ESTONIAN UNIVERSITY OF LIFE SCIENCES
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THE EFFECT OF MONENSIN ON THE METABOLISM OF TRANSITION DAIRY COW

MONENSIINI MÕJU PIIMALEHMA AINEVAHETUSELE ÜLEMINEKUPERIOODIL

Final Thesis
Curriculum in Veterinary Medicine

Supervisor: Priit Karis, *PhD*

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Abstract of Final Thesis

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The aim of this final thesis was to find out if monensin influences metabolites non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), aspartate aminotransferase (AST) and glucose dynamics during transition period. Research was done on a commercial Estonian dairy farm. There were 206 cows in the experiment. Randomly selected cows belonged either Control-group (no monensin) or cows were administered continuous release monensin capsule about three weeks before expected parturition (Kex-group). Blood samples were taken from both groups from the day of enrolment till 6 weeks after parturition. Due to the limitations of the testing software, there was 81 randomly selected cows in both groups. Blood samples were analyzed for BHB, NEFA, AST and glucose values. Cow was considered to be hyperketonemic if the BHB concentration was ≥1.2 mmol/L at least once during 6 weeks after parturition. Cows with NEFA concentration ≥0.7 mmol/L at least once during 6 weeks after parturition, were considered having elevated concentrations of NEFA. Results show that monensin did lower the BHB and NEFA values but did not have significant effect on AST and glucose. In addition, monensin lowered the incidence of ketosis as well as the incidence of elevated NEFA.

Keywords: monensin, ketosis, β-hydroxybutyrate, non-esterified fatty acids

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Käesoleva lõputöö eesmärk oli välja selgitada monensiini mõju metaboliitide esterdamata rasvhappeid (NEFA), β-hüdroksübutüraadi (BHB), aspartaataminotransferaasi (AST) ja glükoosi konvtratsioonile üleminekuperioodil. Uuringud tehti Eesti tootmisfarmis. Katses osales 206 lehma. Juhuslikult jagati lehmad kas kontrollrühma (ilma monensiinita) või katserühma. Katserühma lehmadele manustati lehmadele umbes kolm nädalat enne eeldatavat poegimist monensiini pidevalt vabastav vatsabool. Vereproovid võeti mõlemas rühma loomadelt alates katsesse võtmisest kuni kuuenda poegimisjärgse nädalani. Statistilise analüüsi programmi piiratuse tõttu võrdsustati rühma loomade arv 81 juhuslikult valitud lehmani. Vereproovideste analüüsiti BHB, NEFA, AST ja glükoosi väärtusted. Lehma loeti ketoosis olevaks kui BHB kontsentratsioon oli ≥1,2 mmol/L vähemalt korra 6 nädala jooksul pärast poegimist. Lehmadel, kelle NEFA kontsentratsioon oli ≥0,7 mmol/L vähemalt üks kord 6 nädala jooksul pärast poegimist, loeti kõrge NEFA kontsentratsiooniga lehmaks. Tulemused näitavad, et monensiin alandas BHB ja NEFA väärtusi, kuid ei avaldanud olulist mõju AST-le ja glükoosile. Samuti vähendas monensiin ketoosi ja kõrge NEFA-ga loomade arvu.

Märksõnad: monensiin, ketoos, β-hüdroksübutüraat, esterdamata rasvhapped

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LIST OF ABBREVIATIONS

ACAC	Acetoacetate
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BHB	β-hydroxybutyrate
DMI	Dry matter intake
NEFA	Non esterified fatty acids
SARA	Subacute rumen acidosis
VFA	Volatile fatty acids
VLDL	Very-low-density-lipoproteins

INTRODUCTION

Ruminant digestive tract and metabolism has their own peculiarities, that needs to be taken into consideration during the transition period of a dairy cow. Ruminants have four stomachs, from which the rumen is the important fermentation vat where the ruminal microbiota live. Ruminants eat their feed fast and spend a lot of time ruminating to produce adequate amount of saliva to buffer the organic acids produced by fermentation. Roughage based feed needs an effective way to utilize the energy. Ruminal microbiota is responsible for that. Microorganisms provide adenosine triphosphate for energy to themselves by fermenting carbohydrates in anaerobic conditions. Microorganisms excrete the energy in the form of volatile fatty acids (VFA). These VFA (acetate, propionate and butyrate) are the precursors for the gluconeogenesis in the liver and the main sources for digestible energy for the cow. From these VFA propionate is glucogenic and acetate and butyrate are ketogenic. If these are produced in relative excess, they are further metabolized to ketone bodies (acetone, acetoacetate and β-hydroxybutyrate).

During the transition period dairy cow has first suffered from decreased appetite and then after parturition it needs to be able to consume large quantities of feed to meet the demands for the beginning of the lactation. This leads to physiologic lipolytic state and rapid lipid mobilization which results in the increase of the amount of non-esterified fatty acids (NEFA) in blood. In normal conditions NEFA would be re-esterified into triglycerides. But in case of negative energy balance, liver is not able to re-esterify these and excess NEFA are metabolized to ketone bodies.

Changes in feeding and metabolism can lead to different kind of health problems like rumen acidosis, abomasal displacement and ketosis. Ketosis is a very common disease in high yielding dairy cow. Especially when the cow has high body condition score at the time of parturition. Monensin could be one option to prevent ketosis after parturition as it has properties that can alter the ruminal microbiota to be preferable for propionate producing bacteria, instead of bacteria that produces acetate and butyrate.

