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ORIGINAL ARTICLE

Metabolomic analysis of amino acid metabolism in colitic rats supplemented with lactosucrose

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Abstract Intestinal inflammation causes metabolic disorders. The purpose of this study was to determine the effect of dietary supplementation with lactosucrose (LS) on the serum metabolome and intestinal luminal content of fatty acids in colitic rats. Colitis was induced in rats using trinitrobenzene sulfonic acid. Subsequently, rats received intragastric administration of either 250 mg LS/kg body weight or saline (the control group) every day for 5 weeks. Short-chain fatty acids in the intestinal lumen, blood profile, and metabolites in serum were measured, respectively, using gas chromatography, biochemistry analyzer, and nuclear magnetic resonance-based metabolomics combined with multivariate statistics. Metabolic effects of LS included: (1) decreases in concentrations of branched-chain amino acids (isoleucine and valine), alanine, citric acid, trimethylamine oxide and taurine, and the abundance of aspartate aminotransferase in serum; (2) increases in concentrations of glucose metabolites (including succinate) in serum; and (3) altered concentrations of butyrate in the cecal content and of butyrate and acetate in the colon content. The results indicate that LS supplementation to colitic rats affects whole-body metabolism of amino acids and release of aspartate aminotransferase and alkaline phosphatase from tissues into the blood circulation, and enhances the production of short-chain fatty acids in the intestinal lumen.

Keywords Amino acids · Metabolites · Lactosucrose · Inflammation · Nuclear magnetic resonance spectroscopy

Abbreviations

CMDI	Colonic mucosal damage index
IBD	Inflammatory bowel disease
LS	Lactosucrose

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NMR	Nuclear magnetic resonance
PCA	Principal component analysis
SCFA	Short-chain fatty acids
TCA	Tricarboxylic acid
TNBS	Trinitrobenzene sulfonic acid

Introduction

Chronic inflammation is associated with metabolic syndromes, including abdominal obesity, hyperlipidemia and type 2 diabetes (Alemany et al. 2012; Hotamisligil et al. 2006; Ren et al. 2013). Intestinal inflammation leads to disorders in nutrient metabolism (Mercier et al. 2002). Furthermore, Guillet et al. (2012) reported that impairment in the regulation of protein metabolism was linked to obesity and metabolic inflammation. There is also growing interest in the use of new biomarkers (e.g., isoleucine or histidine decarboxylase in plasma) of inflammatory bowel disease (IBD) to diagnose and evaluate its prognosis (Roda et al. 2010). In diabetic humans, the concentrations of 19 metabolites in plasma were increased, including leucine, lysine, phenylalanine, eight acylcarnitines, six lysophosphatidylcholines, and two lysophosphatidylethanolamines (Ha et al. 2012, Zhou et al. 2012). Patients with polycystic ovary syndrome have perturbations in amino acid metabolism, tricarboxylic acid (TCA) cycle, and the gut microflora (Sun et al. 2012). Although previous studies of intestinal inflammation have focused on lipid-related metabolites and cytokines, little attention has been directed to amino acids.

Available evidence shows that inflammation in tissues is associated with altered metabolism of amino acids (Wu 2013b). For example, glutamate is involved in intestinal and cerebral inflammation responses (Xu et al. 2005). Van Meijl et al. (2010) found that glutamine, leucine glycine, and proline attenuated IL-8 production, probably through inhibition of NF-kappaB in HepG2 cells (Yin and Tan 2010). These functional amino acids have multiple regulatory functions in cells (Kim et al. 2007; Li et al. 2007;Wang et al. 2013; Wu et al. 2011a, b, 2013; Wu 2013a). Chitosan oligosaccharide may be effective in the treatment of IBD through inhibition of the NF-kappa B signaling and apoptosis of intestinal epithelial cells (Yousef et al. 2012; Huang et al. 2005, 2007; Tang et al. 2005; Yin et al. 2004). Results of preclinical studies indicate that L-arginine and its family amino acids supplementation could be a potential therapy for IBD through enhancement of iNOS activity (Coburn et al. 2012; Wu et al. 2012; Liu et al. 2012a; Tan et al. 2010; Kong et al. 2012). Oligosaccharides are attracting increasing interest as prebiotic functional food ingredients to treat certain clinical conditions (Kong et al. 2007a, b, c, 2009; Li et al. 2009; Deng et al. 2007; Yin et al. 2008, 2009), including colon cancer, IBD, and mineral malabsorption (Rastall 2010). Treatment with fructo-oligosaccharide (FOS) (days 10–19) produced a faster recovery from intestinal damage with the outcomes of increased crypt depth and crypt area in a mouse model of colitis (Winkler et al. 2007). There are reports about the effect of prebiotic oligosaccharides or dietary amino acids on intestinal inflammation (Hou et al. 2012; Rhoads and Wu 2009). However, there is lack of data concerning the effect of dietary oligosaccharide on amino acid metabolism in the intestine under inflammatory conditions.

