Polish Journal of Veterinary Sciences Vol. 23, No. 3 (2020), 373–381

DOI 10.24425/pjvs.2020.134681

Original article

Characterization of serum adiponectin and leptin in healthy perinatal dairy cows or cows with ketosis, and their effects on ketosis involved indices

L. Shen[1], B. Qian[1], J. Xiao[1], Y. Zhu[1], S. Hussain[1], J. Deng[1], G. Peng[1] , Z. Zuo[1], L. Zou[1], S. Yu[1], X. Ma[1], Z. Zhong[1], Z. Ren[1], Y. Wang[1], H. Liu[1], Z. Zhou[1] , D. Cai[1], Y. Hu[1], X. Zong[2], S. Cao[1†]

[1] Sichuan Agricultural University – Chengdu Campus,
The Key Laboratory of Animal Disease and Human Health of Sichuan Province,
The Medical Research Center for Cow Disease Chengdu, Sichuan, 611130, China
[2] Sichuan Agricultural University – Chengdu Campus,
Academic Affairs Office, Chengdu, Sichuan, 611130, China

Abstract

We investigated changes in concentrations of ADP (adiponectin), LEP (leptin), BHBA (beta-hydroxybutyric acid), NEFA (non-esterified fatty acid), Glucose (Glu) and INS (insulin) in serum of healthy perinatal dairy cows and cows with ketosis. Twenty-one healthy cows and seventeen cows with ketosis from a herd of a total 60 Holstein cows (near dry period i.e. 56 days antepartum) were selected. Blood was collected through the tail vein every 7 days, from 56 day antepartum to 56 day postpartum. Serum ADP, LEP, BHBA, NEFA, Glu, and INS concentrations were determined, and ketosis was diagnosed through serum BHBA (≥1.2 mmol/L). We showed the concentration of serum adipokines and energy balancing indices were stable during antepartum period. However, ADP concentration increased while LEP decreased, and there were a significant increase in cows with ketosis compared to that of in healthy cows. Serum BHBA and NEFA concentrations increased significantly at first, and then gradually decreased in both healthy cows and cows with ketosis. However, cows with ketosis showed higher concentrations of BHBA and NEFA which restored later. The serum concentration of Glu in both healthy dairy cows and cows with ketosis showed a decreasing trend. INS concentration in healthy cows was decreased while it was increased in cows with ketosis. The results reflect the extent of hypoglycemia and lipid mobilization postpartum, suggest IR exists in cows with ketosis while serum ADP and LEP might play roles in the development of ketosis.

Key words: adiponectin, dairy cows, ketosis, leptin, perinatal period

Correspondence to: L. Shen, S. Cao, e-mail: Shenlh@sicau.edu.cn, Suizhongcao@sicau.edu.cn
L. Shen and B. Qian contributed equally to this work.

Introduction

The perinatal period is defined as the time interval from day 21 antepartum to day 21 postpartum in dairy cows. It is one of the most important phases in the productivity of dairy cows, and also is a high-risk period of metabolic and inflammatory diseases. Recently, ketosis has become a global problem in dairy production because of its high incidence, less obvious symptoms, high risks of lactation decrease and secondary diseases (Ruprechter et al. 2018). It is believed that ketosis in dairy cows is caused by negative energy balance (NEB), the disorder in glucose and lipid metabolism, high concentration of non-esterified fatty acid (NEFA), β-hydroxybutyric acid (BHBA), and insulin resistance (IR) (Li et al. 2014, Ježek et al. 2017). Adipokines regulate oxidative stress, inflammatory response and energy balance by influencing insulin sensitivity and regulating glucose and lipid metabolism (Yu-Duan et al. 2013, Stawerska et al. 2016), indicating that NEB is closely associated with ketosis in dairy cows. Recently, researchers found that adiponectin (ADP) and leptin (LEP) play important roles in regulation of energy balance, INS sensitivity and appetite (López-Jaramillo et al. 2014). Dairy cows experience great changes in glucose and lipid metabolism during the perinatal period. To monitor the dairy herd health and predict the disease risk in perinatal dairy cows, metabolic indices and their changes (in this period) were monitored (Ospina et al. 2010). Glucose (Glu), NEFA, and BHBA have been widely used in the prediction of diseases in dairy cows. Furthermore, a single biomarker may not predict diseases precisely, hence, a combined indices monitoring method can provide better diagnostic information (Ospina et al. 2010, Zhang et al. 2013). There are few reports focused on serum adipokines concentrations during the perinatal period in dairy cows. Therefore, in this study, we investigated the indicators which are closely related to the energy balance in dairy cows, including adipokines (ADP, LEP), lipid metabolism indices (BHBA, NEFA) and glucose metabolism indices (Glu, INS), and provided the basis for how adipokines affect energy metabolism in these animals. Furthermore, the study revealed the potential of adipokines as the prediction and analysis indices of clinical ketosis.

