CHOLINE CHLORIDE POSSIBLE EFFECT ON THE BLOOD SERUM OF COWS

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Abstract. In order to know choline chloride effect on blood serum‘s hydroxybutyrate (BHBA), glucose (GLU), insulin (INSUL), triglycerides (TG), cholesterol (T-CHO) concentration of dairy cows, we did research in one of Lithuania's milk farm. The research took place in the „X“ farm and Cathedral of non-infectious disease of LUHS in 2015. 40 cows were selected using analogical reasoning in their 3 or 4 lactation period. All cows 3 weeks before calving were divided into two groups – experimental (n=20) and control (n=20). Calving feeds of experimental cows were added with cholin supplement (dose – 100g/d) before 30 days. Control group cows were given its usual feeds according to the balanced diet ratio. The research results were assesed 21 d.p.p of following parametres: urea (BUN), triglycerides (TG) cholesterol (T-HO), insulin (INSUL) of cows who were fed with cholin supplement (100g/d). It was determined that urea’s concentration after 21 days from the beginning of the research increased from 2,92±0,04 mmol/l to 5,47±0,06 mmol/L of experimental group, while control group‘s were increased from 2,74± mmol/l to 6,02±0,1 mmol/L. Triglyceride level in the control group from the beginning to the end decreased by 0.036 ± 0.002 mmol /L, compared with the experimental group triglyceride level decreased from 0,306 ± 0,023 mmol/L to 0.281 ± 0,016 mmol/L. Insulin level in the experimental group from the beginning increased from 1.748 ± 0.023 μU/ml to 2,435 μU/ml, in the control group decreased from 3.245 ± 0.016 μU/ml to 2,993 ± 0.01 μU/ml. Beta-hydroxybutyrate in the experimental and control groups from the beginning increased equally by 0.87 mmol/L.