This final thesis is a controlled trial to find out effects of monensin to β-hydroxybutyrate (BHB), NEFA, aspartate aminotransferase (AST) and glucose concentrations of a modern dairy cows. Topic is important, because ketosis affects greatly to cows' performance, and can be problem in many high yielding dairy cows. It is also very costly for the farmer, according to Cainzos *et al.* (2022) one incidence of ketosis can cost up to €812, so preventing ketosis can have big

financial impact. According to the study of Loiklung *et al*. (2022) prevalence of subclinical ketosis in dairy cows globally was 22,7% (95% CI 21,2-24,3%). There was no significant difference between different cut off values (BHB ≥1,0, ≥1,2 and ≥1,4 mmol/L), sample types (milk and blood), parity, diagnostic techniques or geographical regions. That study also observed that prevalence in Holsteins (19,8%) was significantly lower than in other breeds (23,7 %).

Effects of monensin have been studied in several trials around the world, but in less extent in European countries. This thesis consists of a brief literature review about metabolism and common metabolic diseases of a dairy cow, and monensin. Data was collected from commercial Estonian dairy farm during years 2016-2018. Analysis of variance and dynamics were used to find out if there was a significant positive effect in using monensin. Hypothesis is that monensin improves energy metabolism during early lactation.

1 LITERATURE REVIEW

Modern dairy cows are expected to have high milk yield and good health. This is a very difficult equation to solve due to the limitations of cows' metabolism and capability to utilize high grain rations. During transition period, defined as a period three weeks before and after calving, dairy cow is preparing for the next lactation by several changes in their energy metabolism. High post-partum demand of energy for milk production brings along a negative energy balance that may lead to increased production of ketone bodies to the blood and as a result subclinical or clinical ketosis. Subclinical ketosis is defined as β-hydroxybutyrate (BHB) concentration in blood above 1,2 mmol/L and clinical ketosis occurs when there are clinical signs, such as inappetence, decrease in the rumen movements, decrease in milk production, depression, weight loss, acetone smell in breathing and mucus covered stool, appear (Issi *et al*., 2016).

Monensin is an ionophore antibiotic that alters the microbiota of the rumen so that the propionate-producing Gram-negative bacteria will overdrive the butyrate and acetate producing Gram-positive bacteria and therefore reduce the incidence of ketosis. Monensin disrupts the ion balance of the bacterial cell wall and inhibits the bacteria (Russell and Strobel, 1989).

1.1 Metabolism of a dairy cow

1.1.1 Ruminant digestion

Webster (2020) describes that ruminant digestive tract differs greatly from that of non-ruminant animals. The roughage-based diet needs a different approach to utilize all nutrients needed for the energy production than in non-ruminant animals. Ruminants have four stomachs (rumen, reticulum, omasum and abomasum. Rumen and reticulum act as reservoir for feed, saliva and other secretions, that are mixed and fermented by micro-organisms. The main role of omasum is to absorb water from the ingesta. Abomasum is somewhat like non-ruminant stomach and has two distinct sections: the fundus, where hydrochloric acid and enzymes are secreted, and pylorus, which collects ingesta before it is passed to the duodenum. Ingesta is neutralized and further degraded by enzymes in the duodenum and passed to the jejunum and ileum, where the absorption of nutrients majorly takes place. Large intestine contains cecum and colon and acts as secondary site for microbial fermentation, absorption of water and mineral salt.

1.1.2 Eating and rumination

When ruminants eat, they ingest feed as quickly as possible and later spend a lot of time to ruminate. Cattle are not selective for what they eat, although they may have preferences for certain kind of feeds. Type of feed also affects the chewing and salivation, roughage containing

long fibers is chewed much longer time than cereals and pelleted feed. During rumination cow can produce saliva 150 ml/min, and per day it makes 140 liters (Webster, 2020).

Rumination is crucial part of the digestion in ruminants. During rumination animal produces saliva which dilutes the rumen contents and mechanical breakdown of the feed particles makes feed easier to pass further to abomasum. Saliva contains sodium bicarbonate that buffers the pH of rumen by neutralizing the organic acids that fermentation has produced. Animal needs to ruminate adequate time to produce enough saliva for maintaining ideal pH (6-7) and healthy digestion. Type of feed affects a great deal the rumination time. There comes the difficulty in feeding, concerning of the high yield dairy cow, especially during the transition period. Webster (2020) says that cereal-based feed is rapidly fermented and contains little material to stimulate rumination and salivation, rumination time may be only few hours. In comparison, a cow fed with long hay can ruminate 8-10 hours per day. At dry period, cow has been fed with roughage-based feed and rumination and digestion are adequate. When changing to the high cereal diet, rumination diminishes, rumen contents are fermented fast, and pH lowers, and ruminal microbiota are changed. The balance between the roughage and grains is crucial to keep up the adequate rumination. Grains are used in ration to increase the energy density of the feed and daily intake of dry matter during the increased energy demand of the milking cow. Grains do not have enough fiber to maintain healthy rumen, and lack of fiber may lead to diminished rumination.

1.1.3 Fermentation of carbohydrates in the rumen

As cattle diet consists of lot of roughage, there needs to be effective way to utilize the nutrients and energy from it. In the rumen, bacteria, ciliated protozoa and anaerobic fungi are responsible of doing that. It is said, that feeding the cow is feeding the rumen microbiota. Microorganisms provide adenosine triphosphate (ATP) for fuel to themselves by fermentation of the carbohydrates in the absence of oxygen. Sugars and starches are fermented rapidly, whereas cellulose and hemicellulose are fermented slowly (Webster, 2020).

The ability of the microorganisms to get energy in the form of ATP from anaerobic metabolism is low, hence most of the substrate energy persists and is excreted by the microorganism in the form of volatile fatty acids (VFA). These VFA (acetic, propionic and butyric acid) are the main source of digestible energy for the cow (Webster, 2020) and propionate is the most important precursors for gluconeogenesis in the liver (De Koster and Opsomer, 2013). VFA are in the form of sodium salts in the rumen, and they are absorbed across the rumen wall by the rumen

papillae. Normal function and structure of the rumen papillae are controlled by the rate of production of VFA, especially acetic acid (acetate). The ratio of propionate to acetate is dependent on the balance between forage and cereals. Propionate is glucogenic and acetate and butyrate are ketogenic and if butyrate is produced in relative excess, it is metabolized to ketone bodies.