Materials and Methods

Animals and managements

After observation and diagnosis by the ranch veterinarian, total 38 dairy cows, including 21 healthy animals during the perinatal period with normal parturition and 17 cows showing signs of ketosis (serum BHBA concentration ≥1.2 mmol/L) (Tatone et al. 2017, Hausmann et al. 2018) were chosen from a herd of 68 Holstein cows with weight of 597±53 kg, BCS 3.42±0.37, 2-4 parity, and which were about to enter the perinatal period in a large-scale semi-closed unified feeding farm in Sichuan province. All cows in the experimental groups had free access to fresh water, and a TMR diet was provided at 6:00, 12:00, and 19:00 each day. The feed composition for selected dairy cows during the early prenatal period (60 days to 20 days antepartum), and the prenatal period (20 days antepartum to parturition) is in Table 1.

Equipment and reagents

Equipment

Miniature oscillator (WZS-III), Jincheng instrument factory, Wuxi, China; centrifuge (LD4-2A), Beijing Centrifuge factory, Beijing, China; Microplate reader (ELx800), BioTek Instruments, Inc., Winooski, VT, U.S.A.; Constant temperature incubator (HH-6), Guohua Electric Co., Ltd, Changzhou, China; Spectrophotometer (722), Nanjing Dawei Instrument Co., Ltd, Nanjing, China.

Test Reagents

Bovine ADP (sensitivity 0.1 μg/mL), LEP (0.1 ng/mL), and INS (0.078 uIU/mL) ELISA kit (accuracy > 0.92); NEFA double reagent enzymatic detection kit; Glu oxidase assay kit; BHBA Enzyme Colorimetric Test Kit, all were purchased from Nanjing Jiancheng Biotechnology Co., Ltd.

Ethics approval

Animal care and the experiments were conducted according to the Regulations for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China, revised in June 2004) and approved by Institutional Animal Care and Use Committee of Sichuan Agricultural University.

Experimental methods

Sample collection and process

Ten mL tail venous blood of 60 selected cows were collected at 6:00 am before feeding on 56d, 49d, 42d, 35d, 28d, 21d, 14d, 7d antepartum (-56d, -49d, -42d, -35d, -28d, -21d, -14d and -7d) respectively, day of parturition(0d) and 7d, 14d, 21d,28d, 35d, 42d, 48d, 56d postpartum. According to the postpartum health care procedure of the farm, the daily lactation, intake, body

Table 1. Dietary components of experimental dairy cows in the early and late prenatal period.

Period		Componets	Percentage / % (In DM)
Early Perinatal Period	Ingridients	Formula material	35.07
		Beet meal	2.035
		Cottonseed	2.057
		Corn silage	11.76
		Oat grass	49.07
	Nutrient	DM	52.3
		CP	14.6
		NDF	43.8
		Starch	13.8
		Ca	0.30
		P	0.37
Late Perinatal Period	Ingredients	Formula material	49.34
		Steam-flaked corn	3.318
		Beet meal	2.647
		Cottonseed	7.390
		Corn silage	14.96
	Nutrient	DM	56.7
		CP	17.1
		NDF	33.9
		Starch	23.8
		Ca	0.94
		P	0.45

Both the early and late prenatal period TMR diet were designed by the experimental dairy farm.

temperature, blood ketone concentration, and other indicators of cows with ketosis were measured continuously. Only cows with stable ketosis condition and no other diseases were included in the experimental group. While 21 healthy cows and 17 cows with ketosis were chosen finally. Collected venous blood was placed in a centrifuge tube without anticoagulant and centrifuged at 3000 rpm for 10 min to separate serum (after 1 h rest at room temperature), and then upper serum was transferred to EP tube which was stored at -20°C afterwards.

Quantification of serum indices

Serum ADP, LEP, BHBA, NEFA, TG, Glu and INS concentrations were tested strictly following kit instructions.

Diagnosis and Grouping

Diagnosis of cows suffering from ketosis was made by measuring serum BHBA concentrations and confirmed by postpartum monitoring of ketosis blood test strips. After clinical observation by ranch veterinarian, the cows with serum BHBA concentrations ≥1.2 mmol/L were included into the ketosis group, while the cows without any clinical disease were assigned to the healthy group.

Data analysis

Serum ADP, LEP, BHBA, NEFA, TG, Glu and INS concentrations in each cow in both the ketosis and healthy group were measured and analyzed by ANOVA in SPSS 24.0. The results were represented in ($\overline{X}$±S), taking time as the abscissa and concentration as the ordinate, plotted the changes of ADP, LEP, Glu, NEFA, TG, BHBA and INS concentrations in cows with ketosis and healthy cows. Differences between healthy cows and cows with ketosis were analyzed at specified time points. The difference was considered significant at $p<0.05$.

