A corn straw-based diet increases release of inflammatory cytokines in peripheral blood mononuclear cells of dairy cows^{*}

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Abstract: Recent studies have shown that diet can affect the body's immunity. Roughage of dairy cows consists of a variety of plant materials which make different contributions to health. This study investigated the effect of different roughages on the immunity of dairy cows. Serum, peripheral blood mononuclear cells (PBMCs), and milk samples were collected from 20 multiparous mid-lactation cows fed mixed forage (MF)- or corn straw (CS)-based diets. Expression profile analysis was used to detect the differentially expressed genes (DEGs) from PBMCs. The results showed that milk protein in the MF group increased to 3.22 g/100 ml, while that of the CS group milk was 2.96 g/100 ml; by RNA sequencing, it was found that 1615 genes were differentially expressed between the CS group and the MF group among the 24027 analyzed probes. Gene ontology (GO) and pathway analysis of DEGs suggested that these genes (especially genes coding cytokines, chemokine and its receptors) are involved in the immune response. Results were confirmed at the protein level via detecting the levels of interleukin-2 (IL-2), IL-6, IL-10, IL-12, leptin (LEP), interferon- γ (IFN- γ), transforming growth factor- β 1 (TGF- β 1), and tumor necrosis factor- α (TNF- α) in peripheral blood by enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay analysis. Our data supported the conclusions that the protein content in milk of the MF group was higher than that of the CS group, the CS-based diets induced more release of cytokines than the MF-based diets in dairy cows' PBMCs, and milk protein content may be affected by cytokines.

Key words: Roughage; Immunity; Inflammatory cytokine; Expression profile analysis
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1 Introduction

An increasing number of studies have shown that diet affects the body's immunity (Alemany, 2013; Gotsis et al., 2015). G-protein-coupled receptors are

metabolite sensors (including food metabolites and intestinal bacterial metabolites) that are expressed in immune cells and intestinal epithelial cells, and drive downstream effects in immune pathways and affect the levels of cytokines (Thorburn et al., 2014). Malnutrition can lead to immune suppression, but overnutrition can result in dysfunction of the immune system, chronic inflammation, and cancer (Rathmell, 2012; Sethi et al., 2012).

In many countries, corn straw (CS) is frequently used in the diets of cows because of a lack of quality

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roughage. Qin et al. (2013) reported that cows fed a CS-based diet produced less milk and tended to produce lower milk fat and protein than those with mixed forage (MF)-based diets. Moreover, these authors also reported that the somatic cell count (SCC) of milk in the CS group was significantly higher than that in the MF group, and they demonstrated that cows fed a CS-based diet experienced less severe negative energy balance (NEB) and had lower concentrations of β -hydroxy butyric acid (BHBA) and non-esterified fatty acid (NEFA) than those with an MF-based diet. Weng et al. (2012) reported that cows fed a CS-based diet have decreased dry matter intake (DMI) and milk production.

Most studies focused on the effects of CS-based diets on milk production. However, in the northern part of China, where CS-based diets are extensively used, the relationship between CS-based diets and mammary gland disease, such as mastitis, has not attracted researchers' attention. In addition, in recent years, a growing number of studies suggested that nutrition affects the body's immune status, which closely correlates with some diseases (Olthof et al., 2013; Schwerd et al., 2016).

Expression profile analysis is a high throughput technology used for the identification and quantification of many specific mRNA sequences in complex nucleic acid samples. In this study, the differential gene expression profiles of peripheral blood mononuclear cells (PBMCs) were obtained from dairy cows fed MF- or CS-based diets using gene chips. Cytokines, which are involved in milk protein synthesis in peripheral blood, were evaluated by enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay. In addition, gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of differentially expressed genes (DEGs) were used to reveal the close relationship between peripheral blood and inflammatory response.

2 Materials and methods

2.1 Animals and management

All animal experiments and procedures were approved by the Institutional Animal Care and Use Committee, Jilin University, China and all animals were cared for as per the standards of the Association

for Assessment and Accreditation of Laboratory Animal Care International. This experiment was performed on two dairy cattle farms located in Changchun, China. The CS- and MF-based diets were collected from the cattle farms. The samples were all yellow green and of good texture. There were no obvious differences in color or moisture content, and the samples were free from mould. Then the samples were dried at 65 °C, ground into powder, and screened by a 40-mesh sieve.

A total of 20 multiparous (2–3 parities) mid-lactation non-pregnant dairy cows (days in milk (DIM): (120±20) d; body weight (BW): (551±23) kg) were selected. One group of 10 cows was fed an MF-based diet (MF consisting of Chinese wild rye, alfalfa hay, and corn silage), and the other 10 cows were fed a CS-based diet (CS is the only roughage material of the diet). The composition and nutrient levels of the experimental diets are shown in Table 1. Both diets were provided with the same concentrate to roughage ratio (45:55). The animals were housed in tie-stalls bedded with wood shavings and with free access to water and food. Before the experiment, the two groups of cows were fed the same MF-based diet. The experimental period was 12 weeks, consisting of the pre-feeding period (3 weeks) and feeding period (9 weeks). The serum, PBMCs, and milk samples were collected on 5–7 d in the last week.