Metabolomics has emerged as a powerful discovery tool in nutrition and biomedical research (He et al. 2009; He et al. 2011a, b, c; Lin et al. 2011; Wang et al. 2009). Nuclear magnetic resonance (NMR) spectroscopy-based metabolomics can provide a wealth of metabolite information and metabolic fingerprints about biological samples obtained from humans and other animals under various nutritional and pathological conditions (Ellis et al. 2007). The comprehensive biochemical profiles of low-molecular weight metabolites generated by NMR spectroscopy can be altered in response to various stimuli to maintain homeostasis (Weljie et al. 2007). Therefore, NMR analysis can reveal important information to aid in understanding molecular mechanisms and provide novel insight into the intervention effect or perturbation of diet on nutrient metabolism and health. However, few studies have focused on the effect of prebiotic oligosaccharides on metabolites in humans or other animals with intestinal inflammation.

This study used a ^{1}H NMR-based metabolomic strategy in conjunction with multivariate analysis to investigate metabolic responses to dietary supplementation in lactosucrose colitic rats. Metabolic profiles in the serum and short-chain fatty acids (SCFA) in the colonic lumen of weanling rats were measured to achieve our study objective.

Materials and methods

Rats, diets and experimental design

Sixteen female Sprague–Dawley rats (200 ± 20 g) were obtained from SIPPR-BK Experimental Animal Co. (Shanghai, China). They were housed in a clean, temperature-controlled environment and had free access to the standard rodent diet and drinking water (Ren et al. 2012). Colitis was induced by TNBS (trinitrobenzene sulfonic acid) as described previously (Nieto et al. 1998). After 3 days, colitic rats were randomly assigned into one of the two groups: control and lactosucrose supplemented (six rats for every group). Rats in the control group were fed the diet and received 2 ml of physiological saline per day, whereas rats in the lactosucrose group were fed the same diet and received oral administration of 2 ml of LS solution (250 mg/kg body weight) per day. This study was carried out at the Center for Disease Control and

Prevention of Jiangxi Province (Nanchang, China) and performed in accordance with the Chinese guidelines for the laboratory animals care (Yao et al. 2008).

Collection and storage of serum and large intestinal content

At the end of a 35-day period of supplementation, rats were killed following a 12-h period of food deprivation to avoid a postprandial effect on serum metabolites (Wu et al. 2013). Blood samples (5 mL) and luminal chyme in the cecum and colon were collected. Serum was obtained by centrifugation at 1,000×*g* and 4 °C for 10 min and stored in 2-mL aliquots at −80 °C until NMR analysis (Yin et al. 2010; Liu et al. 2012b). Intestinal content was stored at −80 °C until gas chromatographic analysis (Tan et al. 2009).

Colonic mucosal damage index (CMDI)

Macroscopic colon damage was scored by two independent observers, described as CMDI, according to the following morphological criteria (Mei et al. 2005): 0 = no damage; 1 = mild hyperemia and edema, and no erosion or ulcer existing in the colonic mucosa surface; 2 = moderate congestion and edema, and erosion existing in the mucosa surface; 3 = severe hyperemia and edema, and necrosis, inflammation and ulcer, with maximum longitudinal diameter of ulceration less than 1 cm; 4 = severe hyperemia and edema, and necrosis and ulcer on mucosa, with maximum longitudinal diameter of ulceration more than 1 cm.

Intestinal morphology analysis

Three cross sections (5-μm thick) of each intestinal segment were processed in paraffin and stained with hematoxylin and eosin. The method was according to that described by Kim et al. (2006).

Concentration of short-chain fatty acids in chyme with gas chromatography

Cecal digesta and colon digesta samples (0.1 g) were homogenized with 10 ml of water and centrifuged at 1,000×*g* for 10 min. A mixture of the supernatant fluid and 25 % metaphosphoric acid solution (V/V = 4:1) was prepared for the determination of SCFA (acetic, propionic, and butyric) by gas chromatography with external standard method (Zhou et al. 2011).

Assay of blood clinical chemistry

When all rats were killed, fresh blood samples were obtained from the heart and collected into heparinized tubes. The blood samples were centrifuged at 600×*g* and the supernatant fluid (plasma) was obtained. All samples were stored at −20 °C for analysis.