Results

Serum BHBA and NEFA concentrations in healthy cows and cows with Ketosis

As shown in Table 2, serum BHBA concentration in cows with ketosis slightly fluctuated from -56d to -21d, and no significant differences was detected at each time

Table 2. Serum BHBA and NEFA levels in ketosis and healthy dairy cows at different time points in the perinatal period ($\overline{X}$±S).

Time	BHBA (mmol/L)		NEFA (mmol/L)	
	Ketosis group	Healthy group	Ketosis group	Healthy group
-56d	$0.54±0.13^{de}$	$0.49±0.15^{defg}$	$0.29±0.11^{g}$	$0.32±0.09^{f}$
-49d	$0.46±0.15^{de}$	$0.46±0.08^{efgh}$	$0.39±0.08^{efg}$	$0.43±0.10^{cde}$
-42d	$0.41±0.14^{de}$	$0.44±0.09^{fgh}$	$0.38±0.10^{efg}$	$0.42±0.12^{cde}$
-35d	$0.46±0.16^{de}$	$0.49±0.08^{defg}$	$0.33±0.08^{fg}_{b}$	$0.39±0.10^{def}_{a}$
-28d	$0.56±0.15^{de}$	$0.41±0.10^{fgh}$	$0.35±0.10^{fg}$	$0.36±0.09^{e}$
-21d	$0.36±0.10^{e}$	$0.41±0.13^{fgh}$	$0.35±0.11^{fg}$	$0.39±0.09^{def}$
-14d	$0.52±0.14^{de}_{a}$	$0.37±0.10^{gh}_{b}$	$0.51±0.16^{def}_{A}$	$0.36±0.11^{ef}_{B}$
-7d	$0.52±0.16^{de}_{a}$	$0.36±0.14^{gh}_{b}$	$0.56±0.17^{cde}_{A}$	$0.36±0.10^{ef}_{B}$
0d	$0.67±0.16^{cd}_{A}$	$0.34±0.14^{h}_{B}$	$0.78±0.20^{c}_{A}$	$0.61±0.08^{b}_{B}$
7d	$2.11±0.62^{a}_{A}$	$0.76±0.26^{a}_{B}$	$1.33±0.43^{a}_{A}$	$0.62±0.23^{b}_{B}$
14d	$2.23±1.04^{a}_{A}$	$0.71±0.25^{abc}_{B}$	$1.34±0.48^{a}_{A}$	$0.60±0.17^{b}_{B}$
21d	$1.47±0.53^{b}_{A}$	$0.79±0.29^{a}_{B}$	$1.15±0.38^{ab}_{A}$	$0.80±0.21^{a}_{B}$
28d	$1.24±0.45^{b}_{A}$	$0.72±0.22^{ab}_{B}$	$1.04±0.19^{b}_{a}$	$0.79±0.26^{a}_{b}$
35d	$0.83±0.27^{c}$	$0.80±0.21^{a}$	$1.01±0.37^{b}_{A}$	$0.46±0.09^{cd}$
42d	$0.82±0.22^{c}$	$0.59±0.17^{cde}$	$0.74±0.19^{c}_{A}$	$0.49±0.11^{c}_{B}$
49d	$0.64±0.15^{cde}$	$0.62±0.22^{bcd}$	$0.69±0.22^{cd}_{A}$	$0.48±0.10^{cd}_{B}$
56d	$0.67±0.14^{cd}$	$0.53±0.16^{def}$	$0.66±0.18^{cd}_{a}$	$0.45±0.10^{cd}_{b}$

In the same indices, there are significant differences between different values marked with lower-case superscript letters on the same column ($p<0.05$); there are highly significant differences between different values marked with capital subscript letters on the same line($p<0.01$), significant differences between values marked with different lower-case subscript letters in the same line($p<0.05$).

point ($p>0.05$). The lowest BHBA concentration was observed on -21d and moderately increased thereafter, no significant differences were observed from -21d to 0d ($p>0.05$) on a weekly basis. However, BHBA concentration on 0d was significantly higher than that on -21d ($p<0.05$). Serum BHBA concentration increased rapidly from 0d to 14d and reached peak value on 14d inducing ketosis while BHBA concentrations on 7d and 14d were significantly higher than those at any other time point ($p<0.05$). BHBA concentration declined during 14d to 56d, this weekly decline was significant between 14d to 35d ($p<0.05$), then getting slightly increase during 35d to 56d and finally restored to the postpartum value on 49d.

Serum BHBA concentration in healthy cows was stable antepartum. The lowest value was detected on 0d and showed significant difference on -56d ($p<0.05$), while no other significant differences were found during the antepartum period. BHBA concentrations increased significantly from 0d to 7d ($p<0.05$) and showed no significant differences between 0d to 35d, but BHBA concentrations were still significantly higher than those found in antepartum ($p<0.05$). Serum BHBA concentration declined from 35d to 56d, and dropped significantly between 35d to 42d ($p<0.05$) and then returned to the level of antepartum on 56d.

