



Identification of Differentially Expressed Proteins in Liver in Response to Subacute Ruminal Acidosis (SARA) Induced by High-concentrate Diet

X. Y. Jiang, Y. D. Ni, S. K. Zhang, Y. S. Zhang*, and X. Z. Shen

College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China

ABSTRACT: The aim of this study was to evaluate protein expression patterns of liver in response to subacute ruminal acidosis (SARA) induced by high-concentrate diet. Sixteen healthy mid-lactating goats were randomly divided into 2 groups and fed either a high-forage (HF) diet or a high-concentrate (HC) diet. The HC diet was expected to induce SARA. After ensuring the occurrence of SARA, liver samples were collected. Proteome analysis with differential in gel electrophoresis technology revealed that, 15 proteins were significantly modulated in liver in a comparison between HF and HC-fed goats. These proteins were found mainly associated with metabolism and energy transfer after identified by matrix-assisted laser desorption ionization/time of flight. The results indicated that glucose, lipid and protein catabolism could be enhanced when SARA occurred. It prompted that glucose, lipid and amine acid in the liver mainly participated in oxidation and energy supply when SARA occurred, which possibly consumed more precursors involved in milk protein and milk fat synthesis. These results suggest new candidate proteins that may contribute to a better understanding of the mechanisms that mediate liver adaptation to SARA. (**Key Words:** Liver, Metabolism, High-concentrate, Subacute Ruminal Acidosis [SARA])

INTRODUCTION

In intensive ruminant livestock systems, rapidly fermentable (high grain) diets are commonly utilized to increase energy intake or simply due to a lack of forages at times in China. When ruminants are fed high-grain diets, short chain fatty acid (SCFA) production can exceed absorption and ruminal pH becomes depressed which can cause ruminal acidosis (Bergman, 1990; DeVries et al., 2009). Subacute ruminal acidosis (SARA), which is diagnosed when ruminal pH falls below 5.6 for more than 3 h per day, is common in practical dairy production (Gozho et al., 2005; AlZahal et al., 2007). As a common digestive disorder occurring in ruminants, the consequences of SARA include feed intake depression, reduced fiber digestion, milk fat depression, diarrhea, laminitis (Nocek, 1997; Stone, 2004), liver abscesses, rumen mucosal damage (Kleen et al.,

2003), increased production of bacterial endotoxin and inflammation characterized by increases in acute phase proteins (AlZahal et al., 2007).

Liver is the largest and complex digestive gland in ruminant animals, which is involved in the digestion, absorption, excretion, detoxification, material transportation, energy metabolism and immunity. Nutrients absorbed from digestive tract in dairy cows must pass through liver, and then enter into circulatory system, arrive at mammary gland; therefore, liver plays an important role in nutrient composition of peripheral blood and metabolic integration regulation. Although the physiopathology of SARA has been comprehensively studied (Nocek, 1997; Kleen et al., 2003), there are few reports describing the hepatic metabolic adaption when SARA occurs. Two-dimensional gel electrophoresis (2-DE) is a holistic protein characterization method that might help to decipher proteomic changes in the goat liver. Therefore, the first objective of this study was to develop and characterize a nutritional model for inducing SARA. The second objective was to determine global protein expression changes in the

* Corresponding Author: Y. S. Zhang. Tel: +86-2584396763, E-mail: zhangyuanshu@njau.edu.cn

Submitted Nov. 16, 2013; Revised Jan. 20, 2014; Accepted Mar. 4, 2014

liver in response to SARA.

MATERIALS AND METHODS

Materials

Immobilized pH gradient (IPG) strips (pH 3.0 to 10.0 non-linear (NL); 17 cm), urea, thiourea, dithiothreitol (DTT), 3-([3-cholamidopropyl] dimethyl-ammonio)-1-propane sulfonate (CHAPS), ampharmalyte pH 3 to 10, phenylmethane sulfonyl fluoride (PMSF), iodoacetamide (IAM) were purchased from Bio-Rad (Richmond, CA, USA). Coomassie brilliant blue G-250 was purchased from AMRESCO (Solon, OH, USA). All chemicals for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) were of electrophoresis grade.

Animals and liver sampling

Sixteen mid-lactating Boer crossbred goats (obtained from Zhejiang, China) with rumen fistula, weighed 46.90 ± 3.14 kg, were randomly divided into 2 groups. The experiment periods were 3 weeks in duration following a 14-day adaptation. The animals were fed either a high-forage (HF) diet or a high-concentrate (HC) diet. Goats were fed and milked twice daily (8:00 and 18:00). The HC diet composition was selected to induce SARA (Brossard et

al., 2003). Composition and nutrient levels of experimental diets are given in Table 1. All goats were housed in individual stalls and had free access to drinking water. The animal care and use protocol was approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University.

