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Original article

The concentration of free amino acids in blood serum of dairy cows with primary ketosis

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Abstract

Ketosis is a common condition found in the initial stages of lactation in high-yielding dairy cows. The major cause of ketosis is a negative energy balance. During the energy deficiency, proteolysis processes develop parallel to lipolysis. During proteolysis, muscle tissue can be used as a source of amino acid. To date, the participation of amino acids in gluconeogenesis (glucogenic amino acids) and ketogenesis (ketogenic amino acids) has not been determined in detail. This paper presents the study on determination of the parameters of protein and free amino acid metabolism in blood serum of dairy cows with primary ketosis compared to healthy cows. This study contributes to better understanding of the role of amino acids in pathogenesis of ketosis. A total of 30 cows, divided into two groups: experimental (15 cows with ketosis) and control (15 healthy cows), were included in the study. The concentrations of glucose, β-hydroxybutyrate, total protein, albumin, urea, and free amino acids were determined in peripheral blood. Statistically significantly higher concentrations of glutamine, glutamic acid, isoleucine ($p \leq 0.001$), and tyrosine ($p \leq 0.05$) were found in cows with primary ketosis compared to healthy cows. Significant decrease in the concentrations of asparagine, histidine, methionine, and serine ($p \leq 0.001$), alanine, leucine, lysine and proline ($p \leq 0.05$) was observed. Significant increase of total ketogenic and glucogenic amino acids ($p \leq 0.05$), and an increased ratio of total ketogenic and glucogenic amino acids to total amino acids ($p \leq 0.001$) were noted in cows with ketosis. In our study, the changes, in particular observed in amino acid concentration in cows with primary ketosis, indicate its intensive use in both ketogenesis and gluconeogenesis processes. Therefore, a detailed understanding of the role that amino acids play in gluconeogenesis and ketogenesis will improve ketosis diagnostics and monitoring the course of a ketosis episode. Perhaps, the prevention of this disease is possible by balancing the appropriate feed ration in terms of amino acid content.

Key words: dairy cows, ketosis, glucogenic amino acids, ketogenic amino acids

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Introduction

Ketosis is a common condition occurring in the initial stages of lactation in high-yielding dairy cows. This disease is a result of nutritive deficiency that leads to an increased susceptibility to infectious and metabolic diseases, as well as fertility disorders (Baird 1982, Grummer 1993, Duffield et al. 2009, Zhang et al. 2009, McArt et al. 2012). Ketosis may occur in the primary, secondary and alimentary form.

The primary cause of ketosis is a negative energy balance (NEB) postpartum, which results in lipolysis of fat reserves with concurrent increase in ketone bodies production. Pathogenesis of this disease combines several different interrelated metabolic processes such as glycolysis, lipolysis, proteolysis, gluconeogenesis, as well as metabolism of amino acids and fatty acids. Milk production in the initial lactation period increases the need for glucose. Glucose is essential for the proper functioning of CNS cells, red blood cells and the synthesis of milk components. In the process of glycolysis, glucose is degraded to pyruvate. Depending on the conditions, pyruvate may be converted into acetyl-CoA and could enter the Krebs cycle, or may be converted into lactate or alanine – which are the precursors of glucose in the process of gluconeogenesis. Glycolysis results in the decomposition of glucose and formation of energy in the form of ATP.

Increased production of milk in the initial lactation period and insufficient energy consumption causes NEB. The NEB leads to lipolysis of triglycerides and degradation of body proteins and to an increase in gluconeogenesis. Increase in NEFA in blood that flows into the liver is a consequence of lipolysis. In the liver, after the conversion into acetyl-CoA, NEFA may be fully oxidized or transformed into ketone bodies (β-hydroxybutyrate, acetoacetate) during the process of ketogenesis (Duffield 2000, Duffield et al. 2009, Gonzales et al. 2011). During the esterification process, NEFA may also be converted again into triglycerides, for which the excessive amounts are accumulated in hepatocytes.

