Journal of Veterinary Medicine and Animal Health Vol. 4(9), pp. 124-129, December 2012
Available online at http://www.academicjournals.org/JVMAH
DOI: 10.5897/JVMAH11.042

Full Length Research Paper

Effect of the periparturient period on blood free amino acid concentration in dairy cows/healthy cows

Yousuke Maeda, Hiromichi Ohtsuka and Masa-aki Oikawa*

Large Animal Medical Center, School of Veterinary Medicine, Kitasato University, 35-1 Higashi 23 Ban-cho, Towada, Aomori 034-8628, Japan.

Accepted 26 September, 2012

A negative energy balance (NEB) attributed to sugar and lipid metabolic disorders are common in periparturient cows. The relationship between periparturient disorders and amino acid metabolism is unclear. The present study was aimed to observe the kinetics of blood free amino acids healthy periparturient cows simultaneously with the concentrations of biochemical constituents in plasma. The changes in the concentrations of plasma biochemical constituents and blood free amino acids were monitored in healthy periparturient cows. There were three types of amino acid; a group of amino acids (Val, Leu, Ile, Trp, Thr, Arg, Lys, Tyr, Ala, Pro, Asn, Orn) whose blood concentrations decreased because their consumption is accelerated under periparturient energy-shortage state, a group of AAs (Gly, Ser) whose concentrations increased because they are mobilized to compensate for periparturient energy shortage, and a group of amino acids (Glu, Gln) that are taken into the mammary gland tissues and mobilized from the start of lactation after delivery. The blood free amino acid concentrations in periparturient cows indicated the various kinetics of amino acid concentration corresponding to changes in energy generation. Analysis of amino acid metabolism should be entertained in the pathogenesis of NEB-related periparturient disorders.

Key words: Cow, serum free amino acid concentration, periparturient, metabolism of amino acid.

INTRODUCTION

Cows are likely to develop a number of periparturient diseases. For example, NEB attributed to sugar and lipid metabolic disorders is common in periparturient cows. A significant correlation between these metabolic disorders and left displaced abomasum (LDA) is well known (Bell, 1995; Doepel et al., 2002). When viewed from the stand-point of individual cows (that is, biological factors), FAs and AAs from adipose and muscle tissues are mobilized as a result of the rapid growth and development of foetuses and the uterus, and the nutrient requirement increases in preparation for lactation at the end of pregnancy, which leads to enhanced gluconeogenesis and protein production in the liver (Goff and Horst, 1997; Grummer et al., 1990) and hence, an energy imbalance. It is speculated that the amplified energy imbalance is a risk factor in animals that contributes to the development of a number of periparturient diseases (Meijer et al., 1995).

*Corresponding author. E-mail: oikawa@vmas.kitasato-u.ac.jp. Tel: +81-176-24-9438. Fax: +81-176-23-8703.

Abbreviations: AA, Amino acid; **Ala,** alanine; **ALB,** albumin; **Arg,** arginine; **Asn,** asparaginic acid; **Asp,** asparagines; **AST,** asparate aminotransferase; **BHB,** β-hydroxybutyrate; **EAA,** essential amino acid; **FAAs,** free amino acids; **FAs,** fatty acids; **FFAs,** free fatty acids; **GL,** glucose; **Gln,** glutamic acid; **Glu,** glutamine; **Gly,** glysine; **His,** histidine; **Ile,** isoleucine; **Leu,** leucine; **Lys,** lysine; **Met,** methionine; **Orn,** ornithine; **Phe,** phenylanine; **Pro,** proline; **Ser,** serine; **T-AA,** total amino acid; **TChl,** total cholesterol; **T-EAA,** total essential amino acid; **Thr,** threonine; **T-NEAA,** total non-essential amino acid; **TP,** total protein; **Trp,** tryptophane; **Tyr,** tyrosine; **Val,** valine; **γ-GTP**, γ-glutamyl transpeptidase.

When viewed from the standpoint of environmental factors (that is, non-biological factors), it is speculated that the feeding method during the periparturient period, such as feeding in excess of the metabolic capacity of cows or a lack of feeding, consequently serves as a dietary risk factor that jeopardizes the maintenance of homeostasis in the cows (Doepel et al., 2002). Many examples develop, owing to pathological conditions induced by an imbalance between the input and output of metabolites in the body (Bell, 1995; Doepel et al., 2002; Itoh et al., 1998; Meijer et al., 1995). In other words, abnormal changes in the homeostasis of metabolism during the periparturient period act as predictors in cows with a genetic predisposition for super efficient milk-producing ability, and are under the control of the periparturient feeding method (that is, environmental factors) (Doepel et al., 2002).

