. The importance of epigenetics as a topic can be appreciated by the fact that in 2024 Lithuanian Scientific Award for Medicine was awarded for the work cycle of prof. R. Navakauskienė, prof. Dr. D. Navakauskas, Dr. V. Borutinskaitė and prof. Dr. Dalius Matuzevičius „Epigenetic and genetic biomarkers for diagnostics and personalized therapy: research of mechanisms, development of methods and applications (2009–2023)“ [189–192]. Though humans were the main subject of their work, hopefully this could give the required push to investigate the epigenetics of livestock in more detail.

As a lot of internal and external factors influencing the health status and the risk of negative energy balance in cows cannot be controlled, one target that can be managed is nutrition and supplementation during prepartum, which can also have a positive impact on calf health as well. Such factors as age of animals, heat stress, previous lactation milk yield are not influenced by the farmer or are time sensitive and their effects are only visible after a longer time, for example genetic selection or improvement in housing. In comparison nutrition is more manageable, flexible and its effects can be evaluated [193].

Monensin supplementation has been one of the main methods of managing negative energy balance through prophylaxis in the prepartum, but new EU policies will encourage the scientific community and industry to investigate other options. Such prophylactic strategy was to be more thoroughly investigated in this thesis and scientific studies, but EU policies had to prohibit certain products and the study design had to be adapted. There are numerous studies about the benefits of monensin supplementation that are expanded upon in the literature review, together with the reasons why supplemental products have been restricted. However supplementation during prepartum in order to minimize negative energy balance is still a topic that is being researched and there are good studies on its effect on calves health as well.

Controlling and managing the immune response and supplementing the immune system in the dry period might increase the chances of both healthy dam and calf. An anti-inflammatory sodium salicylate was provided for dry cows in one study and provided good insight into the relationship between metabolism and immune response [194].

Methionine supplementation during prepartum can reduce negative energy balance and improve both cow and calf health. In the work of Osorio et al. methionine supplemented prepartum cows had increased milk yield, milk fat and protein content. By delivering higher concentration of methionine to the methionine cycle in the liver it ensures synthesis of other active molecules like glutathione (antioxidant), S-adenosyl methionine (active in DNA methylation) and phosphatidylcholine (used in fatty acid synthesis) [195, 196]. Rumen protected methionine supplementation during prepartum has been shown to improve dry matter intake and in return minimizing the negative effects of negative energy balance state [197, 198]. Methionine supplementation before parturition was described in the work of Altharti et al. [199]. This showed a positive effect on the whole blood phagocytosis in the test group, therefore it acts as an immune modulator – by downregulating IL-1β (interleukin-1 beta) and NOS2 (nitric oxide synthase 2) mRNA. In the work of Tabor (2024), pregnant heifer supplementation with methionine during heat stress conditions only slightly improved the immune response indicated by lower concentrations of IL-6 (interleukin-6) in calf samples. The authors suggest that further studies concerning the methionine supplementation effect on calf neutrophil function and development of specific cell cultures should be carried out [200–202].

Amino acids supplementation can be used to pronounce the secretion of some hormones and enzymes, for example, l-arginine promotes the secretion of insulin, growth factor, glucagon, prolactin and placental lactogen 173, 203]. Concerning amino acid supplementation in hopes of supporting the developing fetus, thorough studies should be examined and carried out, since the metabolism and synthesis of amino acids during prepartum is very specific. As was stated in the work of Lemons et al. [204], sheep fetus does not take up glutamate and aspartate from the placenta but synthesizes them and returns them to the placenta. Therefore, enriching a pre partum cow's feed with these amino acids might not give the awaited results [204]. Also, higher concentrations of glutamate can be neurotoxic for the fetus, as shown in some experiments with mice. Mice supplemented with high doses of glutamate for an extended period had worse results compared to control groups in cognitive tests [205]. Another hurdle in amino acid supplementation is the fact, that transport channels for amino acids in the placenta are not all specific and with a variety of maternal amino acids in the circulation an inhibitory process occurs thus the uptake of these acids by the fetus can either increase, stay the same or even decrease [206].

In order to mitigate the level of negative energy balance experienced by the pre partum and postpartum cows, their dry matter intake can be controlled through correcting dry cow feed rations. One task of cattle nutritionists is to increase the nutrient density in feed, by changing the balance between protein, carbohydrates and fats and to maintain or increase dry matter intake. Some studies indicate, that by adding 17.8% crude protein (consisting of 6.7% rumen undegradable protein and 11.1% rumen degradable protein) to the feed, cows maintain their daily dry matter intake when compared to feed with only 13.3% of crude protein (consisting of 4.8% rumen undegradable protein and 8.5% rumen degradable protein) [207]. Dry matter was also not affected in a study of Hartwell et al., where cows were offered feed containing 4.0% rumen undegradable protein and 6.0% [208]. Hayirli et al. showed similar results with dry matter intake not being affected even with cows receiving diets consisting of different parts of crude protein (13.3% ± 0.6, 15.2% ± 0.8, and 17.7% ± 1.5) [209]. Methionine together with histadine are the only amino acids with an increased uptake for in the liver as it plays an integral role in gluconeogenesis during prepartum, but delivery of these amino acids is complicated as they are degradeable in the rumen [210, 211]. However, increasing protein concentration in feed causes an influx of nitrogen into the liver, leading to a higher demand for it's utilization and then urinal or fecal excretion, which may lead to a diminished health status and negative environmental effects and economic loss [212].

Nutrient density can also be improved by modifying the contents of carbohydrates in the feed. It not only improves rumen papillae growth and enchances absorption of nutrients but also has beneficial effects on the microbiota [213, 214]. When fed a diet containing more carbohydrate 1.58 Mcal kg^{-1} (40% non-detergent fiber and 38% non-fiber carbohydrates) or 1.70 Mcal NE_L kg^{-1} (32% non-detergent fiber and 44% non-fiber carbohydrates), Rabelo et al. registered a 19.8% dry matter intake increase [215]. Non-detergent fiber concentration influences the dry matter intake a lot, as described in the study of Minor et al., where cows receiving ration with 1.63 Mcal NE_L kg^{-1} dry matter (43.8% non-fiber carbohydrates and 29.5% non-detergent fiber) had higher dry matter intake than those fed 1.34 Mcal NE_L kg^{-1} (23.5% non-fiber carbohydrates and 48.9% non-detergent fiber) [116]. Colostrum insulin concentration can also be increased by supplementing prepartum cows with more starch [216]. A downside of carbohydrate supplementation when it comes to newborn health is lower IgG concentration registered in colostrum [217]. Increased concentration of starch also increased the colostrum lactose concentration which in turn increased its volume. Higher volume colostrum tends to dilute other essential components of colostrum such as fats [216, 218]. Supplementing the feed ration with carbohydrates poses some risk and most importantly plays a huge role in the development of rumen and metabolic acidosis [219]. Increasing grain content in feed results in sudden changes of rumen environment, causing rumen microorganisms to release more lipopolysacharides which initiate inflammation. Not only that, but the dry matter intake is reduced, potentially diminishing the goal of controlling negative energy balance, and more acute phase proteins can be sampled from blood [220]. Researchers state that even short periods of high grain diets are able to innitiate acute phase response, therefore adaptation to lactating cow feed should not be sudden [221].

Increasing the contents of fat in feed ration can increase nutrient density, but it negatively impacts the dry matter intake. When opting for fat supplementation during prepartum it aims to better prepare the cow for fat metabolism, enchancing β-oxidation activity and decreasing triacylglycerol concentration in liver [222]. Even though cow metabolic parameters of NEFA were increased during prepartum in the fat supplemented group, the same group registered lower concentrations of blood NEFA and liver triglycerides in postpartum, compared to cows not supplemented with fat in prepartum. It is important to note that type of fat is important as positive results were achieved by supplementing saturated fats in prepartum [222]. Possible mechanism for this phenomenon and adaptation was suggested by Grum et al. where cows receiving high fat diet during prepartum were found to have decreased liver triglyceride concentration, lower NEFA and a lower body condition score when compared to cows fed normal or high grain diets. The authors do emphasize that decrease in body condition score was attributed to lower dry matter intake in high fat diet cow group [133, 222]. Diets high in fat, supplemented with oleic and palmitic acids stimulate β-oxidation capacity for long chain fatty acids and decrease liver triacylglycerol concentration [222, 223]. A direct effect of fatty acid supplementation for liver and metabolism parameters was described in a study of Mashek et al. where fasted cows received intravenous infusions of linseed oil and it decreased their blood NEFA and BHB levels with lower concentrations of liver triacylglycerol [224]. In monogastric animals linolenic acid stimulates peroxisomal proliferator activated receptor mRNA in liver tissues therefore increasing β-oxidation of long chain fatty acids [225]. Studies in ruminants reveal that only about 10% bypasses the rumen, and stearic acid with palmitic acid are best used to increase β-oxidation [222, 226]. In a study of Karimian et al., cows were separated into four groups: receiving higher fat content feed prepartum and postpartum, receiving higher fat content prepartum and lower fat content postpartum, receiving lower fat content prepartum and higher postpartum and finally cows receiving lower fat content prepartum and postpartum. Prepartum dry matter intake was reduced by 4 kg/day in cows fed higher fat compared to lower fat rations. Milk yield postpartum was higher in cows that received higher fat content prepartum and low fat postpartum compared to cows that received higher fat feed ration. Low fat prepartum group had higher blood cholesterol concentration in postpartum compared to cows receiving high fat content prepartum (5.16 compared to 3.74 mmol/L respectively) [227]. Benefit of increasing fat contents of feed ration include alleviation of heat stress compared to carbohydrate and protein diets, control of dust and fine particles separating away from mixed feed and providing the required energy without the need for higher volumes of carbohydrate [193]. Relling et al. state that reduction in dry matter intake is initiated due to inhibited fiber fermentation in the rumen and a decrease in feed palatability for the cows [228].

Even with mixed results from different studies and researchers, it is still not exactly clear the relationship between prepartum cow and her calf, but our study and work of other colleagues described above give good ground to claim that the metabolic state of prepartum cow can affect the calf health parameters, either it be through colostrum composition, modified gene expression or metabolic adaptation. To summarize the results of both studies, there is evidence of the impact of the cows' prepartum period, especially it's metabolic state, on the health of not only the cow, but the calf as well. It is important to note that the metabolic state in the prepartum can be managed by prophylactic supplementation to minimize the hazardous effects of negative energy balance experienced in the early lactation period. By managing the negative energy balance state in cows, it is possible to ensure an improved health status for their offspring. More investigation into the prepartum period, internal and external factors influencing it and the relationship between cows and calves, can help achieving better productivity results and improved health status. By studying this important part of the productive cycle, more certain targets and mechanisms can be found, that could help modify and improve animal husbandry. Therefore this relationship should be further studied in order to clearly identify the mechanisms leading to a wanted outcome.

This study presents tools and methods on how the impact of the prepartum period can be brought to light through the use of herd health management system parameters and calf health evaluation systems together. This could also help when deciding on better methods and products for supplementation. Moreover, dairy cattle housing and management could be more easily improved if the benefits of doing so will be backed with evidence from scientific studies.

CONCLUSIONS

1. Prophylaxis during prepartum diminished the negative energy balance state during early lactation period and had a positive effect on udder health. Test group cows compared to control group cows had a lower risk of negative energy balance indicated by a 10.25% higher milk yield ($p < 0.001$) and their body condition score was higher on the 15th (+0.24, $p = 0.003$) and on the 30th (+0.52, $p < 0.001$) test days and remained in the physiological range throughout the study period. Test group cow lactate-dehydrogenase activity remained stable throughout the study period, whereas Control group registered an increase of 19.15% from the 1st to 15th days post partum ($p < 0.001$);
2. Lactation number of cows had no effect on their calf health status. There was no statistically significant difference in distribution of calf health risk groups between primiparous and multiparous cow groups ($p > 0.05$). Multiparous cow group had a 16% lower blood glucose concentration compared to primiparous cow group, but blood β-hydroxybutyrate concentration did not differ between groups;
3. Cow metabolism has an influence on calf metabolism. A negative moderate correlation between cow and calf blood β-hydroxybutyrate concentrations was registered ($r = -0.476$, $p < 0.01$). This indicates an adaptation by the fetus to use β-hydroxybutyrate as an energy source while in the womb;
4. Cows at a higher risk of negative energy balance, evaluated by herd health system parameters, had calves that were more at risk of disease. Cows of High calf score group calves compared to cows of Low calf score group calves had 8% higher milk fat to protein ratio, 1.07% lower milk lactose, 13% higher milk yield coupled with 16% longer rumination time ($p < 0.001$).

RECOMMENDATION

1. Herd management and evaluation systems and protocols should be modified to incorporate calf health monitoring systems paired with changes in herd health system parameters since there is evidence of a relatioship forming during prepartum between cow and its unborn calf. Evaluating changes in calf rectal temperature, mucus out of nose and eyes paired with ear drooping monitoring can help better identify sick calves. According to our data, cows of such calves are more at risk of negative energy balance and concurrent diseases, therefore they should also be monitored or preventative care should be administered. Doing so can improve early disease diagnostics on farms and diminish animal susceptibility to disease.
2. Specific changes in herd health system parameters can be used to identify cows more at risk of negative energy balance. Higher milk fat to protein ratio, lower milk lactose concentration, higher milk yield and longer rumination time are good indicators of such metabolic state. Calves born to such cows should be included in a monitoring system as they are more at risk of disease.
3. Since monensin prophylaxis against negative energy balance has been restricted due to health concerns, more research and development can be carried out in providing a suitable alternative. Studies indicate amino acid supplementation being a viable option.

SUMMARY IN LITHUANIAN

ĮVADAS

Europos Sąjungos (ES) pienininkystės sektorius išlieka vienas iš didžiausių pieno produktų gamintojų pasaulyje, nepaisant visų šiai industrijai gresiančių iššūkių. Europos Sąjungos valstybės 2023-iais metais pagamino ir į pieno perdirbimo įmones pristatė 144,9 tūkst. tonų žalio pieno, o 2024-ais metais šis skaičius išliko panašus – 144,5 tūkst. tonų. Be didelių žalio pieno produkcijos apimčių, ES vis dar išlieka didžiausia sūrio eksportuotoja pasaulyje [1, 2]. Iš vienos karvės primelžiamo pieno vidurkis siekė apie 7 791 kg (didžiausias pieno kiekis registruotas Estijoje – 10 728 kg, mažiausias Rumunijoje – 3 425 kg). Tuo tarpu Lietuvoje pieno primilžio iš vienos karvės vidurkis siekė 6 724 kg [3]. Nuo 2013 iki 2023-čių metų Lietuvos karvių skaičius sumažėjo 19 proc., o Europos Sąjungos vidurkis per tą patį laikotarpį sumažėjo 9,3 proc. Tačiau nors karvių skaičius mažėja, primilžiai iš karvės Europos Sąjungoje padidėjo 20,3 proc. [4, 5]. Tokios tendencijos, kai karvių produktyvumas smarkiai išaugo, fiksuojamos ne tik Europoje, bet ir visame pasaulyje. Nepaisant to, jog karvių produktyvumas nuolatos auga, prognozuojama, jog pieno produktų poreikis nuo 2024 iki 2033-čių metų turėtų padidėti apie 21,5 proc., tuo tarpu pieno produkcija turėtų išaugti tik apie 18,5 proc. [3]. Tokios prognozės siunčia signalą pieno gamintojams, jog pieno poreikis išliks aukštas ir karvių produktyvumą reikia didinti. Kadangi karvių skaičius mažėja, kiekvienos karvės vertė išauga, o ją taip pat didina išoriniai faktoriai kaip didėjančios kuro ir trąšų kainos (įtakojamos geopolitinių neramumų ir klimato kaitos), su gamtosauga susiję mokesčiai ir ūkinės veiklos apmokestinimas [6, 7]. Vis stipriau jaučiama klimato kaita prisideda prie naujų infekcinių ligų plitimo ir protrūkių (snukio ir nagų, mėlynojo liežuvio ligų), kurios dar labiau sumažina jau ir taip besitraukiantį karvių skaičių [8, 9]. Genetiškai užprogramuojamas aukštas karvių produktyvumas lemia, jog vidutinis karvių amžius ūkyje trumpėja, kadangi didesnė dalis vyresnių karvių yra išbrokuojama dėl organizmo negebėjimo palaikyti metabolinius ir hormoninius procesus. Todėl pienininkystės ūkis siekia užtikrinti produktyvią kiekvieną laktaciją bei galvijo sveikatingumą [10]. Šie iššūkiai dabar labiau nei bet kada verčia pieno gamintojus investuoti į pakaitinių telyčių veisimą ir išsaugojimą siekiant didesnio produktyvumo [6].