Gluconeogenesis occurs in parallel to the lipolysis processes, i.e., the formation of glucose from fatty acids of rumen and non-carbohydrate compounds as well. After feeding and in the period of high energy consumption, gluconeogenesis using short chain fatty acids (propionate, valerate, and isobutyrate) of rumen is the highest. A decreased feed intake observed in the course of ketosis involves the use of non-carbohydrate compounds for gluconeogenesis. In this case, substrates for gluconeogenesis are lactic acid, glycerol, and amino acids such as Glu, Asp, Gly, His, Pro, Gln, and Val (Aschenbach et al. 2000).

For energy depletion, in addition to the mobilization of fat and glycogen, we also observed the mobilization of protein reserves. The greatest source of protein for the processes of proteolysis are the skeletal muscles and skin to a lesser extent. Amino acids derived in the process of proteolysis are differently, but intensively, used for milk protein synthesis, direct oxidation, or gluconeogenesis.

In the primary ketosis, lipolysis is accompanied by proteolysis processes, when muscle tissue is used as a source of amino acids actively contributing in gluconeogenesis (Shibano et al. 2005, Akamatsu et al. 2007, Kamiya et al. 2008, Kuhla et al. 2011, Van der Drift et al. 2012). High concentration of 3-methylhistidine, a parameter determining tissue protein decomposition, was noted in cows with ketosis, which confirms the presence of proteolysis and amino acids in pathogenesis of ketosis (Akamatsu et al. 2007). To date, amino acid participation both in the processes of gluconeogenesis and ketogenesis has not been determined in detail. Studies of many authors show clear evidence of the use of amino acids in pathogenesis of ketosis in dairy cows (Li et al. 2014, Sun et al. 2014). Li et al. (2014) demonstrated higher concentrations of valine and glycine and lower concentrations of arginine, leucine, isoleucine, tryptophan, and lysine in dairy cows with primary ketosis compared to healthy cows. Changes in the concentrations of free amino acids in the primary ketosis are different from those observed during the secondary ketosis. The secondary ketosis in the course of left displacement of abomasum (LDA) (Hamana et al. 2010) and fatty liver syndrome was demonstrated by other authors (Pechova et al. 2000, Shibano and Kawamura 2006, Hidiroglou and Veira 2008, Imhasly et al. 2014) concurrently with low concentrations of glucogenic amino acids (asparagine, glutamine, glycine, methionine) and essential amino acids (arginine, phenylalanine, threonine) and high concentration of ketogenic amino acids (leucine, lysine). The studies conducted on dairy cows with the primary ketosis also demonstrated some changes in the concentration of amino acid metabolism (2-piperidinecarboxylic acid, 3-hydroxyisovaleric acid, 4-aminobutyric acid), which indicates their active participation in the ketosis pathogenesis (Zhang et al. 2013, Sun et al. 2014).

The aforementioned results suggest that both lipolysis and proteolysis are implicated in the development of ketosis. The detailed identification of amino acid's share in the gluconeogenesis and ketogenesis processes may have a large application value in monitoring the course of ketosis. Perhaps, also the prevention of this disease is possible by appropriate feed ration balancing in terms of amino acid content.

The aim of the study was to determine the protein profile and changes in free amino acids concentration in blood serum of dairy cows with primary ketosis, compared to healthy cows. This study may be helpful in the determination of the role of amino acids in the pathogenesis of ketosis.

Materials and Methods

Animals

The study was approved by the Local Ethics Committee at the University of Life Sciences in Lublin (No. 41/2014). The study included 30 dairy cows of Holstein-Friesian breed (HF), originating from a herd of 84 cows. Dairy cows were in the period of 2-5 lactations, BCS in cows determined was between 3.5 and 4.0 and the body weight was between 450 and 550 kg. In dairy cows with ketosis, daily yield was 29.78 ± 5.43 kg milk with a fat content of 4,91 ± 0.43% and the protein content of 3.15 ± 0.27%. In healthy cows, daily milk yield was 32.57 ± 6.36 kg milk with fat content of 4.44 ± 0.61% and the protein content of 3.35 ± 0.47%.