1.1.4 Dietary protein and fat

Cows get amino acids from the microorganisms that are digested in the abomasum and duodenum. Microbial protein synthesis requires nitrogen, and dietary proteins are one source of it. Rumen microbiota do not necessarily need amino acids from true proteins as dietary proteins, but they can use more simpler nitrogenous compounds like ammonia. Also, urea is one important substrate for microbial protein synthesis (Webster, 2020).

Fermentation rate of the carbohydrates is closely linked to the microbial protein synthesis rate. From one kilo of fermented organic matter (mainly carbohydrates) cow can get up to 50 g of microbial protein nitrogen. Cow cannot utilize all microbial protein produced in the rumen, only the portion that is passed into the duodenum. On the other hand, ingested carbohydrates and VFA produced from them, are readily available and absorbed directly across the rumen wall to be used as energy. To sustain high milk yields in dairy cows, it is important to keep supply of quickly fermented energy and quickly degradable nitrogen in balance in the diet (Webster, 2020).

According to the Webster (2020) yielding ATP from fats in the anaerobic conditions in rumen is impossible. Fats can even inhibit the fermentation process, slow down the digestion and reduce appetite. Digestion of fats takes place post-ruminally with the action of lipase from the pancreas in the duodenum. If fats are used for energy supply, the fat needs to be protected from microbial action by coating it. In the ruminant diet, fats can be used in small extent as energy source, but fats do not work as energy supply for microbes.

1.2 Changes of metabolism during transition period

During the transition period, from late pregnancy to the onset of lactation, dairy cow undergoes changes in their energy metabolism. Appetite is suppressed during the last week of gestation and after calving the dry matter intake (DMI) recovery may take a week (Sordillo and Raphael, 2013). Cows are in lipolytic state during the periparturient period (Allen and Piantoni, 2013). Rapid mobilization of lipids and proteins begins to ensure the high milk production. Amount of non-esterified fatty acids (NEFA) increase in the blood due to decrease in lipogenesis and

increase in hormone-sensitive lipase activity. Also, insulin secretion decreases prior to lactation. Dairy cows do not need insulin for glucose uptake into mammary gland and lowering the insulin production results in bigger amounts of glucose available to for use of udder for lactose production and less is used in peripheral tissues (McArt *et al*., 2018).

In normal energy equilibrium cow will re-esterify the NEFAs in the liver and they are re-secreted as very-low-density lipoproteins (VLDL). In case of energy deficit liver is not able to esterify these excess NEFA, and they are therefore oxidized to ketone bodies. In dairy cows, negative energy balance occurs during transition period due to increased need of energy for milk production and reduced dry matter intake (DMI). Nutrient intake decreases during the last weeks of gestation due to the decreased rumen capacity as growing uterus occupies caudal abdominal cavity (McArt *et al*., 2018).

High yielding cow requires several times more metabolizable energy than non-pregnant or dry cow. One problem arises from the fact that the capacity to eat is restricted by the volume of the upper digestive tract, the rumen. Intake depends on the rate of feed leaving the rumen towards the rest of the digestive tract. Cow needs much more energy and nutrients to fulfill the needed milk production than it can eat. Cow needs highly digestible diet containing lot of starch, but this leads to too rapid fermentation and possibly to rumen acidosis (Webster, 2020).

Parturition and beginning of lactation disturb also mineral balance, especially calcium. Most of the body calcium is stored in the skeletal bones and only small part in the exchangeable pool. In a dry cow, need of calcium is low and therefore also the absorption is low and most of the circulating calcium is returned to the gut and small amount is excreted into the urine. After parturition, cow starts to excrete large amounts of calcium to the milk, and the balance in the body is disturbed. This sudden unbalance and depletion of calcium from exchangeable pool leads to the low calcium levels in the blood and into condition called parturient paresis (Webster, 2020).

1.3 Metabolic diseases and disorders of a dairy cow

1.3.1 Rumen acidosis

When the production of VFA exceeds their absorption rate, the rumen acidity will rise, which means that the pH will fall. Small fall (pH 5.5) will decrease the appetite and reduce the fibre digestion and increase the feed retention time in the rumen. Acetate to propionate ratio decreases and leads to lower milk fat synthesis. Cow is in the condition of subacute rumen acidosis (SARA) where rumen pH is 5,2-5,8. Acute rumen acidosis is when rumen pH has fallen

under 5,2. There is significant destruction of rumen microbiota and acidosis becomes systemic. Rumen wall is destructed, and endotoxins are released to the bloodstream. Acute acidosis is quite rare in dairy cows, but possible if cow gets to for example grain storage and eats grains in excess (Webster, 2020).

SARA is more frequent in dairy cows after parturition, within first weeks of lactation, especially in cows with high body condition score at the time of parturition. In fat cows the appetite is generally low and after parturition they should be able to eat large amounts of feed to fulfil the demands of high milk yield. After parturition the ratio of starch to fibre is much higher than it was before parturition and this is challenging to the rumen, especially if dry matter intake (DMI) has been low before parturition. When the cow is suffering of reduced appetite, the metabolic demand for lactation increases the mobilisation of body fats and the risk of ketosis increases and may even increase the severity of systemic acidosis (Webster, 2020).

1.3.2 Abomasal disorders

Abomasum is the place where microbial protein is digested with acids. In case of incomplete carbohydrate fermentation in rumen, the partially fermented starches go to the abomasum and the pH of the abomasum rises. The rise in pH prevents the acid digestion of the microbiota and the fermentation of the starches continue in the abomasum. Fermentation forms gas inside the abomasum, and as result, abomasum will distend and becomes atonic. After parturition, there is suddenly more space in the abdominal cavity and distended abomasum may be displaced from its original position either on the right side of the rumen, or it can get trapped on the left side of the rumen (Webster, 2020).

