



Relationship between liver and low rumen pH in goat

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ABSTRACT. The aim of this study was to analyze the response of dry goat liver to sub-acute ruminal acidosis induced by a highly concentrated diet. Non-pregnant, non-lactating female Poll-goats (N = 12) were randomly assigned to either a high-concentrate (HG) or a low-concentrate (LG) diet. Low rumen pH was successfully induced with HG (more than 3 h with rumen pH < 5.8). The plasma lipopolysaccharide concentration was significantly decreased in the HG compared with LG group (P < 0.05). Proteomic analysis showed that aldehyde dehydrogenases and microsomal glutathione S-transferase was downregulated in the HG group, whereas aldo-keto reductase was upregulated compared in the LG group. The abundance of mRNA for these proteins were also correspondingly increased (aldehyde dehydrogenases and microsomal glutathione-S-transferase) or decreased (aldo-keto reductase) in the HG group. Malondialdehyde content in the liver was decreased in the HG group compared to the LG group. These data indicate that the expression of hepatic proteins alters the regulation of endogenous lipopolysaccharide during low rumen pH

in dry dairy goats. In particular, the protective effect of the liver may occur through inhibition of aldehyde and/or peroxide formation.

Key words: Lipopolysaccharide; Low rumen pH; Proteomics

INTRODUCTION

Subacute ruminal acidosis (SARA) is a ruminant condition that affects both feedlot and dairy cattle. SARA is highly prevalent in intensive production systems (Krause and Oetzel, 2006) and has economic consequences associated with decreased animal performance (Stone, 2004). Current feeding practices in the dairy and beef industries use highly fermentable diets to maximize energy intake and achieve high milk yield or rapid growth rates, but such diets change the rumen environment and increase the rate of short-chain fatty acid production. When production of these acids exceeds absorption, pH within the rumen liquor is decreased and SARA develops (Müller et al., 2002).

Induction of SARA by feeding high-grain diets increases lipopolysaccharide (LPS) content within the bovine rumen (Emmanuel et al., 2008; Khafipour et al., 2009). This can result in a 20-fold increase in the amount of LPS released into the rumen liquor; this endotoxin is then trans-located into the blood (Andersen et al., 1994a). Accumulation of endotoxin in the rumen adversely affects the rumen micro-flora and digestion, as well as causes papilla damage (Steele et al., 2009). Papilla damage allows the entry of endotoxins into the blood and leads to the development of generalized effects, such as liver inflammation and liver abscesses (Steele et al., 2009). Ultimately, the endotoxemia reduces feed intake and milk production (Stone, 2004; AlZahal et al., 2007; Steele et al., 2009). One aspect of ruminal acidosis that is not well-understood is the considerable variation in susceptibility among individual animals, even when fed a common diet (Bevans et al., 2005). Furthermore, previous studies have been shown that endotoxemia is not necessarily related to the presence of endotoxin in the peripheral blood. This is a result of hepatic metabolism as demonstrated by studies that investigated the clearance rate of LPS from the portal circulation by the liver (Andersen et al., 1994b), while other studies showed that endotoxin transferred from the gut to the blood was cleared by liver macrophages known as Kupffer cells or neutralized by lipoproteins (Harris et al., 2002). These results suggest that the liver plays a central role in clearing toxins translocated into the blood from inflammatory sites as well as in response to inflammatory conditions with a non-specific general response, known as the acute phase response (Jiang et al., 2008). Endotoxin clearing indicates that the liver plays a protective role in preventing tissue damage following SARA. Therefore, in addition to modifying conditions in the rumen, the adverse effect of SARA may also be mitigated through an understanding of the role of liver in response to acidosis. Together, these approaches may help to establish a productive dairy or beef herd under a high-concentrate feeding regime. Therefore, the aim of the present study was to characterize the role of the liver in the detoxification or neutralization of LPS in dry goats under conditions of low rumen pH resulting from highly concentrated diet supplementation.

MATERIAL AND METHODS

Animals and dietary treatments

Twelve non-pregnant, non-lactating female Poll-Goats (obtained from Luhe Animal

Science base of Jiangsu Academy of Agricultural Sciences, Jiangsu Province, China) were used for this study and cared for in accordance with the guidelines established by the People's Republic of China. All procedures were pre-approved by the Animal Care and Use Committee of Nanjing Agricultural University. The goats were de-wormed using 15 mg/kg body weight albendazole orally and 0.2 mg/kg body weight ivermectin subcutaneously, and were kept under uniform management conditions for adaptation to the new environment for 1 week prior to the study and to ensure they were healthy and free from any detectable clinical abnormalities. After adaptation the goats were grouped by age (23-25 months) and body weight (31.8-35.0 kg), and then randomly assigned to 2 treatments: high-concentrate group (HG, treatment) and low-concentrate group (LG, control) fed for 42 days with concentrate-to-forage ratios of 6:4 and 4:6 respectively (Figure 1). Diets were formulated to meet or exceed the minimum nutrient requirements as recommended by the National Research Council (2001) using the Cornell-Penn-Miner System (Table 1). The rumen of these animals was cannulated using the ruminostomy technique in an anaerobic environment maintained using a wooden stopper.