Ruminal liquid samples (10 mL) were taken from the ventral sac of the rumen before morning feeding and at 1, 2, 4, 6, and 10 h after feeding of the last 2 days. The pH of the rumen fluid was immediately determined by means of a portable pH meter (HI8424, Hana Instruments, Milan, Italy). When rumen pH was depressed for prolonged periods each day, e.g. below 5.6 for over 3 h/d, SARA was induced successfully. After ensuring the occurrence of SARA, liver samples were collected at the 3rd day. Samples were collected directly after goats were sacrificed and stored at -80°C .

Protein sample preparation and 2-D gel electrophoresis

Two-dimensional gel electrophoresis (2-DE) was performed on both HF and HC groups with the method as previously described by Chen et al. (2010). Tissues were homogenized in lysis buffer consisting of 7 M urea, 2 M thiourea, 2% (w/v) CHAPS, 50 mM DTT, 0.8% (w/v) ampharmalyte pH 3 to 10 and 1 mM PMSF using a glass homogenization vessel in ice bath. The resulting homogenates were swirled for 30 min followed by a 30 min centrifugation at 15,000 g at 4°C . Supernatants were collected and fractionated in aliquots. Protein concentrations of the final supernatants were measured using bovine serum albumin (BSA) as standard by the Bradford assay (Bradford, 1976).

To ensure reproducibility, the goat liver extract was pooled and run under the same conditions to overcome inter-individual changes in animals. All 2-DE gels were run three times. In brief, protein concentration of the lysate was adjusted to $850 \mu\text{g}/320 \mu\text{L}$ volume of rehydration buffer and the lysate was applied onto IPG strips (pH 3.0 to 10.0 NL; 17 cm) in the protean system (Bio-Rad, Hercules, CA, USA). After 13 h of passive rehydration in the tray, isoelectric focusing was performed with the following programmed voltage parameters: 250 V for 1 h, 500 V for 1 h, 2,000 V for 1 h, 8,000 V for 3 h, and then holding at 8,000 V until a total of at least 60,000 V-h was reached. Then strips were equilibrated with DTT and iodoacetamide. The second dimension was run on a 12.5% polyacrylamide SDS gel (26×20 cm) at 16°C using Multiphor system (15 W/gel) (Amersham Biosciences, Uppsala, Sweden) until the bromophenol blue dye had reached the bottom of the gel. Then Neuhoff's colloidal Coomassie blue G-250 stain was carried out according to the method described by Candiano et al. (2004).

Stained gels were scanned with a high precision scanner

Table 1. Composition and nutrient levels of experimental diets (%)

Item	HF	HC
Ingredient (%)		
Hay	48	32
Alfalfa	12	8
Corn	29.2	23.07
Wheat bran	0	28.3
Soybean meal	8.43	2
Rapeseed meal	0	3.7
Limestone	0.57	1.43
CaHPO ₄	0.9	0.6
Salt	0.4	0.4
Premix ¹	0.5	0.5
Nutrient level (%)		
ME, KJ/kg	18,012	17,828
CP	14.21	15.38
Fat	3.47	3.44
NDF	60.23	50.81
ADF	23.02	17.29
Ca	0.99	1.18
P	0.33	0.46

HF, high-forage; HC, high-concentrate; ME, metabolizable energy; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.

¹ Premix group was divided into: FeSO₄·7H₂O 170 g/kg; CuSO₄·5H₂O 70 g/kg; MnSO₄·5H₂O 290 g/kg; ZnSO₄·7H₂O 240 g/kg; Co₂Cl₆H₂O 510 mg/kg; KI 200 mg/kg; NaSeO₃ 130 mg/kg; vit A 1,620,000 IU/kg; vit D₃ 324,000 IU/kg; vit E 540 IU/kg; vit K₃ 150 mg/kg; vit B₁₂ 0.9 mg/kg; vit B₅ 450 mg/kg; calcium pantothenate 750 mg/kg; folic acid 15 mg/kg.