Keywords: Choline chloride, ketosis, lactating, calving

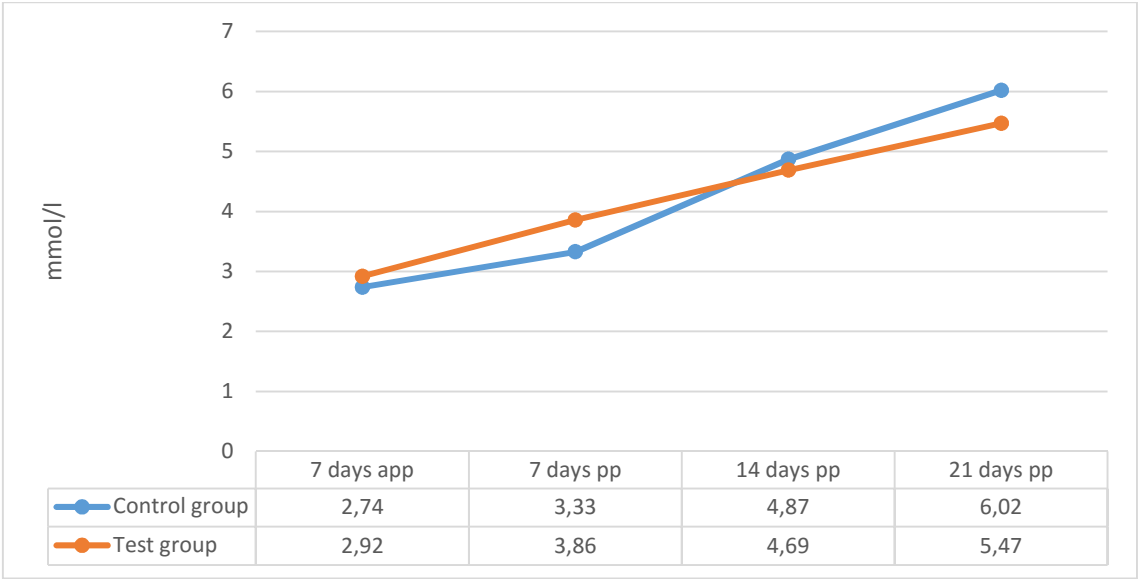
Introduction. One of the reasons for gynecological diseases of dairy cows is acidosis. Acidosis is disfunction of carbohydrates, fat metabolism called ketosis. Ketosis usually occurs to every dairy well-fed cows, 10 days to 6 six weeks after calving. Most of the highly productive cows might get ketosis at the beginning of lactation, because the outcome of the milk is high and the appetite is low. So cows has to rely on it‘s body fat (so-called reserved fat). Some of the foreign experts even claim that about 70-80 proc. ketosis cases are a subclinical (not-recognisable) form, which is why farmers fail to recognize the disease before they are forced to deal with its consequences (weight loss, fertility problems, reduced milk income etc.). It is also believed, if the cow had the subclinical ketosis, its productivity during lactation is decreased by 10-15%., servis period increased twice, decreased the lifetime of the cow and the immunity to diseases weakened. (Minkevičius, Pikelis, 2006). If questioned metabolism condition can be checked via various laboratory tests. In the case of a bad metabolism, all the organs share the changes of morphological, physiological and chemical genesis; a standard biological matter oxidative and reductive reaction worsens as well as deactivating ferments. Ferments happens to be in the cells of a healthy mammal, extracellular and in other biological liquids. In case of dysfunctional organ- the cell elements are damaged, some of it- cytoplasm and mitochondria, which grants some of the ferments the access to blood. Only after identifying what kind of and what ferments are in the blood the condition of the organ can be judged. (Rajala-Schultz et al., 1999; Žilaitis and others, 2006). Blood serum is used to indicate: blood serum hydroxybutyrate (BHBA), triglycerides (TG), urea (BUN), and insulin (INSUL), glucose (GLU) and cholesterol (T-CHO) concentration. The previous articles are also tested in order to prove the feed supplement choline has influence regarding cows health prevention of the ketosis. Dairy cows NEFA and β-hydroxybutyrate testing helps to control subclinical ketosis and negative energy balance strategies. (Contreras, O'Boyle, Herdt, & Sordillo, 2010). According to R. Antanaitis and other scientists (2010), in order to prevent the „ post-calving disease complex“, the measures of early herds health testing must be taken. One of the ways to do so are various blood and milk tests. It shows energy balance and it is an important matter in order to prevent the disease since cows blood biochemical index best reflects the condition of nutrition and metabolism.

Objectives and tasks: to indicate cholines effect on blood serum‘s hydroxybutyrate, glucose, insulin, triglycerides, cholesterol concentration of dairy cows.

Methods and materials. The research took place in the „X“ farm and cathedral of non-infectious diseases of LUHS in 2015. 40 cows were selected using analogical reasoning in their 3 or 4 lactation period. All cows 3 weeks before calving were divided into two groups – experimental (n=20) and control (n=20). Feeds of experimental cows were added with cholin supplement (dose – 100g/day) 30 days before calving. Control group cows were given its usual feed according to the balanced diet ratio. A blood samples were taken according to all the requirements against sepsis, e.g. the area of the puncture is disinfected. (Sutkevičius, 2003). The blood from the tail is taken with vacutainer and vacuum tubes help. The blood was taken from a tail‘s vein (*v. coccygeal*) before the tail is lifted and the area is disinfected, vacutainer‘s needle is penetrated in the middle of root area of the tail. In order to trace every sample, all the tubes were id-marked. Then the blood serum was used to do the biochemical testing. The blood serum is extracted after blood centrifugation for couple of minutes with a speed of 2000-3000rpm. (Sutkevičius, 2003).

The blood samples of cows groups biochemical testing were performed by intervals: 7 days until calving, 7 days after calving, 14 days after calving and 21 days after calving. The blood serum was tested by „Spotchem II aia-360“ which indicated beta-hydroxybutyrate (BHBA), triglycerides (TG), urea (BUN), and insulin (INSUL), glucose (GLU) and cholesterol (T-CHO) concentration in the groups.