Vienas iš būdų užtikrinti didžiausią produkciją iš karvės ir kartu sulaukti sveikos pakaitinės telyčios yra užtikrinti stabilų užtrūkinimo laikotarpį [11]. Šiame gyvenimo etape galvijo fiziologija turi prisitaikyti prie būsimų pokyčių – pieno liauka ruošiasi būsimai laktacijai, prasideda kolostrogenezė, augantis vaisius turi būti aprūpinamas jam reikalingomis maisto medžiagomis, kai tuo pačiu metu kaupiamas riebalinis sluoksnis ir prisitaikoma prie kintančio pašaro ir energijos poreikio [12–15]. Per paskutinius tris veršingumo mėnesius, vaisius sparčiai auga ir jo organų sistemos galutinai išsivysto taip pasiruošdamas veršiavimuisi [14]. Dėl šios priežasties pienininkystės sektoriuje dirbantys veterinarijos gydytojai ir šios srities profesionalai deda daug pastangų, jog suvaldytų užtrūkinimo laikotarpį. Keisdami pašaro racioną, stebėdami galvijų kūno masės pokyčius jie stengiasi užkirsti kelią galimoms ligoms laktacijos periode ir tuo pačiu nori užtikrinti gerą būsimo veršelio sveikatos būklę [16–18].

Iki šiol daug dėmesio buvo skiriama užtrūkinimo laikotarpio valdymui siekiant pagerinti laktacijos laikotarpį, tačiau pastaruoju metu vis labiau atkreipiamas dėmesys į užtrūkinimo laikotarpio svarbą būsimo veršelio sveikatingumui ir produkcijai [19]. Užtrūkinimo laikotarpį veikiantys veiksniai, nuo išorinių ir su galvijo organizmo veikla susijusių, turi didelę įtaką vaisiaus sveikatingumui ir netgi jo prognozuojamai produkcijai [20]. Tokie atradimai leidžia daryti prielaidą apie esamą ryšį tarp karvės ir jos veršelio, tad mokslininkų bendruomenė aktyviai tiria šiuos vidinius procesus ir kaip jie valdomi. Identifikavus parametrus ir veiksnius, kurie gali paveikti vaisiaus vystymąsi ir veršelio sveikatingumą, būtų galima geriau užtikrinti veršelių gerovę ir pasiekti juose užkoduotą genetinį potencialą bei gauti daugiau produkcijos garantuojant daugiau laktacijų [26].

Mokslinis naujumas

Užtrūkinimo laikotarpis, kaip didelė dalis tranzitinio laikotarpio, buvo tyrinėjama per jo įtaką būsimai laktacijai. Mokslinių tyrimų pagalba padarytos išvados tik patvirtino prielaidą, jog užtrūkinimo laikotarpio teisingas valdymas gali labai nulemti būsimą karvės laktaciją. Tačiau dabar į užtrūkinimo laikotarpį bandoma žiūrėti ir per kitą prizmę – kaip jis gali turėti įtakos būsimo veršelio sveikatingumui. Tiksliau, kaip išoriniai ir vidiniai veiksniai paveikdami veršingą karvę gali nulemti veršelio sveikatos būklę.

Vienas iš pagrindinių procesų prasidedančių užtrūkinimo laikotarpyje yra neigiamas energijos balansas, kurio neigiamos pasekmės yra sumažinamos taikant profilaktiką prieš karvėms apsiveršiuojant. Atliktuose tyrimuose modifikavome užtrūkinimo laikotarpio profilaktikos poveikio prieš neigiamą energijos balansą vertinimą analizuojant ne tik tipinius bandos valdymo sistemos parametrus susijusius su šiuo procesu, tačiau ir pieno liaukos sveikatingumo parametrus. Atskleista karvių energinės būklės artima sąsaja su karvių sveikatingumu, ypatingai su pieno liaukos sveikatos būkle.

Tyrimuose atskleistas ryšys tarp karvės ir jos veršelio tiriant jų metabolinius parametrus ir vertinant jų sveikatingumą. Naudota veršelių sveikatingumo vertinimo schema derinta kartu su laktuojančių karvių bandos valdymo programos duomenimis leido identifikuoti didesnėje sergamumo rizikoje esančius veršelius ir atrasti sąsajas su jų karvėmis, kurios turėjo išreikštus patiriamo neigiamo energijos balanso būklės požymius, apibūdintus pieno kokybės ir karvės sveikatingumo parametrais.

Praktinė reikšmė

Naudojant aprašytus karvių ir veršelių vertinimo metodus – kraujo BHB ir Glu koncentracijų tyrimą, bandos valdymo programos duomenų ir pieno liaukos susirgimų indikatorių registravimą bei veršelių sveikatingumo vertinimo schemos pritaikymą – tyrėjai, ūkiuose dirbantys veterinarijos gydytojai ir ūkių valdytojai gali įsivertinti savo ūkio galvijų sveikatingumo situaciją.

Turint pagrįstus duomenis, kurie indikuoja apie užtrūkinimo laikotarpio poveikį ne tik karvės bet ir veršelio sveikatingumui, suteikiama galimybė lengviau nuspręsti dėl užtrūkusių karvių ir veršelių auginimo ir laikymo sąlygų gerinimo, raciono papildymo ar naudojamos profilaktikos tinkamumo. Taip bus užtikrinamas gyvūnų ilgaamžiškumas ir sveikesnė bei didesnė produkcija.

Hipotezė

Karvių ir jų veršelių sveikatingumą bei metabolinius parametrus lemia karvės užtrūkinimo laikotarpio veiksniai, energinė būklė bei taikoma profilaktika.

Tikslas ir uždaviniai

Tyrimo tikslas yra įvertinti užtrūkinimo laikotarpyje patiriamos neigiamo energijos balanso būklės ir jos profilaktikos poveikį karvių ir jų veršelių metaboliniams bei sveikatingumo parametrams po apsiveršiavimo

Tikslui įgyvendinti išsikėlėme šiuos uždavinius:

1. Įvertinti užtrūkinimo laikotarpyje karvėms taikomos profilaktikos prieš neigiamos energijos balanso būklės ir dėl to pasireiškiančio pieno liaukos uždegimo efektyvumą panaudojant bandos valdymo programų rodiklius: įmitimą, primelžiamo pieno kiekį, pieno β-hidroksibutirato koncentraciją bei pieno laktatdehidrogenazės (LDH) fermento aktyvumą;

2. Įvertinti užtrūkusių karvių laktacijos skaičiaus poveikį jų bei veršelių metaboliniams kraujo β-hidroksibutirato (BHB) ir gliukozės (Glu) parametrams bei sveikatingumui;
3. Įvertinti metabolinį ryšį susidarantį tarp užtrūkusių karvių ir jų veršelių analizuojant kraujo BHB ir Glu koncentraciją po apsiveršiavimo bei veršelio sveikatingumo stebėjimo schemos duomenis;
4. Įvertinti užtrūkusių karvių neigiamo energijos balanso poveikį veršelių sveikatingumui panaudojant karvių bandos valdymo programos ir veršelių sveikatingumo vertinimo schemos parametrus po apsiveršiavimo.

TYRIMO METODIKA

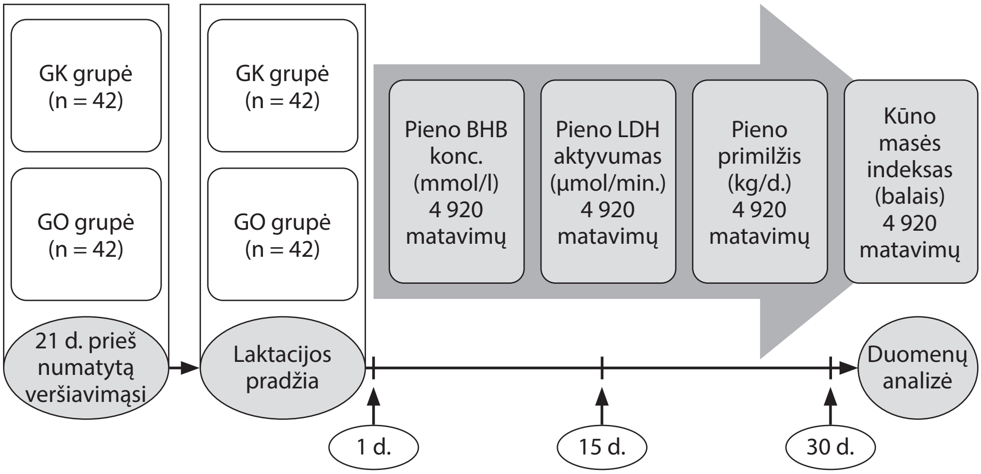
Užtrūkinimo laikotarpyje taikomos profilaktikos poveikio neigiamo energijos balanso būklei ir sveikatingumui įvertinimo tyrimas pasitelkiant bandos valdymo programos duomenis

Pieno ūkio ir gyvūnų atranka

Tyrimas atliktas 2021.03.01–2021.09.30 viename Lietuvos pieno ūkyje laikančiame 550 pieninio tipo karvių. Visos karvės buvo 2-os ar 3-os laktacijos dėl didesnės neigiamo energijos balanso rizikos palyginus su pirmaveršėmis ir šeriamos subalansuotu pašaru 5:00 ir 17:00 valandomis.

Tiriamosios ir kontrolinės grupių atranka

Jonoforo monenzino lėtojo dozavimo kapsulės (Kexxtone Elanco GmbH, Bad Homburg, Vokietija) buvo naudojamos šiame tyrime. Jų paskirtis – su specialia dėtuve į stemplę įstumiama kapsulė, kuri patekusi į didįjį prieskrandį, lėtai ir per ilgą laiką išskiria 335 mg monenzino. Šių kapsulių naudojimas buvo atliekamas tiksliai laikantis gamintojo instrukcijų. Tyrimui atlikti buvo suformuotos dvi grupės: 1. GK – karvių grupė, kurioms užtrūkinimo laikotarpyje buvo taikyta profilaktika prieš neigiamo energijos balanso būklės pasireiškimą pasitelkiant monenzino jonoforo nuolatinio atpalaidavimo didžiajame prieskrandyje priemonę (n = 42) ir 2. GO – karvių grupė, kurioms užtrūkinimo metu buvo per *os* įvedama tuščia monenzino kapsulė (n = 42) (1 pav.). Abiem grupėms jų atitinkamos kapsulės buvo įdedamos 21 d. prieš numatytą veršiavimosi datą, vadovaujantis gamintojo instrukcijomis. Abi grupės buvo laikomos vienodomis sąlygomis ir po apsiveršiavimo buvo pervedamos į melžiamų karvių grupę, kur melžimas vykdomas melžimo robotų pagalba. Robotuose integruota bandos valdymo programa kiekvieno melžimo metu registravo karvių sveikatingumo ir kokybės parametrus, o kiekvienas galvijas buvo vertinamas 30 laktacijos dienų.



1 pav. *Pirmojo tyrimo metodikos schema*

GK grupė – karvės, kurioms taikyta profilaktika užtrūkinimo laikotarpyje; GO grupė – karvių grupė, kurioms užtrūkinimo laikotarpyje profilaktika netaikyta; n – gyvūnų skaičius grupėje; BHB – β-hidroksibutiratas; LDH – laktatdehidrogenazė; matavimų – per tyrimo laikotarpį užregistruotų parametrų verčių skaičius.

Bandos valdymo programos registruojamų duomenų analizė

Pasitelkiant Delaval melžimo robotus (DeLaval Inc., Tumba, Švedija) ir Herd Navigator analizatorių (Lattec I/S, Hillerød, Danija) kiekvieno melžimo metu buvo registruojami pieno primilžio ir pieno β-hidroksibutirato (BHB) koncentracija (mmol/l) ir laktatdehidrogenazės (LDH) aktyvumo (µmol/min.) rodikliai. Tyrimo laikotarpiu atlikta 4 920 matavimų kiekvieno parametro reikšmėms abejose grupėse esantiems gyvūnams. Pieno primilžis buvo apskaičiuojamas akredituotu optiniu davikliu. LDH aktyvumas (µmol/min.) buvo automatiškai apskaičiuojamas LDH koncentraciją padalinant iš primilžio. LDH aktyvumo fiziologinė norma, kuri naudota rezultatų vertinimui, aprašyta Torben Larsen tyrime [118]. Pieno BHB koncentracija buvo įvertinama Herd Navigatoriaus pagalba pasitelkiant „sausos lazdelės“ metodą, o kaip fiziologinė norma laikyta reikšmė įvardinta De Jong ir kt. atliktame tyrime [104].

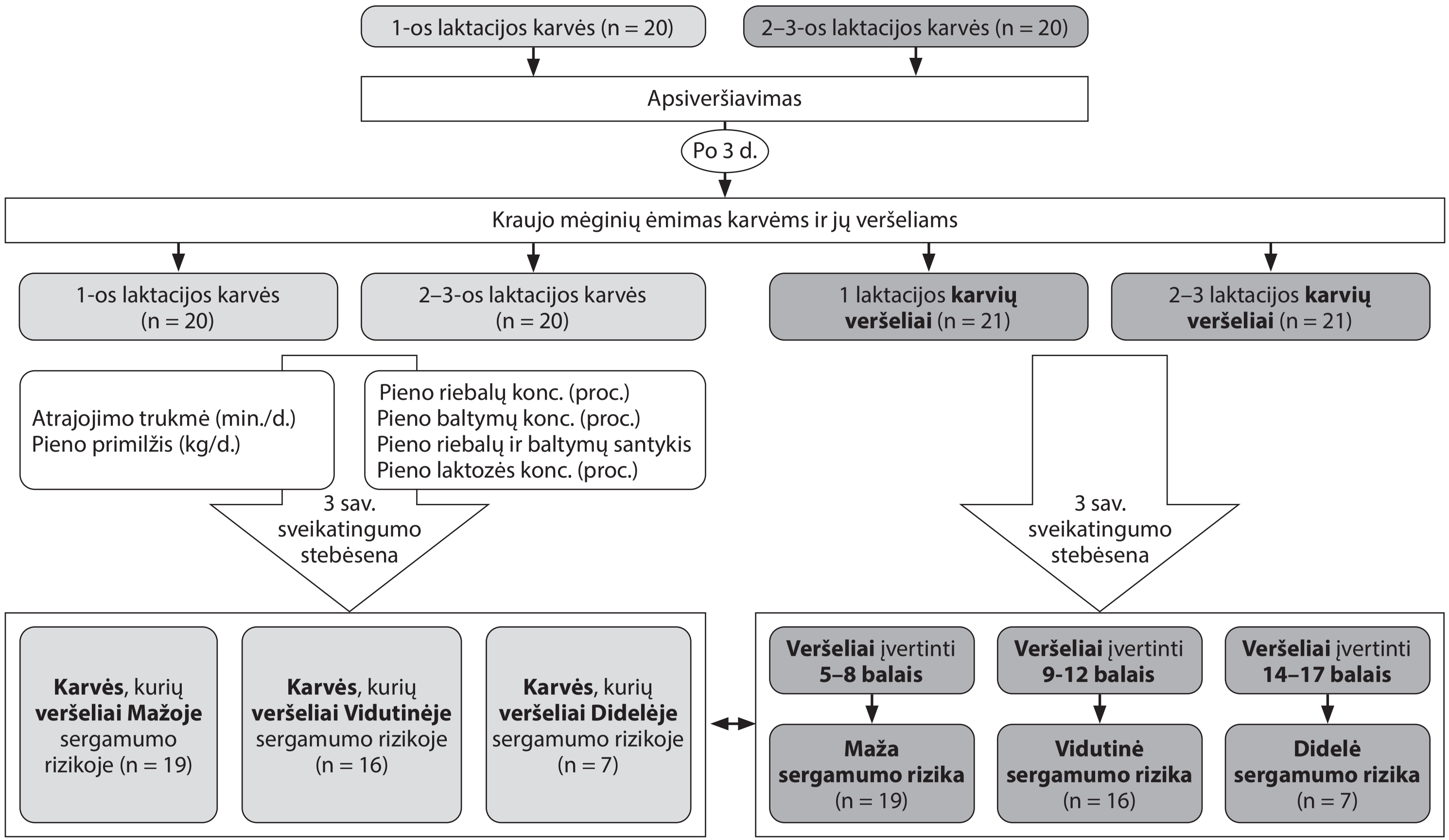
Prieš kiekvienai karvei įeinant į melžimo robotą, kiekvienos karvės kūno įmitimas buvo įvertinamas 3D kūno būklės vertinimo kamera (DeLaval International AB, Švedija). Galvijai buvo filmuojami iš viršaus ir buvo fokusuojama į galinę kūno dalį (nuo liemens iki uodegos), o toliau galvijo įmitimas buvo įvertinamas skaitinėje skalėje. Kiekvieną kartą galvijui praėjus pro robotą, jis yra filmuojamas ir pagal nufilmuotos medžiagos kokybę ir ryškumą bei įvertinimą buvo apskaičiuojamas kūno būklės dydis tai dienai. 3D kamera pasitelkia šviesos kodavimo technologiją, kuri ant galinės galvijo dalies spinduliuoja infraraudonuosius spindulius. Spinduliai ant galvijo kūno kameros matomi taško pavidalu ir sistema apskaičiuoja atstumus tarp šių taškų. Pagal šių taškų padėtį 3D plotmėje yra suformuojamas kompiuterinis galvijo kontūras ir algoritmo pagalba individui yra priskiriamas kūno būklės įvertinimas. Algoritmas naudoja galvijo įmitimo vertinimo skalę nuo 1 iki 5 [119]. Šioje skalėje 1-tas skiriamas labai išliesėjusiam gyvūnui, 5-tas – apkūniam. Roche ir kt. straipsnyje nurodoma, jog ankstyvajame laikotarpyje po apsiveršiavimo karvių įmitimas turi būti 3–3,5 ir tai naudota kaip fiziologinė norma rezultatų įvertinimui [119].