(Versa Doc 3000, Bio-Rad, Hercules, CA, USA) and analyzed using PDQuest8.1.0 software (Bio-Rad, Hercules, CA, USA). After alignment, spots between gels were automatically matched first. The matched spots were re-examined manually to ensure accuracy. Generally, only those spots of more than 3-fold difference in both dietary modes were chosen for further analysis. Spot quantity normalization was conducted in the 'total quantity valid spots' mode.

Matrix-assisted laser desorption ionization/time of flight analysis

The differentially expressed protein spots were excised from gels and conserved in Ependoff tubes, followed by matrix-assisted laser desorption ionization/time of flight analysis which was carried out by BoYuan Biological Technology Company in Shanghai, China. Protein digested by trypsin was used to calibrate the mass instrument using the internal calibration mode. Parent mass peaks had a mass range of 800-3,500 Da. Protein data searches were performed by search engine of Mascot.

RESULTS

Dynamic change of ruminal pH

The pH data were expressed as mean±standard deviation, and differences were considered significant when $p < 0.05$, which were tested by one-way analysis of variance (ANOVA) followed by a multiple range test using SPSS16.0 software (SPSS, Chicago, IL, USA). As expected, goats fed the HF diet displayed steady ruminal pH traces

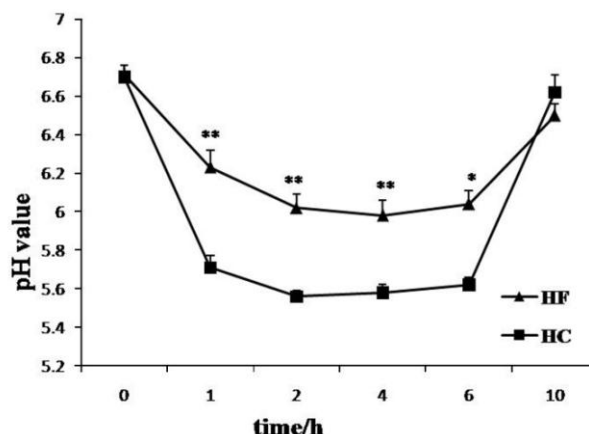


Figure 1. Effect of different forage to concentrate ratio diet on pH changes in rumen in lactating goats. Treatment consisted of HF (■) and HC (▲) for 3 weeks. The ruminal liquid samples (10 mL) were taken from the ventral sac of the rumen through a cannula at before feeding and at 1, 2, 4, 6, 10 h after the morning feeding at the end of experiment. The pH of the rumen fluid was immediately determined by means of a portable pH meter. * and ** indicate significant ($p < 0.05$) or markedly significant ($p < 0.01$) differences between 2 groups ($n = 8$). HF, high-forage; HC, high-concentrate.

with no hours below pH 5.6 (Figure 1). In contrast, SARA was diagnosed in the HC group as ruminal pH was depressed below 5.6 for about 4 h (Figure 1).

Comparison of proteomic patterns

We performed comparative visual and software-guided analysis of representative 2-DE proteome profiles of the 2 groups. There was good separation of protein spots seen in

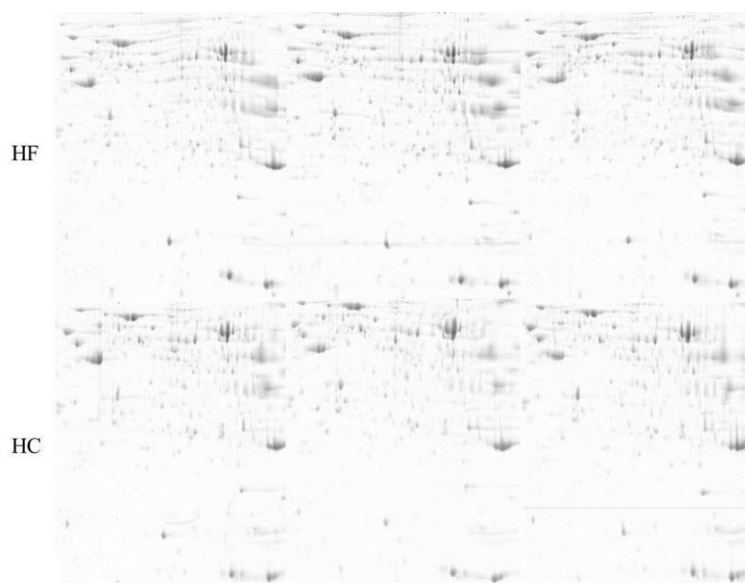


Figure 2. Representative spot maps of HF and HC groups. HF stands for high-forage and HC stands for high-concentrate. Equal amounts of protein (850 µg) were loaded and separated on 17 cm IPG strips (pH 3-10), followed by electrophoresis on 12.5% SDS-PAGE gels for second dimension electrophoresis. The gels were stained with CCB G250. IPG, Immobilized pH gradient; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