1.3.3 Ketosis

According to Opsomer (2015) “Ketosis is caused by an insufficient supply of blood glucose to support metabolic needs associated with the sudden onset of high milk secretion after calving when feed intake is not in accordance with production.”

Ketosis is a condition associated with negative energy balance and where ketone bodies (β-hydroxybutyrate, acetoacetate and acetone) are produced from excess NEFA and released to the blood stream. The concentration of circulating NEFA reflects the mobilization of fat from storages and therefore it indirectly mirrors DMI. The level of β-hydroxybutyrate (BHB) reflects how completely fat is oxidized in the liver (Opsomer, 2015). Small increase in blood ketone bodies is normal after calving, because lipid mobilization and NEFA oxidation is physiologic response to changes in insulin sensitivity and plasma insulin concentration at the time of

parturition (Allen and Piantoni, 2013). In the dairy cow, most of the glucose is produced in the liver, and it originates mainly from propionic acid, which is produced by the rumen microflora. When the blood glucose levels decrease, it induces a decline in plasma insulin, and as a result causes an increase in fat mobilization from adipose tissue, and therefore the condition aggravates even more (Opsomer, 2015).

According to Opsomer (2015) ketosis can be classified as clinical or subclinical based on ketone body levels in the blood, urine and milk and by the presence or absence of clinical signs. Clinical signs of ketosis include reduced appetite, loss of body weight, reduced milk yield, odor of ketone in the breath or milk, dry or hard feces, activity reduction and in severe cases also nervous signs (Issi *et al*., 2016; Opsomer, 2015). Usually, these clinical signs can be difficult to observe, as they can be very variable, subtle and nonspecific. It is even more complicated if the individual feed intake is difficult to measure (e.g. free stall housing).

1.4 Monensin

Monensin is a polyether ionophore, more specifically part of carboxylic subgroup. It is a natural fermentation product produced by bacteria *Streptomyces cinnamonensis*. Monensin acts as promoting the growth of Gram-negative bacteria which produce propionate, the gluconeogenic precursor, by inhibiting the growth of Gram-positive bacteria which produce butyrate and acetate. It binds to cell membranes and interferes with ion exchange that these Gram-positive bacteria needs for thriving. Due to the different and more complex outer cell membrane Gram-negative bacteria are resistant to effects of monensin. As the bacterial population in the rumen changes, the efficiency of the dairy cow's energy metabolism is improved, by reduced level of blood ketones and increased serum glucose, and as a result, this reduces incidence of ketosis (Duffield *et al*., 2008; European Medicines Agency, 2021).

McGarvey *et al.* (2019) found that monensin has dosage dependent effect on ruminal microbiota. They used dosages 0 mg, 175 mg, 368 mg and 518 mg per cow per day for 20 days. Use of dose 368 mg was found most efficient in increasing gram-negative bacteria from phyla *Bacteroidetes* and *Proteobacteria*. This dosage also was effective at reducing methanogenic archaea in the rumen.

According to Mutsvangwa *et al.* (2002) monensin did not increase the ruminal pH during subacute ruminal acidosis (SARA), but it is possible that there is an interaction between monensin and SARA, and that monensin-treated cows adapted faster to additional grain feeding and due to that maintained DMI and milk yield.

1.4.1 Monensin affecting on blood ketone bodies

According to the study by Heuer *et al.* (2001), use of monensin resulted as decreased beta-hydroxybutyrate (BHB) and acetoacetate (ACAC) concentrates in blood. In cows that received monensin 4 to 6 weeks after calving, BHB level was lower in the first months after starting the treatment and both BHB and ACAC were significantly lower three months after starting the treatment. Lower BHB and ACAC concentrations were detected before and up to two months after calving, when treatment with monensin was started before calving.

1.4.2 Monensin affecting on glucose metabolism

In ruminants up to 90% of necessary glucose must come from gluconeogenesis (Young, 1977). Monensin increases the efficiency of gluconeogenesis by increasing contribution of propionate to plasma glucose and it decreases glucose input rates from other sources than propionate (i.e., lactate and dietary starches). Monensin also reduces the glucose disposal rate, which indicates that glucose utilization efficiency is improved in body tissues of cows in transition period (Markantonatos and Varga, 2017).

1.4.3 Other effects of monensin

According to the study by Heuer *et al*. (2001) monensin reduced the incidence of non-infectious lameness, when administered before parturition. Non-infectious lameness was referred as laminitis, which is the consequence of metabolic disturbances affecting the structures of the hooves.

2 AIM OF THE STUDY

The thesis tests a hypothesis that monensin improves energy metabolism in dairy cattle during early lactation. Therefore, the aim of the thesis is to assess the effect of monensin on the dynamics of blood parameters describing energy metabolism at early lactation in Estonian Holstein cows. We are testing if monensin influences metabolites NEFA, BHB, AST and glucose concentration in blood during transition period.

3 MATERIAL AND METHODS

3.1 Experimental design

The blood and milk samples for the study were collected on a commercial dairy farm in Estonia between September 2016 and January 2018. Cows were loose housed, fed grass-clover silage-based total mixed ration, and milked three times per day. There were ~600 Estonian Holstein dairy cows with average lactation milk yield of 11,350 kg/cow in 2017. Cows were fed with identical rations from the close-up dry period, 3 weeks before expected calving until 6 weeks postpartum.