Karvės metabolinės būklės poveikio veršelio sveikatingumui tyrimas

Pieno ūkio ir gyvūnų atranka

Siekiant ištirti ryšį tarp karvės ir jos veršelio užtrūkinimo laikotarpyje, 2022 metais pasirinkome ūkį, kuriame melžiama apie 1 200-tai Holšteinų-Fryzų veislės karvių. Tai palaido laikymo ūkis, kuriame melžimas vykdomas Lely Astronaut 3 melžimo robotais (Lely, Maassluis, Nyderlandai).

Siekiant įvertinti ar laktacijos skaičius turės įtakos ryšiui tarp karvės ir veršelio, 20 veršelių buvo parinkta iš pirmaveršių karvių, bei 20 iš vyresnės laktacijos karvių (tik antros ir trečios laktacijos) (2 pav.).



2 pav. *Antrojo tyrimo metodikos schema*

n – gyvūnų skaičius grupėje; balais – veršelių sveikatingumo schemoje nurodytas skaitinis vienetas naudojamas klinikinių simptomų ryškumui įvertinti.

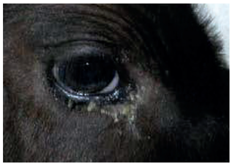
Į tyrimą įtraukti tik nuo kovo 25-tos iki balandžio 25-tos gimę veršeliai, siekiant sumažinti skirtingų klimatinių sąlygų įtaką gyvūnams ir rezultatams. Tam, kad veršelis galėtų dalyvauti tyrime, jis turėjo atitikti kelis kriterijus: 1. Atvedimas buvo lengvas ir nereikėjo veterinarijos gydytojo pagalbos; 2. Prieš veršiavimąsi karvė buvo nesirgusi; 3. Veršelis gavo geros kokybės krekenis iš savo motinos. Krekenų kokybė buvo įvertinama MS Colostro Balls (MS Schippers, Kerken, Vokietija) pagalba – tai skirtingų spalvų tabletės, kurių tankis tarpusavyje skiriasi. Kaip nurodyta instrukcijoje, krekenys tabletėmis buvo vertinamos tinkamoje 20–30 °C temperatūroje. Apmokytas darbuotojas atvestam veršeliui per pirmą valandą sugirdydavo 4l krekenų ir po to girdymą kartodavo po 12 val. Pagal gamintojų pateiktą instrukciją – jeigu visos tabletės iškyla į krekenų paviršių, vertinama, jog krekenų tankis siekia 1075 g/dm^3 ir tai vertinama kaip „geros kokybės krekenys“ [120, 121]. Krekenų tankio ribinės vertės 1 075 g/dm^3 vienetus konvertavus į santykinį tankį, gaunama 1,075. Krekenys laikomos geros kokybės, kai jų santykinis tankis > 1,047. Šis krekenų kokybės įvertinimo rodiklis artimai koreliuoja su IgG koncentracijos nustatymo metodais, radialine imunodifuzija ($r = 0,77$) bei ELISA (imunofermentinės analizės reakcija) ($r = 0,79$) [122–124].

Po krekenų sugirdymo veršeliai buvo atskiriami į vienviečius gardelius ir maitinami pienmilčiais (Sprayfo Yellow, Trouwnutrition, Putten, Nyderlandai) bei koncentruotu pašaru.

Veršelių tyrimo grupių sudarymas ir sveikatingumo vertinimas

Trečią dieną po apsiveršiavimo visiems tinkamiems veršeliams buvo atliekamas išsamus klinikinis tyrimas. Tyrimą atliko ir vėliau sveikatingumą vertino vienas ir tas pats apmokytas veterinarijos gydytojas su 5-ių metų darbo patirtimi. Tyrimui atrinkta $n = 42$ veršeliai, kadangi dvi karvės atsivedė dvynukus (patikslinta, nes veršelių skaičius didesnis už tyrimui atrinktų karvių skaičių). Sveikatos būklės įvertinimo eiga ir schema panaudota pagal McQuirck [125] aprašytą metodologiją, veršelių vertinimas susidarė iš nosies bei akių išskyrų įvertinimo, ausų padėties, rektinės temperatūros bei diarėjos ar pneumonijos ženklų įvertinimo (3 pav.). Pagal naudotą schemą, 0-io įvertinimas reiškia, jog nėra matomų klinikinių simptomų ir pakitimų – tad gyvūnas yra sveikas, o klinikinių simptomų būvimas indikuoja apie susirgimą.

School of Veterinary Medicine
UNIVERSITY OF WISCONSIN–MADISON

Veršelių sveikatingumo vertinimo kriterijai			
0	**1**	**2**	**3**
Rektinė temperatūra, °C			
37,7–38,2	38,3–38,8	38,8–39,3	≥ 39,4
Kosulys			
Kosulio nėra	Sudirginus kosteli kartą	Sudirginus kosteli kelis kartus arba stebint spontaniškai kosėja	Pasikartojantis spontaniškas kosulys
Išskyros iš nosies			
Skaidrios seroziškos išskyros	Negausus kiekis neskaidrių išskyrų iš vienos šnervės	Išskyros iš abiejų šnervių, neskaidrios arba gausios	Iš abiejų šnervių gausios mukopurulentinės išskyros
			
Akių būklės įvertinimas			
Be pakitimų	Negausios išskyros iš akių	Ryškiai matomos išskyros iš abiejų akių	Gausios išskyros iš akių
			
Ausų būklės įvertinimas			
Be pakitimų	„Karpymas“ ausimis arba galvos kratymas	Viena ausis truputį nuleista	Galva pakreipta arba abi ausys nuleistos
			

3 pav. *Veršelių sveikatingumo vertinimo schema*

Adaptuota pagal McGuirk SM, Peek SF. Timely diagnosis of dairy calf respiratory disease using a standardized scoring system. Animal Health Research Reviews. 2014;15(2):145–147.

Tyrimui tinkami veršeliai buvo vertinami kas 2–3 dienas kol būdavo 3 sav. amžiaus. Po trijų savaičių laikotarpio veršelių simptomų balų skaičius buvo susumuojamas. Surinkus visų veršelių duomenis ir susumavus balus, pagal apskaičiuotą bendrą dalmenį „4“, veršeliai buvo suskirstyti į 3 grupes –

Mažo sergamumo veršeliai (LCS) surinkę tik 5–8 balus (n = 19), vidutinio sergamumo veršeliai (MCS) surinkę 9–12 balų (n = 16) ir didelio sergamumo veršeliai (HCS) surinkę 14–17 balų (n = 7). Grupių paskirstymo metodika adaptuota pagal Maier ir kolegų taikytą metodiką [126].

Karvių ir veršelių kraujo mėginių ėmimas ir vertinimas

Prieš pilnai veršelius įtraukiant į tyrimą, trečią gyvenimo dieną iš jų jungo venos buvo paimamas kraujo mėginys į mėgintuvėlį skirtą kraujo biocheminiam tyrimui (BD Vacutainer Red, Mississauga, Kanada). Kraujo lašas iš mėgintuvėlio buvo panaudojamas gliukozės ir BHB koncentracijoms nustatyti pasitelkiant rankinį matuoklį (CentriVet GK, Acon, San Diego, USA).

Nucentrifuguotas serumas būdavo toliau tiriamas, siekiant įvertinti jo bendrą baltymų kiekį ir patvirtinti, jog veršeliai gavo geros kokybės krekenis. Tam tikslui serumas buvo vertinamas rankiniu refraktometru (RHC200, YHequipment, Shenzhen, Kinija). Pagal Renaud ir kt. [127], žemiausia koncentracija, iki kurios laikoma, jog krekenys įsisavintos tinkamai yra < 5,2 g/dl. Visi veršeliai gavo geros kokybės krekenis ir laiku, kadangi visų veršelių serumo baltymų koncentracija siekė apie 7,6 g/dl.

Tą pačią dieną, kai kraujo mėginys buvo imama veršeliui, tas pats buvo atliekama ir su to veršelio karve. Taip pat kaip ir veršeliams, kraujo lašas iš mėgintuvėlio buvo naudojamas gliukozės (mmol/l) ir BHB (mmol/l) koncentracijai nustatyti su rankiniu matuokliu (CentriVet GK, Acon, San Diego, JAV). Mėginys karvei bei jos veršeliui buvo imamas tik kartą.

Karvių tyrimo grupių paskyrimas ir bandos valdymo programos duomenų įvertinimas

Po trijų savaičių, kai buvo įvertinami jų veršeliai ir priskiriami grupei pagal susumuotus sergamumo balus, jų motinos būdavo priskiriamos tai pačiai grupei (LCS, MCS ir HCS). Karvės kurių veršeliai buvo mažoje, vidutinėje arba didelėje sergamumo rizikos grupėje. Po to šių karvių duomenys, kurie buvo renkami Lely Astronaut A3 (Lely, Maassluis, Nyderlandai) melžimo robotų pagalba tris savaites po apsiveršiavimo, buvo perkeliami iš bandos valdymo programos. Sistema pateikė šiuos parametrus: atrajojimo trukmė (atrajojimui skirtas laikas minutėmis per dieną, 1 200 matavimų), dienos pieno kiekis (kilogramai pieno per dieną, 2 400 matavimų), pieno baltymų koncentracija (proc., 2 400 matavimų), pieno riebalų koncentracija (proc., 2 400 matavimų), pieno laktozės koncentracija (proc., 2 400 matavimų) ir pieno riebalų baltymų santykis (2 400 matavimų). Duomenys buvo registruojami kiekvieną dieną per trijų savaičių laikotarpį. Jie buvo susisteminti, išvesti vidurkiai ir palyginti tarp grupių.

STATISTINĖ ANALIZĖ

SPSS 26,0 (SPSS Inc., Čikaga, JAV) programa buvo naudojama statistinei analizei atlikti. Analizuojant galvijų ir jų veršelių kraujo tyrimų duomenis, Shapiro–Wilko testas buvo naudojamas duomenų normalaus išsidėstymo įvertinimui, kadangi tiriamųjų skaičius buvo mažas. Karvių bei veršelių kraujo BHB koncentracijos duomenys nebuvo normaliai išsidėstę. Kraujo gliukozės koncentracijos duomenys karvių bei veršelių mėginiuose buvo normaliai išsidėstę, tad vienfaktorinis ANOVA testas buvo panaudotas įvertinti vidurkių skirtumus tarp skirtingų laktacijų karvių duomenų. Analizės rezultatai pateikti kaip vidurkiai su standartiniu nuokrypiu. Pirsono koreliacija buvo atliekama nustatyti ryšį tarp duomenų, kurių išsidėstymas buvo normalus. Koreliacijos stiprumas įvertintas pagal šią skalę: |0,1–0,3| = koreliacija silpna, |0,3–0,5| = vidutiniška ir |0,5–1,0| = koreliacija stipri. Nenormaliai išsidėstę duomenys buvo vertinami Mano–Vitnio testu, o veršelių sergamumo grupių išsidėstymas tarp skirtingų laktacijų karvių buvo įvertintas Pirsonos Chi kvadrato (χ^2) testu.

Kolmogorovo–Smirnovo testas buvo naudojamas įvertinti normalų pasiskirstymą duomenų gautų iš automatinių bandos valdymo sistemų, kadangi duomenų skaičius buvo didelis. Atrajojimo laiko, pieno primilžio, pieno baltymų, riebalų ir laktozės koncentracijų bei pieno riebalų baltymų santykio duomenys buvo normaliai pasiskirstę. Dėl šios priežasties Stjudento t-testas buvo panaudotas palyginti šių duomenų vidurkius tarp pirmaveršių ir vyresnės laktacijos karvių grupių. Vienfaktorinis ANOVA testas buvo panaudotas įvertinti ar buvo statistiškai patikimų skirtumų tarp šių rodiklių vidurkių lyginant tarpusavyje tarp karvių suskirstytų pagal jų veršelių sveikatingumo grupes. Fišerio mažiausiai reikšmingo skirtumo aposteriorinis testas sekė vienfaktorinį ANOVA testą, siekiant nustatyti tarp kurių tiksliai grupių šie skirtumai buvo statistiškai patikimi. Rezultatai pateikti vidurkiais ir standartine paklaida. Skirtumai buvo laikomi statistiškai patikimais, kai patikimumas buvo $p < 0,05$.

Tyrime dėl monenzino naudojimo užtrūkinimo laikotarpyje, karvių įmitimo ir pieno parametrų duomenys buvo tikrinami dėl normalaus išsidėstymo pasitelkiant Shapiro–Wilk testą. Dėl LDH duomenų skaitinės vertės, jie buvo pakeisti logaritmine išraiška ($\log_{10}$). Duomenų vertinimui naudotas apibendrintasis tiesinis pakartotinių reikšmių modelis. Parametrai buvo lyginami tarp GK ir GO grupių 1-ą, 15-tą ir 30-tą laktacijos dienomis. Rezultatai pateikti vidurkiais ir standartine paklaida (SEM) bei 95 proc. patikimumo intervalu vidurkiui. Bonferoni testas buvo naudojamas palyginti duomenis pagal laktacijos dienas ir pagal grupes. Linijinės ir antrojo lygmens kintamojo regresijos modeliai buvo naudojami išanalizuoti pokytį kintamuosiuose parametruose –

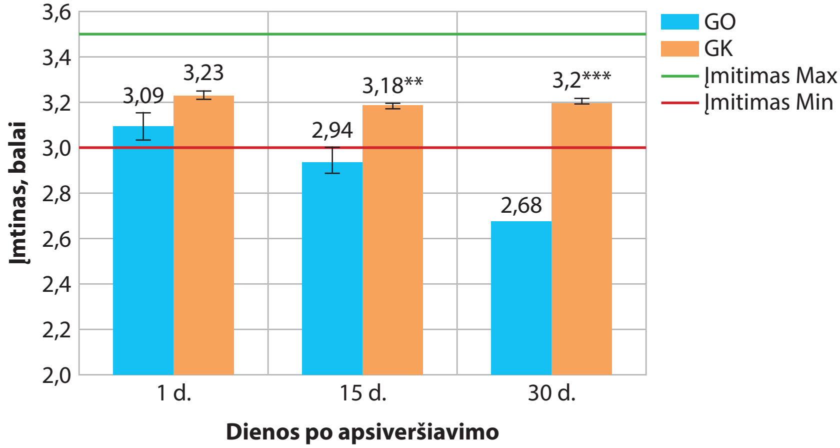
karvių įmitimo ir pieno kokybės rodikliuose. Pieno BHB duomenys buvo suskirstyti pagal atskaitos taškus (0,04, 0,05 ir 0,06 mmol/l). Fisherio testas buvo naudojamas įvertinti ryšį tarp BHB lygio bei kitų parametrų.

