Journal of Advanced Veterinary Research

http://advetresearch.com/index.php/avr/index

Comparison between Serum and Saliva Biochemical Constituents in Dairy Cows during Lactation and Dry Period

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ARTICLE INFO

Original Research

Accepted:
24 June 2015

Keywords:

Cows
Dry period
Lactation
Milk
Serum
Saliva

ABSTRACT

The present study was undertaken to compare serum and salivary biochemical constituents during lactation and dry period in dairy cows. Also, the present study evaluated for the first time the salivary biochemical constituents in dairy cows. The study was carried out using 45 healthy multiparous Holstein cows maintained in dairy farms located in Morioka city (Iwate prefecture, Japan). Cows were classified into groups based on the month of lactation. Serum, saliva and milk samples were collected and analyzed. Data were statistically analyzed and the variation in serum and salivary biochemical constituents during lactation and dry period were discussed. From the present study, it could be concluded that the 1st month of lactation has the highest levels for serum free fatty acids (FFA), β- Hydroxy butyric acid (BHBA) and aceto Acetic acid (ACAC). The dry period has the highest serum glucose level and the lowest serum FFA, BHBA and aspartate aminotransferase levels. Both serum and salivary FFA showed the highest value during the 1st month of lactation. Saliva contains a high level of gamma glutamyl transferase. The level of ammonia in saliva is higher than its serum level during all months of lactation and dry period. Most of the biochemical constituents in saliva change in different way from serum during lactation and dry period. Milk protein/fat ratio of 0.7 may be not indicative for subclinical ketosis.

Introduction

At the onset of lactation, the nutrients requirements increase faster than the increase in feed intake. Thus energy balance represents a challenge for dairy cows in early lactation. Most metabolic diseases in dairy cows occur during the first two weeks postpartum (Goff and Horst, 1997), and a wide range of factors (animal, management and feed) may lead to such problems. Negative energy balance, fat mobilization and consequent increase in ketone bodies concentrations play a contributing role in fatty liver syndrome, clinical ketosis, and abomasal displacement. A negative energy balance may also increase the risk of retained placenta, metritis, and mastitis through impaired immune function. In addition, nitrogen balance and calcium homeostasis are disrupted through parturition. Therefore, measuring biochemical parameters may be useful for monitoring cows during the lactation period (Carson, 2008).

Blood profiles have frequently been used to assess nutrient status of cows during lactation and transition period (Payne *et al*., 1970; Blowey, 1975; Ingraham and Kappel, 1988; Ward *et al*., 1995; Kida, 2002). Blood profiles included glucose, proteins, minerals, free fatty acids (FFA) and β-hy-

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ISSN: 2090-6277/2090-6269/

droxybutyrate (BHBA), which are considered useful to identify nutritional shortcomings even before the productivity is impaired (Whitaker, 2004). Such profiles have also been used to monitor herd health and to find subclinical disease, to predict risk of ketosis or abomasal displacement as well as investigate herd problems with metabolic disorders (Geishauser *et al*., 1997; Oetzel, 2004; LeBlanc *et al*., 2005).

Metabolic profile is usually conducted on blood samples. According to our knowledge, measuring the biochemical variables for the metabolic profile in saliva have not been done before in cattle. Saliva offers an alternative to serum as a biologic fluid that can be analyzed for diagnostic purposes. Whole saliva contains locally produced as well as serum-derived components that have been found to be useful in the diagnosis of a variety of systemic disorders. This study aimed to compare serum and salivary biochemical constituents during lactation and dry period in dairy cows.

Materials and methods

The study was carried out on 45 healthy multiparous Holstein cows maintained in dairy farms located in Morioka city (Iwate prefecture, Japan). The animals were divided into eight groups. The first group (n.=6) comprised cows during the first month of lactation. The second group (n.=5) included cows during the second month of lactation, with an average milk yield 36.94±10.32 kg/animal/day. The third group (n.=6) included cows during the 3rd month of lactation with an average milk yield 41.92±7.56 kg/animal/day. The fourth group (n.=5) included cows during the 4th to 5th month of lactation with an average milk yield 37.72±6.47 kg/animal/day. The fifth group (n.=4) comprised cows during the 6th to 7th month of lactation with an average milk yield 43.07±8.49 kg/animal/day. The sixth group (n.=3) comprised cows during the 8th month of lactation with an average milk yield 31.20±8.40 kg/animal/day. The seventh group (n.=5) comprised cows during the 8th month of lactation with an average milk yield 30.22±4.67 kg/animal/day. The eighth group (n.=11) included cows during the dry period.