However, because there is less knowledge about AA metabolism in relation to energy metabolism in clinically healthy cows than there is about sugar and lipid metabolism, the relationship between periparturient diseases and AA metabolism is unclear (Hamana et al., 2010). The purpose of this study was to observe the kinetics of blood FAAs in healthy periparturient cows simultaneously with the concentrations of biochemical constituents in plasma. The data obtained will be used as the basis for further studies of various diseases frequently observed in periparturient cows from the viewpoint of AA metabolism.

DESIGN AND METHODS

Experimental period and cows used in this study

The experiment was conducted from October 2004 to June 2005. Six Holstein cows, considered to be clinically healthy (57.7 ± 24.5 months old) with no feed leavings from 1 month before to 2 months after delivery were chosen from among 55 female cows. Cows were raised on a farm with a herd-corrected milk yield of 10,000 kg in Maebashi City, Gunma Prefecture, using the tie-stall system.

Feeding conditions

The feeding status of the cows on the farm is shown in Table 1. Concentrate feeds were separately given to the cows twice per day in accordance with their milk yield during their lactating period. While lactating, 7 kg of grass hay and 3 kg of grain feeding were given to the all the cows. Minerals were also provided once per day. During the first half of the non-lactating period, 10 kg or more of grass hay was given to the cows. During the second half of this period, concentrate feeds were given from 3 weeks before the estimated date of delivery with gradual increases to 2 to 3 kg, in addition to the roughage feed.

Collection of blood samples and their treatment

Blood samples were collected from the jugular vein on seven times occasions 30, 20, and 10 days before the estimated date of delivery (herein after referred to as "day -30," "day -20, " and "day-10", respectively), and 1, 15, 30, and 60 days after the delivery (herein after referred to as "day +1," "day +15," day "+30," and "day +60", respectively). Blood samples were collected between 3:00 and 4:00 p.m. The blood drawn from a vein was dispensed into a heparin-containing vacuum blood collection tube and a vacuum blood collection tube with sodium fluoride and ethylenediamine tetraacetic acid (EDTA•Na-F) (for blood sugar measurement). These test tubes were placed in ice-cold water immediately after blood collection until before plasma separation.

The plasma was separated by centrifugation at 3,000 rpm for 10 min within 45 min of blood collection and dispensed into plastic containers. The plasma and serum were then frozen at -80°C until analysis with the use of the method described by Hamana et al. (2010).

Table 1. Feed salary situation of the dairy farm.

Parameter	Weight of ration (kg)	
	Close-up dry	Lactation
Ingredients of diet	Period	Period
Timothy Hay	10	4[1]
Alfalfa Hay		3
Brewer's grain		3
Beet pulp		3
Concentrate mix[2]	2-3	14
Supplement mix[3]		1
Mineral[4]		0.15

Body weight: 650 kg, milk yield: 40 kg/day.[1]Satiation state; [2]TDN/DM 72.0%, CP/DM 16.5%; [3]TDN/DM 75.0%, CP/DM 25.0% and [4]Ca/DM 22.5%, P/DM 18.0%.

Measurement of biochemical constituents in plasma

Biochemical constituents in the plasma measured using an automatic blood analyser (Hitachi 7170, Hitachi, Limited, Tokyo). The analysed blood components included AST, γ-GTP, GL, FFAs, T-chl, BHB, TP and ALB.

Measurement of blood FAA concentrations

The blood FAA concentrations were measured by high-performance liquid chromatography using a reverse-phase distribution column with an automatic AA analyser (JIC-300, JEOL Limited, Tokyo). The concentrations of the following fractionated AAs in blood were determined: ten essential AAs, namely, Val, Leu, Ile, Trp, Phe, Met, Thr, Arg, His, and lLys, and 10 non-essential AAs, namely, Tyr, Ala, Gly, Ser, Pro, Glu, Gln, Asp, Asn, and Orn. In addition, T-AA, T-EAA, and T-NEAA concentrations were determined.