REZULTATAI

Užtrūkinimo laikotarpyje taikomos profilaktikos neigiamo energijos balanso būklei tyrimo rezultatai

Tyrime, kuriame vertinta profilaktikos taikymo užtrūkinimo laikotarpyje poveikis neigiamo energijos balanso būklės pasireiškimui nustatyta, jog karvių grupėje, kuriai buvo taikyta profilaktika, karvių įmitimas turėjo tendenciją didėti bet viso tyrimo laikotarpiu išliko fiziologinės normos ribose, o kontrolinės grupės karvių įmitimas tuo tarpu mažėjo. GK grupėje kūno įmitimo skalės reikšmės buvo statistiškai patikimai didesnės palyginus su GO grupe 15-tą (+0,24, p = 0,003) ir 30-tą (+0,52, p < 0,001) laktacijos dienomis.

GO grupės kūno įmitimo reikšmė visą tyrimo laikotarpį mažėjo (p < 0,001), o tuo tarpu GK grupėje įmitimo vertės sumažėjo 15-tą dieną (–1,58 proc., p = 0,005), bet 30-tą dieną buvo padidėjęs (+0,56 proc., p = 0,040).

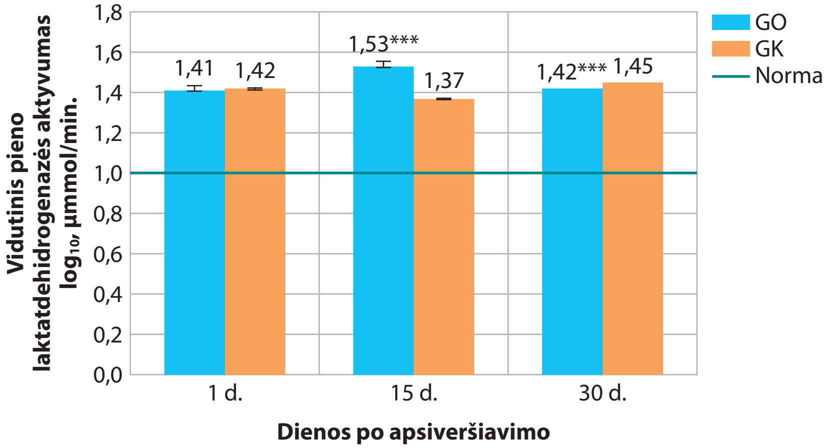


***4 pav.** Karvių kūno įmitimo pokytis ir palyginimas tarp grupių tyrimo laikotarpiu*

GO – karvių grupė, kuriai užtrūkinimo laikotarpiu netaikyta profilaktika; GK – karvių grupė, kuriai taikyta profilaktika užtrūkinimo laikotarpyje; įmitimas Max – karvės įmitimo normos ankstyvajame laikotarpyje po apsiveršiavimo viršutinė riba; įmitimas Min – karvės įmitimo normos ankstyvajame laikotarpyje po apsiveršiavimo žemutinė riba; **p = 0,003, ***p < 0,001.

LDH aktyvumas abejose grupėse statistiškai patikimai nesiskyrė pirmąją dieną po apsiveršiavimo, tačiau sekančiuose tyrimo etapuose GK grupės LDH aktyvumas buvo statistiškai patikimai žemesnis už GO grupės ($p < 0,001$).

GO grupės LDH koncentracija piene nuo tyrimo pradžios iki pabaigos padidėjo 19,15 proc. ($p < 0,001$), tuo tarpu GK grupėje šio rodiklio koncentracijos augimas sustojo.



5 pav. *Pieno LDH aktyvumo pokytis ir palyginimas tarp grupių tyrimo laikotarpiu*

GO – karvių grupė, kurioms užtrūkinimo laikotarpyje netaikyta profilaktika; GK – karvių grupė, kurioms taikyta profilaktika užtrūkinimo laikotarpyje; Norma – fiziologinė laktatdehidrogenazės aktyvumo norma; ***$p < 0,001$.

GO grupės pieno BHB koncentracija statistiškai patikimai buvo didesnė už GK grupės vidurkį visu tyrimo laikotarpiu (skirtumai siekė nuo 0,005 iki 0,012 mmol/l, $p < 0,001$), tačiau skirtumas tarp grupių mažėjo laktacijos dienų skaičiui didėjant. GK grupės pieno BHB koncentracija tyrimo metu mažėjo nežymiai, tačiau GO grupėje matosi aiškus vidurkio mažėjimas (nuo 0,060 iki 0,054 mmol/l, $p < 0,001$)

Išanalizavus duomenis nustatyta, jog GK grupėje, karvių skaičius, kurių pieno BHB koncentracija siekė 0,06 mmol/l, tyrimo laikotarpiu sumažėjo 4,70 proc. (nuo 19,00 proc. iki 14,30 proc.).

1 lentelė. *Pieno BHB koncentracijos (mmol/l) tarp grupių palyginimas skirtingomis tyrimo dienomis*

Dienos po apsiveršiavimo	**Grupė**	**M**	**SEM**	**95 proc. CI**		**p tarp grupių**
				Mažiausia reikšmė	**Didžiausia reikšmė**	
1	GO	0,060[a]	0,001	0,059	0,061	< 0,001
	GK	0,048[A]	0,001	0,047	0,049	
15	GO	0,057[b]	0,001	0,055	0,058	< 0,001
	GK	0,049[A]	0,001	0,047	0,050	
30	GO	0,054[b]	0,001	0,052	0,055	< 0,001
	GK	0,049[A]	0,001	0,047	0,050	

GK – monenzino papildą gavusių karvių grupė; GO – kontrolinė grupė; M – pieno BHB koncentracijos vidurkis (mmol/l); SEM – standartinė paklaida; 95 proc. CI – patikimumo intervalas; [a,b,c] skirtingos raidės nurodo statistiškai patikimus skirtumus tarp skirtingų laktacijos dienų GO grupei [A,B,C] skirtingos raidės nurodo statistiškai patikimus skirtumus tarp skirtingų laktacijos dienų GK grupei.

Karvės metabolinės būklės ryšio su veršeliu ir įtakos veršelio sveikatingumui tyrimo rezultatai

Kraujo gliukozės koncentracija statistiškai patikimai skyrėsi tarp pirmaveršių ir vyresnės laktacijos karvių. Pirmaveršės turėjo 16 proc. aukštesnę kraujo gliukozės koncentraciją palyginus su vyresnės laktacijos karvėmis (3,03 mmol/l, std. paklaida ±0,093 ir 2,61 mmol/l, std. paklaida ±0,102, atitinkamai) ($p < 0,01$). Tačiau lyginant kraujo gliukozės koncentracijas tarp veršelių iš šių karvių – statistiškai patikimo skirtumo nerasta ($p > 0,05$).

Taip pat kai vertinome veršelių sergamumo grupių pasiskirstymą tarp skirtingų laktacijų karvių, statistiškai patikimų skirtumų neregistravome ($\chi^2(2) = 1,588$, $p > 0,05$) (2 lentelė). Statistiškai patikimo skirtumo tarp Mažos sergamumo rizikos ir Vidutinės sergamumo rizikos ($p > 0,05$) bei tarp Didelės sergamumo rizikos grupių ($p > 0,05$) nebuvo apskaičiuota. Tarp Vidutinės sergamumo rizikos ir Didelės sergamumo rizikos grupių skirtumas taip pat nepatikimas ($p > 0,05$).

2 lentelė. *Veršelių sergamumo rizikos grupių pasiskirstymas tarp skirtingų laktacijų skaičiaus karvių grupių*

Laktacijos skaičius	**Rodiklis**	**Veršelio sergamumo rizikos grupė**		
		Mažos	**Vidutinės**	**Didelės**
Pirmaveršės	n	10	9	2
	proc.	47,6	42,9	9,5
Vyresnės laktacijos	n	9	7	5
	proc.	42,9	33,3	23,8

$\chi^2 = 1{,}588$, df = 2, p > 0,05; Mažos – Mažo sergamumo rizikos balo veršelių grupė per tyrimo laikotarpį surinkusi 5–8 balus, laikoma, jog linkusi mažiausiai sirgti; Vidutinės – Vidutinio sergamumo rizikos balo veršelių grupė per tyrimo laikotarpį surinkusi 9–12 balų. Linkusi vidutiniškai sirgti; Didelės – Didelės sergamumo rizikos balo veršelių grupė per tyrimo laikotarpį surinkusi 14–17 balų. Dažnai sirgusių veršelių grupė; n – veršelių skaičius iš tos laktacijos karvių pasiskirsčiusių veršelių sergamumo grupėje.

Statistiškai patikima vidutinio stiprumo neigiama koreliacija apskaičiuota tarp karvių BHB ir karvių kraujo gliukozės koncentracijų (r = −0,353, p < 0,05).Tarp karvių kraujo BHB koncentracijos ir veršelių kraujo BHB koncentracijos nustatyta statistiškai patikimas vidutinio stiprumo neigiama (r = −0,476, p < 0,01) (3 lentelė).

3 lentelė. *Koreliacijos koeficientų reikšmės tarp karvių ir veršelių kraujo parametrų reikšmių*

	Karvių BHB	**Karvių Glu**	**Veršelių BHB**	**Veršelių Glu**	**Veršelių sergamumo balas**
	Koreliacijos koeficientas				
Karvių BHB		−0,353*	−0,476**	−0,147	−0,05
Karvių Glu	−0,353*		0,2	0,183	−0,164
Veršelių BHB	−0,476**	−0,353*		0,096	−0,207
Veršelių Glu	−0,147	0,183	0,096		0,111
Veršelių sergamumo balas	−0,05	−0,164	−0,207	0,111	

* Koreliacijos statistinis patikimumas 0,05 (2-tailed); ** Koreliacijos statistinis patikimumas 0,01 (2-tailed); Karvių BHB – β-hidroksibutirato koncentracija karvių kraujo serume; Karvių Glu –karvių kraujo serumo gliukozės koncentracija; Veršelių BHB – β-hidroksibutirato koncentracija veršelių kraujo serume; Veršelių Glu – veršelių kraujo serumo gliukozės koncentracija; Veršelių sergamumo balas – balas apskaičiuotas veršeliui pagal klinikinių simptomų pasireiškimą ir stiprumą.

Post hoc duomenų analizė indikuoja, jog bandos valdymo programos parametrų vidurkiai statistiškai patikimai skiriasi tarp karvių, kurių veršeliai yra skirtingose sergamumo rizikos grupėse (4 lentelė). Ilgiausia atrajojimo trukmė nustatyta karvėms, kurių veršeliai buvo Didelio sergamumo rizikos grupėje ir buvo 16 proc. ilgesnė palyginus su karvėmis, kurių veršeliai buvo Mažoje sergamumo rizikos grupėje ($p < 0,001$). 8 proc. skirtumas taip pat nustatytas ir su karvių grupe, kurių veršeliai buvo Vidutinėje sergamumo rizikos grupėje ($p < 0,001$). Statistiškai patikimai reikšmingas skirtumas taip pat nustatytas tarp karvių, kurių veršeliai buvo Mažoje sergamumo rizikos grupėje ir Vidutinio sergamumo rizikos grupėje ir siekė 8 proc. ($p < 0,001$).

4 lentelė. *Bandos valdymo programos registruojamų karvių rodiklių vidurkių palyginimas pagal veršelių sergamumo rizikos grupes*

Rodiklis	**Veršelių grupės**	**n**	**Vidurkis**	**Standartinis nuokrypis**	**F**	**p**
Atrajojimo trukmė (min./d.)	Mažo sergamumo rizikos	19	461,94	134,80	61,86	< 0,001
	Vidutinio sergamumo rizikos	16	505,56	108,54		
	Didelio sergamumo rizikos	7	550,79	110,67		
Primilžis (kg/d.)	Mažo sergamumo rizikos	19	38,83	10,70	52,42	< 0,001
	Vidutinio sergamumo rizikos	16	36,31	11,62		
	Didelio sergamumo rizikos	7	44,80	14,84		
Pieno baltymų koncentracija (proc.)	Mažo sergamumo rizikos	19	3,84	0,34	29,17	< 0,001
	Vidutinio sergamumo rizikos	16	3,78	0,25		
	Didelio sergamumo rizikos	7	3,68	0,36		
Pieno riebalų koncentracija (proc.)	Mažo sergamumo rizikos	19	4,28	0,70	11,45	< 0,001
	Vidutinio sergamumo rizikos	16	4,25	0,61		
	Didelio sergamumo rizikos	7	4,47	0,65		

4 lentelės tęsinys

Rodiklis	Veršelių grupės	n	Vidurkis	Standartinis nuokrypis	F	p
Pieno riebalų baltymų santykis	Mažo sergamumo rizikos	19	1,11	0,16	47,56	< 0,001
	Vidutinio sergamumo rizikos	16	1,12	0,14		
	Didelio sergamumo rizikos	7	1,21	0,16		
Pieno laktozės koncentracija (proc.)	Mažo sergamumo rizikos	19	4,66	0,10	38,76	< 0,001
	Vidutinio sergamumo rizikos	16	4,62	0,11		
	Didelio sergamumo rizikos	7	4,61	0,08		

Mažo sergamumo rizikos – veršelių grupė per tyrimo laikotarpį surinkusi 5–8 balus, laikoma, jog linkusi mažiausiai sirgti; Vidutinio sergamumo rizikos – veršelių grupė per tyrimo laikotarpį surinkusi 9–12 balų. Linkusi vidutiniškai sirgti; Aukšto sergamumo rizikos – veršelių grupė per tyrimo laikotarpį surinkusi 14–17 balų. Dažnai sirgusių veršelių grupė; n – karvių skaičius; F – F dydis; p – patikimumas.

Didžiausias primilžis nustatytas karvėms, kurių veršeliai buvo Didelėje sergamumo rizikos grupėje. Šioje grupėje primilžis buvo 19 proc. didesnis palyginus su karvėmis, kurių veršeliai buvo Vidutinio sergamumo rizikos grupėje ($p < 0,001$) ir 13 proc. didesnis palyginus su karvėmis, kurių veršeliai buvo Mažoje sergamumo rizikos grupėje ($p < 0,001$). Tarp pastarųjų grupių taip pat nustatytas 6 proc. statistiškai patikimas vidurkių skirtumas ($p < 0,001$).

Didžiausia pieno baltymų koncentracija nustatyta karvėms, kurių veršeliai buvo Mažoje sergamumo rizikos grupėje. Šioje grupėje koncentracija buvo 1,5 proc. didesnė palyginus su karvių, kurių veršeliai buvo Vidutinėje sergamumo rizikos grupėje ($p < 0,001$) ir 4 proc. didesnė palyginus su karvėmis, kurių veršeliai buvo Didelėje sergamumo rizikos grupėje ($p < 0,001$). Pieno riebalų koncentracijos vidurkio reikšmė didžiausia buvo karvių grupėje, kurių veršeliai priklausė Didelei sergamumo rizikos grupei. Ši reikšmė buvo 4 proc. didesnė palyginus su karvių, kurių veršeliai buvo Mažoje sergamumo rizikos grupėje ($p < 0,001$) ir 5 proc. didesnė palyginus su karvėmis, kurių veršeliai buvo Vidutinėje sergamumo rizikos grupėje ($p < 0,001$). Nors karvių, kurių veršeliai buvo Mažoje sergamumo rizikos grupėje, pieno riebalų koncentracija buvo didesnė palyginus su karvėmis, kurių veršeliai buvo Vidutinėje sergamumo rizikos grupėje, statistiškai patikimo skirtumo tarp šių vidurkių ($p > 0,05$). Pieno riebalų ir baltymo santykis buvo didžiausias karvių grupėje, kurių veršeliai priklausė Didelei sergamumo rizikos grupei ir buvo 7 proc. didesnis palyginus su karvėmis, kurių veršeliai buvo Vidutinėje sergamumo rizikos grupėje ($p < 0{,}001$) bei 8 proc. didesnis palyginus su karvėmis, kurių veršeliai buvo Mažoje sergamumo rizikos grupėje ($p < 0{,}001$). Statistiškai patikimo skirtumo tarp pastarųjų grupių nenustatyta ($p > 0{,}05$). Karvės, kurių veršeliai buvo Mažoje sergamumo rizikos grupėje, turėjo didžiausią pieno laktozės koncentraciją. Ji buvo 1 proc. didesnė palyginus su karvėmis, kurių veršeliai buvo Vidutinėje sergamumo rizikos grupėje ($p < 0{,}001$) ir 1,07 proc. didesnė palyginus su karvėmis, kurių veršeliai buvo Didelėje sergamumo rizikos grupėje ($p < 0{,}001$). Statistiškai patikimo skirtumo tarp karvių, kurių veršeliai buvo Didelėje sergamumo rizikos grupėje ir Vidutinio sergamumo rizikos grupėje, nebuvo ($p > 0{,}05$).