Sample collection and analytical procedures

Blood samples were obtained from the jugular vein in evacuated (Vacutainer) tubes without anticoagulant. Tubes were allowed to clot at room temperature for 30 minutes before centrifugation at 2000 rpm for 20 min (Coles, 1986), serum samples were harvested in eppendorf tubes and frozen at -80 ºC until analysis.

Saliva samples were collected from all cows in a clean, sterile and dry plastic tube from the buccal cavity. The saliva samples were centrifuged at 10,000g for 30 minutes at 4 ºC and then the supernatant was stored at -80 °C until analysis (Rai *et al*., 2010).

Milk samples were obtained in sterile tubes, and analyzed at the same day of collection.

Measurement of serum constituents

Serum metabolic profile included measurement of FFA (µEq/l), glucose (mg/dl), total cholesterol (mg/dl), phospholipids (PL, mg/dl), low density lipoprotein (LDL-C, mg/dl), β- Hydroxy butyric acid (BHBA, mmol/L), aceto Acetic acid (ACAC, mmol/L), albumin (g/dl), blood urea nitrogen (BUN, mg/dl), calcium (mg/dl), aspartate aminotransferase (AST, I.U./l), gamma glutamyl transferase (GGT, I.U./l), ammonia (NH3, µg/dl) and lactic acid (mg/dl). Serum analyses were carried out using Autoanalyzer (Automatic autoanalyzer 7060, Hitachi, Japan).

Measurement of saliva biochemical variables

Saliva biochemical constituents included measurement of FFA, PL, BHBA, ACAC, albumin, BUN, calcium, AST, GGT, NH3 and lactic acid. Saliva analyses were carried out using Autoanalyzer (Automatic autoanalyzer 7060, Hitachi, Japan).

Analyses of milk

Milk samples were collected by hand milking. The compositions of the milk (Fat%, protein%, protein/fat (P/F) ratio, somatic cells count) were analyzed using infrared spectroscopy (Milko-Scan Sys4000, Foss, Denmark). The use and care of all animals in this study were approved by the Animal Care and Use Committee of Iwate University, Morioka, Japan.

Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA). Groups were tested for difference using analysis of variance LSD Post-hoc test. Comparison of biochemical constituents in serum and saliva was carried out using one way analysis of variance. Statistically significant differences were determined at $P \leq 0.05$.

Results

Serum metabolic profile during lactation and dry period

During the 1st month of lactation, serum FFA level showed a highly significant increase than its level during other months of lactation and dry period. The lowest serum FFA level (57.8±6.7 µEq/l) was recorded during the 9th month of lactation. The highest serum glucose concentration (66.3±6.8 mg/dl) was observed during the dry period, which was significantly higher than serum glucose during lactation. The lowest glucose concentration was observed during the 1st and 3rd month of lactation. Serum total cholesterol, PL and LDL-C levels started to increase from the lowest level (75.3±17.9, 104.3±33.7 and 5.2±3.1 mg/dl respectively) on the 1st month of lactation, and reach the highest levels (244.0±55.2, 258.4±54.5 and 17.1±4.1 mg/dl respectively) at the 6th -7th month of lactation.

Serum BHBA and ACAC levels were significantly higher at the 1st month of lactation (1039.3±218.9 and 68.16±25.69 mmol/l respectively) than the other investigated lactation period and also higher than their levels during the dry period. The lowest serum BHBA and ACAC levels were observed during the dry period (476.7±137.3 and 14.6±7.0 mmol/l respectively). Serum AST level during the dry period (64.8±11.4 I.U./l) showed a significant decrease when compared with level during the 1st, 3rd, 4th and 5th months of lactation. The highest serum level (87.7±28.9 I.U./l) of AST was observed during the 1st month of lactation. Serum level of GGT was significantly higher at 4th-5th month of lactation when compared with its level during the 1st month of lactation and during the dry period.

Serum NH_3 level was significantly higher at the 1st month of lactation than its level during other lactation period except during the 2nd month of lactation and during the dry period. The highest serum level of lactic acid (9.2±7.2) was observed during the 6th-7th month of lactation, which was significantly higher than its serum level during the 2nd and 3rd month of lactation (Table 1).