Hematological parameters, alkaline phosphatase (ALP), total protein, albumin, urea nitrogen, low-density lipoprotein, and cholesterol were determined using a biochemistry analyzer (Beckman, CA, USA). Alanine transaminase (ALT) and aspartate aminotransferase (AST) were determined by spectrophotometry using commercial kits (Nanjing Jiancheng Bio., Nanjing, China).

^{1}H-NMR spectroscopic analysis of serum

For ^{1}H NMR spectroscopy experiments, serum (210 μL) was mixed with 420 μL saline (0.9 % NaCl containing 50 % D_2O). D_2O provided a field frequency lock. ^{1}H NMR spectra were acquired on a Bruker Avance DRX-600 spectrometer (Bruker Biospin, Rheinstetten, Germany) operating at a proton frequency of 599.97 MHz and a temperature of 298 K, using a cryogenic high-resolution probe. For each sample, the 90° pulse length (~10.0 μs) was adjusted separately. A total of 32 transients were averaged and collected into 32 K data points for each spectrum, using a 15-ppm spectral width and relaxation delay of 2 s. There were three ^{1}H NMR spectra for each sample. The standard one-dimensional NMR spectrum (NOESY), which is a general depiction of the total biochemical composition, was acquired using the NOESY pulse sequence [90°-t1-90°-tm-90°-acq] and applied to suppress the residual water signal (Nicholson et al. 1995). The tm was 100 ms, the t1 was set at 3 μs, and weak irradiation of the water resonance was achieved during tm and RD (18B). The spectra of samples were acquired through a Carr–Purcell–Meiboom–Gill (CPMG) spin-echo pulse experiment, which was performed using the CPMG pulse sequence [90°-(T-180°-T)n-acq] (He et al. 2009). Spectra were obtained with a total spin–spin relaxation delay ($2n\tau$) of 200 ms. The diffusion-edited NMR spectrum, which was measured using the bipolar-pair longitudinal-eddy-current (BPP-LED) pulse sequence [RD-90°-G_1-τ-180°-G_2-τ-90°-D-90°-G_3-τ-180°-G_4-τ-90°-T_e-90°-acq] (Wu et al. 1995), was used to selectively measure the large macromolecules. The experimental conditions included: the duration of 2.5 ms and a delay (τ) of 400 μs, as well as a delay Te of 5 ms and a diffusion time (Δ) of 100 ms. For selected serum samples, signal assignment was achieved using two-dimensional (2D) total correlation spectroscopy (TOCSY) and literature values.

Data analysis

All NMR spectra were phase adjusted and baseline corrected with MNova-6.1.1 (MestreLab, Santiago de

Compostela, Spain). The chemical shift was compared to the reference of the lactate doublet at δ1.33. Each spectrum was bucketed by contiguous segments having an equal bin size of 0.005 ppm and integrated over the range of δ0.5–9.0. The region δ4.6–5.2 was removed to avoid the influence of the water signal. Each integral region was then normalized to the sum of total integral regions. Then the data were exported as an Excel file and input into SIMCA-P 12.0 as variables. The resulting data of principal component analysis (PCA) were intuitionistic by the PC score plots and loading plots. From the data and NMR spectra, classification of samples and the metabolites can be listed separately (Yang et al. 2007).

All experimental results are expressed as the mean ± standard deviation (SD). The significance of the CMDI data was determined by the Kruskall–Wallis test using the SPSS 16.0 software (SPSS Inc., Chicago, USA). One-way analysis of variance was performed to test initially for differences in the treatment (Wei et al. 2012). The unpaired *t* test was conducted to examine significant differences between the two groups (Fu et al. 2010). For all analyses, *P* values less than 0.05 were taken to indicate statistical significance.

Results

Colonic damage in inflammatory rats and effects of lactosucrose supplementation on intestinal morphology

Using rats with TNBS-induced inflammatory bowel, we observed that animals had a substantially damaged colonic mucosa (Fig. 1a, b). The histological appearance of the colon demonstrated distinct atrophy and a loosely arrayed epithelium (Fig. 1b). HE staining of specimens from the ileum of inflammatory rats revealed that their villi were blunt and the brush border was discontinuous (Fig. 1c). The rats supplemented with LS exhibited improvements in the colon and ileum (Fig. 1). In particular, treatment with LS reduced intestinal mucosal damage.

Effects of lactosucrose supplementation on short-chain fat acids in the luminal content of the large intestine

Rats in the LS group exhibited increases in concentrations of butyrate ($P < 0.05$) in the lumen content of the cecum, compared with the control group (Table 1). Similar results were obtained for butyrate in the colon chyme. The concentration of propionate in the colon or cecum content did not differ ($P > 0.05$) between the control and LS-supplemented rats.