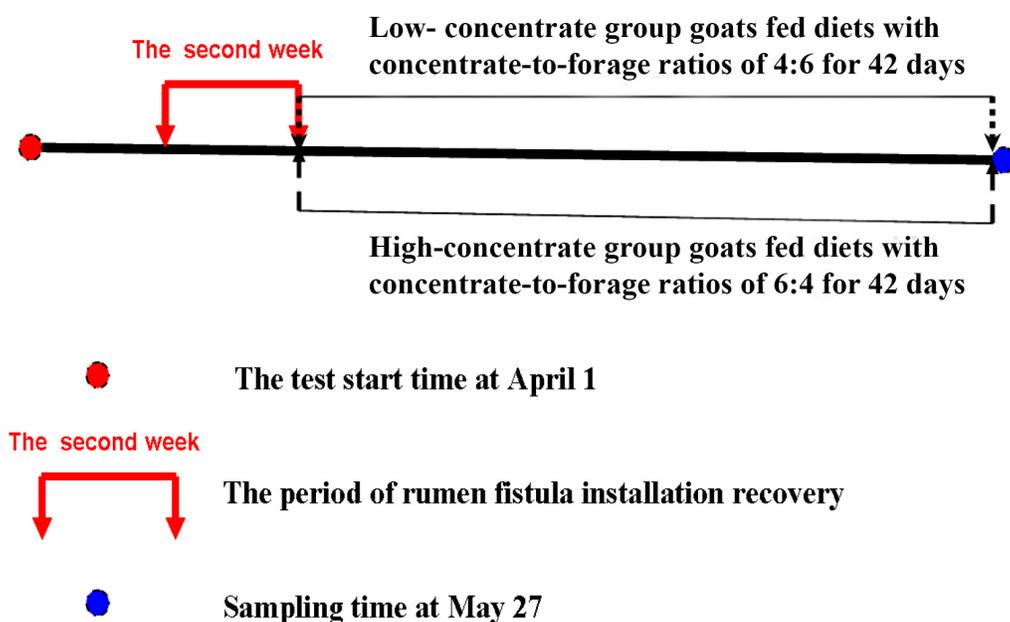


Figure 1. Experiment protocol (year, 2012).

Rumen fluid and rumen pH

Appropriate representative samples of ruminal fluid were collected at hourly intervals and strained through a cotton cloth and the pH was measured. The data were summarized by calculating the average pH, the length of time below pH 5.8, and the area (time x pH) below pH 5.8. A rumen pH below 5.8 was selected because microbial fiber digestion and nutrient digestibility are reduced below this pH value (Calsamiglia et al., 2002). Beauchemin et al. (2003) used rumen pH depression <5.8 as a threshold for SARA.

Table 1. Ingredient and nutrient composition of the low (LG) and high concentrate (HG) diets.

	Diets	
	HG	LG
Ingredients		
Hay	32.00	48.00
Purple medic	8.00	12.00
Corn	43.17	28.18
Soybean meal	12.40	8.75
Limestone	1.35	0.97
Calcium hydrogen phosphate	1.73	1.20
Salt	0.60	0.40
Premix ¹	0.75	0.50
Nutrients composition		
Net energy (MJ/kg)	5.89	5.40
CP (%)	13.75	12.24
NDF (%)	27.69	36.55
ADF (%)	17.54	24.04
Ca (%)	1.05	0.87
P (%)	0.51	0.40

¹Provided per kg of premix: Vitamin A, 6000 U; Vitamin D, 2500 U; Vitamin E, 80 mg; Cu, 6.25 mg; Fe, 62.5 mg; Zn, 62.5 mg; Mn, 50 mg; I, 0.125 mg; Co, 0.125 mg; Mo, 0.125 mg. CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber.

Blood sampling and analysis

Blood samples were collected using a jugular catheter at 0, 2, and 4 h post-feeding. Heparin was used as an anticoagulant for the preparation of plasma by centrifugation at 3000 g at 4°C for 10 min within 20 min of collection. Samples were stored at -20°C.