Salivary biochemical constituents during lactation and dry period

The highest salivary FFA level was reported during the 1st month of lactation. There was a significant difference in the salivary FFA level only between the 1st and 8th month of lactation. The highest salivary level (7.9±6.5 mg/dl) of PL was observed during the 9th month of lactation, which was significantly higher than the observed levels during other months of lactation and during the dry period. Salivary PL level was significantly higher at the 3rd month of lactation (4.2±3.2 mg/dl) than its level during the 6th – 7th months of lactation and during the dry period.

Salivary BHBA and ACAC levels were significantly higher at the 2nd month of lactation (38.3±16.8 and 39.7±28.1, respectively) than the other lactation period and also higher than their levels during the dry period. The highest salivary BUN level was observed during the 1st month of lactation, which was significantly higher than the measured levels at the 2nd and 9th months of lactation and also during the dry period. Salivary calcium was the highest (19.5±6.7 mg/dl) during the 1st month of lactation, which was significantly higher than its level during other lactation period and during the dry period.

At the 2nd month, salivary AST showed the highest significant increase (11.2±8.2 I.U/l) than its level during lactation and dry periods. Salivary GGT level showed the highest value (28.2±14.5 I.U./l) during the dry period, which was significantly higher than its level during the 4th- 5th month of lactation.

The highest level for NH_3 level in saliva was observed during the 1st month of lactation (891.6±677.0 ug/dl), which was significantly higher than salivary levels during the 4th-5th and 8th month of lactation and also during the dry period. The highest salivary level of lactic acid was observed during the 1st month of lactation (4.3±3.3

Table 1. Serum and salivary biochemical constituents in lactating cows during lactation and dry period