Serum BHBA concentrations in cows with ketosis were significantly higher than those in healthy cows from -14d to -7d ($p<0.05$), and they were significantly higher than that of in healthy cows from 0d to 28d ($p<0.01$) while no significant differences were observed between healthy cows and cows with ketosis during -56d to -14d as well as during 28d to 56d.

Healthy cows and cows with ketosis showed similar levels of serum BHBA concentrations, but the rate of change in serum BHBA concentration in cows with ketosis was remarkably greater than that of in healthy cows.

Table 2 also shows no significant difference in serum NEFA concentrations from -56d to -21d ($p>0.05$) and they were increased from -21d to 14d, reaching the highest level on 14d while no significant differences were found during -21d to 0d on a weekly basis($p>0.05$). Furthermore, serum NEFA concentration increased rapidly from 0d to 14d, while the concentrations on 7d and 14d were significantly higher than that of at any other time points ($p<0.05$). We also observed a decreasing trend of serum NEFA concentration from 14d to 56d, and it declined significantly in a week from 35d to 42d ($p<0.05$), but the concentration on 56d was still significantly ($p<0.05$) higher than that on -56d to -21d.

Healthy cows showed stable NEFA concentrations from -56d to -7d, no significant difference was found except on -56d which was significantly lower than that on -49d and -42d ($p<0.05$). Serum NEFA concentra-

Table 3. Serum Glu and INS levels in ketosis and healthy dairy cows at different time points in the perinatal period ($\overline{X}$±S).

Time	Glu (mmol/L)		INS (μIU/mL)	
	Ketosis group	Healthy group	Ketosis group	Healthy group
-56d	$3.76±0.49^{a}$	$3.95±0.68^{a}$	$5.67±1.06^{b}$	$5.90±1.07^{abc}$
-49d	$3.35±0.60^{abcd}$	$3.58±0.77^{ab}$	$5.20±1.25^{b}$	$5.32±1.05^{bcdef}$
-42d	$3.61±0.49^{ab}$	$3.48±0.48^{b}$	$5.43±1.22^{b}$	$5.32±1.18^{cdef}$
-35d	$3.47±0.70^{abcd}$	$3.41±0.44^{b}$	$5.70±0.75^{b}$	$5.43±1.02^{bcdef}$
-28d	$3.32±0.60^{bcd}$	$3.52±0.90^{b}$	$5.65±0.85^{b}$	$5.42±1.07^{abcdef}$
-21d	$3.42±0.49^{abcd}$	$3.46±0.83^{b}$	$5.47±0.83^{b}$	$5.52±0.99^{abcdef}$
-14d	$3.50±0.34^{abc}$	$3.27±0.59^{bcde}$	$5.42±0.87^{b}$	$5.61±1.26^{abcdef}$
-7d	$3.07±0.41^{cdef}_{b}$	$3.51±0.81^{b}_{a}$	$5.46±0.70^{b}$	$5.77±0.92^{abc}$
0d	$3.27±1.01^{bcde}$	$3.36±0.67^{bc}$	$5.19±1.03^{b}$	$5.13±1.21^{def}$
7d	$2.52±0.73^{gh}$	$2.89±0.65^{e}$	$5.76±1.13^{a}_{A}$	$5.06±0.75^{ef}_{B}$
14d	$2.35±0.62^{h}_{b}$	$2.92±0.79^{de}_{a}$	$6.06±1.23^{a}_{A}$	$4.85±0.87^{f}_{B}$
21d	$2.39±0.83^{h}_{b}$	$2.96±0.72^{cde}_{a}$	$5.80±0.94^{ab}$	$5.33±0.83^{bcdef}$
28d	$2.78±0.66^{fgh}_{b}$	$3.29±0.64^{bcde}_{a}$	$5.12±0.80^{b}$	$5.50±0.82^{abcdef}$
35d	$3.03±0.86^{def}$	$3.31±0.90^{bcde}$	$5.28±0.90^{b}$	$5.59±0.93^{abcdef}$
42d	$2.87±0.59^{efg}_{b}$	$3.40±0.78^{b}_{a}$	$5.53±1.06^{b}$	$5.67±0.76^{abcd}$
49d	$3.08±0.16^{cdef}$	$3.34±0.61^{bcd}$	$5.39±0.76^{b}$	$5.92±1.08^{ab}$
56d	$3.18±0.53^{bcdef}$	$3.51±0.63^{b}$	$5.63±1.58^{b}$	$6.02±0.86^{a}$

In the same indices, there are significant differences between different values marked with lower-case superscript letters on the same column ($p<0.05$); there are highly significant differences between values marked with different capital subscript letters in the same line($p<0.01$), significant differences between different values marked with lower-case subscript letters in the same line($p<0.05$).

tions increased from -7d to 28d and reached the highest level on 21d, in which a stabilization period existed from 0d to 14d which revealed no significant difference between the time points but the concentrations was still significantly higher than that on -7d ($p<0.05$) and significantly lower than that on 21d and 28d ($p<0.05$). The concentration of NEFA in healthy cows decreased significantly during 28d to 35d ($p<0.05$), then slightly declined and returned to antepartum concentrations on 56d.