Plasma LPS was determined using an ELISA Assay Kit purchased from ShangHai Lengton Bioscience Co., Ltd. (Shanghai, China) and used according to manufacturer instructions after 1:3 sample dilution in pyrogen-free water. Plasma LPS determination was performed using a 96-well microplate at an absorbance of 450 nm on a microplate reader (RT-6000, RayTo, Shenzhen, China). The maleic dialdehyde (MDA) concentrations in the plasma and liver tissue were assayed using commercial assay kits purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Liver sampling, protein sample separation, and 2-dimensional gel electrophoresis (2-DE)

The goats were sacrificed by captive bolt stunning followed by exsanguination. The abdominal cavity was opened; the liver was removed and washed twice with cold physiological saline solution (0.9% NaCl solution) to remove the blood and other possible contaminants. The left lobe of the liver was separated, frozen in liquid nitrogen, and stored at -80°C.

For proteomic analyses, similar amounts of liver tissue from all animals within a group were combined and then homogenized in clearance buffer consisting of 7 M urea (Bio-Rad, USA), 2 M thiourea (Bio-Rad, USA), 2% (w/v) CHAPS (Bio-Rad, USA), 50 mM dithiothreitol (Bio-Rad, USA), 0.8% (w/v) ampharmalyte (Bio-Rad, USA), pH 4-7, and 1 mM phenylmethylsulfonyl fluoride (Bio-Rad, USA) using a glass homogenization vessel in an ice bath. The resultant homogenate was swirled for 20 min and centrifuged for 20 min at 15,000 g at 4°C. The supernatant was collected, aliquoted, and stored until use. The protein concentra-

tion in the supernatants was measured using the Bradford assay with bovine serum albumin used as the standard.

Iso-electrophoresis using immobilized pH gradient strips (pH 3.0-10.0 NL, 11 cm; Bio-Rad, USA) was used to separate 200 mg of each protein extract in the protean system (Bio-Rad, USA). Iso-focusing was performed by increasing the voltage in a step-wise manner as follows: 4 h at 500 V, 1 h at 1000 V, 1 h at 2000 V, 1 h at 4000 V, and 2.5 h at 8000 V, and then holding at 8000 V until a total of at least 60,000 Vh was reached. The second dimension was run on a 12.5% polyacrylamide sodium dodecyl sulfate gel using the Multiphor system (Amersham plc, Amersham, UK). The 2-DE for each sample was repeated 3 times. Neuhoff's colloidal Coomassie Blue G-250 (Amresco, USA) stain was carried out according to the method described by Candiano et al. (2004). Stained gels were scanned and analyzed using the PDQuest-7.3 software (Bio-Rad). After alignment, spots between gels were first automatically matched. The matched spots were then re-examined manually to ensure accuracy. Generally, only spots with a quality value over 50 were chosen for further analysis. Spot quantity normalization was conducted in the 'total quantity of valid spots' mode.

Matrix-assisted laser desorption/ionization-time of flight-mass spectrometry analysis and databases queries

In-gel trypsin digestion of protein spots (Promega, USA) and matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (Bruker Daltonic Inc., USA) analyses were based on procedures described by Wang et al. (2005). Mass spectrometry fingerprinting data searches were performed using the search engines ProFound and MS-fit (<http://www.matrixscience.com>) against the NCBI nr database in the taxa of Ruminant (goat or cattle) with the parameter sets of trypsin digestion, 2 missed cleavages, complete modification of iodoacetamide (Cys), partial modification of methionine oxidation, protein mass $\pm 20\%$ of the observed protein mass, pI = ± 1 of observed pI, and a mass tolerance for monoisotopic data of 100 ppm. Protein identification was assigned when the following criteria were met: at least 4 matching peptides and $>20\%$ sequence coverage (Huang et al., 2010).

Functional annotation of identified proteins

Functions of the proteins identified through MS fingerprinting data were annotated by querying against the protein function database Pfam (<http://www.sanger.ac.uk/Software/Pfam/>) or Inter-Pro (<http://www.ebi.ac.uk/interpro/>).