Effects of lactosucrose supplementation on blood profile

Dietary supplementation with LS decreased ($P < 0.05$) the activity of AST in serum and increased ($P < 0.05$) the activity of ALP in serum, compared to the control group (Table 2). The increase in the activity of serum AST suggests the exacerbation of liver injury. The decrease in the activity of AST in the serum of the LS group, compared to the control group, showed that LS treatment attenuated tissue injury and inflammation. The results are consistent with those in Fig. 1.

^{1}H-NMR spectrum of serum

A typical one-dimensional ^{1}H NMR CPMG (Fig. 2a), NOESY (Fig. 2b), and BPP-LED (Fig. 2c) spectrum obtained from the rat serum are displayed in Fig. 2. From these spectra, 40 metabolites were clearly assigned. Their

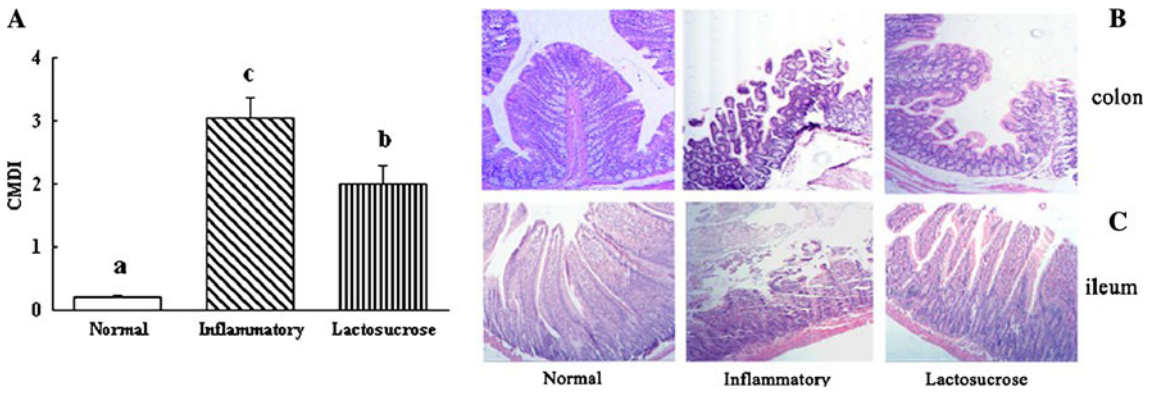

Fig. 1 Colonic damage in inflammatory rats and effects of lactosucrose supplementation on intestinal morphology. **a** Colonic mucosal damage index; *letters a*, *b* and *c* in the column indicate significant differences ($P < 0.05$). **b** Representative figures for the colon stained with hematoxylin and eosin. **c** Representative figures for the ileum stained with hematoxylin and eosin. All pictures are shown with magnification ×100

Table 1 Concentrations of short-chain fat acids in cecal and colon chyme of colitic rats

Treatment groups	Acetate (mmol/L)	Propionate (mmol/L)	Butyrate (mmol/L)	Acetate:propionate:butyrate
Colon chyme				
Control	1.63 ± 0.11[a]	0.34 ± 0.06[a]	0.64 ± 0.08[a]	62.5:13.0:24.5
Lactosucrose	1.26 ± 0.08[a]	0.38 ± 0.06[a]	0.75 ± 0.02[b]	52.7:15.9:31.4
Cecal chyme				
Control	7.87 ± 0.62[a]	3.07 ± 0.20[a]	3.57 ± 0.20[a]	54.2:21.2:24.6
Lactosucrose	11.02 ± 0.94[b]	2.87 ± 0.36[a]	7.35 ± 0.42[b]	51.9:13.5:34.6

Values are mean ± SD, $n = 6$

Within a column for each variable, superscript letters, a and b, indicate significant differences ($P < 0.05$)

Table 2 Effects of lactosucrose supplementation on blood profile in TNBS-induced colitic rats

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/L)	ALB (g/L)	BUN (mmol/L)	LDL (mmol/L)	CHO (mmol/L)
Control	42.2 ± 6.4[a]	19.8 ± 5.9	41.0 ± 15.4[b]	65.4 ± 3.3	30.8 ± 1.9	6.48 ± 0.4	0.43 ± 0.0	1.25 ± 0.1
Lactosucrose	32.2 ± 3.3[b]	15.0 ± 2.8	67.6 ± 17.7[a]	67.6 ± 4.9	31.4 ± 2.8	5.99 ± 1.1	0.46 ± 0.0	1.27 ± 0.4

Values are mean ± SD, $n = 6$

Within a column for each variable, superscript letters, a and b, indicate significant differences ($P < 0.05$)

ALT alanine transaminase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *TP* total protein, *ALB* albumin, *BUN* urea nitrogen, *LDL* low-density lipoprotein, *CHO* cholesterol

chemical shifts and peak multiplicity are given in Table 3, along with the corresponding ^{1}H NMR chemical shifts and signal multiplicities. Assignment of metabolites was made by comparison with literature values (Liao et al. 2007) and confirmed by 2D ^{1}H–^{1}H COSY and TOCSY methods (data not shown).