Results and discussions. During the research, the level of urea in the blood serum increased in a linear regression model every 7 days. The urea increased equally in both groups 7 days before calving and 7 days after calving. On the 14th and 21st day after calving urea in experimental group decreased faster than it did in the control group. It is recorded that 21st day after calving in experimental group urea‘s concentration was 0,55 mmol/L less than it was in the control group. When the feed supplement is used more of the feed-protein is absorbed, that helped to decrease urea (as protein dividing product) quantity in the blood serum. This is how steady fat-protein ratio is kept and the acidosis risk is reduced. Milk and blood urea is the final product of the protein oxidative process in the organism and is very important indicator, that can give us the infomration about protein based feed. Increased urine concentration in the blood or in the milk is the indicator, that cow might have a problem getting impregnated. (Gustafsson, Carlsson, 1993).



	7 days app	7 days pp	14 days pp	21 days pp
Control group	2,74	3,33	4,87	6,02
Test group	2,92	3,86	4,69	5,47

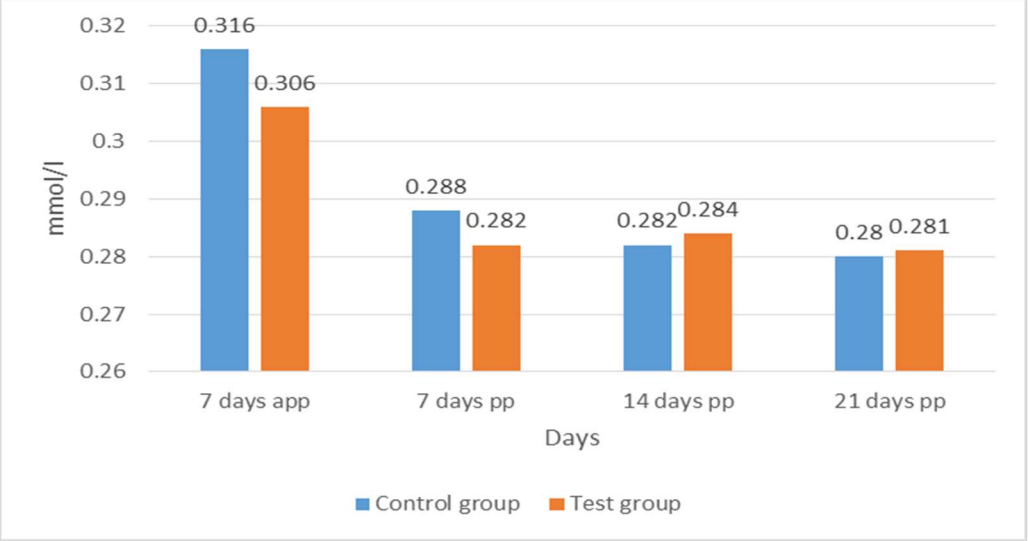
1 picture. ***Control and experimental groups ureas changing tendencies and regressive analysis score***

Triglycerides are neutral fats consisted of glycerol fatty acid ester. It is very important for the organism because it has the ability to accumulate pure and non-hydrated. (Praškevičius and others, 2002). Triglycerides are the main energy source. The energy accumulates double during its degradation than out of carbohydrates. (Robertson, 2003).