Serum NEFA concentrations in healthy cows and cows with ketosis showed similar changes before -21d, but NEFA concentration in cows with ketosis increased more early and rapidly than in healthy cows and had a higher value than antepartum concentration.

Changes of Glu and INS in healthy and cows with ketosis

As shown in Table 3, serum Glu concentrations showed a decreasing trend but no significant differences during the antepartum period when monitored on a weekly basis, and concentrations at -7d and 0d were significantly lower than that on -56d ($p<0.05$). Serum Glu concentrations declined from 0d to 21d, showed a significant difference on 0d and 7d ($p<0.05$) and reached the lowest level on 14d. Serum Glu concentrations were increasing moderately between 21d and 56d and reached the similar level of antepartum concentration at 49d.

Healthy cows showed no significant differences in serum Glu concentration during the antepartum period, but Glu level was significantly decreased from 0d to 7d ($p<0.05$), and gradually returned to the antepartum level after 28d and became stable within next two weeks.

Table 3 shows that serum INS concentration stably declined during the antepartum period, and began to increase after 14d; INS concentrations at 7d and 14d were significantly higher than during antepartum period and at the day of parturition ($p<0.05$), and finally decreased to the antepartum level at 28d.

Serum INS concentration in healthy cows showed no significant differences in the antepartum period, and began to decrease at -7d and became significantly lower than that during the antepartum on 7d and 14d ($p<0.05$). These INS concentrations began to increase after 14d, showed no significant difference during antepartum on 21d, and a moderate increase was observed till 56d.

There was no significant difference in serum INS concentrations between healthy cows and cows with ketosis ($p>0.05$), except on the 7d and 14d, when INS concentrations in cows with ketosis were significantly higher than those in healthy cows ($p<0.05$).

Table 4. Serum ADP and LEP levels of ketosis and healthy dairy cows at different time points in the pernatal period($\overline{X}$±S).

Time	ADP (mg/L)		LEP (ng/mL)	
	Ketosis group	Healthy group	Ketosis group	Healthy group
-56	25.10±7.34^{de}	25.35±8.67^{bc}	4.11±0.99^{a}	3.89±1.85^{ab}
-49	25.17±8.25^{de}	27.52±7.36^{abc}	3.95±1.22^{ab}	3.88±1.31^{ab}
-42	25.77±9.70^{de}	26.92±8.30^{abc}	3.70±1.10^{abc}	3.81±1.28^{ab}
-35	25.10±7.25^{de}	25.61±7.01^{bc}	3.81±1.04^{abc}	3.93±1.96^{a}
-28	25.11±9.42^{de}	25.25±9.11^{bc}	3.51±0.84^{abcd}	3.72±1.85^{abc}
-21	26.03±4.58^{de}	26..34±8.53^{abc}	3.75±1.57^{abc}	3.54±1.93^{abc}
-14	28.63±8.08^{cde}	25.74±7.08^{bc}	3.47±1.06^{abcd}	3.62±1.46^{abc}
-7	28.74±7.93^{cde}	26.32±5.78^{abc}	3.30±0.90^{bcde}	3.67±0.92^{abc}
0	31.14±7.30^{bc}	29.11±5.59^{ab}	2.84±0.74^{def}	3.12±0.99^{abc}
7	37.32±6.67$^{a}_{A}$	30.76±5.95$^{a}_{B}$	2.62±0.49^{ef}	2.89±0.99^{c}
14	35.53±5.63$^{ab}_{A}$	28.28±7.29$^{abc}_{B}$	2.55±0.60^{f}	3.02±1.10^{bc}
21	28.93±6.88^{cd}	25.42±7.56^{bc}	2.73±0.74^{ef}	3.22±1.36^{abc}
28	26.89±8.56^{cde}	25.45±7.44^{bc}	2.89±0.77^{def}	3.26±1.37^{abc}
35	25.39±8.20^{de}	24.35±7.84^{c}	3.20±0.92^{cdef}	3.70±1.48^{abc}
42	25.23±7.67^{de}	24.88±6.78^{bc}	3.45±1.05^{abcd}	3.79±1.25^{ab}
49	25.40±6.12^{de}	26.67±8.53^{abc}	3.69±1.38^{abc}	3.87±1.55^{ab}
56	23.79±8.18^{e}	25.38±8.10^{bc}	3.66±1.56^{abc}	3.79±1.64^{ab}

In the same indices, there are significant differences between different values marked with lower-case superscript letters in the same column (p<0.05); there are highly significant differences between different values marked with capital subscript letters in the same line(p<0.01), significant differences between different values marked with lower-case subscript letters in the same line(p<0.05).