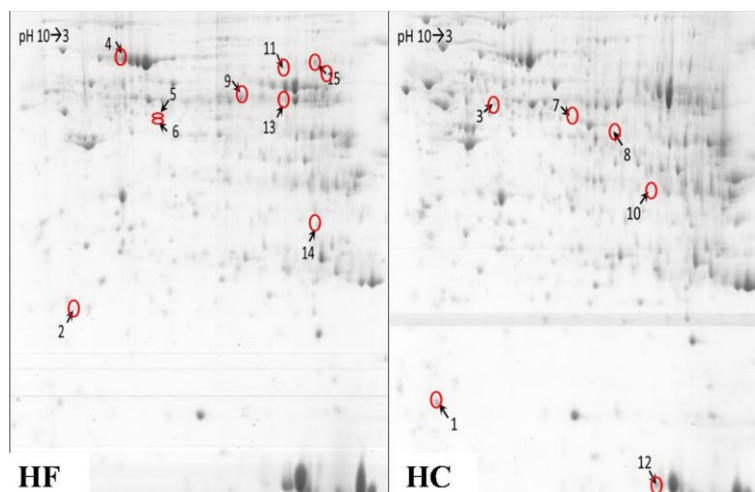


Figure 3. Effect of different forage to concentrate ratio diet on the protein/enzyme profiles of liver. The HC diet (diet forage to concentrate ratios was 60:40) was selected to induce subacute ruminal acidosis (SARA) with mid-lactating goats. After SARA occurred, the 3rd day liver samples were collected. The total liver proteins of 2 groups were extracted and separated by 2-DE (two dimensional gel electrophoresis). The arrows indicate the proteins with at least 3.0-fold change. HF, high-forage; HC, high-concentrate.

the 2-D gel electrophoresis except in the base area (Figure 2). After auto-matching and manual quality check of the detected spots, 495 ± 23 valid spots were identified in this experiment series.

In general, the protein profiles of the 2 feeding conditions detected by 2-DE were similar to each other, suggesting that few proteins were differentially expressed in the two groups. A total of 15 protein spots, however, showed up to a 3-fold difference in volume when comparing the 2 groups (Figure 3). Positive identifications were obtained for these 15 spots, including 10 up-regulated

and 5 down-regulated in SARA group, and protein identities of the 15 spots are listed in Table 2.

In order to assess functional relevance of changes in the differentially expressed proteins, the proteins were classified according to their primary functions. These proteins were mainly related to metabolism and energy transfer. When SARA was induced, acyl-CoA synthetase (ACS) and cytochrome *b5* involved in lipid metabolism were up-regulated, phosphoenolpyruvate carboxykinase (PEPCK) involved in glucose metabolism was down-regulated, while enolase was up-regulated. At the aspect of

Table 2. MAIDI-TOF/TOF identification of differentially expressed proteins in liver in response to SARA

No. ¹	Protein name	Accession number ²	pI	Molecular weight	Matched peptide sequences (m/z) ³	Up or down ⁴
Glucose metabolism						
7	Alpha enolase	AAD33073	6.44	47,589	VVIGMDVAASEFYR (1571.7654) AAVPSGASTGIYEALELR (1803.9366) LAMQEFMILPVGAEENFR (1996.9750) AGYSDKVVIGMDVAASEFYR (2193.0412) DATNVGDEGGFAPNILENKEALELLK (2756.3868)	Up
15	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial	NP_083270	7.88	74,226	IFHVNWFR (1117.5821) FLWPGFGENAR (1292.6302) EVLAELEALEGR (1327.6983) GVPLVYEAFNWR (1449.7405)	Down
Lipid metabolism						
1	Cytochrome <i>b5</i>	1106188A	5.15	11,044	STWLILHYK (1159.6390) YYTLEEIQK (1185.5917) TFIIGELHPDDR (1411.7096) FLEEHPGGEEVLR (1510.7416) EQAGGDATENFEDVGHSTDAR (2204.9207)	Up
11	Acyl-coenzyme A synthetase ACSM1, mitochondrial	NP_777107	7.84	65,706	EGWLDLFR (921.4345) ATIDEEGYFWFLGR (1702.7991) TLDPMIAIFFTSGTTGFPK (1945.9496) IKPTRPIGLFMYEENPEK (2325.1827)	Up
Protein metabolism						
4	Serum albumin precursor	NP_001009376	5.80	71,139	YLYEVAR (912.4705) HPEYAVSVLLR (1282.7034) TVMENFVAFVDK (1414.6803) HGEYGFQNALIVR (1502.7630) DVFLGSFLYEYSR (1594.7668)	Down