Statistical analyses

Data are expressed as the mean ± standard deviation. The concentration on day -30 was used as a reference (day -30: reference point) and compared with that for each subsequent blood collection.

For comparison, analysis of variance was performed after confirmation of the normality and equality of variance of each measured value, and multiple comparisons were carried out using Tukey's method with a significance level of less than 5%.

Table 2. Change of plasma biochemistry component concentration in the periparturient healthy cow.

Item	AST (IU/L)	γ-GTP (IU/L)	GL (mg/dl)	FFA (μEq/L)	T-chl (mg/dl)	BHB (μmol/L)	TP (g/dl)	ALB (g/dl)
-30days	58.4±11.9[a]	25.1±6.2	61.7±2.5[a]	107.5±38.0[a]	115.8±48.1[a]	460.2±149.4[a]	7.3±0.5[a]	3.4±0.3
-20days	54.3±9.9	24.0±5.7	60.8±2.9	185.2±241.6	88.2±8.6	500.3±136.1	7.1±0.5	3.3±0.3
-10days	53.8±7.3	22.4±6.4	62.2±3.0	211.5±225.9	75.3±6.6	523.0±57.5	7.0±0.5	3.4±0.3
+1day	69.2±8.0	22.2±5.9	62.3±6.7	352.5±169.4[b]	59.3±4.3	492.7±86.9	6.4±0.4[b]	3.3±0.3
+15days	92.4±23.3[c]	27.6±7.2	54.2±3.5[b]	361.5±141.0[b]	111.5±54.0	841.2±330.1[c]	7.1±0.7	3.3±0.3
+30days	78.8±17.4[b]	29.2±8.6	58.5±4.5	157.2±64.1	188.5±56.3[b]	557.0±140.1	7.6±0.5	3.5±0.2
+60days	72.4±12.3	30.1±9.7	61.5±2.7	99.5±26.9	248.2±56.73[c]	543.3±150.1	7.5±0.5	3.5±0.3

Days in periparturient period: -30; 30 days before parturition, +30; 30 days after parturition. Values are expressed as mean ± SD, Tukey's method (a to b: $P < 0.05$, a to c: $P < 0.01$)

RESULTS

Concentrations of plasma biochemical constituents

Concentrations of plasma biochemical constituents during periparturient period were shown in Table 2.

Aspartate aminotransferase (AST)

The plasma AST concentration tended to be constant before delivery, relative to the reference point. However, it tended to increase from day +10 and was significantly higher on days +15 ($p < 0.01$) and +30 ($p < 0.05$) and then was slightly higher (p = 0.10) on day +60.

Glucose (GL)

The plasma GL concentration was almost constant from the reference point to day +1 but was significantly lower on day +15 ($p < 0.05$), followed by slightly higher (p = 0.10) levels on days +30 and +60.

Free fatty acid (FFA)

The plasma FFA concentration increased gradually as time passed and was significantly higher on days +1 and +15 ($p < 0.05$), after which it tended to decrease until day +60.

Total cholesterol (T-chl)

The plasma T-chl concentration decreased gradually as time passed, reaching the lowest level on day +1, after which it tended to increase and became significantly higher on days +30 ($p < 0.05$) and +60 ($p < 0.01$).

β-Hydroxybutyrate (BHB)

The plasma BHB concentration increased gradually as time passed and was significantly higher on day +15 ($p < 0.01$), after which it tended to decrease until day +60.

Total protein (TP)

There were no marked changes in the periparturient plasma TP concentration other than it being significantly lower on day +1 ($p < 0.05$).

γ-Glutamyl transpeptidase (γ-GTP) and albumin (ALB)

No significant changes were observed in the periparturient γ-GTP or ALB concentrations in the plasma.

Blood free fatty acids (FAA) concentrations

Blood FAA concentrations during periparturient period were shown in Table 3.

Total amino acid (T-AA) and total essential amino acid (T-EAA)

The blood T-AA and T-EAA concentrations decreased

Table 3. Blood free amino acids concentration in the periparturient cow.