Serum INS concentrations in healthy cows and cows with ketosis showed similar changes during antepartum period, but INS level in cows with ketosis increased after parturition while that in healthy cows declined.

Changes in serum ADP and LEP concentrations in healthy cows and in cows with ketosis

As shown in Table 4, serum ADP concentrations showed no significant differences during the antepartum period, but were increasing moderate from -21d and became higher at 0d than those at any other time points before -21d (p<0.05). ADP level reached the the highest level on the 7d postpartum, significantly higher than that on 0d, then remained higher till 14d and began to decline significantly until 21d (p<0.05), finally returned to antepartum level on 56d.

Serum ADP concentration showed no significant difference weekly (p>0.05), but increased from -14d to 7d, reached the highest level at 7d, those ADP concentration was significantly higher than that on -56d and -14d (p<0.05). Furthermore, from 7d to 21d, serum ADP level decreased and returned to the antepartum level on the 21d, being significantly lower than that at 7d (p<0.05).

Healthy cows and cows with Ketosis revealed a similar trend of change in serum ADP concentration during the perinatal period, but cows with ketosis showed significantly higher serum ADP level than healthy cows during 7d to 14d (p<0.05) and experienced a great change within the period from 0d to 21d.

Table 4 also shows that serum LEP concentrations in cows with ketosis showed a moderate decreasing trend, and on -7d it was significantly lower than that on -56d (p<0.05) while at 0d it was significantly lower than that before -35d (p<0.05). Serum LEP level continued to decrease and reached the lowest level on 14d, which was significantly lower than that at any time point in the antepartum period (p<0.05), then it started increasing. It recovered to antepartum concentration on 42d, and there was no significant change during the next 14 days (p<0.05).

Healthy cows showed no significant difference in serum ADP level during the antepartum period (p>0.05) but showed a decreasing trend near to parturition, and continued to decrease till 14d when concentrations at 7d and 14d were significantly lower than its highest concentration found on -35d (p<0.05), and finally returned to the normal level on 21d.

Discussion

Changes in serum BHBA and NEFA concentrations in perinatal healthy cows and cows with ketosis

Blood NEFA concentration in dairy cows is an indicator of body fat mobilization (Ospina et al. 2010) and has a negative correlation with the energy balance. Energy requirement and DMI in dairy cows decreases by 30% during the dry period and leads to antepartum NEB (Mann et al. 2015), and causes an increase in NEFA level, which enhances body fat mobilization (Tatone et al. 2017). Increased BHBA level leads to a higher risk of fatty liver (Kalaitzakis et al. 2010), severely decreasing hepatic fat metabolism. Serum BHBA and NEFA concentrations were significantly increased after parturition in both healthy cows and cows with ketosis ($p<0.05$) and maintained for about 30d before restoring to the antepartum level, indicating postpartum body fat mobilization. This mobilization continued until DMI restored or perinatal period was finished, reducing body fat deposits, raising hepatic burden, and increasing the risk of metabolic diseases. Serum BHBA level reached the highest level between 7d to 14d and began to fall, but was still significantly higher than in healthy cows in this period ($p<0.01$). This shows that ketosis in dairy cows mainly occurs in the perinatal period and has a long auto-recovery period, inducing potential lactation loss and a higher risk of secondary metabolic diseases.

Changes in Glu and INS concentrations in perinatal healthy cows and cows with ketosis

During the perinatal period, Glu consumption can reach up to 500g per day postpartum (Liu 2003). Ketones and BHBA have a negative effect on Glu concentration in the blood by downregulating serum Glu precursor concentration, hence worsen the situation (Cao et al. 2017). In our study, serum Glu concentrations were significantly decreased after parturition ($p<0.05$), but this decline in Glu concentration in healthy cows was slight and returned to the normal level on 28d, whereas it was more obvious in cows with ketosis and took longer time to recover.

Increased fetal amino acid (AA) and protein requirement during early lactation leads to decline in serum AA concentration in a cow, which largely interferes the INS synthesis (Zhang et al. 2013). Therefore, persistently high NEFA concentration can cause apoptosis of islet cells, reducing INS secretion (Mao et al. 2017). In our study, serum INS and Glu concentrations were stable before parturition. In consistent with Glu serum level INS began to fall from -7d and was maintained in a lower level until 21d in healthy cows, because of 3 reasons: (1) Decreased blood glucose level in postpartum cows reduced the islet B cell stimulation and hence, decreased the INS secretion; (2) During last trimester, protein requirement increases and INS synthesis is reduced in dairy cows; (3) Body fat is mobilized to balance energy requirement, hence BHBA and NEFA concentrations was increased and inhibited INS secretion. Researches have shown that IR occurs in dairy cows with type II ketosis, and its severity is significantly correlated with serum NEFA and BHBA concentrations (Gheise et al. 2018). Our research showed that serum INS concentration is significantly higher in cows with ketosis than in healthy cows, indicating type II ketosis may had happened in this herd, showing high serum INS concentrations, and exacerbating NEB condition.