Assessment of aldehyde dehydrogenase (ALDH), aldo-keto reductase (AKR), and microsomal glutathione S-transferase (MGST) mRNA

The methods for total RNA extraction and RNase protection have been described elsewhere (Katsumata et al., 2000). Total RNA was isolated from 0.5 g liver tissue using TRIZOL (Invitrogen, USA) and quantified by measuring absorbance at 260 nm in a Nano-Drop ND-1000 Spectrophotometer (Thermo Scientific, USA) (Desjardins and Conklin, 2011). cDNA was converted by M-MLV reverse transcriptase (Promega, USA) as indicated by the manufacturer protocol (heated to 95°C for 2 min, incubated for 5 min at 70°C, and then chilled

on ice; cDNA was generated for 1 h at 37°C). Real-time PCR was performed using a SYBR Green PCR Master Mix (Roche, Basel, Switzerland) in a Bio-Rad MyiQ™ Detection System, according to manufacturer instructions. The thermal cycling conditions were 2 min at 50°C, 10 min at 95°C, followed by 40 repeats at 95°C for 20 s, 60°C for 45 s, and 72°C for 30 s in a Bio-Rad MyiQ™ Detection System.

The relative amount of mRNA for each target gene was determined from the ratio against the mRNA of β -actin. According to the comparative threshold cycle (Ct) method, the amount of target mRNA normalized to β -actin and relative to an internal control was calculated by $2^{-\Delta\Delta Ct}$; $\Delta\Delta Ct = (Ct_{i,j} - Ct_{b-actin,j}) - \text{mean}(Ct_{i,k} - Ct_{b-actin,k})$, where $Ct_{i,j}$ and $Ct_{b-actin,j}$ are the Ct for gene *i* and for β -actin in a sample (the sample named *j*) and where $Ct_{i,k}$ and $Ct_{b-actin,k}$ are the Ct for gene *i* and for β -actin in sample (the sample named *k*), expressed as the standard. The primers (Invitrogen) for ALDH, AKR, and MGST are shown in Table 2.

Table 2. Nucleotide sequences of specific primers used in the study.

Gene	GenBank accession No.	Primers (from 5' to 3')	Product size (bp)
β -actin	JN033788	F: CGGGA TCCAT CCTGC GTCTG GACCT G R: GGAAT TCGGA AGGAA GGCTG GAAGA G GGAATTCGGAAGGAAGGCTGGAAGAG	261
ALDH	NM_001046423.1	F: TGTCT TCACC AGGGA CATCC R: ATCTC CACAC ACACG GTCTT	195
AKR	XM_001787874.3	F: AGATC CTGAA CAAG CTGGGG R: AGAGC TGCCG TTTTC TTGTG	238
MGST	AF140223.2	F: ACATG AAGGA GAGAG CCCTG R: TAGTC TTGTC CGTGG CTCTT	187

Statistical analysis

The effect of diets on LPS and MDA content were analyzed by 1-way analysis of variance (ANOVA) using the general linear model. Differences among individual means were evaluated. ANOVA and correlation coefficient estimates for protein expression changes were evaluated using JMP 5.1 (SAS Institute, Cary, NC, USA). The results are reported as means \pm standard error of the mean and differences were considered to be significant when $P < 0.05$ as tested by ANOVA.

RESULTS

Ruminal fluid pH

The mean ruminal pH values were lower and were below 5.8 for a longer period of time in HG goats (Figure 2 and Table 3). Over the 12h measurement period, the average ruminal pH was below 5.8 (but above pH 5.6) for 4 h in HG animals, whereas the pH was always above pH 5.8 in LG goats (Figure 2).

LPS and MDA content

As shown in Figure 3, at all sample times the LPS concentrations in peripheral plasma were lower in the HG compared with the LG animals, particularly at 0 and 2 h ($P < 0.05$). Interestingly, the plasma concentration of LPS was highest at 0 h in both groups of goats. The LPS concentration was decreased at 2 and 4 h compared with 0 h in the HG group ($P < 0.05$),

but was similar between 2 and 4 h ($P > 0.05$).

MDA content in the liver was greater for HG compared with LG ($P < 0.05$; Figure 4), although these differences were not reflected in the plasma concentrations ($P > 0.05$). Importantly, MDA concentration in the plasma of HG animals was lower than that of corresponding liver values ($P > 0.05$).