Table 2. MAIDI-TOF/TOF identification of differentially expressed proteins in liver in response to SARA (Continued)

No. ¹	Protein name	Accession number ²	pI	Molecular weight	Matched peptide sequences (m/z) ³	Up or down ⁴
Protein metabolism						
5	Adenosylhomocysteinase	NP_001029487	5.88	48,120	IILLAEGR (883.5491) AGIPVYAWK (1003.5491) YPQLLSGIR (1045.5920) WLNENAVEK (1101.5454) VADISLAAWGR (1157.6193) EGPFPKPDHYRY (1407.6571) GISEETTTGVHNLK (1647.8104)	Up
6	26S protease regulatory subunit 7	NP_150239	5.59	48,943	DIRFELLAR (1131.6400) FDDGAGGDNEVQR (1378.5750) KIEFSLPDLEGR (1402.7456) LREVVETPLLHPER (1686.9417)	Down
8	Elongation factor Tu, mitochondrial precursor	NP_776632	6.72	49,709	YEEIDNAPEER (1363.5891) QIGVEHVVVYVVK (1482.8195) LLDAVDTYIPVPTR (1571.8559) DLEKPFLLPVESVYSIPGR (2158.1674)	Up
13	Glutamate dehydrogenase 1, mitochondrial	XP_001925088	8.03	61,668	NLNHVSYGR (1058.5257) DDGSWEVIEGYR (1424.6208) HGGTIPIVPTAEFQDR (1736.8846) DSNYHLLMSVQESLER (1935.8996) ELEDFKLQHGTLGFPK (1971.0466) KGFIPGIDVPAPDMSTGER (2059.0045)	Up
Electron transfer						
1	Cytochrome b5	1106188A	5.15	11,044	STWLILHYK (1159.6390) YYTLEEIQK (1185.5917) TFIIGELHPDDR (1411.7096) FLEEHPGGEEVLR (1510.7416) EQAGDATENFEDVGHSTDAR (2204.9207)	Up
9	Retinal dehydrogenase 1	NP_001009778	6.37	55,417	TIPMDGNFFTYTR (1561.7235) IFINNEWHSSVSGK (1616.7947) ELGEYGFHEYTEVK (1699.7729) LFVEESIYDEFVRR (1800.9046) GYFIQPTVFSVDVTDMMR (2005.9092)	Up
14	Electron transfer flavoprotein subunit alpha, mitochondrial precursor	NP_001069290	8.77	35,282	LDVAPISDIIAIK (1366.8071) ASSTSPVGISEWLDQK (1703.8366) GLLPEELTPLILATQK (1735.0131) LLYDLADQLHAAV GASR (1811.9530) DPEAPIFQVADYGIVADLFK (2207.1150)	Down
Others						
2	PREDICTED: glyoxalase I-like	XP_001250503	5.81	21,007	VAWVFSR (863.4654) SLDFYTR (900.4341) KSLDFYTR (1028.5291) GFGHIGIAPDVHVGACK (1733.8672) FSLYFLAYEDKNDIPK (1961.9774)	Down
3	Regucalcin	NP_776382	5.54	33,857	QGSLSLFPDHHVEK (1755.8580) YFAGTMAEETAPAVLER (1870.8771)	Up
10	PREDICTED: aldo-keto reductase family 1, member C1	XP_001250576	6.60	37,644	FAIEVGFR (937.5022) QTPALVALR (967.5815) REDIFYTSK (1157.5717) SHDIVLVAYGALGAQR (1668.8947) VWSTFLRPELVRPALEK (2040.1520) LNDGHFIPVLGFGTFAPPEVVK (2351.2314)	Up
12	Glutathione S-transferase Mu 1	NP_787019	6.90	25,789	RPWFAGDK (975.4926) LTQSNAILR (1014.5822) LLELYTDTNYEER (1657.7835)	Up

MAIDI-TOF/TOF, Matrix-assisted laser desorption ionization/time of flight; SARA, subacute ruminal acidosis.