Day	Amino acid (AA) (μmol/L)		
	T-AA	T-EAA	T-NEAA
-30	1944.5±84.9[a]	872.0±44.2[a]	1072.5±80.6[a]
-20	1923.3±158.5	842.0±76.5	1081.3±98.8
-10	1894.1±117.7	815.2±68.2	1079.0±82.5
+1	1815.9±317.2	642.6±160.7[b]	1173.2±168.0
+15	2204.5±169.3	924.2±114.6	1280.3±99.2[b]
+30	2284.1±383.9	1023.7±211.1	1260.4±196.2
+60	2424.2±105.0[c]	1191.9±76.5[c]	1232.3±53.2

	Essential amino acid (EAA) (μmol/L)				
	Val	Leu	Ile	Trp	Phe
-30	236.4±21.1[a]	135.2±8.5[a]	111.7±8.0[a]	36.5±5.5[a]	52.3±5.4
-20	226.7±19.5	129.2±11.8	112.4±12.5	36.6±5.4	49.2±2.7
-10	228.5±20.1	127.5±14.7	111.9±12.2	34.2±7.0	50.6±4.8
+1	140.2±37.9[c]	90.7±24.8[b]	81.4±23.4	33.9±6.0	43.3±8.4
+1	259.9±24.5	148.5±24.6	140.2±41.5	37.4±6.8	47.4±7.7
+30	287.9±50.3	155.8±38.9	137.1±33.2	44.8±7.5[b]	52.8±13.2
+60	335.9±52.5[c]	194.3±16.4[c]	158.1±16.2[b]	53.3±7.1[c]	58.5±7.1

	Essential amino acid (EAA) (μmol/L)				
	Met	Thr	Arg	His	Lys
-30	26.7±5.6	53.1±10.2[a]	68.4±7.3	68.2±3.0	83.5±9.5
-20	26.6±3.9	47.6±11.9	65.4±8.3	64.2±9.3	84.1±18.7
-10	25.5±1.9	46.4±4.1	62.3±10.7	61.5±5.8	66.8±18.6
1	26.7±6.2	49.8±21.9	54.6±18.4	59.6±20.3	62.4±19.9
15	25.9±6.6	69.2±18.4	62.1±9.2	58.5±8.0	75.0±20.9
30	29.5±5.6	91.7±26.5[c]	75.7±19.1	58.8±15.6	89.6±19.3
60	29.1±3.7	103.9±16.5[c]	87.9±8.2	67.4±10.6	103.6±7.4

	Non-essential amino acid (NEAA) (μmol/L)				
	Tyr	Ala	Gly	Ser	Pro
-30	51.5±6.8[a]	216.6±17.8	218.3±39.5[a]	65.0±10.2[a]	64.1±7.6[a]
-20	48.3±4.0	208.8±37.7	244.2±39.7	68.5±9.5	60.1±10.3
-10	46.6±6.5	200.6±28.1	258.4±47.3	72.9±13.4	57.3±6.2
1	41.7±10.6	168.3±52.9	347.2±105.0[b]	113.6±21.2[c]	54.6±16.8
15	52.8±9.7	203.4±62.3	498.6±85.6[c]	111.1±17.8[c]	76.4±18.9
30	67.8±19.4	272.5±69.9	379.0±69.4[c]	99.7±23.7[b]	92.4±25.1[b]
60	73.1±8.2[c]	288.6±65.8	310.8±38.3	89.3±11.7	96.5±15.9[c]

	Non-essential amino acid (NEAA) (μmol/L)				
	Glu	Gln	Asp	Asn	Orn
-30	65.4±13.0[a]	319.7±35.0[a]	5.0±0.9	23.6±3.9[a]	43.4±5.1[a]
-20	67.4±19.2	316.1±45.3	5.1±1.1	23.9±2.3	38.8±7.3
-10	62.9±17.5	318.0±44.9	4.5±1.2	21.6±3.9	36.2±5.5
+1	30.5±15.5[c]	364.0±38.6	5.5±1.9	28.5±10.6	19.4±7.1[b]
+15	26.6±3.7[c]	236.5±46.9[b]	6.5±2.4	40.3±10.4[b]	28.2±6.3[c]
+30	26.5±5.3[c]	236.0±39.0[b]	4.8±1.2	46.5±16.2[c]	35.3±7.0
+60	26.8±5.4[c]	250.2±1.2	3.9±0.6	49.4±6.7[c]	43.7±5.5

Date are expressed as mean ± SD, Tukey's method (a to b: $P < 0.05$, a to c: $P < 0.01$).

gradually as time passed, reaching the lowest on day +1 (T-EAA: $p < 0.05$). However, after that, these concentrations increased and were higher on days +15 and +30 than those at the reference point and was significantly higher on day +60 than at the reference point and were significantly higher on day +60 than at the reference point ($p < 0.01$).