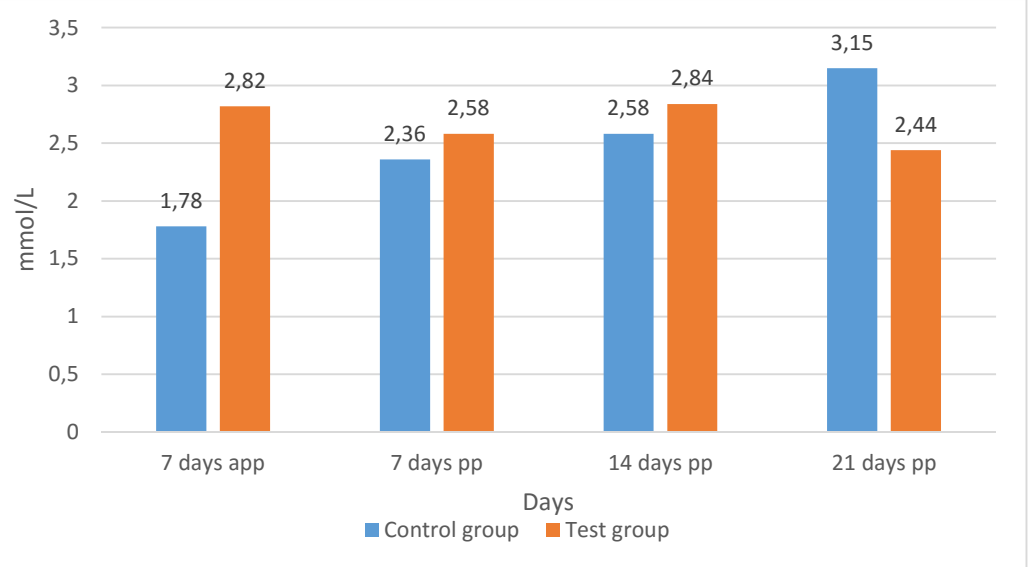
Cows have a decreased risk of ketosis (7 d.p.p decreases TG conc. after calving also decreases concentration of BHBA, increased outcome of milk quantity). Increased triglycerides (TG) concentration in the blood indicates that cow might have fatty liver, ketosis or it may have DA(Herd Health, Hoard, 1993). 1 picture shows triglycerides concentration comparison between control and experimental groups.

It was discovered that using choaline supplement 7 days before calving triglycerides concentration was 0,17 mmol/L ($p>0,05$) less than control groups(see pic. 2). This tendency was recorded until 14th day post calving, where the experimental group had TG concentration slightly increased, but on the 21st day after calving T.G concentration was stable ($p>0,05$) compared to the control group.

Glucose (GLU) is the most important carbohydrate in the organism and it is the main source of energy. When blood lacks carbohydrates , fat cannot perform a full-scale degradation which is why the blood gets increased number of ketone bodies. That is the main reason how cows get sick with ketosis. Blood glucose concentration changes very slowly, but if it is low, we might suspect the subclinical ketosis. (Hutjens,2005)



2 picture. *Triglycerides concentration changes in control and experimental groups*



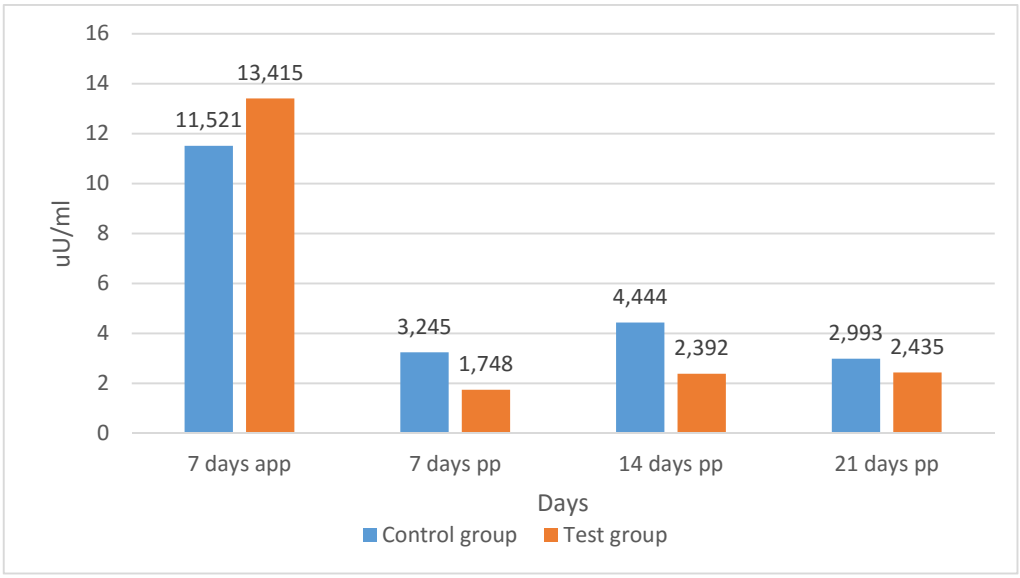
3 picture *Concentration of blood glucose comparison between control and experimental groups*