2.2 Milk and blood sample collection and analytical methods

Milk samples ($n=10$ per treatment) were collected three times daily at the indicated time into a sampling tube prepared with 0.1 g of potassium dichromate, and the samples were mixed as one sample for nutrient content analysis using an automatic milk composition analyser (Gerber Inc., Berlin, Germany).

Blood samples ($n=10$ per treatment) were obtained quickly before the morning feeding from the tail vein. The serum was prepared by centrifugation of the blood at 5000g for 5 min at 4 °C and stored at –80 °C until analysis. The levels of interleukin-2 (IL-2), IL-6, IL-10, IL-12, leptin (LEP), interferon- γ (IFN- γ), adiponectin (ADPN), insulin (INS), and transforming growth factor- β 1 (TGF- β 1) in the serum were measured by ELISA kits (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. The concentration of tumor necrosis factor- α (TNF- α) in the serum was measured using a

Table 1 Composition and nutrient levels of experimental diets

Item	Composition and nutrient levels (DM basis)	
	MF	CS
Alfalfa hay (%)	10.88	0.00
Corn silage (%)	14.21	0.00
Corn straw (%)	0.00	26.24
Soybean meal (%)	14.88	14.65
Rapeseed meal (%)	5.34	5.26
Cottonseed meal (%)	2.61	2.57
Extruded soybean (%)	2.64	2.60
Fuzzy cottonseed (%)	13.36	13.16
Maize meal (%)	32.64	32.14
Limestone (%)	0.95	0.93
Salt (%)	0.59	0.58
Premix ¹ (%)	0.71	0.70
Dicalcium phosphate (%)	1.18	1.17
Net energy for lactation (MJ/kg)	7.57	7.32
Crude protein (g/kg)	21.26	20.26
Ether extract (g/kg)	4.58	4.60
Neutral detergent fiber (g/kg)	26.98	32.07
Acid detergent fiber (g/kg)	17.69	18.87
Ash (g/kg)	4.69	4.68
Ca (g/kg)	0.72	0.78
P (g/kg)	0.43	0.39

¹ Premix contains (per kg DM): Vitamin A 2000000 IU, Vitamin D 600000 IU, Vitamin E 10800 mg, Fe 5500 mg, Cu 4080 mg, Mn 4989 mg, Zn 17500 mg, I 180 mg, Se 110 mg, and Co 8805 mg. DM, dry mass; MF, mixed forage; CS, corn straw

radioimmunoassay kit from Sigma Diagnostics (St. Louis, MO, USA) in the Department of Clinical Laboratory at the First Hospital of Jilin University, Changchun, China.

2.3 Expression profile analysis

PBMCs were isolated from cows using lymphocyte separation medium from TBD (Tianjin, China) according to the manufacturer's protocol. Total RNA of PBMCs was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol and was further purified and evaluated for purity. The quality and integrity of the RNA samples were assessed by agarose gel electrophoresis.

Global gene expression between cows fed the MF- or CS-based diet was identified using microarray analysis with the Affymetrix Bovine Genome Array (Affymetrix, Inc., CA, USA). The design of the array

was based on the content from UniGene and GenBank mRNAs. The Bovine Genome Array was developed through the GeneChip Consortia Program, and contains 24027 probe sets representing more than 23000 transcripts and includes approximately 19000 UniGene clusters.

A comprehensive bioinformatics analysis approach was used to enrich the dataset for genes that were most likely to be associated with the balance of several systemic hormones and cytokines. The following approaches were used: DEG analysis, GO analysis, GO map analysis and pathway analysis. Genes differentially expressed in the CS and MF groups were identified using the two classification difference genetic screening (TwoclassDif) method. Genes were deemed significantly differentially expressed with a *P*-value of ≤ 0.05 and a false discovery rate (FDR) of ≤ 0.01 in sequence counts across libraries. GO analysis, the key functional classification of National Center for Biotechnology Information (NCBI), was applied to analyze the main functions of the DEGs. Fisher's exact test and multiple test were used to classify the GO categories, and the FDR was calculated to correct the *P*-value. Low FDRs indicate small errors in judging the *P*-value. Enrichment provides a measure of the significance of the function. As the enrichment increases, the corresponding function is more specific, which helps to identify the GO categories with more concrete functional descriptions in the experiment. Pathway analysis was used to identify significantly different regulatory pathways according to the KEGG, Biocarta, and Reactome. Fisher's exact test and a χ^2 test were used to select each significant pathway, and the threshold of significance was defined by the *P*-value and FDR.

2.4 Validation of DEG data by qPCR

Total RNA was isolated using TRIzol reagent (Invitrogen, USA) as per the manufacturer's instructions. *β-actin* is a housekeeping gene for analysis (Bougarn et al., 2011). The target gene expression levels were quantified relative to the expression of *β-actin* by employing the optimized comparative threshold cycle ($\Delta\Delta C_T$) value method. Relative differences between the groups were calculated and defined as the relative increase, and the MF group mean values were set at 1. The primers used are listed in Table 2.