¹ Number of the protein spots in the gel.² Accession number in National Center for Biotechnology Information.³ The sequences of all the identified peptides with the corresponding m/z ratio in brackets.⁴ The expression of high-concentrate group compared with high-forage group.

protein metabolism, elongation factor Tu (EF-Tu), glutamate dehydrogenase 1 (GLDH), glutathione S-transferase and adenosylhomocysteinase were up-regulated while 26S protease regulatory subunit 7 and serum albumin precursor were down-regulated in HC group. At the same time, glutathione S-transferase and cytochrome b5 were also involved in oxidative stress. These results hinted that SARA had a key effect on substance metabolism in liver.

DISCUSSION

In ruminants, the change of rumen pH is related to prevalent consumption of rapidly fermentable carbohydrates, and authors generally define SARA based on ruminal pH values (DeVries et al., 2009). But the average pH over a day is sometimes considered as a poor indicator of SARA (Sauvant et al., 1999), because it does not reflect the large daily fluctuations in pH (Dijkstra et al., 1993; Dragomir et al., 2008). Several authors have pointed out the necessity of analyzing pH fluctuations (Nocek, 1997; Beauchemin et al., 2001), and the time spent under a pH threshold. We opted to use time below pH 5.6 as the cut-off point for SARA, as in Krause and Oetzel, because it allowed us to detect SARA in goats which were fed with a concentrate: forage ratio of 60:40, in line with previous studies using similar diets (Krause and Oetzel, 2005). The goats ate all the feeds provided, thus keeping concentrate: forage ratios of 60:40 and 40:60. We recorded that the time when pH <5.6 was about 4 h in goats fed with HC diet over 2 successive days. So we determined that SARA was induced successfully by a concentrate: forage ratio of 60:40.

The SARA has a prolonged influence on production performance. It has long been assumed that SARA causes milk fat depression (Nocek, 1997; Kleen et al., 2003; Stone, 2004). Experimentally induced SARA, either by adding grain pellets to the diet or by replacing alfalfa hay with alfalfa pellets, reduced milk fat percentage but increased milk protein percentage (Fairfield et al., 2007). Currently, the research concerning SARA are focused on the physiopathology of SARA (Nocek, 1997; Kleen et al., 2003). Our study applied 2-DE technology to examine liver protein profiles of mid-lactating goats under different dietary patterns and identified 15 differentially expressed proteins by MAIDI-TOF/TOF. The results indicated that glucose, lipid and protein catabolism were enhanced when SARA occurred. Changes of some of the proteins were well correlated with previous findings by different means. For example, alpha enolase (spot 7) is the glycolytic enzyme that catalyzes the production of phosphoenolpyruvate from 2-phosphoglycerate. Phosphoenolpyruvate carboxykinase (spot 15) catalyzes the rate-limiting step in hepatic gluconeogenesis and adipose glyceroneogenesis. The up-

regulation of alpha enolase and the down-regulation of PEPCK which are involved in glycolysis and gluconeogenesis showed that gluconeogenesis was inhibited and glycolysis was strengthened, namely carbohydrate mainly participated in oxidation and energy supply in liver when SARA occurred.

This study also inferred that lipid catabolism could be enhanced when SARA occurred, as shown by the profiles of spots 1 and 11 (Table 2 and Figure 3). Cytochrome b5 is known to function as an electron transfer component in a number of oxidative pathways, including the anabolic metabolism of fats and steroids. The cytochrome b5 electron-transport system has recently been implicated in fatty acid desaturation reactions in isolated microsomes (Porter, 2002; Schenkman and Jansson, 2003). Acyl-CoA synthetase is a crucial enzyme that facilitates the uptake and permits the metabolism of intracellular lipids. And the enzyme catalyzes the first step of fatty acid metabolism by converting inactive fatty acids into active acyl CoA derivatives (Schoonjans et al., 1995). Thus it was possible that more energy was required and the mobilization of liver fat was enhanced when SARA occurred.