The spectra from all serum samples contained resonances from amino acids, organic acids, albumin, lipids, and unsaturated lipids, as well as choline and creatine. TCA cycle metabolites, including succinate, citrate and fumarate, were also detected by ^{1}H NMR spectroscopy.

Effects of lactosucrose supplementation on metabolites in the serum of colitis rats

To detect more subtle treatment-related metabolic differences, pattern recognition techniques were applied (He et al. 2011). The PCA score plot of the ^{1}H NMR serum data is shown in Fig. 3a. This plot shows the first two PCs and accounts for 75.7 % of the variation in the samples. The corresponding loading plot (Fig. 3b) indicated decreases in serum NMR peaks of valine, isoleucine, alanine, citric acid, trimethylamine oxide, and taurine in LS-supplemented rats, compared with the control group. Increases in the peaks of succinic acid and α-glucose are the major contributors to the separate clustering of the groups (Table 4). In addition, decreases in serum leucine, β-hydroxybutyrate, creatine, arginine, glycine, threonine, and betaine were observed in the LS-supplemented rats (Table 3).

Discussion

In IBD, the intestine often exhibits epithelial injury, which causes ulcerations and dysfunction of digestion and impaired transcellular transport (Boutry et al. 2012; Wehkamp et al. 2005). Therefore, alterations in the levels of metabolites including amino acids and fatty acids may aid in developing dietary interventions to treat the metabolic syndrome (Dai et al. 2013; He et al. 2011; Li et al. 2011; Wu et al. 2012). Information on concentrations of metabolites in the serum of animals can be obtained in a noninvasive means to provide insight into changes in whole-body metabolism of nutrients (Wang et al. 2013; Wu 2009, 2010a, b).

A novel and unexpected finding from this work is that dietary supplementation with LS affects the circulating levels of intermediates in the TCA cycle. Specifically, oral administration of LS to young rats increased the concentration of succinic acid, while decreasing the concentration of citric acid in serum (Table 3). Succinate can be derived from the catabolism of many amino acids, including glutamate, glutamine, histidine, isoleucine, methionine, arginine, ornithine, proline, and valine (Lei et al. 2012; Rezaei et al. 2013a, b; Wu 2013b). Increases in the degradation of amino acids (e.g., arginine and branched-chain amino acids) via inter-organ cooperation may lead to reductions in their concentrations in plasma or serum (Dai et al. 2012a, b, c; Tan et al. 2012; Yao et al. 2012). This notion is consistent with the findings from the present study. A lowered level of citrate in serum may reflect a reduced