Apibendrinant tyrimuose gautus rezultatus, nustatytas užtrūkinimo laikotarpio, ypatingai karvės metabolinės būklės šiuo laikotarpiu, poveikis ne tik karvės, bet ir jos veršelio sveikatingumui. Ankstyvajame laktacijos laikotarpyje karvių patiriamas neigiamas energijos balansas yra suvaldomas būtent užtrūkinimo laikotarpyje taikant profilaktikos priemones. Kadangi užtrūkinimo laikotarpyje prasidedanti neigiamo energijos balanso būklė nulemia karvės metabolizmą, tai turi neigiamą poveikį veršelio sveikatingumui. Dėl šios priežasties profilaktika užtrūkinimo laikotarpyje yra gera strategija užtikrinti ne tik karvės, bet ir jos veršelio sveikatingumą.

Įvertinus užtrūkinimo laikotarpio svarbą, atsiranda poreikis platesniems tyrimams susijusiems su vidiniais ir išoriniais šio laikotarpio veiksniai, ryšiu tarp karvių ir jų veršelių. Išsamesni tyrimai įsigilinantys į šį ryšį ir laikotarpyje prieš veršiavimąsi vykstančius procesus leistų pasiekti geresnių produktyvumo rezultatų ir didesnio sveikatingumo, o kartu leistų įsivertinti poreikį koreguoti užtrūkinimo laikotarpio valdymą.

Tyrimuose pasitelktos metodikos ir sistemos, kaip bandos valdymo programų registruojami duomenys bei veršelių sveikatingumo įvertinimo schemos, leidžia įsivertinti ir nustatyti užtrūkinimo laikotarpio ir jo profilaktikos reikšmę produkcijai ir sveikatos būklei. Šių metodikų ir sistemų naudojimas pritaikomas ir įvertinant naudojamos profilaktikos efektyvumui. Surinkus tikslius duomenis ir remiantis atliktais moksliniais tyrimais šia tema, galima pagrįsti poreikį modifikuoti užtrūkinimo laikotarpyje taikomą profilaktiką, šėrimą ar gyvūnų laikymo sąlygas.

IŠVADOS

1. Užtrūkinimo laikotarpyje taikyta profilaktika sumažino neigiamo energijos balanso ir pieno liaukos uždegimo riziką laktacijos pradžioje. Tiriamosios grupės karvės palyginus su kontrolinės grupės karvėmis turėjo mažesnę neigiamo energijos balanso riziką vertinant, jog 15-tą tyrimo dieną jų pieno produkcija buvo 10,25 proc. didesnė ($p < 0,001$), o įmitimas buvo didesnis 15-tą (+0,24, $p = 0,003$) bei 30-tą (+0,52, $p < 0,001$) tyrimo dienomis ir tyrimo laikotarpiu išliko fiziologinės normos ribose. Tiriamosios grupės LDH aktyvumas išliko stabilus tyrimo laikotarpiu, o kontrolinės grupės aktyvumas nuo tyrimo pradžios iki 15-tos dienos padidėjo 19,15 proc. ($p < 0,001$);
2. Karvių laktacijos skaičius neturėjo įtakos veršelių sveikatingumui, kadangi veršelių skirtingose sergamumo grupėse pasiskirstymas tarp karvių laktacijų grupių buvo vienodas ($p > 0,05$). Nors vyresnės laktacijos karvių kraujo gliukozės koncentracija buvo 16 proc. žemesnė palyginus su pirmaveršėmis, tačiau β-hidroksibutirato koncentracija nesiskyrė nei tarp karvių, nei tarp jų veršelių;
3. Karvės metabolizmas lemia veršelio metabolizmo parametrus. Užfiksuota vidutinė neigiama koreliacija tarp karvių ir jų veršelių β-hidroksibutirato koncentracijos ($r = -0,476$, $p < 0,01$). Koreliacija tarp karvių ir veršelių gliukozės koncentracijos parametrų nebuvo nustatyta. Tai indikuoja apie veršelio organizmo prisitaikymą prie β-hidroksibutirato kaip energijos šaltinio dar besivystant gimdoje;
4. Karvių metabolinė būklė turi įtakos veršelio sveikatingumui. Bandos valdymo programos parametrais įvertintos didesnėje neigiamo energijos balanso būklės rizikoje karvės atsivedė veršelius didesne sergamumo rizika. Karvės, kurių veršeliai buvo didelėje sergamumo rizikos grupėje, palyginus su karvėmis, kurių veršeliai buvo mažoje sergamumo rizikos grupėje pasižymėjo 8 proc. didesniu pieno riebalų baltymų santykiu, 1,07 proc. žemesne pieno laktozės koncentracija, 13 proc. didesniu primilžiu bei 16 proc. ilgesne atrajojimo trukme ($p < 0,001$).

REKOMENDACIJOS

1. Bandos sveikatingumo vertinimas turi būti papildomas veršelių sveikatingumo stebėjimo schemos duomenimis derinant ją su karvių bandos valdymo programos parametrų pokyčiais. Veršelių sveikatos būklė gali būti įvertinama pagal rektinės temperatūros pokyčius, išskyrų kiekį iš nosies ir akių bei vertinant ausų nusvirimą. Identifikavus sergančius veršelius, jų karvės yra didesnėje NEB ir su šia būkle susijusių ligų rizikoje, todėl jos turėtų būti stebimos bandos valdymo programos pagalba. Taip padidinama tikimybė ankstyvai ligų diagnostikai ir sergamumo sumažinimui.
2. Bandos valdymo programos rodiklių pokyčiai – padidėjęs pieno riebalų baltymų santykis, sumažėjusi pieno laktozės koncentracija, didesnis galvijo produktyvumas bei ilgesnė atrajojimo trukmė, turi būti naudojami kaip neigiamo energijos balanso rizikos indikatoriai. Padidėjusios rizikos karvių veršeliai taip pat turėtų būti įtraukiami į sveikatingumo stebėjimo schemą, siekiant kuo anksčiau diagnozuoti galimus susirgimus.
3. Dėl galimos žalos aplinkai ir kitiems gyvūnams, apribojus monenzino preparatų, kaip profilaktikos priemonės prieš NEB naudojimą, reikalinga ieškoti alternatyvų. Vienas iš galimų variantų yra amino rūgščių papildų naudojimas užtrūkinimo laikotarpyje.

REFERENCES

1. OECD-FAO Agricultural Outlook 2023–2032. OECD; 2023.
2. Publications Office of the European Union. European Commission: Eurostat, Key figures on Europe : 2024 edition [Internet]. 2024 [cited 2025 May 7]. Available from: https://op.europa.eu/s/z5gv
3. Eurostat. Milk and milk product statistics [Internet]. 2024 Nov [cited 2025 Feb 9]. Available from: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Milk_and_milk_product_statistics
4. Piesarskas E. UAB „Ekonominės konsultacijos ir tyrimai". Pieno sektoriaus apžvalga. Pasaulis. Europos Sąjunga. Lietuva [Internet]. 2025 Jan [cited 2025 Feb 9]. Available from: https://www.ekt.lt/wp-content/uploads/2025/01/EKT_Pieno_sektoriaus_apzvalga_santrauka_2025.01.29-1.pdf#page=18.00
5. ŽŪDC. Pieno gamybos ir realizavimo metinės deklaracijos už 2023–2024 m. [Internet]. 2024 [cited 2025 Feb 9]. Available from: https://zudc.lt/pieno-gamybos-ir-realizavimo-metines-deklaracijos-uz-2023-2024-m/
6. De Vries A. Economic trade-offs between genetic improvement and longevity in dairy cattle. J Dairy Sci. 2017 May;100(5):4184–92.
7. Arndt C, Diao X, Dorosh P, Pauw K, Thurlow J. The Ukraine war and rising commodity prices: Implications for developing countries. Glob Food Sec. 2023 Mar;36:100680.
8. Commission Implementing Decision (EU) 2025/186 of 24 January 2025 concerning certain emergency measures relating to foot and mouth disease in Germany and repealing Implementing Decision (EU) 2025/87 (notified under document C(2025) 654) [Internet]. Jan 24, 2025. Available from: http://data.europa.eu/eli/dec_impl/2025/186/oj
9. Komunikat Głównego Lekarza Weterynarii dotyczący stwierdzenia na terytorium RP pierwszego ogniska choroby niebieskiego języka (BT) [Internet]. 2024 Nov [cited 2025 Feb 9]. Available from: https://www.wetgiw.gov.pl/main/komunikaty/Komunikat-Glownego-Lekarza-Weterynarii-dotyczacy-stwierdzenia-na-terytorium-RP-pierwszego-ogniska-choroby-niebieskiego-jezyka-BT/idn:2679#:~:text=Zgodnie%20z%20prawodawstwem%20UE%2C%20choroba,nie%20stanowi%20zagro%C5%BCenia%20dla%20ludzi.
10. Schuster JC, Barkema HW, De Vries A, Kelton DF, Orsel K. Invited review: Academic and applied approach to evaluating longevity in dairy cows. J Dairy Sci. 2020 Dec;103(12):11008–24.
11. Kerwin AL, Burhans WS, Mann S, Nydam DV, Wall SK, Schoenberg KM, et al. Transition cow nutrition and management strategies of dairy herds in the northeastern United States: Part II – Associations of metabolic- and inflammation-related analytes with health, milk yield, and reproduction. J Dairy Sci. 2022 Jun;105(6):5349–69.
12. Chen J, Kok A, Remmelink GJ, Gross JJ, Bruckmaier RM, Kemp B, et al. Effects of dry period length and dietary energy source on lactation curve characteristics over 2 subsequent lactations. J Dairy Sci. 2016 Nov;99(11):9287–99.
13. Baumrucker CR, Bruckmaier RM. Colostrogenesis: IgG1 Transcytosis Mechanisms. J Mammary Gland Biol Neoplasia. 2014 Mar 29;19(1):103–17.
14. Green JC, Meyer JP, Williams AM, Newsom EM, Keisler DH, Lucy MC. Pregnancy development from day 28 to 42 of gestation in postpartum Holstein cows that were

either milked (lactating) or not milked (not lactating) after calving. Reproduction. 2012 May;143(5):699–711.
15. Redifer CA, Duncan NB, Meyer AM. Factors affecting placental size in beef cattle: Maternal and fetal influences. Theriogenology. 2021 Oct;174:149–59.
16. Lemley CO, Littlejohn BP, Burnett DD. Fetal programming. In: Bovine reproduction. Wiley; 2021. p. 339–46.
17. Janovick NA, Drackley JK. Prepartum dietary management of energy intake affects postpartum intake and lactation performance by primiparous and multiparous Holstein cows. J Dairy Sci. 2010 Jul;93(7):3086–102.
18. Drackley JK, Cardoso FC. Prepartum and postpartum nutritional management to optimize fertility in high-yielding dairy cows in confined TMR systems. Animal. 2014;8:5–14.
19. Reynolds LP, Borowicz PP, Caton JS, Crouse MS, Dahlen CR, Ward AK. Developmental programming of fetal growth and development. Veterinary Clinics of North America: Food Animal Practice. 2019 Jul;35(2):229–47.
20. Laporta J, Ferreira FC, Ouellet V, Dado-Senn B, Almeida AK, De Vries A, et al. Late-gestation heat stress impairs daughter and granddaughter lifetime performance. J Dairy Sci. 2020 Aug;103(8):7555–68.
21. Meyer AM, Redifer CA. The curse of the firstborn: effects of dam primiparity on developmental programming in ruminant offspring. Anim Reprod Sci. 2024 Jun;265:107469.
22. Herdt TH. Ruminant adaptation to negative energy balance. Veterinary Clinics of North America: Food Animal Practice. 2000 Jul;16(2):215–30.
23. Steinhoff-Wagner J, Görs S, Junghans P, Bruckmaier RM, Kanitz E, Metges CC, et al. Maturation of endogenous glucose production in preterm and term calves. J Dairy Sci. 2011 Oct;94(10):5111–23.
24. Abuelo A, Hernández J, Benedito JL, Castillo C. Redox biology in transition periods of dairy cattle: role in the health of periparturient and neonatal animals. Antioxidants. 2019 Jan 13;8(1):20.
25. Mammi LME, Guadagnini M, Mechor G, Cainzos JM, Fusaro I, Palmonari A, et al. The use of monensin for ketosis prevention in dairy cows during the transition period: a systematic review. Animals. 2021 Jul 2;11(7):1988.
26. Vautier AN, Cadaret CN. Long-term consequences of adaptive fetal programming in ruminant livestock. Frontiers in Animal Science. 2022 Apr 25;3.
27. Laporta J, Ferreira FC, Ouellet V, Dado-Senn B, Almeida AK, De Vries A, et al. Late-gestation heat stress impairs daughter and granddaughter lifetime performance. J Dairy Sci. 2020 Aug;103(8):7555–68.
28. Trevisi E, Cattaneo L, Piccioli-Cappelli F, Mezzetti M, Minuti A. International symposium on ruminant physiology: the immunometabolism of transition dairy cows from dry-off to early lactation: lights and shadows. J Dairy Sci. 2025 Jan;
29. De Koster JD, Opsomer G. Insulin resistance in dairy cows. Veterinary Clinics of North America: Food Animal Practice. 2013 Jul;29(2):299–322.
30. Ling T, Hernandez-Jover M, Sordillo LM, Abuelo A. Maternal late-gestation metabolic stress is associated with changes in immune and metabolic responses of dairy calves. J Dairy Sci. 2018 Jul;101(7):6568–80.
31. Castillo C, Hernandez J, Bravo A, Lopez-Alonso M, Pereira V, Benedito JL. Oxidative status during late pregnancy and early lactation in dairy cows. The Veterinary Journal. 2005 Mar;169(2):286–92.

32. Sordillo LM, Aitken SL. Impact of oxidative stress on the health and immune function of dairy cattle. Vet Immunol Immunopathol. 2009 Mar;128(1–3):104–9.
33. Abuelo A, Hernández J, Benedito JL, Castillo C. Association of oxidative status and insulin sensitivity in periparturient dairy cattle: an observational study. J Anim Physiol Anim Nutr (Berl). 2016 Apr 14;100(2):279–86.
34. Sordillo LM, Raphael W. Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders. Veterinary Clinics of North America: Food Animal Practice. 2013 Jul;29(2):267–78.
35. Burton JL, Madsen SA, Chang LC, Weber PSD, Buckham KR, van Dorp R, et al. Gene expression signatures in neutrophils exposed to glucocorticoids: a new paradigm to help explain "neutrophil dysfunction" in parturient dairy cows. Vet Immunol Immunopathol. 2005 May;105(3–4):197–219.
36. Kimura K, Goff JP, Kehrli ME, Harp JA, Nonnecke BJ. Effects of mastectomy on composition of peripheral blood mononuclear cell populations in periparturient dairy cows. J Dairy Sci. 2002 Jun;85(6):1437–44.
37. Nonnecke BJ, Kimura K, Goff JP, Kehrli ME. Effects of the mammary gland on functional capacities of blood mononuclear leukocyte populations from periparturient cows. J Dairy Sci. 2003 Jul;86(7):2359–68.
38. Lee JY, Plakidas A, Lee WH, Heikkinen A, Chanmugam P, Bray G, et al. Differential modulation of toll-like receptors by fatty acids: preferential inhibition by n-3 polyunsaturated fatty acids. J Lipid Res. 2003 Mar;44(3):479–86.
39. de Heredia FP, Gómez-Martínez S, Marcos A. Obesity, inflammation and the immune system. Proceedings of the Nutrition Society. 2012 May 20;71(2):332–8.
40. Raphael W, Sordillo L. Dietary polyunsaturated fatty acids and inflammation: the role of phospholipid biosynthesis. Int J Mol Sci. 2013 Oct 22;14(10):21167–88.
41. Serhan CN. Systems approach to inflammation resolution: identification of novel anti-inflammatory and pro-resolving mediators. Journal of Thrombosis and Haemostasis. 2009 Jul;7:44–8.
42. Silvestre FT, Carvalho TSM, Crawford PC, Santos JEP, Staples CR, Jenkins T, et al. Effects of differential supplementation of fatty acids during the peripartum and breeding periods of Holstein cows: II. Neutrophil fatty acids and function, and acute phase proteins. J Dairy Sci. 2011 May;94(5):2285–301.
43. Contreras GA, Mattmiller SA, Raphael W, Gandy JC, Sordillo LM. Enhanced n-3 phospholipid content reduces inflammatory responses in bovine endothelial cells. J Dairy Sci. 2012 Dec;95(12):7137–50.
44. Bertoni G, Trevisi E, Han X, Bionaz M. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J Dairy Sci. 2008 Sep;91(9):3300–10.
45. Ohtsuka H, Koiwa M, Hatsugaya A, Kudo K, Hoshi F, Itoh N, et al. Relationship between serum TNF activity and insulin resistance in dairy cows affected with naturally occurring fatty liver. Journal of Veterinary Medical Science. 2001;63(9):1021–5.
46. Bradford BJ, Mamedova LK, Minton JE, Drouillard JS, Johnson BJ. Daily injection of tumor necrosis factor-α increases hepatic triglycerides and alters transcript abundance of metabolic genes in lactating dairy cattle. J Nutr. 2009 Aug;139(8): 1451–6.
47. O'Boyle NJ, Contreras GA, Mattmiller SA, Sordillo LM. Changes in glucose transporter expression in monocytes of periparturient dairy cows. J Dairy Sci. 2012 Oct; 95(10):5709–19.