Variables	Sample	Months of Lactation							Dry period
		1st (n.=6)	2nd (n.=5)	3rd (n.=6)	4th-5th (n.=5)	6th-7th (n.=4)	8th (n.=3)	9th (n.=5)	(n.=11)
Free Fatty Acids (µEq l)	Serum	497.5±266.5a	131.4±53.4b	119.2±29.9b	71.4±9.7b	70.25±12.82b	99.3±4.5b	57.8±6.7b	77.7±22.7b
	Saliva	48.0±28.0a**	36.0±12.8ab**	37.5±5.9ab**	39.0±9.0ab**	48.8±15.8ab**	20.7±6.4b**	42.7±23.9ab	41.6±13.5ab**
Glucose (mg dl)	Serum	53.8±6.8a	60.5±4.0b	52.8±5.4a	57.8±2.1ab	59.2±3.3ab	61.4±5.3b	56.1±3.9ab	66.3±6.8d
	Saliva	-	-	-	-	-	-	-	-
T. Cholesterol (mg dl)	Serum	75.3±17.9a	169.4±9.9b	197.8±36.7b	242.0±58.8cd	244.0±55.2d	187.3±22.1b	193.8±40.8b	88.7±6.6a
	Saliva	-	-	-	-	-	-	-	-
Phospholipids (mg dl)	Serum	104.3±33.7a	182.6±17.5b	216.7±37.8bc	258.4±54.5cd	268.5±66.9d	215.0±20.1c	214.4±43.7bc	107.7±14.8a
	Saliva	3.7±2.1ab**	1.8±1.9ab**	4.2±3.2a**	2.1±0.9ab**	0.8±0.6b**	0.9±1.3ab**	7.9±6.5c**	0.9±0.7b**
LDL-C (mg dl)	Serum	5.2±3.1a	6.5±2.6e	8.8±4.1ae	16.9±6.5c	17.1±4.1c	10.7±0.8de	13.26±3.16cd	8.4±2.9ae
	Saliva	-	-	-	-	-	-	-	-
BHBA (mmol L)	Serum	1039.3±218.9a	681.2±267.9b	726.0±174.0b	730.4±92.4b	709.5±133.3b	640.7±118.1bc	697.2±202.5b	476.7±137.3c
	Saliva	8.7±4.5a**	38.3±16.8b**	1.9±1.8a**	0.0±0.0a**	0.0±0.0a**	0.0±0.0a**	4.1±1.6a**	1.4±1.2a**
ACAC (mmol L)	Serum	68.16±25.69a	35.9±19.5b	30.6±19.0b	29.1±6.9b	26.8±12.2bc	14.0±3.2bc	27.3±13.9bc	14.6±7.0c
	Saliva	0.0±0.0a**	39.7±28.1b	6.7±4.7a**	4.5±2.4a**	8.8±5.7a**	0.0±0.0a**	15.1±7.3ac	0.0±0.0ad**
Albumin (g dl)	Serum	3.5±.6a	3.4±0.6a	3.6±.2a	3.8±0.3a	4.0±0.2a	3.9±0.1a	3.8±0.1a	3.8±0.3a
	Saliva	0.3±0.0a**	0.3±0.1a**	0.4±0.1a**	0.3±0.1a**	0.4±0.1a**	0.3±0.0a**	0.3±0.0a**	0.3±0.0a**
BUN (mg dl)	Serum	11.1±4.3a	8.8±2.9a	11.3±2.3a	12.0±2.5a	12.5±2.4a	13.7±7.9ab	12.1±2.7a	8.8±2.2ac
	Saliva	16.0±7.4ac	5.1±2.1b	8.9±4.8abc	8.5±1.7abc	9.7±5.2abc	14.0±10.7c	7.1±.4b	6.6±1.9b
Calcium (mg dl)	Serum	9.8±1.0a	9.5±0.2ac	9.8±0.4a	10.0±0.7a	10.4±0.6ab	10.5±0.3ab	9.8±0.4a	9.9±0.3a
	Saliva	19.5±6.7a**	1.3±0.5b**	5.7±3.9b	10.0±0.0c	3.2±2.4b**	4.1±3.7b**	8.8±7.3c	3.9±2.1b**
AST (I.U./l)	Serum	87.7±28.9a	80.0±13.1ab	84.8±23.1a	88.2±12.3a	85.8±6.3a	84.7±11.5ab	77.2±8.9ab	64.8±11.4bc
	Saliva	3.1±3.1a**	11.2±8.2b**	4.3±2.6a**	2.1±0.9a**	5.5±3.4a**	2.5±1.8a**	5.8±3.4ab**	3.9±1.6a**
GGT (I.U./l)	Serum	21.5±11.9a	31.6±17.9ab	26.0±6.4abc	33.2±5.9b	30.0±3.5abc	31.0±3.0abc	24.8±6.1abc	21.9±3.4ac
	Saliva	28.5±14.3ab	20.9±7.9ab	12.5±13.3ab	5.8±3.2a**	24.2±13.8ab	24.5±20.5ab	22.7±18.2ab	28.2±14.5b
NH_3 (µg dl)	Serum	37.1±19.7ac	26.1±5.7ad	23.1±10.0b	21.9±2.5b	13.9±5.0db	21.3±21.1b	22.2±4.1b	31.1±5.3cb
	Saliva	891.6±677.0a**	608.4±427.7abc**	482.6±360.9abc**	222.0±12.6b**	649.3±281.9abc**	316.2±220.9bc**	753.9±307.0ac**	337.2±131.7bc**
Lactic acid (mg dl)	Serum	6.1±3.3ab	4.3±2.3a	3.6±0.6a	5.2±3.3ab	9.2±7.2b	7.6±1.4ab	6.9±1.9ab	6.8±3.1ab
	Saliva	4.3±3.3a	0.9±0.8b**	1.5±1.7b	0.4±0.2b**	1.6±1.6b**	2.3±1.4b**	1.4±0.9b**	1.1±0.7b**

Data were expressed as mean±SD,
In each row, data followed by different letters means significant difference
**: ($P<0.01$), refers to significant variations between serum and saliva

mg/dl), which was significantly higher than the observed levels during other months of lactation and during the dry period (Table 1).

Comparison of serum and Salivary biochemical constituents during lactation and dry period

Serum levels of FFA, PL, BHBA, albumin and AST were significantly higher (P<0.01) than their corresponding levels in saliva during all lactation months and also during the dry period. Serum ACAC level was significantly higher (P<0.01) than its level in saliva except during the 2nd month of lactation. There was insignificant change in BUN levels between serum and saliva. Serum calcium level was significantly higher (P<0.01) than saliva level except during the 1st, 3rd-5th and 9th month of lactation. Serum GGT level was significant (P<0.01) only during the 4th-5th month of lactation. On the other hand, there was a significant increase (P<0.01) in salivary NH_3 level when compared with its level in serum. Serum lactic acid was significantly higher (P<0.01) in serum than its level in saliva except during the 1st and 3rd months of lactation (Table 1).