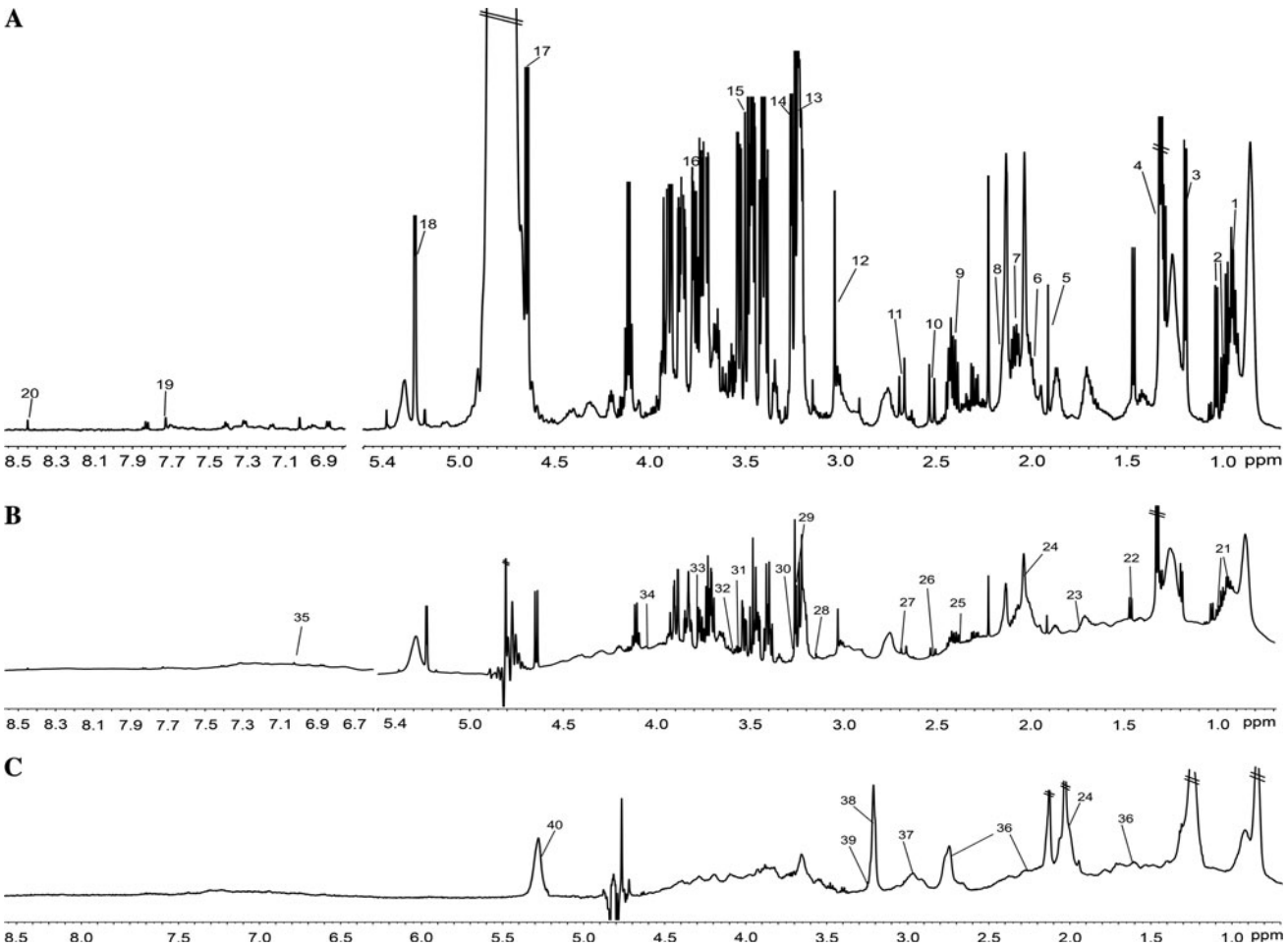

Fig. 2 Typical 600 MHz ^{1}H NMR CPMG (**a**), NOESY (**b**) and BPP-LED (**c**) spectra of serum taken from a colitic rat

leakage of the mitochondrial and plasma membranes of cells in the body. In addition, the concentrations of α-glucose and β-glucose in serum were markedly higher in LS-supplemented rats than in the inflammatory group (Table 3). This result may be explained by reduced utilization of glucose by cells of the immune system (Li et al. 2007), likely due to reduced number of lymphocytes and macrophages, as well as a reduced activity of these cells.

Amino acids actively contribute to nitrogen recycling in the large intestine (Bergen and Wu 2009). Intestinal bacteria extensively catabolize dietary amino acids and modulate the pattern of amino acids in the lumen of the gut (Dai et al. 2010, 2011). Except for glutamate and glutamine, concentrations of all protein amino acids in serum were reduced in LS-supplemented rats, compared with the control (inflammatory) group. These results may be explained by the following putative mechanisms. First, LS may reduce the digestion of dietary protein and the absorption of amino acids from the lumen of the small intestine by shortening the transit time of digesta through the gut (Kong et al. 2011). This can beneficially decrease the production of ammonia by extra-intestinal tissues in colitic rats due to the reduced availability of amino acids in the circulation (Geng et al. 2011; Hou et al. 2011; Rezaei et al. 2011). Second, LS may stimulate microbial activity in the lumen of the small intestine and the large intestine, thereby increasing the catabolism of dietary amino acids by the gut. In support of this view, the concentrations of trimethylamine oxide [exclusively metabolites of microbial metabolism (Wu 2013b) in serum were lower in LS-supplemented than in inflammatory rats.

Citrulline and ornithine were virtually absent in the diet (Gao et al. 2012; Go et al. 2012; Li et al. 2011). These two amino acids can be synthesized from glutamine and glutamate by both enterocytes and bacteria in the small intestine (Wu et al. 1994, 2011a, b). Thus, a decrease in serum concentrations of both citrulline and ornithine suggests a reduced activity of intestinal microbes. Third, LS supplementation may increase the synthesis of both glutamate and glutamine by intestinal bacteria, leading to elevated levels in the circulation. Catabolism of amino acids by intestinal bacteria results in the production of ammonia,

Table 3 Changes in relative concentrations of serum metabolites in lactosucrose-supplemented rats on the basis of chemical shifts relative to the methyl group of lactate at δ1.33