48. Calder PC, Dimitriadis G, Newsholme P. Glucose metabolism in lymphoid and inflammatory cells and tissues. Curr Opin Clin Nutr Metab Care. 2007 Jul;10(4):531–40.
49. Hammon DS, Evjen IM, Dhiman TR, Goff JP, Walters JL. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet Immunol Immunopathol. 2006 Sep;113(1–2):21–9.
50. Suriyasathaporn W, Heuer C, Noordhuizen-Stassen EN, Schukken YH. Hyperketonemia and the impairment of udder defense: a review. Vet Res. 2000 Jul;31(4):397–412.
51. Farney JK, Mamedova LK, Coetzee JF, KuKanich B, Sordillo LM, Stoakes SK, et al. Anti-inflammatory salicylate treatment alters the metabolic adaptations to lactation in dairy cattle. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2013 Jul 15;305(2):R110–7.
52. Windeyer MC, Leslie KE, Godden SM, Hodgins DC, Lissemore KD, LeBlanc SJ. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. Prev Vet Med. 2014 Feb;113(2):231–40.
53. Lillycrop KA, Slater-Jefferies JL, Hanson MA, Godfrey KM, Jackson AA, Burdge GC. Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. British Journal of Nutrition. 2007 Jun 1; 97(6):1064–73.
54. Kwon H, Ford SP, Bazer FW, Spencer TE, Nathanielsz PW, Nijland MJ, et al. Maternal nutrient restriction reduces concentrations of amino acids and polyamines in ovine maternal and fetal plasma and fetal fluids. Biol Reprod. 2004 Sep 1;71(3):901–8.
55. Houin SS, Rozance PJ, Brown LD, Hay WW, Wilkening RB, Thorn SR. Coordinated changes in hepatic amino acid metabolism and endocrine signals support hepatic glucose production during fetal hypoglycemia. American Journal of Physiology-Endocrinology and Metabolism. 2015 Feb 15;308(4):E306–14.
56. Battaglia FC, Regnault TRH. Placental transport and metabolism of amino acids. Placenta. 2001 Feb;22(2–3):145–61.
57. Houin SS, Rozance PJ, Brown LD, Hay WW, Wilkening RB, Thorn SR. Coordinated changes in hepatic amino acid metabolism and endocrine signals support hepatic glucose production during fetal hypoglycemia. American Journal of Physiology-Endocrinology and Metabolism. 2015 Feb 15;308(4):E306–14.
58. Martin-Gronert MS, Ozanne SE. Experimental IUGR and later diabetes. J Intern Med. 2007 May 10;261(5):437–52.
59. Ma Y, Zhu MJ, Uthlaut AB, Nijland MJ, Nathanielsz PW, Hess BW, et al. Upregulation of growth signaling and nutrient transporters in cotyledons of early to mid-gestational nutrient restricted ewes☆. Placenta. 2011 Mar;32(3):255–63.
60. Ford SP, Hess BW, Schwope MM, Nijland MJ, Gilbert JS, Vonnahme KA, et al. Maternal undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring1. J Anim Sci. 2007 May 1;85(5):1285–94.
61. Duarte MS, Gionbelli MP, Paulino PVR, Serão NVL, Nascimento CS, Botelho ME, et al. Maternal overnutrition enhances mRNA expression of adipogenic markers and collagen deposition in skeletal muscle of beef cattle fetuses1. J Anim Sci. 2014 Sep 1;92(9):3846–54.

62. do Amaral BC, Connor EE, Tao S, Hayen MJ, Bubolz JW, Dahl GE. Heat stress abatement during the dry period influences metabolic gene expression and improves immune status in the transition period of dairy cows. J Dairy Sci. 2011 Jan;94(1):86–96.
63. Vidarsson G, Dekkers G, Rispens T. IgG Subclasses and allotypes: from structure to effector functions. Front Immunol. 2014 Oct 20;5.
64. Godden SM, Haines DM, Hagman D. Improving passive transfer of immunoglobulins in calves. I: Dose effect of feeding a commercial colostrum replacer. J Dairy Sci. 2009 Apr;92(4):1750–7.
65. Laporta J, Ferreira FC, Ouellet V, Dado-Senn B, Almeida AK, De Vries A, et al. Late-gestation heat stress impairs daughter and granddaughter lifetime performance. J Dairy Sci. 2020 Aug;103(8):7555–68.
66. Tao S, Monteiro APA, Thompson IM, Hayen MJ, Dahl GE. Effect of late-gestation maternal heat stress on growth and immune function of dairy calves. J Dairy Sci. 2012 Dec;95(12):7128–36.
67. Monteiro APA, Tao S, Thompson IM, Dahl GE. Effect of heat stress during late gestation on immune function and growth performance of calves: Isolation of altered colostral and calf factors. J Dairy Sci. 2014 Oct;97(10):6426–39.
68. Laporta J, Fabris TF, Skibiel AL, Powell JL, Hayen MJ, Horvath K, et al. In utero exposure to heat stress during late gestation has prolonged effects on the activity patterns and growth of dairy calves. J Dairy Sci. 2017 Apr;100(4):2976–84.
69. Monteiro APA, Guo JR, Weng XS, Ahmed BM, Hayen MJ, Dahl GE, et al. Effect of maternal heat stress during the dry period on growth and metabolism of calves. J Dairy Sci. 2016 May;99(5):3896–907.
70. Dado-Senn B, Laporta J, Dahl GE. Carry over effects of late-gestational heat stress on dairy cattle progeny. Theriogenology. 2020 Sep;154:17–23.
71. Reynolds LP, Ferrell CL, Robertson DA, Ford SP. Metabolism of the gravid uterus, foetus and utero-placenta at several stages of gestation in cows. J Agric Sci. 1986 Jun 27;106(3):437–44.
72. Lucy MC, Butler ST, Garverick HA. Endocrine and metabolic mechanisms linking postpartum glucose with early embryonic and foetal development in dairy cows. Animal. 2014;8:82–90.
73. Lucy MC, Green JC, Meyer JP, Williams AM, Newsom EM, Keisler DH. Short communication: glucose and fructose concentrations and expression of glucose transporters in 4- to 6-week pregnancies collected from Holstein cows that were either lactating or not lactating. J Dairy Sci. 2012 Sep;95(9):5095–101.
74. Wooding FBP, Fowden AL, Bell AW, Ehrhardt RA, Limesand SW, Hay WW. Localisation of glucose transport in the ruminant placenta: implications for sequential use of transporter isoforms. Placenta. 2005 Sep;26(8–9):626–40.
75. Regnault TRH, de Vrijer B, Galan HL, Davidsen ML, Trembler KA, Battaglia FC, et al. The relationship between transplacental O 2 diffusion and placental expression of PlGF, VEGF and their receptors in a placental insufficiency model of fetal growth restriction. J Physiol. 2003 Jul;550(2):641–56.
76. Thureen PJ, Trembler KA, Meschia G, Makowski EL, Wilkening RB. Placental glucose transport in heat-induced fetal growth retardation. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 1992 Sep 1;263(3):R578–85.

77. do Amaral BC, Connor EE, Tao S, Hayen J, Bubolz J, Dahl GE. Heat-stress abatement during the dry period: Does cooling improve transition into lactation? J Dairy Sci. 2009 Dec;92(12):5988–99.
78. Bazer FW, Spencer TE, Wu G, Cudd TA, Meininger CJ. Maternal nutrition and fetal development. J Nutr. 2004 Sep;134(9):2169–72.
79. Ahmed BMS, Younas U, Asar TO, Monteiro APA, Hayen MJ, Tao S, et al. Maternal heat stress reduces body and organ growth in calves: Relationship to immune status. JDS Communications. 2021 Sep;2(5):295–9.
80. Yin T, Halli K, König S. Direct genetic effects, maternal genetic effects, and maternal genetic sensitivity on prenatal heat stress for calf diseases and corresponding genomic loci in German Holsteins. J Dairy Sci. 2022 Aug;105(8):6795–808.
81. Ellett MD, Daniels KM, Hanigan MD, Corl BA, Perez-Hernandez G, Parsons CLM, et al. Tissue specific responses to oxidative fuel source preference during heat stress in lactating dairy cows. JDS Communications. 2024 Sep;
82. Monteiro APA, Tao S, Thompson IMT, Dahl GE. In utero heat stress decreases calf survival and performance through the first lactation. J Dairy Sci. 2016 Oct;99(10): 8443–50.
83. Lawrence P. Reynolds, Alison K. Ward, Joel S. Caton. Biology of Domestic Animals. 1st ed. Scanes CG, Hill RA, editors. Boca Raton, FL: CRC Press, 2017. | "A science publisher's book.": CRC Press; 2017. 82–119 p.
84. Skibiel AL, Peñagaricano F, Amorín R, Ahmed BM, Dahl GE, Laporta J. In utero heat stress alters the offspring epigenome. Sci Rep. 2018 Oct 2;8(1):14609.
85. Feil R, Fraga MF. Epigenetics and the environment: emerging patterns and implications. Nat Rev Genet. 2012 Feb 4;13(2):97–109.
86. Bestor TH, Edwards JR, Boulard M. Notes on the role of dynamic DNA methylation in mammalian development. Proceedings of the National Academy of Sciences. 2015 Jun 2;112(22):6796–9.
87. Edwards JR, O'Donnell AH, Rollins RA, Peckham HE, Lee C, Milekic MH, et al. Chromatin and sequence features that define the fine and gross structure of genomic methylation patterns. Genome Res. 2010 Jul;20(7):972–80.
88. Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, et al. Human DNA methylomes at base resolution show widespread epigenomic differences. Nature. 2009 Nov 19;462(7271):315–22.
89. Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, et al. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature. 1998 May;393(6683):386–9.
90. Jones PL, Veenstra GJC, Wade PA, Vermaak D, Kass SU, Landsberger N, et al. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat Genet. 1998 Jun;19(2):187–91.
91. Mee JF. State-of-the-art sensors to monitor/manage dairy calf birth and calf health. Large Animal Review. 2024;30(2):61–6.
92. Braun U, Tschoner T, Hässig M. Evaluation of eating and rumination behaviour using a noseband pressure sensor in cows during the peripartum period. BMC Vet Res. 2014 Dec 4;10(1):195.
93. Miller GA, Mitchell M, Barker ZE, Giebel K, Codling EA, Amory JR, et al. Using animal-mounted sensor technology and machine learning to predict time-to-calving in beef and dairy cows. Animal. 2020;14(6):1304–12.

94. Kaufman EI, LeBlanc SJ, McBride BW, Duffield TF, DeVries TJ. Association of rumination time with subclinical ketosis in transition dairy cows. J Dairy Sci. 2016 Jul;99(7):5604–18.
95. Cocco R, Canozzi MEA, Fischer V. Rumination time as an early predictor of metritis and subclinical ketosis in dairy cows at the beginning of lactation: systematic review-meta-analysis. Prev Vet Med. 2021 Apr;189:105309.
96. Thorup VM, Nielsen BL, Robert PE, Giger-Reverdin S, Konka J, Michie C, et al. Lameness affects cow feeding but not rumination behavior as characterized from sensor data. Front Vet Sci. 2016 May 10;3.
97. King MTM, Pajor EA, LeBlanc SJ, DeVries TJ. Associations of herd-level housing, management, and lameness prevalence with productivity and cow behavior in herds with automated milking systems. J Dairy Sci. 2016 Nov;99(11):9069–79.
98. Ceciliani F, Lecchi C, Urh C, Sauerwein H. Proteomics and metabolomics characterizing the pathophysiology of adaptive reactions to the metabolic challenges during the transition from late pregnancy to early lactation in dairy cows. J Proteomics. 2018 Apr;178:92–106.
99. Carvalho MR, Peñagaricano F, Santos JEP, DeVries TJ, McBride BW, Ribeiro ES. Long-term effects of postpartum clinical disease on milk production, reproduction, and culling of dairy cows. J Dairy Sci. 2019 Dec;102(12):11701–17.
100. Paudyal S, Maunsell FP, Richeson JT, Risco CA, Donovan DA, Pinedo PJ. Rumination time and monitoring of health disorders during early lactation. Animal. 2018; 12(7):1484–92.
101. Hadrich JC, Wolf CA, Lombard J, Dolak TM. Estimating milk yield and value losses from increased somatic cell count on US dairy farms. J Dairy Sci. 2018 Apr;101(4): 3588–96.
102. Kunes R, Bartos P, Iwasaka GK, Lang A, Hankovec T, Smutny L, et al. In-line technologies for the analysis of important milk parameters during the milking process: a review. Agriculture. 2021 Mar 12;11(3):239.
103. 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):1676–89.
104. De Jong E, Rijpert-Duvivier A, Veldman H, Steeneveld W, Jorritsma R. Milk β-hydroxybutyrate metrics and its consequences for surveillance of hyperketonaemia on commercial dairy farms. Front Vet Sci. 2023 Nov 8;10.
105. Klein R, Nagy O, Tóthová C, Chovanová F. Clinical and diagnostic significance of lactate dehydrogenase and its isoenzymes in animals. Vet Med Int. 2020 Jun 15;2020: 1–11.
106. King MTM, Duffield TF, DeVries TJ. Short communication: assessing the accuracy of inline milk fat-to-protein ratio data as an indicator of hyperketonemia in dairy cows in herds with automated milking systems. J Dairy Sci. 2019 Sep;102(9):8417–22.
107. Portnoy M, Barbano DM. Lactose: use, measurement, and expression of results. J Dairy Sci. 2021 Jul;104(7):8314–25.
108. Televičius M, Juozaitiene V, Malašauskienė D, Antanaitis R, Rutkauskas A, Urbutis M, et al. Inline milk lactose concentration as biomarker of the health status and reproductive success in dairy cows. Agriculture. 2021 Jan 8;11(1):38.
109. Duffield TF, Rabiee AR, Lean IJ. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 1. Metabolic Effects. J Dairy Sci. 2008 Apr;91(4):1334–46.