Annual milk yield determined in 305 days' lactation was 7 669 – 8 670 kg of milk, with fat content of 4.90 – 4.48% and protein content of 3.36 – 3.42%. Cows' feeding was based on Total Mixed Ration (TMR) system including 19 kg of silage maize, 8 kg of haylage, 1.5 kg of hay, 5 kg of concentrated feed, 0.5 kg of molasses and 0.2 kg of mineral-vitamin premix. The feeding was adjusted to the actual needs and lactation period of the cows.

Ketosis was preliminarily diagnosed in 15 cows based on a field test aimed at quick ketone body detection in urine, Testoket (Biowet, Puławy, Poland), and these cows constituted the study group. Ketosis occurred in cows spontaneously, without the presence of other diseases. The control group also consisted of 15 cows, which were in the same stage of lactation and were free of ketosis. Ketosis, or its absence, was additionally determined in all animals selected for the study based on the concentration of β-hydroxybutyrate (BHBA) in blood serum. According to the literature data, the animals in which BHBA in blood serum was >1.2 mmol/L were recognized as cows with ketosis (McArt et al. 2012). Cows in which the concentration of BHBA was 1.0-1.2 mmol/L were considered to be at risk of diseases associated with postpartum period and were excluded from our studies.

The animals in which BHBA concentration was lower than 1.0 mmol/L were recognized as healthy cows (Oetzel 2004, Seifi et al. 2011, McArt et al. 2012). The study was conducted during the period of the highest number of calvings in the herd (March – June) in 2014.

All the cows included in the study were in the period from 5 to 28 days' postpartum and were on a farm during the whole period of experiments. Except the concentration of BHBA, blood biochemical examinations, including basic parameters of protein metabolism and free amino acid concentration, were performed in all the selected cows.

Sampling

Blood samples (9 mL) were collected from the external jugular vein into clot activator tubes (Vacutest Kima srl, Arzergrande (PD), Italy). Blood samples were collected once in the morning, before the morning feeding. Then, the blood samples were centrifuged at 2,500 × g for 10 minutes at 4°C, the serum was collected, transferred to 2 mL microcentrifuge tubes and then frozen and stored at -80°C until analysis.

Glucose (GLUC), total protein (TP), albumin (ALB), and urea (UREA) content in blood serum used was assayed using Accent-200 Glucosum, Accent-200 Total Protein, Accent-200 Albumin, Accent-200 Urea (PZ Cormay S.A., Lomianki, Poland) kits. The analyses were performed using a BS-160 analyzer (Mindray Medical International Limited, Shenzhen, China). The concentration of globulins (GLOB) was calculated based on a difference between TP and ALB.

The concentration of BHBA in blood serum was determined using colorimetric method with reagents kit (Ranbut, Randox Laboratories, Crumlin, Antrim, UK). The absorbance readings and subsequent calculations of final concentrations were performed on an automatic microplate reader (Asys Expert Plus, Biochrom Ltd., Cambridge, England) at 450 nm and 630 nm.

The determination of serum concentration of free amino acids was performed using ion-exchange chromatography in Ingos AAA-400 apparatus for automatic analysis of amino acids (Ingos s.r.o., Praha, Czech Republic). One milliliter of serum was added to 1 mL of 6.0% buffered sulfosalicylic acid, pH 2,9. Then the sample was centrifuged for 15 min at 12,000 rpm using the centrifuge MPW 250 (MPW Med. Instruments, Warsaw, Poland). The obtained supernatant was used for the determination of free amino acid contents. Amino acids were separated on analytic column (Ostion LG FA, 3 mm × 200 mm). During the separation, five lithium citrate buffers of different pH (2.9, 3.1, 3.35, 4.05, and 4.9) were used. The amino acids were derivatized with ninhydrin and their identi-

Table 1. The results of biochemical blood analysis of dairy cows with ketosis and of healthy cows.

Parameters	Dairy cows with ketosis	Healthy dairy cows
BHBA [mmol/L]	2.98 ± 1.66**	0.75 ± 0.24
GLUC [mmol/L]	2.03 ± 0.7**	3.20 ± 0.37
TP [g/L]	76.66 ± 7.64	74.75 ± 6.21
ALB [g/L]	38.84 ± 3.24	40.06 ± 2.76
GLOB [g/L]	36.25 ± 6.08	35.73 ± 2.44
UREA [mmol/L]	2.33 ± 0.83	2.53 ± 1.20

Mean ± SD. * – significant difference between two group by Student's t-test (p≤0.05). ** – significant difference between two groups by Student's t-test (p≤0.001)

Table 2. The concentration of free amino acids in blood of dairy cows with ketosis and of healthy cows [µmol/L].