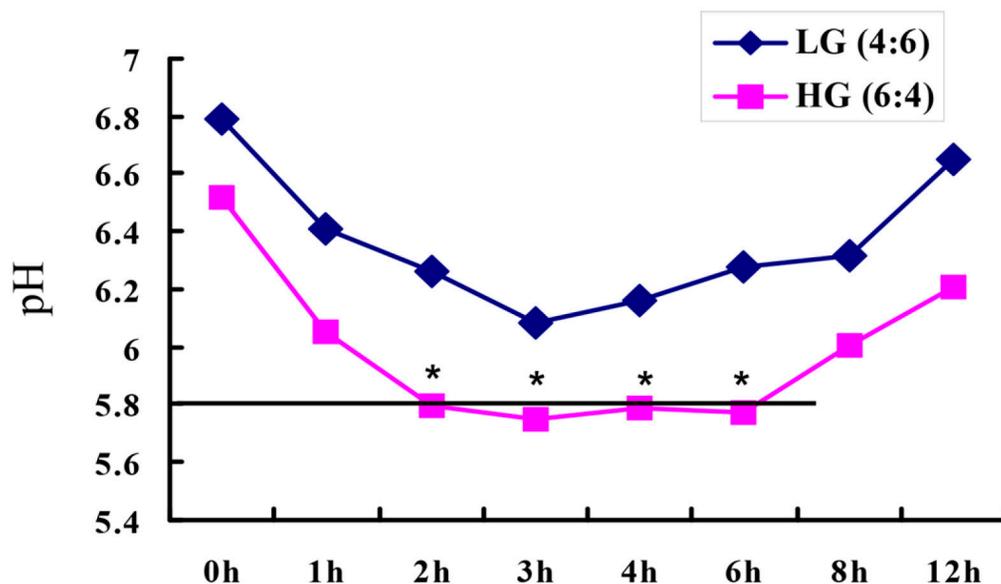


Figure 2. Rumen fluid pH over 12 h from groups of dry Poll dairy goats fed either a high-concentrate (HG, square) or a low-concentrate (LG, diamond) diet. Values are reported as means \pm SE, N = 6. * $P < 0.05$ between groups.

Table 3. Effect of low (LG) and high concentrate (HG) diets on ruminal pH of dry dairy goats.

	Treatment		P value ¹
	LG	HG	
Mean rumen pH ²	6.37 \pm 0.085	5.98 \pm 0.096	<0.05
Ruminal pH < 5.8	0	240 \pm 5.76	<0.001
Duration (minutes/12 h)	0	17.4 \pm 0.3	<0.001
Area [times (h) \times pH]	0		

Values are mean \pm SE. ¹P value indicates statistical differences between HG and LG treatments. ²Ruminal pH data were collected during 12 h.

Comparison of hepatic proteomic patterns between the high concentrate and low concentrate diets

Good separation of proteins was observed on 2-DE, except in the base area. A total of 18 spots were identified that differed between diet treatments. Of these, 15 showed good repeatability between gels for the same sample. Spot-to-spot correlation coefficients were estimated and were found to vary from 0.83-0.91, with all significant at $P \geq 0.0001$. In each sample, at least 1 of the 3 correlation coefficients was >0.85 , while the difference in spot volume among replicates was not significant according to ANOVA.

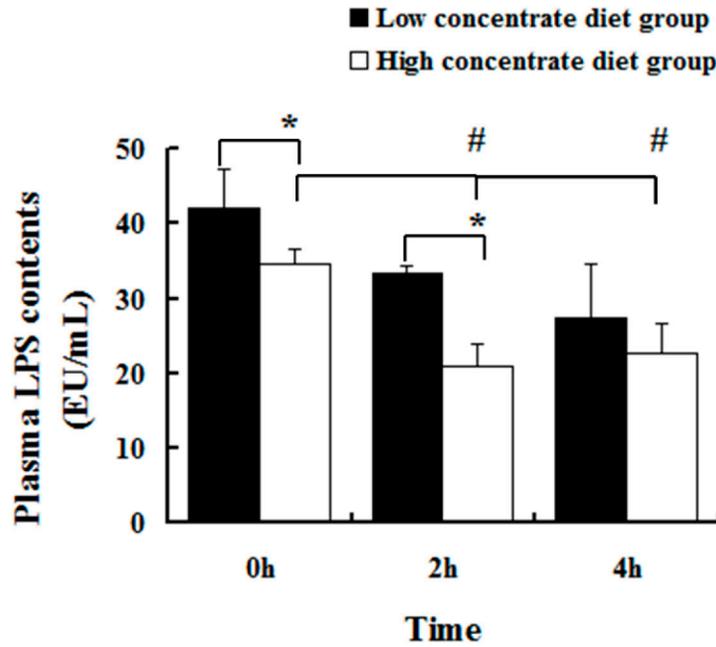


Figure 3. Effect of high concentrate diet supplementation on plasma lipopolysaccharide concentrations in dry goats in the 4 h after being fed either a high concentrate or low concentrate diet. Values are reported as means \pm SE, N = 6. *P < 0.05 between diets at each time point; #P < 0.05, within diet compared with 0 h.