The results after evaluating both groups were that glucose level in the control group increased steadily. 7 d.p.p glucose concentration was 1,04 mmol/L ($p<0,01$) or 21,24 % lower than the experimental group and it was increasing until 21 d.p.p. In the experimental group glucose concentrations was increasing until 14 d.p.p, and then it had decreased by 0,71 mmol/L compared to the control group.

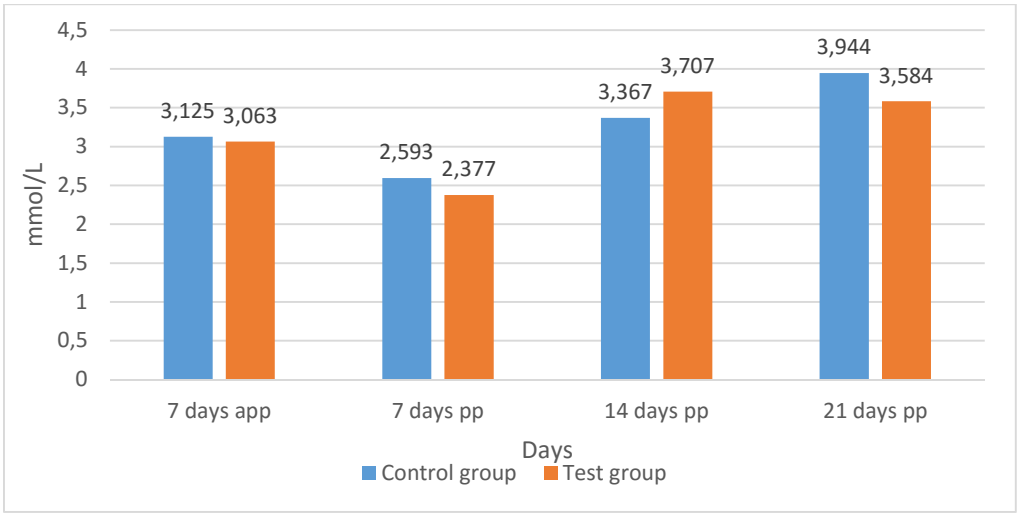
The biggest increase of the insulin concentration in blood serum is recorded 7 days before calving in the experimental group, which had insulin concentration higher by 1,89 uU/ml ($p>0,05$) (4 pic.) than in the control group, but 7 days after calving insulin concentration kept decreasing steadily, while control group did not maintain a concentration level so steady. After 7 d.p.p control group insulin concentration results were higher than in the experimental group.

Insulin stimulates fatty acid and lipid synthesis, inhibits the degradation of fat and reduces the formation of ketone substances in the liver (Sojke et al., 2008). Lipogenesis activates glucose uptake in fat cells and its degradation: 3 – glycerophosphate and acetil-CoA formation that are necessary for fatty acid and triglyceride synthesis (Kitazava et al., 2011). Insulin inhibits lipolysis (Caltabilota et al., 2009) in the liver and adipose tissue. It stops the hormone sensitive lipase - triglyceryphasis, activating it dephosphorylation (Caltabilota et al., 2009). By blocking the release of free fatty acids from fat tissue decreases the production of ketone bodies in the liver (ketogenesis) (Pan et al., 2011).