Amino acid	Dairy cows with ketosis	Healthy dairy cows
Ketogenic amino acids		
Leu	63.73 ± 18.50*	85.82 ± 21.66
Lys	60.18 ± 8.50*	74.36 ± 7.79
Glucogenic amino acids		
Ala	148.55 ± 26.61*	190.91 ± 45.28
Arg	153.36 ± 27.93	140.55 ± 19.56
Asn	4.0 ± 2.86**	11.09 ± 3.94
Asp	19.09 ± 5.38	19.75 ± 7.42
Gln	369.36 ± 65.77**	248.18 ± 35.66
Glu	122.82 ± 23.78**	74.45 ± 16.43
Gly	474.64 ± 64.32	554.91 ± 127.88
His	32.82 ± 14.30**	54.36 ± 11.95
Met	14.18 ± 2.52**	20.09 ± 2.39
Orn	25.82 ± 5.74	31.55 ± 7.19
Pro	27.72 ± 24.94*	59.10 ± 27.73
Ser	62.0 ± 17.57**	106.18 ± 26.87
Thr	73.55 ± 15.55	62.45 ± 14.58
Val	174.82 ± 30.18	180.45 ± 25.91
Glucogenic and ketogenic amino acids		
Ile	108.18 ± 24.91**	79.55 ± 16.54
Phe	48.27 ± 6.53	47.42 ± 6.89
Trp	18.00 ± 14.16	12.64 ± 6.61
Tyr	43.00 ± 9.85*	31.55 ± 7.17

Mean ± SD. * – significant difference between two group by Student's t-test (p≤0.05). ** – significant difference between two group by Student's t-test (p≤0.001)

fication was performed on the basis of retention time in comparison to the standards using photocell combined with a computer. Analytical separation of acidic and alkaline amino acids was performed at 38-39°C, while neutral amino acids were separated at 59-60°C. The original software MICRO version 1.8.0 (Ingos Corp., Czech Republic) was used for amino acid evaluation.

The amino acids determined were as follows: essential amino acids – valine (Val), leucine (Leu), isoleucine (Ile), tryptophan (Trp), phenylalanine (Phe), methionine (Met), threonine (Thr), arginine (Arg), histidine (His), lysine (Lys), non – essential amino acids – tyrosine (Tyr), alanine (Ala), glycine (Gly), serine (Ser), proline (Pro), glutamine (Gln), glutamic acid (Glu), asparagine (Asp), aspartic acid (Asn) and ornithine (Orn).

Table 3. The concentration of amino acids groups in blood of healthy cows and of cows with ketosis [µmol/L].

Amino acid	Dairy cows with ketosis	Healthy dairy cows
T-KAA	131.91 ± 22.66	149.64 ± 34.35
T-GAA	1702.27 ± 127.96	1754.00 ± 170.80
T-GKAA	214.09 ± 39.90*	172.00 ± 26.25
T-EAA	751.73 ± 84.78	755.55 ± 60.55
T-NEAA	1296.55 ± 97.93	1327.64 ± 180.52
T-AA	2083.18 ± 161.62	2048.27 ± 158.23
BCAA	346.83 ± 58.20	345.82 ± 39.27
T-KAA / T-AA ratio	0.064 ± 0.009	0.072 ± 0.019
T-GAA / T-AA ratio	0.841 ± 0.025	0.831 ± 0.019
T-GKAA / T-AA ratio	0.104 ± 0.015**	0.083 ± 0.014

Mean ± SD. * – significant difference between two groups by Student's t-test ($p \leq 0.05$). ** – significant difference between two groups by Student's t-test ($p \leq 0.001$)
T-KAA – total ketogenic amino acids, T-GAA – total glucogenic amino acids, T-GKAA – total glucogenic and ketogenic amino acids, T-EAA – total essential amino acids, T-NEAA – total non-essential amino acids, T-AA – total amino acids, BCAA – branched-chain amino acids.