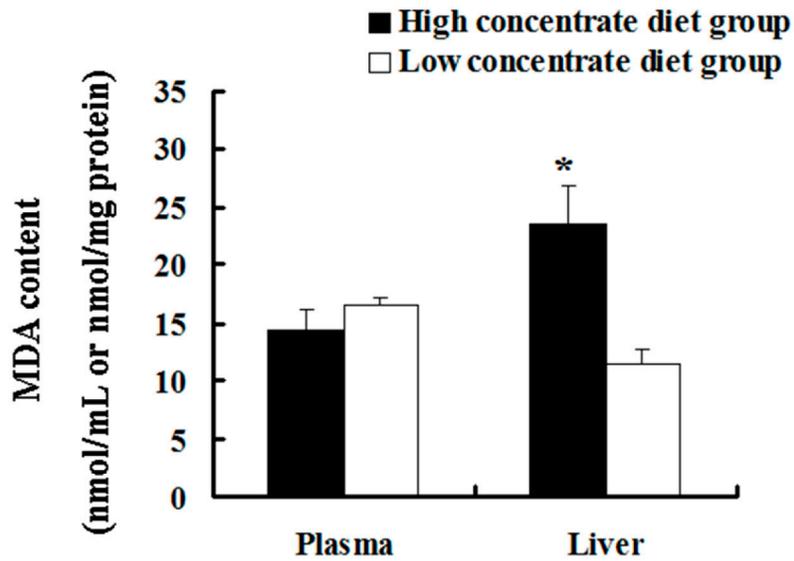


Figure 4. Effect of high concentrate diet supplementation on the concentration of maleic dialdehyde (MDA) in plasma and liver of dry goats fed either a high concentrate or low concentrate diet. Plasma MDA concentration as nM; liver MDA concentration as nmol/mg protein. Values are reported as means \pm SE, N = 6. *P < 0.05, compared within sample site between diets.

The 18 spots that differed by at least 2-fold in abundance between the high and low concentrate treatments are shown in Figure 5. These proteins were subjected to in-gel digestion and analyzed by matrix-assisted laser desorption/ionization-time of flight-mass spectrometry and then the MS fingerprint data were compared against the Swiss-Prot, NCBI nr protein, and Polypeptide databases. All proteins were identified successfully and sorted into 6 main functional classes based on KOG (Eukaryotic orthologous groups of proteins, <http://www.hsls.pitt.edu/obrc/index.php?page=URL1144075392>) database comparison. A group of proteins (spots 3, 13, and 17) were categorized as important in carbohydrate metabolism, 4-trimethylaminobutyraldehyde dehydrogenase, aldehyde dehydrogenase, and the aldo-keto reductase family. Others proteins (spots 5, 9, 15, and 18) were involved in signal transduction and anti-oxidative stress pathways, and included catalase and heat shock protein beta-1. The remaining proteins were not related to any KOG-based function (Table 4).

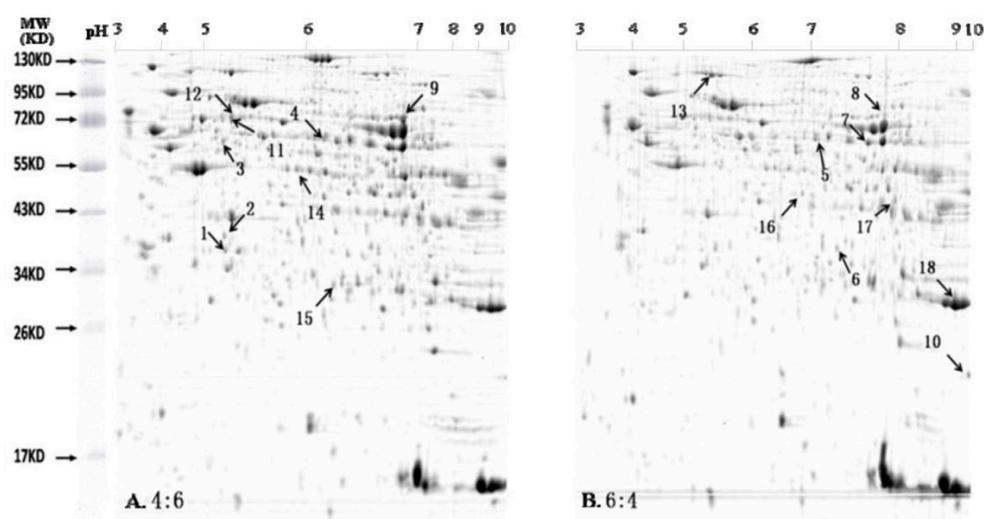


Figure 5. 2-D proteomic profile liver proteins from dry goats fed either a high-concentrate or low-concentrate diet. **A.** Low-concentrate diet treatment (4:6). **B.** High-concentrate diet treatment (6:4). Arrows indicate the proteins with at least a 2-fold change between the 2 diets. Numbers refer to proteins identified in Table 4.