Total non-essential amino acid (T-NEAA)

The blood T-NEAA concentration was almost constant before delivery tended to increase from day +1, and peaked on day +15 ($p < 0.05$). After that, the blood T-NEAA concentration tended to decrease but remained slightly higher on days +30 and +60 than at the reference point.

Val, Leu, Ile, Trp, Thr, Arg, Lys, Tyr, Ala, Pro, Asn, and Orn

The blood concentrations of these AAs showed changes similar to those for the blood T-AA concentration.

Glutamine (Glu) and glutamic (Gln)

The blood Glu and Gln concentrations were almost constant before delivery. However, on day +1, the blood Glu concentration significantly decreased to approximately 50% of that at the reference point ($p < 0.01$) and remained significantly lower until day +60 ($p < 0.01$). The blood Gln concentration was higher on day +1 by approximately 10% of that at the reference point. The blood Gln concentration was significantly lower from days +15 to +60 ($p < 0.05$) and remained lower until day +60 relative to the concentration at the reference point.

Glysine (Gly) and serine (Ser)

The blood Gly and Ser concentrations increased gradually compared with those at the reference point as time passed, peaking on day +1 (Gly: $p < 0.05$; and Ser: $p < 0.01$). After day +1, they tended to decrease until day +60. However, the blood Gly and Ser concentrations remained significantly higher on days +15 (Gly and Ser: $p < 0.01$) and +30 (Gly: $p < 0.01$; and Ser: $p < 0.05$) than those at the reference point.

Asp, Phe, Met, and His

No significant changes were observed in the blood Asp, Phe, Met, or His concentration.

DISCUSSION

The plasma FFA concentration was higher and the plasma TP concentration was lower on day +1 than on day -30. The plasma GL concentration was lower and the plasma FFA and BHB concentrations were higher on day +15 than on day -30, and the plasma T-chl concentration was higher on days +30 and +60 than on day -30. The changes in the plasma FFA, TP, BHB and T-chl concentrations suggest that the nutritional condition of the cows declined gradually as time passed relative to that on day -30. The cows continued to be in the low-energy state from day +1 to day +30 after delivery and recovered to the high-energy state on day +60 is the same as on day -30. These findings are similar to the findings of the concentrations of the plasma GL (Doepel et al., 2002; Meijer et al., 1995; Ohtsuka et al., 2006; Park et al., 2010), the plasma T-chl (Ohtsuka et al., 2006) and the plasma BHB (Doepel et al., 2002) in healthy periparturient cows.

Regarding the changes in the periparturient blood FAA concentrations, the concentrations of T-AA, T-EAA, Val, Leu, Ile, Trp, Thr, Arg, Lys, Tyr, Ala, Pro, Asn, and Orn decreased during the second half of the non-lactation period, and these concentrations increased after delivery. These changes in the periparturient blood AA concentrations are thought to be due to the increased AA requirement due to the rapid growth of foetuses and the placenta at the end of pregnancy and a decrease in feed consumption (Goff and Horst, 1997). It was suggested that decreased T-AA was resulting from enhancement of requirement of fetus growth and depressed feed intake in the current study (Goff and Horst, 1997).

On the other hand, after delivery, homeostatic activity in the body in response to an energy shortage and the increased amount of feed consumed corresponding to the increase in lactation were considered to have contributed to the increase in the blood AA concentrations (Reis and Tunks, 1979; Rodwell, 2006). Certain EAAs and NEAAs that exhibited the above-mentioned changes as shown in Table 3 are considered to be consumed at an accelerated rate during the energy shortage state during the periparturient period, leading to a decrease in the concentrations of these AAs in the blood.

In contrast to the above mentioned changes as shown in Table 3, the concentrations of Gly and Ser, which are NEAAs, began to increase from before delivery and became higher after delivery than before delivery. The increase in the blood Gly concentration from before delivery is considered to be due to changes in the compensation for AA shortfalls associated with increased energy requirements owing to the utilisation of energy from muscle tissue (Rodwell, 2006; Shibano et al., 2006). Ser is synthesized from 3-phosphoglyceric acid, a glycolic intermediate, similar to Gly, and there is a reversible catabolic response between Gly and Ser. Hence, similar changes were considered to have been exhibited (Stengärde et al., 2008). Regarding the concentrations of blood Gly and Ser on day +15, the blood Gly and Ser concentrations accounted for higher percentages of the T-NEAA concentration than other NEAAs.