Key	Metabolites	Moieties	δ^1H (ppm) and multiplicity	Lactosucrose
1	Leucine	αCH, δCH_3, δCH_3	3.72(t), 0.91(d), 0.96(d)	↓
3	β-Hydroxybutyrate	γCH_3	1.22(d)	↓
4	Lactate	αCH, βCH_3	4.11(q), 1.33(d)	↑
5	Acetate	CH_2–C=O	1.92(s)	–
6	Proline	βCH_2, γCH_2, δCH_2	2.02–2.33(m), 2.00(m), 3.35(t)	–
7	Glutamate	αCH, βCH_2, γCH_2	3.75(m), 2.08(m), 2.37(m)	↑
8	Glutamine	αCH, βCH_2, γCH_2	3.68(t), 2.15(m), 2.45(m)	↑
9	Succinate	α, βCH_2	2.41(s)	↑
10	Methylamine	CH_3	2.54(s)	–
11	Dimethylamine	CH_3	2.71(s)	↑
12	Creatine	N–CH_3, CH_2	3.04(s), 3.93(s)	↓
13	Arginine	αCH, βCH_2, γCH_2, δCH_2	3.76(t), 1.89(m), 1.63(m), 3.25(t)	↓
14	Betaine	CH_3, CH_2	3.28(s), 3.90(s)	↓
15	Acetoacetate	CH_3, CH_2	2.29(s), 3.49(s)	↑
16	Methionine	αCH, βCH_2, γCH_2, δCH_3	3.78(m), 2.16(m), 2.6(dd), 2.14(s)	↓
17	β-Glucose	2-CH, 1-CH	3.25(dd), 4.65(d)	↑
18	α-Glucose	1-CH	5.24(d)	↑
19	1-Methyl histidine	4-CH, 2-CH	7.05(s), 7.77(s)	↑
20	Formate	CH	8.45(s)	↑
21	Isoleucine	γCH_3, δCH_3	1.01(d), 0.94(t)	↓
22	Alanine	αCH, βCH_3	3.77(q), 1.48(d)	↓
23	Lysine	αCH, βCH_2, γCH_2, δCH_2	3.77(t), 1.89(m), 1.73(m), 1.47(m)	↓
24	Glycoprotein	CH_3–C=O	2.05(s), 2.08(s), 2.15(s)	↓
25	Pyruvate	CH_3	2.37(s)	↓
26	Citrate	CH_2	2.52(d), 2.70(d)	↓
27	Aspartic acid	αCH, βCH_2	3.89(m), 2.68(m), 2.82(m)	↓
28	Citrulline	αCH_2, γCH_2, δCH_2	3.70(m), 1.58(m), 3.15(t)	↓
29	TMAO	CH_3	3.26(s)	↓
30	Taurine	N–CH_2, S–CH_2	3.26(t), 3.41(t)	↓
31	Glycine	CH_2	3.56(s)	↓
32	Threonine	αCH, βCH, γCH_3	3.58(d), 4.25(m), 1.32(d)	↓
33	Ornithine	CH_2, αCH	3.80(s), 3.79(t)	↓
34	Creatinine	CH_3, CH_2	3.05(s), 4.05(s)	↑
35	3-Methyl histidine	4-CH, 2-CH	7.00(s), 7.60(s)	↓
36	Lipids	$CH_3(CH_2)n$, $(CH_2)n$	1.22(m), 1.29(m)	↓
		$CH_2^*CH_2CO$, CH2–C=C	1.58(m), 2.04(m)	↓
		CH_2–C=O, CH–O–CO	2.24(m), 2.75(m)	↑
37	Albumin	Lysyl-CH_2	3.02(s)	↓
38	Choline	N–$(CH_3)_3$, αCH_2, βCH_2	3.2(s), 4.05(t), 3.51(t)	↓
39	GPC	N–$(CH_3)_3$, OCH_2, NCH_2	3.22(s), 4.32(t), 3.68(t)	↓
40	Unsaturated lipids	=C–CH_2–C=, –CH=CH–	5.19(m), 5.31(m)	↓

s Singlet, *d* doublet, *t* triplet, *q* quartet, *m* multiplet, *dd* doublet of doublets, *TMAO* trimethylamine-*N*-oxide, *GPC* glycerophosphorylcholine, ↓ and ↑ the metabolite levels are lower or higher, respectively, compared with the control group, – the metabolite levels are the same as in the control group

which, along with α-ketoglutarate, is converted to glutamate by glutamate dehydrogenase (Wu et al. 2009). In the presence of glutamine synthetase, glutamate reacts with another ammonia molecule to form glutamine (Wu 2013b). Glutamine can beneficially regulate the metabolism of amino acids by intestinal microbes (Dai et al. 2012a, b, c; Xi et al. 2012), improve antioxidative response in intestinal cells (Haynes et al. 2009; Wang et al. 2012), and protect the intestine from apoptosis (Rhoads and Wu 2009).