Table 4. Differential-expression of liver proteins identified by PMF query from goats fed low (LG) and high concentrate (HG) diets.

Spot No.	gi	Up or Down	Identified protein name	MW (kDa)/pI	Functional classification	Accession No.
3	114051782	Down	4-trimethylaminobutyraldehyde dehydrogenase	54854/5.84	Catalytic activity	NP_001039888
5	115496214	Down	Aldehyde dehydrogenase, mitochondrial precursor	57073/7.55	Catalytic activity; signal transduction	NP_001068835
9	78369302	Down	Catalase	60106/6.78	Catalytic activity; signal transduction	NP_001030463
13	156718104	Down	Aldehyde dehydrogenase family 1 member L1	99531/5.53	Catalytic activity	NP_001096557
15	71037405	Down	Heat shock protein beta-1	22722/5.77	Signal transduction	NP_001020740
17	194685192	Up	PREDICTED: aldo-keto reductase family 1, member C1	37214/7.61	Catalytic activity; enzyme regulation activity, anti-oxidant	XP_0011787926
18	76876909	Down	Microsomal glutathione S-transferase	25385/8.87	Cellular detoxification	AAD42800

ALDH, MGST, and AKR gene mRNA expression in liver

The ALDH, MGST, and AKR mRNA abundances in the liver are summarized in Figure 6. When compared with LG goats, decreases in ALDH (Figure 6A) and MGST (Figure 6B) mRNA level were observed for HG goats ($P < 0.05$), while AKR mRNA abundance was markedly increased ($P < 0.05$, Figure 6C).

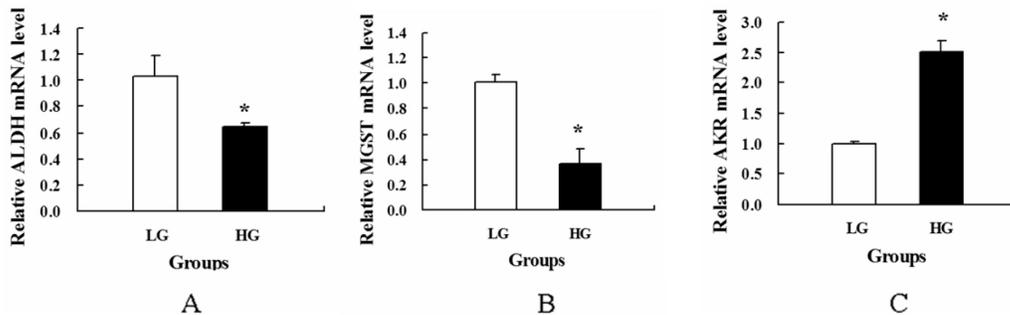


Figure 6. Expression of ALDH, MGST, and AKR mRNA in liver from goats fed either a high concentrate or low concentrate diet. **A.** ALDH mRNA; **B.** MGST mRNA; **C.** AKR mRNA. RNA extracted from liver tissue was reverse-transcribed to cDNA and analyzed by real-time polymerase chain reaction. Values are reported as means \pm SE, $N = 6$. * $P < 0.05$, difference between diets for each mRNA species.

DISCUSSION

Data from this study is in agreement with those of previous reports showing that high concentrate diets decrease ruminal pH (Sutton et al., 2003). In our study, the depression in ruminal pH below 5.8 persisted for 4 h (Table 3). Previous reports demonstrated a higher LPS concentration in peripheral plasma during acidosis in feedlot and dairy cows fed diets containing higher proportions of grain (Andersen, 2003). Similarly, switching the animal diet from all-forage to one with 61% concentrate caused a 3-fold increase in LPS concentration during SARA (Gozho et al., 2006). In contrast, plasma LPS concentrations were decreased in dairy cows when the grain content of the diet was increased (Gozho et al., 2007). Our data support these latter observations in which plasma LPS concentrations were reduced in goats fed the high concentrate diet compared with those fed the low concentrate ration. This result was consistent with that of a previous study showing that LPS was cleared by the liver and transferred to the blood in a form neutralized by lipoproteins (Harris et al., 2002). These findings suggest that high concentrate diet supplementation alters specific protein or enzyme expression in the liver of dry goats as part of the mechanism for clearing and detoxifying LPS.