110. Rezaei Ahvanooei MR, Norouzian MA, Piray AH, Vahmani P, Ghaffari MH. Effects of monensin supplementation on lactation performance of dairy cows: a systematic review and dose–response meta analysis. Sci Rep. 2023 Jan 11;13(1):568.
111. McGuffey RK, Richardson LF, Wilkinson JID. Ionophores for dairy cattle: current status and future outlook. J Dairy Sci. 2001 Jun;84:E194–203.
112. Brito ESA, Andrade TG, Oliveira CHS de, Moura VMBD de. Outbreak of monensin poisoning in cattle due to supplementation error. Ciência Rural. 2020;50(11).
113. Raboisson D, Barbier M. Economic synergy between dry cow diet improvement and monensin bolus use to prevent subclinical ketosis: an experimental demonstration based on available literature. Front Vet Sci. 2017 Mar 14;4.
114. European Commission DG for H and FS. Kexxtone Article 130(4) procedure – the veterinary medicine Kexxtone suspended across the European Union. European Commission, Directorate-General for Health and Food Safety; May 15, 2024.
115. Carresi C, Marabelli R, Roncada P, Britti D. Is the use of monensin another Trojan Horse for the spread of antimicrobial resistance? Antibiotics. 2024 Jan 28;13(2):129.
116. Minor DJ, Trower SL, Strang BD, Shaver RD, Grummer RR. Effects of nonfiber carbohydrate and niacin on periparturient metabolic status and lactation of dairy cows. J Dairy Sci. 1998 Jan;81(1):189–200.
117. NRC. Nutrient requirements of dairy cattle. 8th ed. Washington, D.C.: National Academies Press; 2021.
118. Larsen T. Determination of lactate dehydrogenase (LDH) activity in milk by a fluorometric assay. Journal of Dairy Research. 2005 May 21;72(2):209–16.
119. Roche JR, Kay JK, Friggens NC, Loor JJ, Berry DP. Assessing and managing body condition score for the prevention of metabolic disease in dairy cows. Veterinary Clinics of North America: Food Animal Practice. 2013 Jul;29(2):323–36.
120. Godden SM, Lombard JE, Woolums AR. Colostrum management for dairy calves. Veterinary Clinics of North America: Food Animal Practice. 2019 Nov;35(3):535–56.
121. Martin CC, Baccili CC, Avila-Campos MJ, Hurley DJ, Gomes V. Effect of prophylactic use of tulathromycin on gut bacterial populations, inflammatory profile and diarrhea in newborn Holstein calves. Res Vet Sci. 2021 May;136:268–76.
122. Fleenor WA, Stott GH. Hydrometer test for estimation of immunoglobulin concentration in bovine colostrum. J Dairy Sci. 1980 Jun;63(6):973–7.
123. Bartier AL, Windeyer MC, Doepel L. Evaluation of on-farm tools for colostrum quality measurement. J Dairy Sci. 2015 Mar;98(3):1878–84.
124. Lemberskiy-Kuzin L, Lavie S, Katz G, Merin U, Leitner G. Determination of immunoglobulin levels in colostrum by using an online milk analyzer. Can J Anim Sci. 2019 Sep 1;99(3):631–3.
125. McGuirk SM. Disease management of dairy calves and heifers. Veterinary Clinics of North America: Food Animal Practice. 2008 Mar;24(1):139–53.
126. Maier GU, Love WJ, Karle BM, Dubrovsky SA, Williams DR, Champagne JD, et al. A novel risk assessment tool for bovine respiratory disease in preweaned dairy calves. J Dairy Sci. 2020 Oct;103(10):9301–17.
127. Renaud DL, Duffield TF, LeBlanc SJ, Kelton DF. Short communication: validation of methods for practically evaluating failed passive transfer of immunity in calves arriving at a veal facility. J Dairy Sci. 2018 Oct;101(10):9516–20.
128. French PD. Dry matter intake and blood parameters of nonlactating Holstein and Jersey cows in late gestation. J Dairy Sci. 2006 Mar;89(3):1057–61.

129. Hayirli A, Grummer RR, Nordheim EV, Crump PM. Models for predicting dry matter intake of Holsteins during the prefresh transition period. J Dairy Sci. 2003 May;86(5): 1771–9.
130. Grummer RR. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J Anim Sci. 1995;73(9):2820.
131. Santos MGS, Mion B, Ribeiro ES. Estimations of prepartum feed intake and its effects on transition metabolism and subsequent milk production. J Dairy Sci. 2024 Nov; 107(11):9786–802.
132. Sordillo LM, Contreras GA, Aitken SL. Metabolic factors affecting the inflammatory response of periparturient dairy cows. Anim Health Res Rev. 2009 Jun 29;10(1):53–63.
133. Grum DE, Drackley JK, Younker RS, LaCount DW, Veenhuizen JJ. Nutrition during the dry period and hepatic lipid metabolism of periparturient dairy cows. J Dairy Sci. 1996 Oct;79(10):1850–64.
134. Odegaard JI, Chawla A. Leukocyte set points in metabolic disease. F1000 Biol Rep. 2012 Jul 2;4.
135. Churakov M, Karlsson J, Edvardsson Rasmussen A, Holtenius K. Milk fatty acids as indicators of negative energy balance of dairy cows in early lactation. Animal. 2021 Jul;15(7):100253.
136. Ranga Niroshan Appuhamy JAD, Strathe AB, Jayasundara S, Wagner-Riddle C, Dijkstra J, France J, et al. Anti-methanogenic effects of monensin in dairy and beef cattle: a meta-analysis. J Dairy Sci. 2013 Aug;96(8):5161–73.
137. Ha S, Kang S, Jung M, Kim SB, Hwang S, Lee J, et al. Changes in haematological and serum biochemical parameter concentrations from the day of calving to ketosis onset in Holstein dairy cows during the postpartum period. Ir Vet J. 2025 Mar 19;78(1):8.
138. Rico JE, Bandaru VVR, Dorskind JM, Haughey NJ, McFadden JW. Plasma ceramides are elevated in overweight Holstein dairy cows experiencing greater lipolysis and insulin resistance during the transition from late pregnancy to early lactation. J Dairy Sci. 2015 Nov;98(11):7757–70.
139. Weber C, Hametner C, Tuchscherer A, Losand B, Kanitz E, Otten W, et al. Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows. J Dairy Sci. 2013 Jan;96(1):165–80.
140. Casaro S, Pérez-Báez J, Bisinotto RS, Chebel RC, Prim JG, Gonzalez TD, et al. Association between prepartum body condition score and prepartum and postpartum dry matter intake and energy balance in multiparous Holstein cows. J Dairy Sci. 2024 Jul;107(7):4381–93.
141. Caixeta LS, Omontese BO. Monitoring and improving the metabolic health of dairy cows during the transition period. Animals. 2021 Jan 31;11(2):352.
142. Richards BF, Vasquez JA, Perfield KL, Kvidera SK, Drackley JK. Rumen effects of monensin in dry cow diets varying in energy density. J Dairy Sci. 2022 Oct;105(10): 8008–15.
143. Khatun M, Thomson PC, García SC, Bruckmaier RM. Suitability of milk lactate dehydrogenase and serum albumin for pathogen-specific mastitis detection in automatic milking systems. J Dairy Sci. 2022 Mar;105(3):2558–71.

144. Hernández-Castellano L, Wall S, Stephan R, Corti S, Bruckmaier R. Milk somatic cell count, lactate dehydrogenase activity, and immunoglobulin G concentration associated with mastitis caused by different pathogens: a field study. Schweiz Arch Tierheilkd. 2017 May 5;159(5):283–90.
145. Dai L, Liu Z, Guo L, Chai Y, Yang Y, Wang Y, et al. Multi-tissue transcriptome study of innate immune gene expression profiling reveals negative energy balance altered the defense and promoted system inflammation of dairy cows. Vet Sci. 2023 Feb 1;10(2):107.
146. Moyes KM, Drackley JK, Morin DE, Rodriguez-Zas SL, Everts RE, Lewin HA, et al. Mammary gene expression profiles during an intramammary challenge reveal potential mechanisms linking negative energy balance with impaired immune response. Physiol Genomics. 2010 Apr;41(2):161–70.
147. Ha S, Kang S, Jung M, Kim SB, Hwang S, Lee J, et al. Changes in haematological and serum biochemical parameter concentrations from the day of calving to ketosis onset in Holstein dairy cows during the postpartum period. Ir Vet J. 2025 Mar 19;78(1):8.
148. Casaro S, Pérez-Báez J, Bisinotto RS, Chebel RC, Prim JG, Gonzalez TD, et al. Association between prepartum body condition score and prepartum and postpartum dry matter intake and energy balance in multiparous Holstein cows. J Dairy Sci. 2024 Jul;107(7):4381–93.
149. Noya A, Casasús I, Ferrer J, Sanz A. Long-term effects of maternal subnutrition in early pregnancy on cow-calf performance, immunological and physiological profiles during the next lactation. Animals. 2019 Nov 8;9(11):936.
150. Toni F, Vincenti L, Grigoletto L, Ricci A, Schukken YH. Early lactation ratio of fat and protein percentage in milk is associated with health, milk production, and survival. J Dairy Sci. 2011 Apr;94(4):1772–83.
151. Heuer C, Schukken YH, Dobbelaar P. Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds. J Dairy Sci. 1999 Feb;82(2):295–304.
152. Krpálková L, O'Mahony N, Carvalho A, Campbell S, Walsh J. Association of rumination with milk yield of early, mid and late lactation dairy cows. Czech Journal of Animal Science. 2022 Mar 15;67(3):87–101.
153. Zhao M, Bu D, Wang J, Zhou X, Zhu D, Zhang T, et al. Milk production and composition responds to dietary neutral detergent fiber and starch ratio in dairy cows. Animal Science Journal. 2016 Jun;87(6):756–66.
154. Andersen JB, Friggens NC, Sejrsen K, Sørensen MT, Munksgaard L, Ingvartsen KL. The effects of low *vs.* high concentrate level in the diet on performance in cows milked two or three times daily in early lactation. Livest Prod Sci. 2003 Jun;81(2–3):119–28.
155. Reist M, Erdin D, von Euw D, Tschuemperlin K, Leuenberger H, Chilliard Y, et al. Estimation of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. J Dairy Sci. 2002 Dec;85(12):3314–27.
156. Haile-Mariam M, Pryce JE. Genetic parameters for lactose and its correlation with other milk production traits and fitness traits in pasture-based production systems. J Dairy Sci. 2017 May;100(5):3754–66.
157. Kamal MM, Van Eetvelde M, Depreester E, Hostens M, Vandaele L, Opsomer G. Age at calving in heifers and level of milk production during gestation in cows are associated with the birth size of Holstein calves. J Dairy Sci. 2014 Sep;97(9):5448–58.

158. Lagranha C, Morel N, Costa-Junior LM, Klafke G, Gallina T, Reck J. Does parity order or cow antibody level influence the humoral immunity of calves for cattle anaplasmosis? Trop Anim Health Prod. 2024 Mar 5;56(2):67.
159. Mashimo R, Ohban H, Kumazaki Y, Ito S, Katagiri T, Kusaba N, et al. Dairy cow parity affects relationships among nutritional parameters in the blood of dams, umbilical cords, and calves and placental development at calving. Journal of Reproduction and Development. 2024;70(5):2024–010.
160. Curtis CR, Erb HN, Sniffen CJ, Smith RD, Kronfeld DS. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. J Dairy Sci. 1985 Sep;68(9):2347–60.
161. Baumrucker CR, Burkett AM, Magliaro-Macrina AL, Dechow CD. Colostrogenesis: mass transfer of immunoglobulin G1 into colostrum. J Dairy Sci. 2010 Jul;93(7): 3031–8.
162. Vasquez JA, McCarthy MM, Richards BF, Perfield KL, Carlson DB, Lock AL, et al. Effects of prepartum diets varying in dietary energy density and monensin on early-lactation performance in dairy cows. J Dairy Sci. 2021 Mar;104(3):2881–95.
163. Janovick NA, Dann HM, Loor JJ, Drackley JK. Prepartum dietary energy intake alters hepatic expression of genes related to peroxisome proliferator-activated receptor and inflammation in peripartal dairy cows. J Dairy Sci. 2022 Oct;105(10):8069–86.
164. Reynolds LP, Caton JS, Redmer DA, Grazul-Bilska AT, Vonnahme KA, Borowicz PP, et al. Evidence for altered placental blood flow and vascularity in compromised pregnancies. J Physiol. 2006 Apr 28;572(1):51–8.
165. Mann S, Sipka A, Leal Yepes FA, Nydam DV, Overton TR, Wakshlag JJ. Nutrient-sensing kinase signaling in bovine immune cells is altered during the postpartum nutrient deficit: A possible role in transition cow inflammatory response. J Dairy Sci. 2018 Oct;101(10):9360–70.
166. Zhang F, Li D, Wu Q, Sun J, Guan W, Hou Y, et al. Prepartum body conditions affect insulin signaling pathways in postpartum adipose tissues in transition dairy cows. J Anim Sci Biotechnol. 2019 Dec 15;10(1):38.
167. Leonardini A, Laviola L, Perrini S, Natalicchio A, Giorgino F. Cross-talk between PPAR γ and insulin signaling and modulation of insulin sensitivity. PPAR Res. 2009 Jan 23;2009(1).
168. Ji P, Osorio JS, Drackley JK, Loor JJ. Overfeeding a moderate energy diet prepartum does not impair bovine subcutaneous adipose tissue insulin signal transduction and induces marked changes in peripartal gene network expression. J Dairy Sci. 2012 Aug;95(8):4333–51.
169. Jaakson H, Karis P, Ling K, Ilves-Luht A, Samarütel J, Henno M, et al. Adipose tissue insulin receptor and glucose transporter 4 expression, and blood glucose and insulin responses during glucose tolerance tests in transition Holstein cows with different body condition. J Dairy Sci. 2018 Jan;101(1):752–66.
170. Zachut M, Honig H, Striem S, Zick Y, Boura-Halfon S, Moallem U. Periparturient dairy cows do not exhibit hepatic insulin resistance, yet adipose-specific insulin resistance occurs in cows prone to high weight loss. J Dairy Sci. 2013 Sep;96(9):5656–69.
171. Barletta RV, Maturana Filho M, Carvalho PD, Del Valle TA, Netto AS, Rennó FP, et al. Association of changes among body condition score during the transition period with NEFA and BHBA concentrations, milk production, fertility, and health of Holstein cows. Theriogenology. 2017 Dec;104:30–6.

172. Li P, Yin YL, Li D, Woo Kim S, Wu G. Amino acids and immune function. British Journal of Nutrition. 2007 Aug 1;98(2):237–52.
173. Coleman DN, Lopreiato V, Alharthi A, Loor JJ. Amino acids and the regulation of oxidative stress and immune function in dairy cattle. J Anim Sci. 2020 Aug 18; 98(Supplement_1):S175–93.
174. Jacometo CB, Alharthi AS, Zhou Z, Luchini D, Loor JJ. Maternal supply of methionine during late pregnancy is associated with changes in immune function and abundance of microRNA and mRNA in Holstein calf polymorphonuclear leukocytes. J Dairy Sci. 2018 Sep;101(9):8146–58.
175. Alharthi AS, Coleman DN, Liang Y, Batistel F, Elolimy AA, Yambao RC, et al. Hepatic 1-carbon metabolism enzyme activity, intermediate metabolites, and growth in neonatal Holstein dairy calves are altered by maternal supply of methionine during late pregnancy. J Dairy Sci. 2019 Nov;102(11):10291–303.
176. Alharthi AS, Lopreiato V, Dai H, Bucktrout R, Abdelmegeid M, Batistel F, et al. Short communication: supply of methionine during late pregnancy enhances whole-blood innate immune response of Holstein calves partly through changes in mRNA abundance in polymorphonuclear leukocytes. J Dairy Sci. 2019 Nov;102(11):10599–605.
177. Jacometo CB, Zhou Z, Luchini D, Trevisi E, Corrêa MN, Loor JJ. Maternal rumen-protected methionine supplementation and its effect on blood and liver biomarkers of energy metabolism, inflammation, and oxidative stress in neonatal Holstein calves. J Dairy Sci. 2016 Aug;99(8):6753–63.
178. Dänicke S, Kowalczyk J, Renner L, Pappritz J, Meyer U, Kramer R, et al. Effects of conjugated linoleic acids fed to dairy cows during early gestation on hematological, immunological, and metabolic characteristics of cows and their calves. J Dairy Sci. 2012 Jul;95(7):3938–53.
179. Collazos C, Lopera C, Santos JEP, Laporta J. Effects of the level and duration of maternal diets with negative dietary cation-anion differences prepartum on calf growth, immunity, and mineral and energy metabolism. J Dairy Sci. 2017 Dec;100(12):9835–50.
180. Cabezas-Garcia EH, Gordon AW, Mulligan FJ, Ferris CP. Revisiting the relationships between fat-to-protein ratio in milk and energy balance in Dairy Cows of different parities, and at different stages of lactation. Animals. 2021 Nov 14;11(11):3256.
181. Cripps RL, Green LR, Thompson J, Martin-Gronert MS, Monk M, Sheldon IM, et al. The effect of maternal body condition score before and during pregnancy on the glucose tolerance of adult sheep offspring. Reproductive Sciences. 2008 May 31; 15(5):448–56.
182. Wallace JM, Milne JS, Adam CL, Aitken RP. Adverse metabolic phenotype in low-birth-weight lambs and its modification by postnatal nutrition. British Journal of Nutrition. 2012 Feb 28;107(4):510–22.
183. Jolazadeh AR, Mohammadabadi T, Dehghan-banadaky M, Chaji M, Garcia M. Effect of supplementation fat during the last 3 weeks of uterine life and the preweaning period on performance, ruminal fermentation, blood metabolites, passive immunity and health of the newborn calf. British Journal of Nutrition. 2019 Dec 28;122(12): 1346–58.
184. Jolazadeh AR, Mohammadabadi T, Dehghan-banadaky M, Chaji M, Garcia M. Effect of supplementing calcium salts of n-3 and n-6 fatty acid to pregnant nonlactating cows on colostrum composition, milk yield, and reproductive performance of dairy cows. Anim Feed Sci Technol. 2019 Jan;247:127–40.