Amino acids were divided into the following groups: T-KAA – total ketogenic amino acids, T-GAA – total glucogenic amino acids, T-GKAA – total glucogenic and ketogenic amino acids, T-EAA – total essential amino acids -T-NEAA – total non-essential amino acids, T-AA – total amino acids (T-EAA+T-NEAA), BCAA – branched-chain amino acids (Hamana et al. 2010).

Statistical analysis

All values are presented as means ± SEM. Statistical analysis was performed using the Statistica software (version 10.0). Data were found to be normally distributed in accordance with the Kolomogorov-Smirnov test and Lilliefors correction. The mean values were compared between healthy and ketosis groups using non-paired Student t-test. $p \leq 0.05$ was considered as statistically significant.

Results

The concentrations of BHBA, GLUC, TP, ALB, GLOB, and UREA in blood serum of the studied dairy cows are presented in Table 1. An increase in the concentration of BHBA ($p \leq 0.001$) and decrease in glucose content ($p \leq 0.001$) were demonstrated in dairy cows with ketosis compared to healthy cows.

The concentration of free amino acids in blood serum of both studied groups of dairy cows are presented in Table 2. Statistically significant decrease in the concentration of Leu and Lys ($p \leq 0.05$) was noted in the group of cows with ketosis compared to healthy cows. The concentrations of Asn, His, Met, and Ser ($p \leq 0.001$) as well as Ala and Pro ($p \leq 0.05$) were statistically significantly lower and the concentrations of Glu and Gln were higher ($p \leq 0.001$) compared to healthy cows. A significant increase in the concentration of Ile ($p \leq 0.001$) and Tyr ($p \leq 0.05$) was noted in dairy cows with ketosis compared to the healthy cows.

Total amino acids content in blood serum of cows with ketosis and healthy cows is presented in Table 3. In dairy cows with ketosis, the values obtained for the whole amino acids groups T-EAA, T-NEAA, and T-AA did not differ compared to healthy cows, however, considerable differences in particular amino acids content were noted between the groups. Among essential amino acids (EAA) in dairy cows with ketosis, the concentrations of Met, His ($p \leq 0.001$), Leu, and Lys ($p \leq 0.05$) were lowered, while the concentration of Ile ($p \leq 0.001$), was elevated compared to the healthy cows. In the group of non-essential amino acids (N-EAA), a significantly lower values of Asn, Ser ($p \leq 0.001$), Ala, Pro ($p \leq 0.05$), and higher values of Glu, Gln ($p \leq 0.001$), and Tyr ($p \leq 0.05$) were demonstrated in dairy cows with ketosis. Also significant increase in T-GKAA ($p \leq 0.05$), as well as higher value of T-GKAA/T-AA ratio ($p \leq 0.001$), were found in dairy cows with ketosis compared to healthy cows.

Discussion

The study evaluated the selected parameters of protein metabolism and changes in free amino acids concentration in peripheral blood of dairy cows diag-

nosed with ketosis in early lactation stage and in healthy cows at the same time after calving. Furthermore, these cows demonstrated a high concentration of β-hydroxybutyrate (BHBA) and low glucose (GLUC) level, which, according to the literature, is characteristic for the primary ketosis (Duffield 2000, Zhang et al. 2009, Gonzales et al. 2011).

The examinations performed in dairy cows with ketosis did not demonstrate any significant changes in TP, ALB, GLOB, and UREA, except a considerable increase in BHBA level. No significant changes in the abovementioned concentrations can result from undisturbed processes of protein metabolism in liver. Changes in these concentrations appear late, when following a long-lasting ketosis process, there is a significant development of hepatocytes injury and liver failure. These results are consistent with the results of other authors, who also demonstrated no significant correlation between the concentration of BHBA and concentration of total protein, albumins, globulins, and urea in cows with ketosis (Akamatsu et al. 2007, Gonzales et al. 2011). Kirovski et al. (2013) demonstrated a decreased concentration of albumins along with no changes in the concentration of total protein and urea in cows with clinical ketosis occurring on 10th day after the birth. According to the authors, the occurrence of clinical ketosis (type II ketosis), low albumin and glucose concentration in this study period, was probably associated with liver failure caused by its fatty liver syndrome.