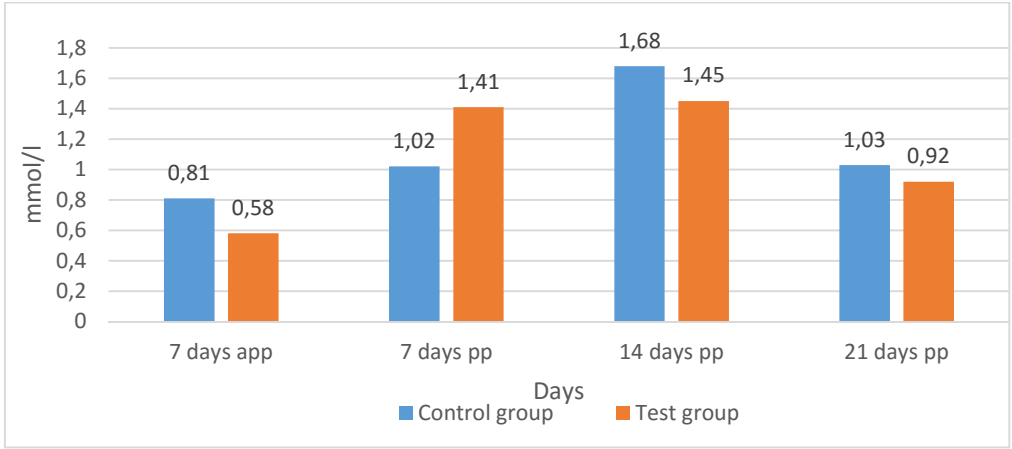
Cholesterol concentration before calving was higher in both groups , however 7 d.p.p cholesterol concentration decreased by 0,73 mmol/L ($p>0,05$) and 21 d.p.p in experimental group cholesterol concentration was 0,36 mmol/L or 9,13 %. ($p>0,05$).



4 picture. *Insulin concentration decreases in control and experimental groups.*



5 pav. *Cholesterol concentration changes in control and experimental groups*



6 picture *Beta- hydroxybutyrate concentration changes in control and experimental groups*

Citil (1999) described increased BHBA concentration in the blood serum from cows that had DA, after 17 days post calving, compared to the healthy cows BHBA. Since most of the DA cases happens to go along with the ketosis , we might suggest that prevention of the ketosis is the key in order to prevent DA (Itoh et al.,1998).

When parameters of the ketonic bodies were compared, it is clear that 7 days before calving BHBA concentration is 0,23 mmol/L ($p>0,05$) less than in the experimental group. 7 days after calving the BHBA is higher than the one in the control group. Though on 14th and 21st day BHBA concentration in the experimental group has started to decrease. 14 d.p.p experimental group‘s BHBA concentration is decreased by 13,7 % compared to the control group‘s BHBA. In conclusion, since choline helps to decrease BHBA concentration and it automatically helps to prevent ketosis.

Conclusions: According to the research results it is clear that adding choline supplement 100g/day to the feed would result in:

It is concluded that experimental groups urea’s concentration after 21 days from the beginning of the research increased from 2,92±0,04 mmol/L to 5,47±0,06 mmol/L, while control group were from 2,74± mmol/L to 6,02±0,1 mmol/L. Triglyceride level in the control group from beginning to the end decreased by 0.036 ± 0.002 mmol/L, while the experimental group triglyceride levels decreased from 0,306 ± 0,023 mmol/l to 0.281 ± 0,016mmol/L. Insulin levels in the experimental group from beginning increased from 1.748 ± 0.023 μU/ml to 2,435 μU /ml, in the control group decreased from 3.245 ± 0.016 μU/ml to 2,993 ± 0.01 μU/ml Beta-hydroxybutyrate in experimental and control group from beginning increased equally by 0.87 mmol/L.

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