Primi- or multiparous cows that received the continuous release monensin capsules (Kexxtone; Eli Lilly and Company Limited and Elanco Animal Health) were randomly selected and administration to the rumen was done approximately 3 weeks before expected calving. There were 206 cows in the experiment. In the original control group, there were 125 animals and 81 in the treatment group. Those cows that did not go through the complete 6 weeks trial were excluded from the study. Treated cows belonged then in the treatment group (Kex; total n=81) and the rest of the cows belonged to the control group (Control; total n=81). Groups were equalized due to the limitations of the statistical tests (Excel Data Analysis add-in ANOVA: two-factor without replication), which were used to test the data. All 81 cows from treatment group were included, and 81 cows from control group were randomly selected using Microsoft Excel function RAND.

Body condition scoring was done according to Ferguson *et al*. (1994). Scoring was done by the same observer about 3 weeks before expected parturition and after that once per week.

3.2 Sample collection and laboratory analysis

Blood samples (n=1900) were taken about three weeks and one week before expected parturition and every day on the first four days after parturition, then on the sixth or seventh day of milking and then two times in the second week, and then once per week until the end of sixth week after parturition. Blood samples were taken into Li-Heparin vacuum tubes (Vacuette 202, Greiner Bio-One International Gmbh) from the coccygeal vein and kept at 4°C, before plasma separation by centrifugation (4000 x g, 20 min at room temperature). Plasma was separated within 4 hours after sampling and stored at -80°C before analysis (automatic analyzer RX Monaco, Randox Laboratories Ltd) for BHB (kinetic-enzymatic UV-method, cat. no. RB 1007; Randox Laboratories Ltd.) and NEFA (enzymatic-colorimetric method, cat. no. FA 115).

Classification to hyperketonemic was done based on the postpartum BHB concentrations, cow was hyperketonemic if the BHB concentration was ≥1,2 mmol/L at least once during 6 weeks after parturition. Cows with NEFA concentration ≥0,7 mmol/L at least once during 6 weeks after parturition, were classified as having elevated concentrations of NEFA (Ospina *et al*., 2013). Normal AST values differ widely during pregnancy and lactation and are dependent of the physiological stage (Issi *et al*., 2016), according to Kaneko *et al*. (2008) normal values of AST in dairy cow are 78-132 U/L. In this study AST values >132 U/L were considered indicating impaired liver function.

3.3 Statistical analysis

To compare the incidence of high NEFA and BHB values in control and treatment group, contingency tables were constructed, and the difference was assessed by the Chi-square test (Excel function CHISQ.TEST). The overall effect of monensin and time of sampling was assessed by the analysis of variance from the Excel Data Analysis add-in (ANOVA: two-factor without replication). To assess the difference of the means of the control and treatment groups at each sampling point two-sided Students t-test was used (Excel function T.TEST). Results were considered statistically significant at $P \leq 0,05$.

4 RESULTS

Testing with the t-test revealed that the BHB concentration was significantly lower in Kex group from 7 days before parturition till 14 days after parturition (Figure 1; P <0,05). For example, on day 4 after parturition the BHB concentration was 0,81 mmol/L (Control) and 0,56 mmol/L (Kex) (P <0,01) and on day 14 after parturition concentration was 0,90 mmol/L (Control) and 0,63 mmol/L (Kex) (P <0,01). Samples taken 21 days after parturition did not show significant difference (P >0,05), the BHB concentrations were 0,77 mmol/L in Control group and 0,68 mmol/L in Kex group. From 28 days till 35 days after parturition difference was again significant (P <0,05). On day 28 BHB concentration was 0,87 mmol/L (Control) and 0,59 mmol/L (Kex) and on day 35 0,71 mmol/L and 0,55 mmol/L respectively (P <0,01). On day 42 there was no significant difference, with BHB concentration 0,56 mmol/L (Control) and 0,53 mmol/L (Kex) (P >0,05).

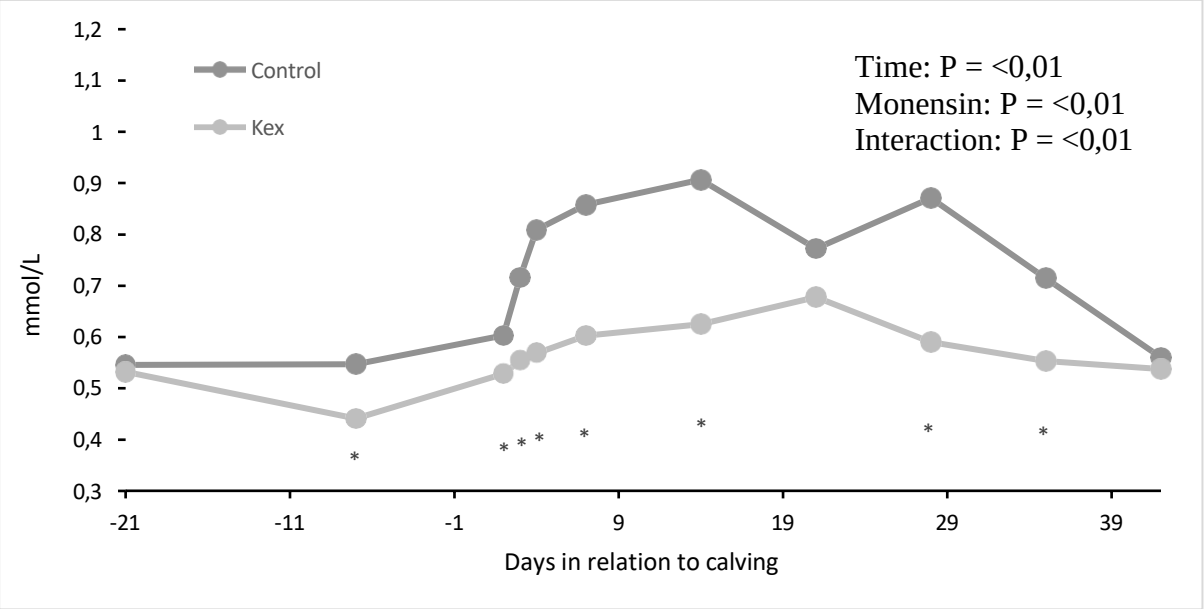

Figure 1. The dynamic of β-hydroxybuturate (BHB) on two study groups. Symbol "*" denotes means that differ.