Our study conducted in dairy cows with the primary ketosis showed significantly lower concentrations of leucine (Leu) and lysine (Lys) compared to healthy cows. These amino acids are found among ketogenic amino acids (KAA), which in the course of metabolism are converted to acetyl-CoA, acetoacetyl-CoA, or acetoacetate, being the precursors of ketone bodies. After the conversion to acetyl-CoA, ketogenic amino acid can be utilized in the Krebs cycle as an energy source. In dairy cows with ketosis, we demonstrated a low concentration of citrate – an intermediate of the Krebs cycle. It suggests that ketotic cows consume large amounts of ketogenic amino acids for energy (Sun et al. 2014).

A decrease in essential amino acid level (Leu, Lys) in dairy cows with ketosis was confirmed by studies of other authors authors (Maeda et al. 2012, Sun et al. 2014). According to the literature data, the confirmation of a significant role of both mentioned amino acids in ketosis pathogenesis is the change in the concentration of their metabolism products. A high increase in the concentration of 2-piperidinecarboxylic acid (2PC) resulting from an intense decomposition of Lys and low concentration of 3-hydroxyisovaleric acid (3HIV) caused by Leu deficiency were noted in subclinical and clinical ketosis (Zhang et al. 2013, Sun et al. 2014).

Lys, Met, and three branched-chain amino acids (Ile, Leu, and Val) are considered amino acids that reduce milk yield. The literature data confirm that the shortage of Leu and Lys leads to reduced milk yield and protein content in milk (Rulquin and Pisulewski 2006). Our observations of dairy cows with ketosis (with a low concentration of Leu, Lys, and Met) showed lower milk yield, lower protein content and higher fat content in milk, in comparison to healthy cows.

In healthy cows, with maintained appetite, the main glucose precursors include propionate (60-74%), L-lactate (16-26%), valerate and isobutyrate (5-6%), glycerol (0.5-3%) and small amounts of glucogenic amino acids (Glu, Asn, Gly, His, Pro, Gln, and Val) (Aschenbach et al. 2000, Larsen and Kristensen 2013, Sun et al. 2014). The absence or decreased appetite in cows suffering from ketosis causes deficiency of the mentioned substrates, and therefore there is an activation of gluconeogenesis using amino acids, which was confirmed by other authors (Li et al. 2014, Sun et al. 2014). In this study, among glucogenic amino acids, a decrease in the concentrations of alanine (Ala), aspartic acid (Asn), histidine (His), methionine (Met), proline (Pro), and serine (Ser) were demonstrated in dairy cows with ketosis. Low concentrations of these amino acids may occur due to their intensive use in the process of gluconeogenesis (Shibano et al. 2006, Maeda et al. 2012). According to the literature data, the intermediates, i.e., pyruvate and keto acids (h-ketoglutarate, succinyl CoA, fumarate, and oxaloacetate) are formed from glucogenic amino acids during metabolic processes. They participate in the metabolic reactions of the Krebs cycle, creating CO_2, H_2O, and energy in the form of ATP. In the case of high demand for glucose, oxaloacetate falls out of the Krebs cycle and is used for glucose synthesis (Xu et al. 2008). In dairy cows with ketosis, due to low concentration of glucogenic amino acids, gluconeogenesis is inhibited. Simultaneously, the lack of glucogenic amino acids leads to the disruption of the Krebs cycle reaction (interruption of the TCA cycle). It is evidenced by the decreased concentration of citrate – intermediate metabolite of the Krebs cycle, among dairy cows with ketosis (Zhang et al. 2013). Low concentration of essential amino acids (Met) demonstrated in cows with ketosis could result from their low content in the feed ration and/or low intake of feed. Met is a methyl donor for the synthesis of phospholipids – essential components of very low-density lipoprotein (VLDL) which is responsible for the removal of triglycerides from the liver. In cows with ketosis, we demonstrated a low concentration of

low density lipoprotein (LDL) and VLDL, which may indicate the disruption of their synthesis caused by, among others, Met deficiency (Sun et al. 2014).