Changes in milk composition during lactation

The lowest volume of milk (30.2±4.7 kg/animal/day) was observed during the 9th month of lactation, which was significantly lower than volume of milk during the 3rd and 6th – 7th month of lactation. Fat % was significantly higher during the 2nd month of lactation than its percent during the 3rd - 5th and 9th months of lactation. Percent of protein during the 9th month of lactation showed a significant increase when compared with protein % during 2nd – 5th months of lactation. Ratio of protein/fat was significantly lower at the 1st month of lactation when compared to its value during the 4th – 7th and 9th months of lactation. The highest somatic cells count was observed during the 8th month of lactation (Table 2).

Discussion

Assessing the metabolic blood profiles may help in investigating the herd problems by demonstrating the severity and timing of disturbance in energy metabolism. In this study, A higher serum FFA level of 497.5±266.5 µEq/l, and the lowest serum glucose level was observed during the 1st month of lactation, the measured FFA level was slightly higher than the reference value of 400 µEq/l used by Whitaker (2004), and indicating that cows subjected to this study were at the border of negative energy balance during the 1st month of lactation. On the other hand, cows at the dry period had the lowest FFA and the highest serum glucose levels. Glucose is the primary metabolic fuel, and is absolutely required for vital organ function, fetal growth, and milk production. In dairy cows, the massive energy demand to support milk production is partly met through gluconeogenesis (Herdt 2000b).

In the present study, the gradual increase in serum total cholesterol, PL and LDL-C levels during lactation, may be attributed to the high demand of the steroidogenic tissues to the cholesterol that being transferred by LDL-C (Strauss *et al*., 1984; Kovanen, 1987).

Results of the present study revealed that the 1st month of lactation had the highest serum levels of FFA, BHBA and ACAC. Circulating concentrations of FFA and BHBA measure the success of adaptation to negative energy balance. Increased levels of ketone bodies indicate that the supply of FFA to the liver exceeds the ability of liver to completely oxidize the fatty acids to supply energy (Herdt, 2000a). However BHBA does not originate

Table 2. Analysis of milk during lactation

	Months of Lactation							Dry period
	1st (n.=6)	2nd (n.=5)	3rd (n.=6)	4th-5th (n.=5)	6th-7th (n.=4)	8th (n.=3)	9th (n.=5)	(n.=11)
Volume (kg/day)	-	36.9±10.3ab	41.9±7.6a	37.7±6.5ab	43.1±8.5a	31.2±8.4ab	30.2±4.7b	-
Fat (%)	-	4.9±1.64a	3.7±0.5b	3.5±0.5b	3.9±1.4ab	3.9±0.0ab	3.6±.4b	-
Protein (%)	-	3.1±0.4a	3.0±.1a	3.1±.3a	3.3±0.3ab	3.4±0.1ab	3.5±0.1b	-
P/F	-	0.7±0.2a	0.8±0.1ab	0.9±0.1b	0.9±0.2b	0.9±0.0ab	0.9±0.1b	-
Somatic Cells (x10^3/ml)	-	74.0±51.7a	34.50±30.6ab	26.0±13.7b	56.0±57.8ab	103.5±62.5ab	47.6±20.5b	-

Data were expressed as mean±SD,
In each row, data followed by different letters means significant difference

only from incomplete oxidation of FFA in the liver but also from butyrate of rumen origin that oxidized to BHBA in the rumen epithelium (Kristensen *et al*., 2000). Although the serum levels of BHBA was the highest at the 1st month of lactation in the investigated animals, but it still below the threshold level for subclinical ketosis (1.4 mmol/l) as suggested by Oetzel (2004). As recorded in this study, the higher metabolism of FFA by the liver was reflected by higher serum AST and NH_3 levels at the 1st month of lactation. AST is an enzyme that becomes elevated with cell damage and may be elevated in cows with fatty liver disease (Geishauser *et al*., 1997). High serum NH_3 level is usually associated with inefficient metabolism by hepatic dysfunction as a result of the fat mobilization during the first month of lactation (Taboada and Dimski, 1995).