Changes in serum ADP concentrations in perinatal healthy cows and cows with ketosis

ADP was firstly discovered in 1995 by Scherer and Lodish. The current research showed that ADP could regulate INS sensitivity, glucose and lipid metabolisms, resist oxidation stress and inflammatory response (Yadav et al. 2013). Nada et al. (Nada et al. 2010) reported that the mRNA level of hormone-sensitive lipase (HSL) in the adipose tissue decreased with the decrease in the concentration of ADP mRNA in antepartum perinatal dairy cows and increased with the rise in the level of ADP mRNA in the postpartum period, suggesting that ADP can regulate lipid metabolism by regulating the expression and secretion of HSL mRNA. This study showed that changes in serum ADP concentrations were similar to those in NEFA concentration. Our results are consistent with those report of Nada and Thompson (2010), indicating that both ADP and NEFA concentration in cow's serum can reflect the body fat mobilization condition in dairy cows. In the condition of ketosis, the level of ADP mRNA is significantly higher than that in postpartum cows (Nada et al. 2010), indicating that metabolic energy disorder can stimulate the expression of ADP to regulate the energy balance. Researches also confirmed that NEFA could promote the expression of ADP mRNA in adipocyte in vitro (Zhang et al. 2008). In this study, Serum ADP concentration in cows with ketosis was significantly higher than that in healthy cows between the 7d to 14d ($p<0.01$), and the results are consistent with the above findings in vitro.

Changes in LEP concentration in perinatal ketosis and healthy cows

LEP, an adipokine encoded by obesity gene and secreted by white adipose tissues, can bind to many receptors like LEP receptors and adrenergic receptors and affects cell multiplication pathways, plays a regulatory role in appetite, fat mobilization and INS sensitivity, as an important regulatory hormone (Chan et al. 2013). Serum LEP concentration is directly proportional to body fat deposits while it is inversely proportional to DMI. Hence it can be the metabolic regulatory hormone of the dairy cow (Zheng et al. 2010, Cao et al. 2017). LEP can inhibit fat synthesis, promote triglyceride decomposition, inhibit fatty acid synthase expression and INS sensitivity of adipose tissue; thus, regulates fat synthesis (Robertson et al. 2008). In our study, serum LEP concentration in both healthy cows and cows with ketosis remained stable and had no significant difference before parturition. However decreased after parturition and recovered within 4 weeks, which may result in body fat mobilization. Lower LEP level can stimulate hypothalamic feeding centre and increase appetite, and can cause autoregulation in the NEB situation in dairy cows. Cows with ketosis showed lower LEP concentrations than healthy cows, which supposedly can be a consequence of ketosis promoting fat mobilisation. Moreover, a lower LEP concentration promotes lipid decompo sition, and the interactive mechanism is still unknown.

Conclusion

BHBA, NEFA, Glu, INS, ADP, and LEP concentrations were similar and stable in both healthy cows and cows with ketosis during the early prenatal period. In the contrast, during the postpartum period, BHBA and NEFA concentrations in both healthy cows and cows with ketosis were initially increased and then decreased, but cows with ketosis showed the much bigger increase and much longer recovery time. Glu concentrations in both healthy cows and cows with ketosis showed a decreasing trend, but INS concentration in cows with ketosis increased while that in healthy cows declined. In the light of the above discussion, we hypothesize that hypoglycemia and body fat mobilization may have occurred in both healthy cows and cows with ketosis during the postpartum period, indicating that IR happened in cows with ketosis. Also, serum ADP concentrations increased while LEP levels declined after parturition and such a change was greater in cows with ketosis, which indicate that ADP and LEP might play a role in the regulation of energy balance, body fat mobilization and occurrence of ketosis.

Acknowledgements

We gratefully acknowledge a financial support from the National Key Research and Development Project of China (2018YFD0500900) and the Sichuan Department of Science and Technology Support Project (2019YJ0650).

References

Cao Y, Zhang J, Yang W, Xia C, Zhang HY, Wang YH, Xu C (**2017**) Predictive value of plasma parameters in the risk of postpartum ketosis in dairy cows. J Vet Res 61: 91-95.

Chan CJ, Summers KL, Chan NG, Hardy DB, Richardson BS (**2013**) Cytokines in umbilical cord blood and the impact of labor events in low-risk term pregnancies. Early Hum Dev 89: 1005-1010.

Gheise NJ, Riasi A, Celi P, Shahneh AZ (**2018**) Effects of dietary supplementation of pioglitazone or walnut meal on metabolic profiles and oxidative status in dairy cows with high pre-calving BCS. J Dairy Res 85: 16-22.