Based on the protective response by the liver against acidosis, we used proteomic analysis to quantify changes in proteins and enzymes associated with putative mechanisms. In particular, 7 proteins that were differentially expressed in the goats fed the high concentrate diet were involved in carbohydrate metabolism and anti-oxidation pathways. In response to the high concentrate diet, 4-trimethyl-amino-butyraldehyde dehydrogenase (4-TABD) was upregulated. This is a key enzyme in carnitine biosynthesis that oxidizes 4-trimethyl-ammonium-butanol to 4-trimethyl-ammonium-butanoic acid. Carnitine is necessary for fatty acid transport from the cytosol to the mitochondria, a key step prior to beta-oxidation within the

citric acid cycle and energy release (Fritz and Yue, 1963; Bremer, 1983). *In vivo* experiments have shown that carnitine displays a variety of protective effects, including de-activation of abnormal metabolites, as an antioxidant, and aids in the repair of cell membranes (Arduini, 1992). Thus, upregulation of 4-TABD expression may permit increased L-carnitine synthesis within the liver of HG goats, leading to a protective effect by neutralizing LPS. However, the relationship between 4-TABD and the antioxidant defense barrier against abnormal metabolites, including LPS, requires further investigation.

ALDHs are a group of NAD(P⁺)-dependent enzymes that oxidize a wide range of aldehydes to their corresponding carboxylic acids. Because aldehydes are highly reactive molecules involved in a broad spectrum of physiological, biological, and pharmacological processes, ALDH plays a key role in reducing their toxic effects on the body (Daiber et al., 2009). Previous studies have shown that ALDH alleviated the toxic effects and oxidative damage associated with aldehydes, and may effectively alleviate acetaldehyde-elicited cell injury and apoptosis through oxidation-reduction signaling pathways involving extracellular signal-related kinases 1/2 and stress-activated protein kinase/c-Jun N-terminal kinase-dependent mechanisms (Lu and Morimoto, 2009). ALDH1, ALDH precursors, and ALDH mRNA levels were all downregulated when the dry goats were fed a high concentrate diet. In contrast, AKR was upregulated in the HG group. AKR plays an important role in the detoxification of aldehydes and ketones (Jez et al., 1997). AKR has high catalytic activity against straight-chain saturated and unsaturated aldehydes and regulates osmosis involved in the metabolism of fructose and steroid hormones (Yabe-Nishimura, 1998). In addition, inhibition of AKR prevented both the early activation of complement in the vessel walls within the retina and decreased the levels of complement inhibitors in diabetic rats, as well as the apoptosis of vascular pericytes and endothelial cells and the development of acellular capillaries (Dagher et al., 2004). In the current study, the increase in liver MDA in HG goats may have induced AKR upregulation as part of the control of aldehyde formation pathways or hepatic detoxification of aldehydes. Thus, the rate of formation of hepatic aldehydes and the associated oxidative stress may be regulated by ALDH and AKR in response to dietary changes within ruminants. These data indicate that altered ALDH and AKR expression may play a key role in the liver by ameliorating the deleterious effects of LPS production induced by low rumen pH.

GST is dimeric, major phase II metabolism enzyme found mainly in the cytosol and is involved in the extensive ligand binding mechanism linked to detoxification in insects (Yu et al., 2008; Yamamoto et al., 2005, 2009), and aquatic animals (Nóvoa-Valinas et al., 2002; Hamed et al., 2004; Sen and Kirikbakan, 2004; Lee et al., 2006). Importantly, the hepatic isoenzyme GST 8-8 is induced in response to the presence of other lipid peroxides (Khan et al., 1995). Other studies showed that GST expression was regulated by the enhancement of c-Fos and c-Jun gene transcription (Bergelson et al., 1994). Both MGST mRNA abundance and MGST protein were downregulated in dry goats fed a high concentrate diet. These data indicate that lipid peroxide formation could be inhibited, alleviating the toxic effects of LPS. Thus, the effects of the high concentrate diet on the combination of 4-TABD, ALDH, AKR, and MGST synthase expression in the liver may play a significant role in the detoxification of deleterious metabolic substances, although the precise mechanisms require further examination.

Overall, our proteomics data suggest that the liver exerts protective effects by inhibiting reactions associated with aldehyde formation. However, understanding the mechanism involved in the release, removal, or neutralization of LPS by the liver in goats fed high con-

centrate diets requires further analysis. Importantly, the protective response of the liver during short-term diet-induced low rumen pH in dry dairy goats will be helpful for preparing the animal for the next lactation.

Conflicts of interest

The authors declare no conflict of interest.

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