185. Woollett LA. Maternal cholesterol in fetal development: transport of cholesterol from the maternal to the fetal circulation. Am J Clin Nutr. 2005 Dec;82(6):1155–61.
186. Garcia M, Greco LF, Favoreto MG, Marsola RS, Wang D, Shin JH, et al. Effect of supplementing essential fatty acids to pregnant nonlactating Holstein cows and their preweaned calves on calf performance, immune response, and health. J Dairy Sci. 2014 Aug;97(8):5045–64.
187. Vedovatto M, Izquierdo V, Palmer E, Oliveira RA, Silva HM, Vendramini JMB, et al. Monensin supplementation during late gestation of beef cows alters maternal plasma concentrations of insulin-like growth factors 1 and 2 and enhances offspring preweaning growth. Transl Anim Sci. 2022 Jul 1;6(3).
188. Lemal P, May K, König S, Schroyen M, Gengler N. Invited review: from heat stress to disease – Immune response and candidate genes involved in cattle thermotolerance. J Dairy Sci. 2023 Jul;106(7):4471–88.
189. Lithuania Scientific Academy Award Commitee. Lithuanian Scientific Academy Lithuania Scientific award [Internet]. 2024 [cited 2025 Feb 23]. Available from: https://www.lma.lt/2024-m-lietuvos-mokslo-premijos-skirtos
190. Navakauskienė R, Navakauskas D, Borutinskaitė V, Matuzevičius D. Protein Phosphorylation in Leukemia. In: Epigenetics and Proteomics of Leukemia. Cham: Springer International Publishing; 2021. p. 165–94.
191. Navakauskienė R, Navakauskas D, Borutinskaitė V, Matuzevičius D. Epigenetic modifiers and their inhibitors in leukemia treatment. In: Epigenetics and Proteomics of Leukemia. Cham: Springer International Publishing; 2021. p. 25–66.
192. Borutinskaitė V, Žučenka A, Vitkevičienė A, Stoškus M, Kaupinis A, Valius M, et al. Genetic and epigenetic signatures in acute promyelocytic leukemia treatment and molecular remission. Front Genet. 2022 Apr 12;13.
193. Hare KS, Fischer-Tlustos AJ, Wood KM, Cant JP, Steele MA. Prepartum nutrient intake and colostrum yield and composition in ruminants. Animal Frontiers. 2023 Jun 14;13(3):24–36.
194. Farney JK, Mamedova LK, Coetzee JF, KuKanich B, Sordillo LM, Stoakes SK, et al. Anti-inflammatory salicylate treatment alters the metabolic adaptations to lactation in dairy cattle. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2013 Jul 15;305(2):R110–7.
195. Osorio JS, Ji P, Drackley JK, Luchini D, Loor JJ. Supplemental smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. J Dairy Sci. 2013 Oct;96(10):6248–63.
196. Vance DE, Walkey CJ, Cui Z. Phosphatidylethanolamine N-methyltransferase from liver. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism. 1997 Sep;1348(1–2):142–50.
197. Zhou Z, Vailati-Riboni M, Trevisi E, Drackley JK, Luchini DN, Loor JJ. Better postpartal performance in dairy cows supplemented with rumen-protected methionine compared with choline during the peripartal period. J Dairy Sci. 2016 Nov;99(11): 8716–32.
198. Batistel F, Arroyo JM, Bellingeri A, Wang L, Saremi B, Parys C, et al. Ethyl-cellulose rumen-protected methionine enhances performance during the periparturient period and early lactation in Holstein dairy cows. J Dairy Sci. 2017 Sep;100(9):7455–67.
199. Alharthi AS, Batistel F, Abdelmegeid MK, Lascano G, Parys C, Helmbrecht A, et al. Maternal supply of methionine during late-pregnancy enhances rate of Holstein calf development in utero and postnatal growth to a greater extent than colostrum source. J Anim Sci Biotechnol. 2018 Dec 23;9(1):83.

200. Dai H, Coleman DN, Hu L, Martinez-Cortés I, Wang M, Parys C, et al. Methionine and arginine supplementation alter inflammatory and oxidative stress responses during lipopolysaccharide challenge in bovine mammary epithelial cells in vitro. J Dairy Sci. 2020 Jan;103(1):676–89.
201. Osorio JS, Ji P, Drackley JK, Luchini D, Loor JJ. Supplemental Smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. J Dairy Sci. 2013 Oct;96(10):6248–63.
202. Tabor EM. Evaluating pre-natal nutritional and post-natal cooling management interventions for heat abatement on the immune function of pre-weaned dairy calves. [Doctoral dissertation]. [Wisconsin]: University of Wisconsin-Madison; 2024.
203. Wu G, Bazer FW, Davis TA, Kim SW, Li P, Marc Rhoads J, et al. Arginine metabolism and nutrition in growth, health and disease. Amino Acids. 2009 May 23;37(1): 153–68.
204. Lemons JA, Adcock EW, Jones MD, Naughton MA, Meschia G, Battaglia FC. Umbilical uptake of amino acids in the unstressed fetal lamb. Journal of Clinical Investigation. 1976 Dec 1;58(6):1428–34.
205. Yu T, Zhao Y, Shi W, Ma R, Yu L. Effects of maternal oral administration of monosodium glutamate at a late stage of pregnancy on developing mouse fetal brain. Brain Res. 1997 Feb;747(2):195–206.
206. Józwik M, Teng C, Battaglia FC, Meschia G. Fetal supply of amino acids and amino nitrogen after maternal infusion of amino acids in pregnant sheep. Am J Obstet Gynecol. 1999 Feb;180(2):447–53.
207. Putnam DE, Varga GA, Dann HM. Metabolic and production responses to dietary protein and exogenous somatotropin in late gestation dairy cows. J Dairy Sci. 1999 May;82(5):982–95.
208. Hartwell JR, Cecava MJ, Donkin SS. Impact of dietary rumen undegradable protein and rumen-protected choline on intake, peripartum liver triacylglyceride, plasma metabolites and milk production in transition dairy cows. J Dairy Sci. 2000 Dec;83(12):2907–17.
209. Hayirli A, Grummer RR, Nordheim EV, Crump PM. Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. J Dairy Sci. 2002 Dec;85(12):3430–43.
210. Larsen M, Kristensen NB. Precursors for liver gluconeogenesis in periparturient dairy cows. Animal. 2013;7(10):1640–50.
211. Dalbach KF, Larsen M, Raun BML, Kristensen NB. Effects of supplementation with 2-hydroxy-4-(methylthio)-butanoic acid isopropyl ester on splanchnic amino acid metabolism and essential amino acid mobilization in postpartum transition Holstein cows. J Dairy Sci. 2011 Aug;94(8):3913–27.
212. Patra AK. Urea/ammonia metabolism in the rumen and toxicity in ruminants. In: Rumen Microbiology: From Evolution to Revolution. New Delhi: Springer India; 2015. p. 329–41.
213. Dieho K, Bannink A, Geurts IAL, Schonewille JT, Gort G, Dijkstra J. Morphological adaptation of rumen papillae during the dry period and early lactation as affected by rate of increase of concentrate allowance. J Dairy Sci. 2016 Mar;99(3):2339–52.
214. Wei Z, Xie X, Xue M, Valencak TG, Liu J, Sun H. The effects of non-fiber carbohydrate content and forage type on rumen microbiome of dairy cows. Animals. 2021 Dec 10;11(12):3519.

215. Rabelo E, Rezende RL, Bertics SJ, Grummer RR. Effects of transition diets varying in dietary energy density on lactation performance and ruminal parameters of dairy cows. J Dairy Sci. 2003 Mar;86(3):916–25.
216. Soufleri A, Banos G, Panousis N, Fletouris D, Arsenos G, Kougioumtzis A, et al. Evaluation of factors affecting colostrum quality and quantity in Holstein dairy cattle. Animals. 2021 Jul 5;11(7):2005.
217. Mann S, Leal Yepes FA, Overton TR, Lock AL, Lamb SV, Wakshlag JJ, et al. Effect of dry period dietary energy level in dairy cattle on volume, concentrations of immunoglobulin G, insulin, and fatty acid composition of colostrum. J Dairy Sci. 2016 Feb;99(2):1515–26.
218. Morin DE, Nelson SV, Reid ED, Nagy DW, Dahl GE, Constable PD. Effect of colostral volume, interval between calving and first milking, and photoperiod on colostral IgG concentrations in dairy cows. J Am Vet Med Assoc. 2010 Aug 15;237(4):420–8.
219. Valente TNP, Sampaio CB, Lima E da S, Deminicis BB, Cezário AS, Santos WBR dos. Aspects of acidosis in ruminants with a focus on nutrition: a review. Journal of Agricultural Science. 2017 Feb 13;9(3):90.
220. Emmanuel DGV, Dunn SM, Ametaj BN. Feeding high proportions of barley grain stimulates an inflammatory response in dairy cows. J Dairy Sci. 2008 Feb;91(2): 606–14.
221. Gott PN, Hogan JS, Weiss WP. Effects of various starch feeding regimens on responses of dairy cows to intramammary lipopolysaccharide infusion. J Dairy Sci. 2015 Mar;98(3):1786–96.
222. Andersen JB, Ridder C, Larsen T. Priming the cow for mobilization in the periparturient period: effects of supplementing the dry cow with saturated fat or linseed. J Dairy Sci. 2008 Mar;91(3):1029–43.
223. Mashek DG, Grummer RR. Effects of long chain fatty acids on lipid and glucose metabolism in monolayer cultures of bovine hepatocytes. J Dairy Sci. 2003 Jul;86(7):2390–6.
224. Mashek DG, Bertics SJ, Grummer RR. Effects of intravenous triacylglycerol emulsions on hepatic metabolism and blood metabolites in fasted dairy cows. J Dairy Sci. 2005 Jan;88(1):100–9.
225. Kerner J, Hoppel C. Fatty acid import into mitochondria. Biochimica et Biophysica Acta (BBA) – Molecular and Cell Biology of Lipids. 2000 Jun;1486(1):1–17.
226. Hagemeister H, Franzen M, Barth CA, Precht D. α-linolenic acid transfer into milk fat and its elongation by cows. Lipid / Fett. 1991 Jan 24;93(10):387–91.
227. Karimian M, Khorvash M, Forouzmand MA, Alikhani M, Rahmani HR, Ghaffari MH, et al. Effect of prepartal and postpartal dietary fat level on performance and plasma concentration of metabolites in transition dairy cows. J Dairy Sci. 2015 Jan; 98(1):330–7.
228. Relling AE, Pate JL, Reynolds CK, Loerch SC. Effect of feed restriction and supplemental dietary fat on gut peptide and hypothalamic neuropeptide messenger ribonucleic acid concentrations in growing wethers1. J Anim Sci. 2010 Feb 1;88(2):737–48.

LIST OF PUBLICATIONS

The PhD thesis is based on these publications:

1. **Urbutis, Mingaudas**; Juozaitienė, Vida; Palubinskas, Giedrius; Džermeikaitė, Karina; Bačėninaitė, Dovilė; Bilskis, R; Baumgartner, W; Antanaitis, Ramūnas. Effect of controlled-release monensin on automatically registered body condition score, milk β-hydroxybutyrate, milk yield and milk lactate dehydrogenase in fresh dairy cows // Polish Journal of Veterinary Sciences. Warszawa : Polska Akad Nauk, Polish Acad Sciences, 2022, vol. 25, no. 4, p. 607–615; doi:10.24425/pjvs.2022.143548; [IF:0,8; 2022]
2. **Urbutis, Mingaudas**; Malašauskienė, Dovilė; Televičius, Mindaugas; Juozaitienė, Vida; Baumgartner, Walter; Antanaitis, Ramūnas. Evaluation of the Metabolic Relationship between Cows and Calves by Monitoring Calf Health and Cow Automatic Milking System and Metabolic Parameters // Animals, 2023, t. 13, nr. 16, p. 1–15, doi:10.3390/ani13162576;[IF: 3,0; 2023]

The following abstracts from international and national conferences were used for the thesis:

1. **Urbutis, Mingaudas**; Juozaitienė, Vida; Palubinskas, Giedrius; Džermeikaitė, Karina; Bačėninaitė, Dovilė; Bilskis, Ronaldas; Baumgartner, Walter; Antanaitis, Ramūnas. Controlled release monensin effect on automatically registered parameters of body condition score and in-line milk parameters // XXII Middle-European Buiatric Congress Stara Zagora : Congress Proceedings : Stara Zagora, [31.05–03.06], 2023 / Trakia University. Faculty of Veterinary Medicine. Stara Zagora : Trakia University, 2023, p. 71-71.
2. **Urbutis, Mingaudas**; Antanaitis, Ramūnas; Malašauskienė, Dovilė; Televičius, Mindaugas; Juozaitienė, Vida. Ryšio tarp karvės metabolizmo, laktacijos skaičiaus ir jos veršelio sveikatingumo įvertinimas pasitelkiant biocheminius kraujo ir automatiškai matuojamus pieno kokybės rodiklius // 11-osios jaunųjų mokslininkų konferencijos Jaunieji mokslininkai – žemės ūkio pažangai : pranešimų tezės : [2022 m. lapkričio 10 d.] / Lietuvos mokslų akademijos Žemės ūkio ir miškų mokslų skyrius; [Leidinį sudarė Reda Daukšienė]. Vilnius : Lietuvos mokslų akademija, 2022.

3. **Urbutis Mingaudas,** Malašauskienė, D., Televičius, M., Juozaitienė, V., Baumgartner, W. and Antanaitis, R. (2024). Evaluation of the metabolic relationship between cows and calves by monitoring calf health and cow automating milking system and metabolic parameters. XXIII Middle European Buiatrics Congress – Proceedings Book : April 23rd–27th 2024, Brno, Czech Republic Illek Josef Et Al., 59–59. https://hdl.handle.net/20.500.12512/244298 [T1e] [M.kr.: A002]

CURRICULUM VITAE

Name, Surname: **Mingaudas Urbutis**
Address: Lithuanian University of Health Sciences, Veterinary Academy, Faculty of Veterinary Medicine, Large Animal Clinics,
Tilžės 18, LT-47181 Kaunas, Lithuania
E-mail: mingaudas.urbutis@lsmuni.lt

Education:

2018 Lithuania University of Health Sciences Veterinary Academy Faculty of Veterinary. Master's thesis on the topic of "Changes in the blood morphological, biochemical and blood gas parameters and their clinical value during the first days in neonate calves". Thesis supervisor prof. Dr. Ramūnas Antanaitis.

2018–2020 Residency of Cattle internal diseases and therapy in Large Animal Clinics of Lithuania University of Health Sciences, Veterinary Academy. Supervisor: prof. Dr. Ramūnas Antanaitis

2020–2024 PhD studies on the topic of "Impact of prepartum cow negative energy balance and its prophylaxis on metabolic and health parameters of cows and their calves". Supervisor prof. Dr. Ramūnas Antanaitis.

ACKNOWLEDGEMENTS

I would like to extend my gratitude to prof. Dr. Ramūnas Antanaitis and my colleagues from the cattle section for all of the support, suggestions and valuable time spent to achieve this result.

I am very thankful for prof. Dr. Walter Baumgartner and his continuous support and valuable knowledge and insight. Your lessons on how to conduct myself, reach one's goals and value others will lead me in my path forward.

I would like to extend my gratitude towards prof. Vytuolis Žilaitis who provided comments and suggestions for the thesis but most of all provided such needed reassurance.

I am also very fortunate to work under our head of clinics doc. Arūnas Rutkauskas – your support and calmness are qualities that were a reasurance in this phase of my career. I only hope that I will manage to put the lessons and experience gained to good use in furthering the interests of the Large animal clinics and our Veterinary academy.

Doc. Sigitas Japertas believed in me, reminded what I am capable of and encouraged me to achieve even more. And that is the best thing a person can hope for in a difficult moment, for which I am truly grateful.

I am forever grateful for my beloved wife Paulina, who probably worried about my work even more than me, but masterfully managed not to reveal it. A gentle touch, a calm word, little acts of kindness and even more of your actions probably unnoticed by me, were the pillars on which I was able to lean during this journey. My wish is that I could return the favor or do even more for you in your time of need.

This step in my journey has shown me how small and seemingly insignificant acts or words of someone can have a tremendous impact on another person's life. A kind word or a hand placed on a shoulder could be all someone besides you needs. I could fill pages on pages of these small gestures with the amount of support I have received and extend my gratitude, but I would rather go out into the world and return the favor by passing this kindness on. I leave these words here for myself as well – don't I dare forget them in the upcoming future.