Hausmann J, Deiner C, Patra AK, Immig I, Starke A, Aschenbach JR (**2018**) Effects of a combination of plant bioactive lipid compounds and biotin compared with monensin on body condition, energy metabolism and milk performance in transition dairy cows. PloS One 13:e0193685-.

Ježek J, Cincović M R, Nemec M, Belić B, Djoković R, Klinkon M, Starič J (**2017**) Beta-hydroxybutyrate in milk as screening test for subclinical ketosis in dairy cows. Pol J Vet Sci 20: 507-512.

Kalaitzakis E, Panousis N, Roubies N, Giadinis N, Kaldrymidou E, Georgiadis M, Karatzias H (**2010**) Clinicopathological evaluation of downer dairy cows with fatty liver. Can Vet J 51: 615-622

Li Y, Xu C, Xia C, Zhang H, Sun L, Gao Y **(2014)** Plasma metabolic profiling of dairy cows affected with clinical ketosis using LC/MS technology. Vet Q 34: 152-158.

Liu Z (2003) Modern Animal Nutrition Metabolism, Chemical Industry Press, Beijing, pp 120-132.

López-Jaramillo P, Gómez-Arbeláez D, López-López J, López-López C, Martínez-Ortega J, Gómez-Rodríguez A, Triana-Cubillos S (**2014**) The role of leptin/adiponectin ratio in metabolic syndrome and diabetes. Horm Mol Biol Clin Investig 18: 37-45.

Mann S, Yepes FA, Overton TR, Wakshlag JJ, Lock AL, Ryan CM, Nydam DV (**2015**) Dry period plane of energy: Effects on feed intake, energy balance, milk production, and composition in transition dairy cows. J Dairy Sci 98: 3366-3382.

Mao X, You Z, Wu C and Huang J (**2017**) Potential suppression of the high glucose and insulin-induced retinal neovascularization by Sirtuin 3 in the human retinal endothelial cells. Biochem Biophys Res Commun 482: 341-345.

Nada SE, Thompson RC, Padmanabhan V (**2010**) Developmental Programming: Differential Effects of Prenatal Testosterone Excess on Insulin Target Tissues. Endocrinology 151: 5165-5173.

Ospina PA, Nydam DV, Stokol T, Overton TR (2010) Evaluation of nonesterified fatty acids and beta-hydroxybutyrate

in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J Dairy Sci 93: 546-554.

Robertson SA, Leinninger GM, Myers MG Jr (**2008**) Molecular and neural mediators of leptin action. Physiol Behav 94: 637-642.

Ruprechter G, Adrien ML, Larriestra A, Meotti O, Batista C, Meikle A, Noro M (**2018**) Metabolic predictors of peri-partum diseases and their association with parity in dairy cows. Res Vet Sci 118: 191-198.

Stawerska R, Szałapska M, Borowiec M, Antosik K, Młynarski W, Lewiński A (**2016**) The influence of INS VNTR class III allele on auxological parameters, glucose, insulin, lipids, and adipocytokines secretion in prepubertal children born small for gestational age. Endokrynol Pol 67: 585-591.

Tatone EH, Duffield TF, LeBlanc SJ, DeVries TJ, Gordon JL (**2017**) Investigating the within-herd prevalence and risk factors for ketosis in dairy cattle in Ontario as diagnosed by the test-day concentration of β-hydroxybutyrate in milk. J Dairy Sci 100: 1308-1318.

Tsai YD, Wang CP, Chen CY, Lin LW, Lin TM, Hsu CC, Chung FM, Lin HC, Hsu HF, Lee YJ, Houng JY (**2013**) Elevated plasma level of visfatin/pre-b cell colony-enhancing factor in male oral squamous cell carcinoma patients. Med Oral Patol Oral Cir Bucal 18: e180-e186.

Yadav A, Kataria MA, Saini V, Yadav A (**2013**) Role of leptin and adiponectin in insulin resistance. Clin Chim Acta 417: 80-84.

Zhang H, Cong L, Zhang C and Wang Z **(2008)** Effects of NEFA and BHBA on the expression of HSL and ADPN mRNA in adipocytes cultured in vitro. Chinese Journal of Veterinary Science 7: 840-843 (in Chinese).

Zhang H, Wu L, Xu C, Xia C, Sun L, Shu S (**2013**) Plasma metabolomic profiling of dairy cows affected with ketosis using gas chromatography/mass spectrometry. BMC Vet Res 9: 186.

Zhang Y, Hai J, Cao M, Zhang Y, Pei S, Wang J, Zhang Q (**2013**) Silibinin ameliorates steatosis and insulin resistance during non-alcoholic fatty liver disease development partly through targeting IRS-1/PI3K/Akt pathway. Int Immunopharmacol 17: 714-720.

Zheng J, Xia C, Zhang H and Xu C (**2010**) Correlation between leptin with energy balance and reproductive function of dairy cows during the early lactation. Journal of China Agricultural University 3: 75-82 (in Chinese).