According to our knowledge, the present study is the first that studied the biochemical constituents in saliva of cattle. We were able to measure serum biochemical variables in saliva except total cholesterol and LDL-C. This study revealed that FFA, PL, BHBA, ACAC, BUN, Calcium, AST, GGT, NH_3 and lactic acids were secreted by the salivary gland in saliva. In human medicine, saliva has become popular as a medium for the measurement of various biomolecules (Tabak, 2001; Lawrence, 2002; Streckfus and Bigler, 2002).

Results obtained from this study revealed that salivary FFA level was the highest during the 1st month of lactation, which indicated that higher serum level of FFA is reflected on increased secretion of FFA in saliva. Lipids and fatty acids were extensively studied in human saliva (Vining and McGinley, 1987; Ellison, 1993; Aardal and Holm, 1995; Castro *et al*., 2000). Salivary PL level was high at the 9th month of lactation, which was significantly higher than its level during the dry period (Table 1). Although, results for serum BHBA and ACAC levels were the highest during the 1st month of lactation, the highest salivary levels for these two variables were observed during the 2nd month of lactation. Urine and milk are the most used fluids for measuring BHBA and ACAC to detect subclinical ketosis (Krogh *et al*., 2011). However, according to Oetzel (2004), milk and urine are lower than blood BHBA in both sensitivity and specificity. It was suggested from results of the present study that salivary BHBA and ACAC levels are not sensitive indicators for blood ketone bodies.

As shown in Table 1, salivary BUN and calcium levels showed the highest level at the 1st month of lactation, salivary AST and GGT levels were higher at the 2nd month of lactation and dry periods respectively, which were different from that observed in serum. However, salivary NH_3 level was behaved the same as in serum. Comparing serum and salivary levels of the studied biochemical parameters revealed that, some of these parameters were significantly higher in serum than saliva as FFA, PL, BHBA, albumin and AST. Results also revealed that ACAC, calcium and GGT showed significant increases in saliva than their levels in serum during certain period of lactation. On the other hand, salivary NH_3 level was significantly higher than serum level during lactation and dry period. From this study, it is not clear the cause that stand behind the variation in the levels of salivary biochemical constituents when compared to corresponding levels in serum.

During this study, the amount of milk produced was increased from the 2nd month of lactation and reached the peak (43.1±8.5 kg/animal/day) at the 6th to 7th month of lactation, and then decreased with progress in lactation till the lowest value (30.2±4.7kg/animal/day) at the 9th month of lactation. The lowest value (0.7±0.2) for milk P/F ratio was observed during the 1st month of lactation. Previous studies had been shown that milk P/F is a useful indicator of subclinical ketosis (Duffield *et al*., 1997). Based on a study by Duffield and Bagg (2002), the risk of ketosis arises when cows had a P/F ratio < 0.75. In the present study, although milk P/F ratio was lower than 0.75, serum BHBA level was lower than the threshold level (1400 µmol/l) for subclinical ketosis as suggested by Oetzel (2004). It was suggested from the present study that a P/F ratio of 0.7 may be not indicative for subclinical ketosis. Results of the current study revealed that the highest somatic cells count (103.5±62.5 x103) was observed during the 8th month of lactation. It was reported that somatic cell count in milk from healthy cow should be lower than 200,000 cells/ ml (Dohoo and Leslie, 1991). Somatic cell count between 200,000 and 300,000 cells/ml is indicative of an initial stages of infection (Smith, 1996). The above two studies indicated the absence of subclinical mastitis during lactation in the investigated cows (Table 2).

Conclusion

The 1st month of lactation has the highest levels for serum FFA, BHBA and ACAC. The dry period has the highest serum glucose level and the lowest serum FFA, BHBA and AST levels. Both serum and salivary FFA showed the highest value during the 1st month of lactation. Saliva contains a high level of GGT. The level of NH_3 in saliva is higher than serum levels during all months of lactation and dry period. Most of the biochemical constituents in saliva change in different way from serum during lactation and dry period. Milk P/F ratio of 0.7 may be not indicative for subclinical ketosis.

Acknowledgement

The authors would like to thank all members at Co-Department of Animal Medicine, Faculty of Agriculture, Iwate University, Japan for providing all the required facilities for research. Many thanks and sincere gratitude for the Ministry of Higher Education, Egypt, for supporting the collaborative research between Assiut University, Egypt and Iwate University, Japan.

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