Many authors reported the low concentration of glucogenic amino acids in dairy cows with ketosis, resulting from their use in the aforementioned metabolic processes (Shibano et al. 2005, Zhang et al. 2013).

A significant increase in the concentration of glutamine (Gln) and glutamic acid (Glu) was found in our study. These amino acids are classified as non-essential amino acids (N-EAA) and can be synthesized in an organism. The presumable reason of an increase in these amino acids level may be muscle protein breakdown, as well as reduced use of Gln and Glu in metabolism and milk protein synthesis. The studies carried out by other authors have confirmed the muscle protein breakdown process in cows in the perinatal period (Meijer et al. 1995, Kuhla et al. 2011, Van der Drift et al. 2012). Gln and Glu, compared to previously discussed amino acids, do not contribute significantly to the pathogenesis of ketosis, which may also contribute to an increase in their concentration in dairy cows suffering from ketosis (Zhang et al. 2013). According to Sun et al. (2014), high concentration of Glu and Gln, and thus the unavailability of gluconeogenic substrates in dairy cows with ketosis, may be one of the important risk factors in the pathogenesis of the disease.

Moreover, these amino acids are included in major components of milk casein. Glu and Gln are mobilized at early stages of lactation and are utilized by mammary gland tissues for the synthesis of milk proteins (Maeda et al. 2012). This means that normal milk production should reduce Gln and Glu concentrations in blood serum for the benefit of their content in milk (Meijer et al 1995, Maeda et al 2012). Described in the literature and observed in our study, decreased milk yield in dairy cows with ketosis may be the reason for reduced use of Gln and Glu for proteins milk synthesis and is also a reason for their increased concentration in blood serum. An additional source of Glu may be other amino acids such as His and Pro. These amino acids, in order to enter the metabolic Krebs cycle, must be converted to Glu. For this reason, in this study we demonstrated a decreased concentration of His, Pro, and increased concentration of Glu.

Apart from the aforementioned significant changes in the concentration of free amino acids in dairy cows with ketosis, an increase in the concentration of isoleucine (Ile) and tyrosine (Tyr) was also demonstrated. Due to a limited literature related to the concentrations of these amino acids in cows, it is difficult to interpreted the increase in these amino acids concentration in blood serum of dairy cows with ketosis, especially, that these amino acids are included into gluco- and ketogenic amino acids (GKAA) and can be used for the synthesis of glucose and ketone bodies. Probably, one of the possible reasons for the increase in Ile and Tyr is their reduced use for milk proteins synthesis, which is due to the significantly reduced milk yield in dairy cows with ketosis (Baird 1982, Duffield 2000, Duffield et al. 2009). In addition, high levels of acetyl-CoA and acetoacetyl-CoA, being the metabolism products of both free fatty acids and amino acids, as well as an increased levels of ketone bodies (acetate, acetone, and 3-hydroxybutyrate) in the blood of dairy cows with ketosis may limit the use of GKAA in the pathogenesis of ketosis as a feedback phenomenon (Xu et al. 2008, Sun et al. 2014). Some authors in their study confirmed an increased concentration of isoleucine and tyrosine with the primary ketosis (Zhang et al. 2013, Sun et al. 2014).

Conclusions

Low levels of ketogenic amino acids (Leu, Lys) and selected glucogenic amino acids (Ala, Asn, His, Met, Pro, and Ser) are observed in dairy cows with the primary ketosis, which indicates their important role in the pathogenesis process of ketosis. In turn, high concentration of glucogenic amino acids (Glu and Gln), as well as GKAA (Ile, Tyr), proves their negligible contribution to the development and course of ketosis in dairy cattle. Therefore, a detailed understanding of the role that amino acids play in gluconeogenesis and ketogenesis will improve ketosis diagnostics and monitoring the course of a ketosis episode. Perhaps, the prevention of this disease is possible by balancing the appropriate feed ration in terms of amino acid content.

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