In Kex group NEFA concentration was significantly lower on day 4 and 7 after parturition (Figure 2; P <0,05), but not in other timepoints. NEFA concentration on day 4 was 0,68 mmol/L (Control) and 0,54 mmol/L (Kex) and on day 7 0,77 mmol/L and 0,64 mmol/l respectively. There was no statistically significant difference between groups in AST and glucose concentrations (Figure 3 and 4; P >0,05).

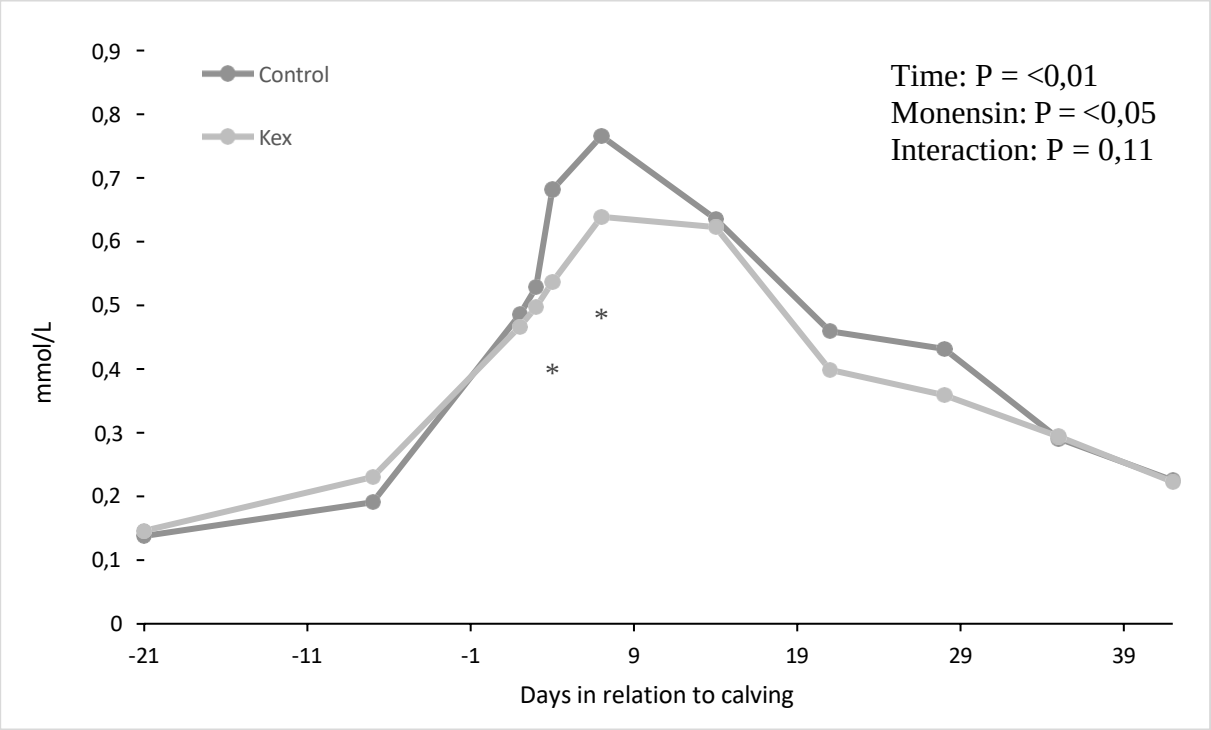

Figure 2. The dynamic of non-esterified fatty acids (NEFA) on two study groups. Symbol "*****" denotes means that differ.