The observation of up-regulation of spots 5, 8, 13 and down-regulation of spots 4 and 6 which participated in protein metabolism in SARA liver was consistent with the previous theories (Lapierre and Lobley, 2001). Elongation factor Tu is a guanosine triphosphate (GTP)-binding protein that is crucial for protein biosynthesis. In the GTP-bound form of the molecule, EF-Tu binds tightly to aminoacyl-tRNA, forming a ternary complex that interacts with the ribosomal acceptor site. During this interaction, GTP is hydrolyzed, and EF-Tu-GDP is released (Kjeldgaard et al., 1993). The catalytic activities of adenosylhomocysteinase have an important effect in controlling the expression of intra-cellular Adohcy as well as regulating transmethylation, transsulfuration and purine metabolism, especially biological methylation. If the activity of adenosylhomocysteinase is decreased or inhibited, the metabolism of protein and nucleic acid will be affected (Palmer and Abeles, 1979). In our experiment, the up-regulation of EF-Tu and adenosylhomocysteinase indicated that liver protein synthesis was enhanced when SARA occurred, which was likely to compete for raw materials of lacto protein. Spot 13 was identified as GLDH, which is the key enzyme of amine acid catabolism. The up-regulation of spot 13 in SARA liver enhanced deamination of glutamic acid, namely, amine acid catabolism was accelerated. This would make oxidation and energy supply of amine acid possible, on the other hand, would degrade the content of lutamic acid and increase the consumption of amine acid.

Calreticulin (spot 3) is a major endoplasmic reticulum Ca²⁺ binding chaperone with multiple functions and is

involved in a variety of cellular signaling pathways, such as those involved in innate immunity, adipocyte differentiation, apoptosis, and cellular stress responses (Obeid et al., 2007; Yan et al., 2011). Calreticulin also plays a crucial role in regulating Ca^{2+} intracellular homeostasis (Wang et al., 2012). Recent study also showed that the overexpression of regucalcin could suppress cell death and apoptosis induced by stimulation of intracellular signaling-related factors in cloned rat hepatoma H4-II-E cells (Izumi and Yamaguchi, 2004). At the same time, some of the spots were matched to proteins related to oxidative stress, such as GST and cytochrome b5. It inferred that anti-oxidative stress of liver occurred in the activation state to resist the injury of SARA. Further studies would be required to discriminate all these processes.

In summary, this proteomic study indicates an adaptation of protein expression in liver to SARA induced by a HC diet. The results suggested that carbohydrate, lipid and amine acid in the liver mainly participated in oxidation and energy supply when SARA occurred, which possibly consumed excessive precursors involved in milk protein and milk fat synthesis. At the same time, characterization of proteome changes in the liver between HF group and HC group could serve as a model for future studies in elucidating the metabolism pathways of liver when SARA occurred.

ACKNOWLEDGMENTS

The authors declare that there is no conflict of interest regarding the publication of this paper. This project was sponsored by grants from the China National Basic Research Program Foundation (Project No. 2011CB100802); Priority Academic Program Development of Jiangsu Higher Education Institutions; and Graduate Students' Innovative Projects of Jiangsu Higher Education Institutions (Project No. CXLX 13-291).