Intestinal microbes convert non-digestible oligosaccharide into SCFA and other nutrients that can be used by the mammalian host (Blachier et al. 2010; Majid et al. 2011).

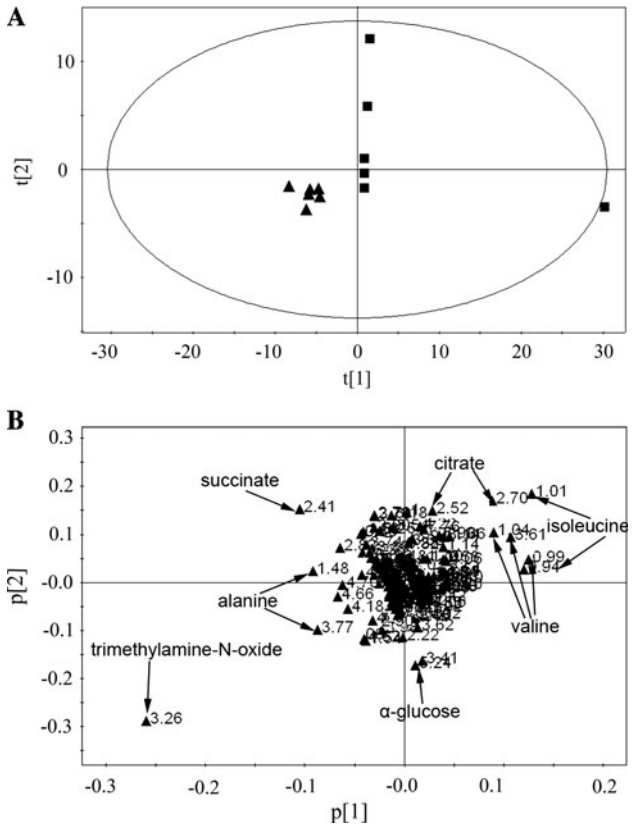

Fig. 3 PCA score plot (**a**) and loading plot (**b**) from ^{1}H NMR spectra of serum obtained from control (*filled triangles*) and lactosucrose-supplemented (*filled squares*) rats

Table 4 Changes in relative concentrations of serum metabolites in lactosucrose-supplemented rats compared to colitic rats

Metabolites	NMR chemical shift (δ)	Change in direction	*P* value
Valine	0.99, 1.04	↓	0.037
Succinic acid	2.41	↑	0.036
α-Glucose	5.24	↑	0.045
Isoleucine	1.01, 0.94	↓	0.031
Alanine	1.48	↓	0.029
Citric acid	2.52	↓	0.027
Trimethylamine oxide	3.26	↓	0.041
Taurine	3.26	↓	0.041

Significant differences are set at $P < 0.05$

FOS formulas increase concentrations of SCFA and fecal bifidobacteria in patients (Lindsay et al. 2006). Dietary oligosaccharides also affect the types of SCFA and microorganisms in the intestinal lumen (Dai et al. 2011). Another interesting finding from this work is that concentration of SCFA in colonic chyme was increased in LS-supplemented rats (Table 1). These changes likely resulted in the increased availability of substrates for the synthesis of acetoacetate and some lipids by gut microorganisms (Table 3), leading to their elevated levels in serum. It is likely that LS is fermented by intestinal microbes to produce SCFA. Acetic acid is the most abundant SCFA produced by raffinose-derived oligosaccharides during the in vitro fermentation process (Hernandez-Hernandez et al. 2011). A change in acetate production in response to LS supplementation may beneficially affect the pH of the colon lumen and metabolic processes in the colon. Butyrate is the second most abundant SCFA produced by the colon and the preferred energy source for large intestinal epithelial cells. Butyrate also regulates nutrient metabolism in colonocytes and gene expression by acting as an inhibitor of histone deacetylase (Wang et al. 2012). Additionally, recent studies reveal that impaired oxidation of butyrate is an important factor for inducing ulcerative colitis (Hamer et al. 2008). Thus, butyrate plays a major role in the physiology of the colonic mucosa and may mediate the pathogenesis of some large intestine diseases.

In conclusion, our results indicate that dietary lactosucrose supplementation affects the serum metabolome in colitic rats. The changes in the circulating levels of amino acids and related metabolites may beneficially protect the host from ammonia toxicity and oxidative injury. To our knowledge, this is the first study describing an NMR-based oligosaccharide intervention for colitis.

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Conflict of interest The authors declare no conflict of interest.

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