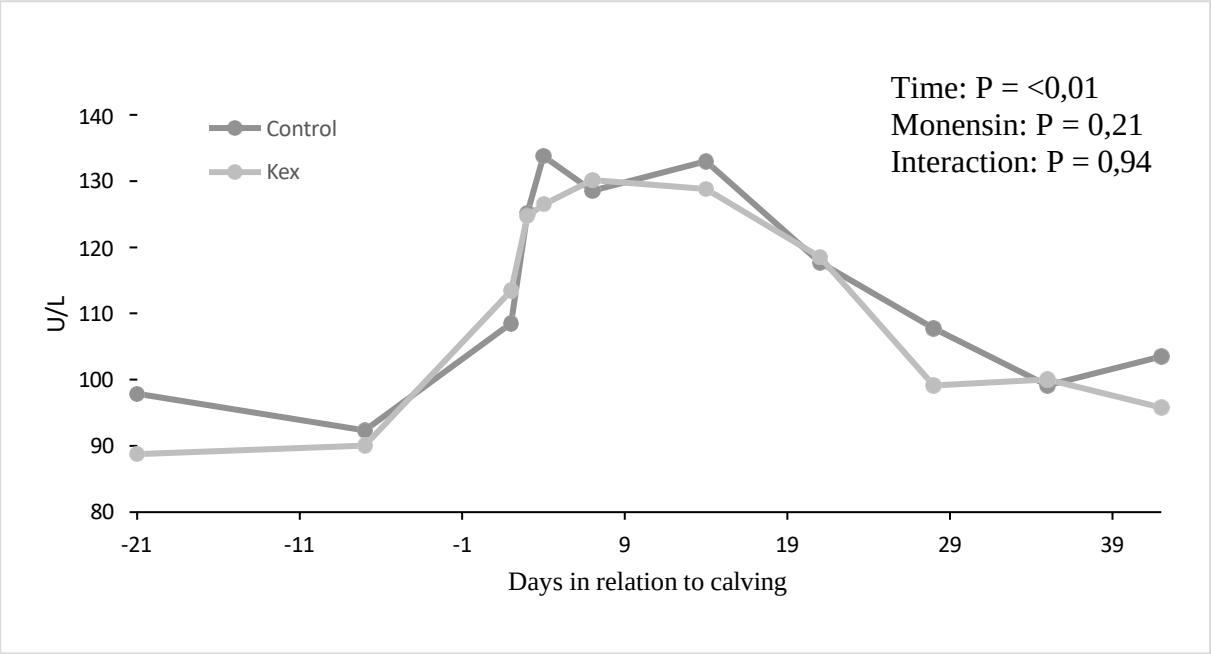

Figure 3. Effect of monensin to aspartate aminotransferase (AST) concentration.

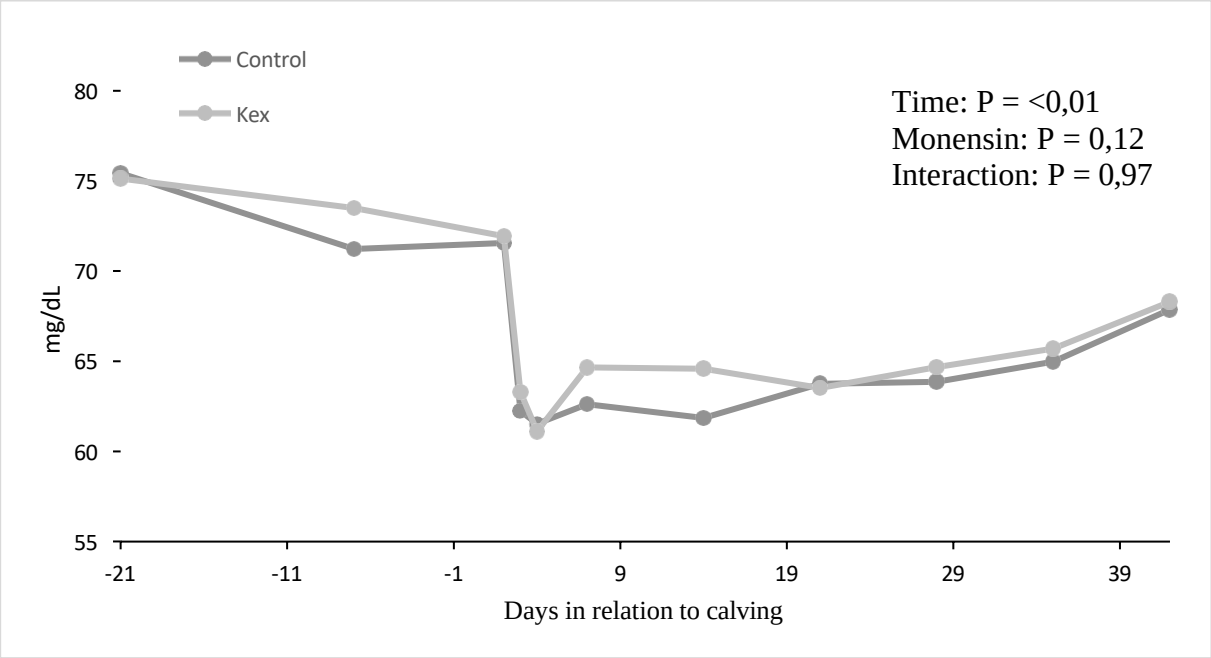

Figure 4. Effect of monensin to blood glucose concentration.

Interpretation of ANOVA-test showed that BHB concentration were significantly lower in the Kex group than in the Control group. Treatment had effect on BHB concentration (P<0,01). In NEFA concentration there was also significant difference and monensin treatment lowered the NEFA concentration significantly in Kex group (P<0,05). Monensin treatment did not have significant effect on AST and glucose concentrations (P>0,05).

With Chi-square test was confirmed, that there is significant difference (P <0,05) between Control and Kex groups in occurrence of BHB concentration >1,2 mmol/L, which can be used as cut off value for subclinical ketosis (Ospina *et al.*, 2013), and NEFA concentration >0,7 mmol/L, which can be considered as high NEFA value. In Control group occurrence of BHB concentration >1,2 mmol/L and NEFA concentration >0,7 mmol/L were significantly higher than in Kex group (Table 1. and 2.). There was no significant difference in occurrence of AST concentration (>132 U/L) between Control and Kex groups (P >0,05) (Table 3.)

Table 1. Prevalence of subclinical ketosis in Control and Kex groups

BHB	<1,2	>1,2	Total
Control	873	80	953
Kex	921	24	945
Total	1794	104	1898

P=<0,01

Table 2. Prevalence of high NEFA concentration in Control and Kex groups

NEFA	<0,7	>0,7	Total
Control	773	174	947
Kex	809	133	942
Total	1582	307	1889

P=<0,02

Table 3. Prevalence of high AST concentration Control and Kex groups

AST	<132	>132	Total
Control	766	187	953
Kex	779	166	945
Total	1545	353	1898

P=0,25

The groups had very similar average BCS (3,13 vs. 3,08) at the time of monensin administration.

5 DISCUSSION

According to McArt *et al*. (2013) slightly increased NEFA and BHB concentrations in the blood are normal physiologic response to the negative energy balance after parturition. Insulin production is decreased after parturition, and this leads to decreased glucose utilization in the tissues (adipose tissue and muscles). In the adipose tissue, the decrease in the availability of glucose increases the amount of NEFA release, which means that animal is using fat as energy instead of glucose. These NEFA are used as fuel for tissues or are delivered to the liver (Herdt, 2000). When there is excess of NEFA, liver fails to oxidize them completely for energy, and NEFA are only partially oxidized forming ketone bodies (McArt *et al*., 2013). Ketone bodies can be used as alternative energy resource in case of negative energy balance, but in severe cases this will lead to ketosis. Excessive NEFA concentrations can also lead to fat accumulation into the liver and disrupt the normal liver function (Ospina *et al*., 2013).