REFERENCES

- AlZahal, O., B. Rustomo, N. E. Odongo, T. F. Duffield, and B.W. McBride. 2007. Technical note: A system for continuous recording of ruminal pH in cattle. *J. Anim. Sci.* 85:213-217.
- Beauchemin, K. A., W. Z. Yang, and L. M. Rode. 2001. Effects of barley grain processing on the site and extent of digestion of beef feedlot finishing diets. *J. Anim. Sci.* 79:1925-1936.
- Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol. Rev.* 70:567-590.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.
- Brossard, L., C. Martin, and B. Michalet-Doreau. 2003. Ruminal fermentative parameters and blood acido-basic balance changes during the onset and recovery of induced latent acidosis in sheep. *Anim. Res.* 52:513-530.
- Candiano, G., M. Bruschi, L. Musante, L. Santucci, G. M. Ghiggeri, B. Carnemolla, P. Orecchia, L. Zardi, and P. G. Righetti. 2004. Blue silver: A very sensitive colloidal coomassie g-250 staining for proteome analysis. *Electrophoresis* 25:1327-1333.
- Chen, J., X. Tang, Y. Zhang, H. Ma, and S. Zou. 2010. Effects of maternal treatment of dehydroepiandrosterone (dhea) on serum lipid profile and hepatic lipid metabolism-related gene expression in embryonic chickens. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* 155:380-386.
- DeVries, T. J., K. A. Beauchemin, F. Dohme, and K. S. Schwartzkopf-Genswein. 2009. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feeding, ruminating, and lying behavior. *J. Dairy Sci.* 92:5067-5078.
- Dijkstra, J., H. Boer, J. Van Bruchem, M. Bruining, and S. Tamminga. 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. *Br. J. Nutr.* 69:385-396.
- Dragomir, C., D. Sauvant, J. L. Peyraud, S. Giger-Reverdin, and B. Michalet-Doreau. 2008. Meta-analysis of 0 to 8 h post-prandial evolution of ruminal pH. *Animal.* 2:1437-1448.
- Fairfield, A. M., J. C. Plaizier, T. F. Duffield, M. I. Lindinger, R. Bagg, P. Dick, and B. W. McBride. 2007. Effects of prepartum administration of a monensin controlled release capsule on rumen pH, feed intake, and milk production of transition dairy cows. *J. Dairy Sci.* 90:937-945.
- Fella, K., M. Gluckmann, J. Hellmann, M. Karas, P. J. Kramer, and M. Kroger. 2005. Use of two-dimensional gel electrophoresis in predictive toxicology: Identification of potential early protein biomarkers in chemically induced hepatocarcinogenesis. *Proteomics* 5:1914-1927.
- Gozho, G. N., J. C. Plaizier, D. O. Krause, A. D. Kennedy, and K. M. Wittenberg. 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. *J. Dairy Sci.* 88:1399-1403.
- Izumi, T. and M. Yamaguchi. 2004. Overexpression of regucalcin suppresses cell death in cloned rat hepatoma h4-ii-e cells induced by tumor necrosis factor-alpha or thapsigargin. *J. Cell. Biochem.* 92:296-306.
- Jianzhen, H., M. Haitian, Y. Liming, and Z. Sixiang. 2007. Developmental changes of protein profiles in the embryonic sanhuang chicken liver. *J. Vet. Med. A. Physiol. Pathol. Clin. Med.* 54:464-469.
- Kleen, J. L., G. A. Hooijer, J. Rehage, and J. P. Noordhuizen. 2003. Subacute ruminal acidosis (sara): A review. *J. Vet. Med. A. Physiol. Pathol. Clin. Med.* 50:406-414.
- Kjeldgaard, M. Nissen, P. Thirup, and S. Nyborg. 1993. The crystal structure of elongation factor EF-Tu from *Thermus aquaticus* in the GTP conformation. *Structure* 1:35-50.
- Krause, K. M. and G. R. Oetzel. 2005. Inducing subacute ruminal acidosis in lactating dairy cows. *J. Dairy Sci.* 88:3633-3639.
- Lapierre, H. and G. E. Lobley. 2001. Nitrogen recycling in the ruminant: A review. *J. Dairy Sci.* 84:E223-E236.
- Nocek, J. E. 1997. Bovine acidosis: Implications on laminitis. *J.*

- Dairy Sci. 80:1005-1028.
- Obeid, M., A. Tesniere, T. Panaretakis, R. Tufi, N. Joza, P. van Endert, F. Ghiringhelli, L. Apetoh, N. Chaput, C. Flament, E. Ullrich, S. de Botton, L. Zitvogel, and G. Kroemer. 2007. Ecto-calreticulin in immunogenic chemotherapy. *Immunol. Rev.* 220:22-34.
- Palmer, J. L., R. H. Abeles. 1979. The mechanism of action of S-adenosylhomocysteinase. *J. Biol. Chem.* 254:1217-1226.
- Porter, T. D. 2002. The roles of cytochrome b5 in cytochrome p450 reactions. *J. Biochem. Mol. Toxicol.* 16:311-316.
- Sauvant, D., F. Meschy, and D. Mertens. 1999. Components of ruminal acidosis and acidogenic effects of diets. *Prod. Anim.* 12:49-60.
- Schenkman, J. B. and I. Jansson. 2003. The many roles of cytochrome *b*₅. *Pharmacol. Ther.* 97:139-152.
- Schoonjans, K., M. Watanabe, H. Suzuki, A. Mahfoudi, G. Krey, W. Wahli, P. Grimaldi, B. Staels, T. Yamamoto, and J. Auwerx. 1995. Induction of the acyl-coenzyme a synthetase gene by fibrates and fatty acids is mediated by a peroxisome proliferator response element in the c promoter. *J. Biol. Chem.* 270:19269-19276.
- Stone, W. 2004. Nutritional approaches to minimize subacute ruminal acidosis and laminitis in dairy cattle. *J. Dairy Sci.* 87:13-26.
- Wang, W. A., J. Groenendyk, and M. Michalak. 2012. Calreticulin signaling in health and disease. *Int. J. Biochem. Cell Biol.* 44: 842-846.
- Yan, Q., J. E. Murphy-Ullrich, and Y. Song. 2011. Molecular and structural insight into the role of key residues of thrombospondin-1 and calreticulin in thrombospondin-1-calreticulin binding. *Biochemistry* 50:566-573.