It is thought that the higher blood T-NEAA concentration on day +15 than on day -30 was mainly due to the higher concentrations of Gly and Ser. Therefore, Gly and Ser were considered to belong to a group of AAs whose concentrations increase to compensate for energy shortages during the periparturient period. On the other hand, the blood concentrations of Glu and Gln, which are NEAAs, continued to be low after delivery. These are AAs taken into the mammary gland tissue (Reis and Tunks, 1979). Because Glu and Gln constitutes 25 to 30% of casein, which is the major milk protein component (Meijer et al., 1995) and these AA are indicative of muscle protein mobilization for supply of the AA to the udder (Doepel et al., 2002). Hence, the changes in the blood Glu and Gln concentrations were considered to reflect the uptake of these AAs into the mammary gland tissue.

There have been reports not only in Japan but also overseas that showed changes in blood AA concentrations after delivery in periparturient cows that are similar to our results (Reis and Tunks, 1979; Shibano et al., 2005). It was confirmed from these reports that the kinetics of AA metabolism in periparturient cows showed a largely similar trend, regardless of the area or environment. The metabolism of AAs for energy generation in periparturient cows showed changes similar to those of sugar and fat.

The results can be a starting point for analyzing the pathogeneses of periparturient diseases that are associated with NEB from the viewpoint of AA metabolism.

REFERENCES

Bell AW (1995). Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804-2819.

Doepel L, Lapierre H, Kennelly J (2002). Peripartum performance and metabolism of dairy cow in response to pre-partum energy and protein intake. J. Dairy Sci. 85:2315-2334.

Goff JP, Horst RL (1997). Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci. 80:1260-1268.

Grummer RR, Bertics SJ, Lacount DW, Snow JA, Dentine MR, Stauffacher RH (1990). Estrogen Induction of fatty liver in dairy cattle. J. Dairy Sci. 73:1537-1543.

Hamana M, Ohtsuka H, Oikawa M, Kawamura S (2010). Blood free amino acids in the postpartum dairy cattle with left displaced abomasum. J. Vet. Med. Sci. 72:1355-1358.

Itoh N, Koiwa M, Hatsugaya A, Yokota H, Taniyama H, Okada H, Kudo K (1998). Comparative analysis of blood chemical values in primary ketosis and abomasal displacement in cows. J. Vet. Med. A. 45:293-298.

Meijer GAL, Van Der Meulen J, Bakker JGM, Van Der Koelen, Van Vuuren AM (1995). Free amino acids in plasma and muscle of high yielding dairy cows in early lactation. J. Dairy Sci. 78:1131-1141.

Ohtsuka H, Watanabe C, Kohiruimaki M, Ando T, Watanabe D, Masui M, Hayashi T, Abe R, Koiwa M, Sato S, Kawamura S (2006). Comparison of two different nutritive conditions against the changes in peripheral blood mononuclear cells of periparturient dairy cows. J. Vet. Med. Sci. 68:1161-1166.

Park AF, Shirley JE, Titgemeyer EC, Cochran RC, DeFrain JM, Wickersham EE, Johnson DE (2010). Characterization of plasma metabolites in Holstein dairy cows during the periparturient period. Int. J. Dairy Sci. 5:253-263.

Reis PJ, Tunks DA (1979). Changes in plasma formaldehyde-treated casein. Aust. J. Biol. Sci. 23:673-680.

Rodwell VW (2006). Catabolism of the carbon skeletons of amino acids. In: Murray RK, Granner DK and Rodwell VW (eds.), Harper's Illustrated Biochemistry, 27th edn. McGraw-Hill, New York. pp. 254-269.

Shibano K, Kawamura S, Hakamada R, Kawamura Y (2006). The relationship between changes in serum glycine and alanine concentrations in non-essential amino acid and milk production in the transition period in dairy cows. J. Vet. Med. Sci. 68:393-396.

Stengärde L, Tråvén M, Emanuelson U, Holtenius K, Hultgren J, Niskanen R (2008). Metabolic profiles in five high-producing Swedish dairy herds with a history of abomasal displacement and ketosis. Acta Vet. Scand. 50:31.