The aim of the thesis was to test a hypothesis that monensin improves energy metabolism during early lactation. BHB, NEFA, AST and glucose metabolism during the trial period in the Control and Kex groups were compared, and there was found evidence, that concentrations of BHB and NEFA were affected by monensin administration. Monensin promotes the growth of Gram-negative bacteria which produce propionate, the main gluconeogenic precursor, and inhibits the growth of Gram-positive bacteria which produce butyrate and acetate.

In Kex group there were significantly lower BHB concentrations, which means that production of propionate has been higher. Results are similar with previous studies made about the effects of monensin in dairy cows for example studies by Heuer *et al*. (2001), and (Duffield *et al*., 2003), and meta-analysis made by Duffield *et al*. (2008). In all those studies use of monensin resulted as lower concentrations of BHB and NEFA in the blood by providing precursor to produce glucose. BHB and NEFA values are connected to each other as NEFA are released from the adipose tissue in case of negative energy balance and through β-oxidation these fatty acids are oxidized eventually to ketones (BHB and ACAC) (Ospina *et al*., 2013). Lower NEFA concentration is the result of decreased mobilization of fat from adipose tissue which leads to lower BHB concentration as well. In this study BHB concentration was significantly lower in all other timepoints except 21 and 42 days after parturition. NEFA values were significantly lower during first week after parturition.

There is insulin resistance during the negative energy balance, which means that the glucose utilization in the tissues is decreased (De Koster and Opsomer, 2013). Monensin has potential

to improve the efficiency of propionate to produce glucose and can also decrease the glucose oxidation in tissues (Markantonatos and Varga, 2017). According to the study by *Chalmeh et al*. (2020), monensin did have minor effect on insulin resistance, but not as significant effect as propylene glycol had. That could be the reason why monensin did not affect significantly to blood glucose concentrations in this study. Similar results were obtained by study of Markantonatos and Varga (2017). According to meta-analysis by Duffield *et al*. (2008) there were no consistency of significant differences in blood glucose levels, although there was indication that monensin can increase the blood glucose concentration.

Fatty liver is an ultimate consequence of excess NEFA, as concentration of NEFA gets too high for liver to cope and triglycerides start to accumulate into the liver. AST can be considered as indicator of liver health. In this study there was no clear evidence of effect of monensin to AST concentration. Most of the cows had normal AST concentration in the beginning and the end of the trial, and changes during the trial can be considered normal variation depending on the cows' physiological status. Effects of monensin to AST concentration and development of fatty liver haven't been studied extensively, but according to the study by Fiore *et al*. (2021) statistically significant difference in AST values were not found between control group and group treated with monensin (Kexxtone) 21 days before parturition. In this study AST levels in Control and Kex groups were very similar, there were no significantly lower values in the Kex group. Overall concentrations of AST were mostly within the reference values. The NEFA concentration was lower in Kex group, but just on two sampling points (on day 4 and 7 of lactation). This short increase in NEFA concentration probably did not cause too high burden on the liver cells on Control cows compared to Kex cows.

According to this study, monensin can improve the energy metabolism during the early lactation and lower the risk of subclinical and clinical ketosis. Prevalence of subclinical ketosis (BHB >1,2 mmol/L) in the Control group was 42% (34 animals out of 81), whereas in the Kex group it was 19% (15 animals out of 81). This can be considered significant difference, as almost half of the cows in Control group had increased BHB concentration. According to the study by Loiklung *et al*. (2022) prevalence of subclinical ketosis in dairy cows globally was 22,7% (95% CI 21,2-24,3%). In Europe prevalence was 20,0% (95% CI 16,6-23,7%), so the prevalence in this study seems to be much higher. Differences with this study may be influenced mostly by sample size. In this study Control group was originally much bigger and due to the limitations of statistical tests groups were equalized by randomly selecting 81 cows from the group.

When we think of the possible financial losses due to subclinical ketosis, there can be very significant impact to farmer. According to the study by Cainzos *et al*. (2022) cost of one incidence of subclinical ketosis can be 812€ in the worst-case scenario (treatment and removal of animal). Calculating the cost for this herd, cost would be nearly 26 000€ in the Control group. Cost in the Kex group would be much less, only 9 700€.

CONCLUSIONS

Periparturient dairy cow can suffer from several problems after parturition. Most of them are related to the changes in the metabolism and feed intake. Accumulation of the ketone bodies due to the negative energy balance and sudden mobilization of body fats is one of the most frequent problems in the dairy cow in transition period. Accumulation of ketone bodies can cause subclinical or clinical ketosis.

In this study was demonstrated, that when monensin was administered as continuous release capsule about three weeks before expected parturition, it affected BHB concentration before and after parturition, and to NEFA metabolism during the first week after parturition by lowering the BHB and NEFA concentrations. Prevalence of subclinical ketosis was clearly lower in the group that was treated with monensin. On the other hand, administration of monensin did not affect to AST values and blood glucose levels.

Monensin has effect and it lowers the prevalence subclinical ketosis. If prevalence of subclinical ketosis is possible to lower with this extent with simple treatment with monensin, I would recommend doing so. Use of monensin can have significant financial benefits, if farmers can prevent cows suffering from subclinical ketosis, or even clinical ketosis.

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APPENDICES

Appendix 1. Non-exclusive licence for depositing the final thesis and opening it for the public and the supervisor's (supervisors') confirmation for allowing the thesis for the defence

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