2.5 Statistical analysis

Milk composition and levels of cytokines were analyzed by one-way analysis of variance (ANOVA) using SPSS software (Version 16.0 for Windows; SPSS Inc., Chicago, IL, USA). *P*-value of <0.05 was considered significant, and *P*<0.01 very significant. The quantitative polymerase chain reaction (qPCR) data were normalized to β -actin using the $\Delta\Delta C_T$ method. The data are expressed as the mean $\pm$ standard error of the mean (SEM) of at least three independent replicates.

3 Results

3.1 Effect of forage type on milk composition

To elucidate the effects of forage type on milk composition, the milk composition was examined

after feeding the cows different diets. As shown in Table 3, the protein content in milk of the MF group was higher than that of the CS group (*P*<0.05). However, the fat and lactose percentages were not significantly different between the two groups (*P*>0.05). In addition, there were no significant differences in the solids-not-fat (SNF), total solids, or fat/protein ratio between the two groups (*P*>0.05).

3.2 GO and KEGG pathway analyses based on expression profiles

Our microarray analysis showed gene expression changes in the PBMCs in cows fed the CS-based diet compared to those fed the MF-based diet as a control. In total, 1615 statistically significant differentially (up-regulated) expressed genes were identified in the CS group compared with the MF

Table 2 Summary of gene targets evaluated by qPCR

Gene name	Amplicon size (bp)	Sequence of primer
<i>IFNG</i>	151	F: 5'-GGTTTTTCTGGTTCTTATGGCC-3' R: 5'-TTTTTTTGTCACTTTTCATCTTTCCA-3'
<i>TNF-α</i>	104	F: 5'-TCTTCTCAAGCCTCAAGTAACAAGC-3' R: 5'-CCATGAGGGCATTGGCATAC-3'
<i>IL-32α</i>	134	F: 5'-GCAGTTCTTGGGCATTCTGC-3' R: 5'-AATCCGTGAGCACTTCCCAG-3'
<i>IL-6R</i>	175	F: 5'-ATTTGGGAATGGCTTGTGGA-3' R: 5'-CACTGGGAGGCTGGTTGTTT-3'
<i>IL-27R</i>	168	F: 5'-GCTGCTTCTATTGTTGCTGTTCA-3' R: 5'-TTGGAATGGTATTTCTGGCTTTG-3'
<i>IL-10</i>	101	F: 5'-GTGATGCCACAGGCTGAGAA-3' R: 5'-TGCTCTTGTTTCGACAGGGCAG-3'
<i>IL-4R</i>	109	F: 5'-GCAGTCAAGGGAGACGATGG-3' R: 5'-GGCAGGTGAGGGCTGAGTAG-3'
<i>CXCR2</i>	131	F: 5'-TCGCCATGACCTTGCCATC-3' R: 5'-GATGCAGGCCAGCAGTAGAA-3'
<i>CCR3</i>	199	F: 5'-CAGAGGATAATGAAAATGCCTGG-3' R: 5'-GGTGTCCAGAAAATAAAGAAGACCA-3'
<i>CSF3</i>	101	F: 5'-CCCCACCTTGACACACTG-3' R: 5'-GAAGCAACCAGGACCCCTC-3'
β -actin	101	F: 5'-GCCCTGAGGCTCTCTTCCA-3' R: 5'-GCGGATGTCGACGTCACA-3'

The cycling conditions consisted of an initial, single cycle of 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. qPCR, quantitative polymerase chain reaction; F, forward; R, reverse

Table 3 Effects of different types of diets on milk composition

Group	SCC ($\times 10^3$ ml ⁻¹)	Milk yield (kg/d)	Fat (g/100 ml)	Protein (g/100 ml)	Lactose (g/100 ml)	SNF (g/100 g)	Total solid (g/100 g)	Freezing point (°C)	Fat/protein (%)
MF	58.90	25.50	3.28	3.22	4.80	8.39	12.18	-0.55	1.03
CS	77.50	23.37	3.37	2.96	4.78	8.38	12.42	-0.55	1.28
SEM	28.89	2.18	0.18	0.05	0.05	0.11	0.04		
<i>P</i> -value	0.16	0.36	0.56	<0.01	0.57	0.55	0.51		

SCC, somatic cell count; SNF, solids-not-fat; MF, mixed forage; CS, corn straw; SEM, standard error of the mean

group ($P < 0.05$), and the number of down-regulated genes was more than that of up-regulated genes. In addition, compared with the MF group, 154 GO categories and 69 signalling pathways were altered in the CS group.

A comprehensive GO analysis was applied to analyze DEGs to gain deeper insight into the main cellular functions that were altered in PBMCs from cows fed different diets. As shown in Fig. 1a, the high-enrichment GO categories included cellular lipid metabolic process, cytokine-mediated signalling pathway, response to progesterone stimulus, positive regulation of cell cycle, small molecule metabolic process, induction of apoptosis by intracellular signals, and type I interferon-mediated signalling pathway. Based on the significant criterion of $P \leq 0.05$ and with an absolute fold change of ≥ 2 , we selected 695 genes that fell in the range of key functional classifications, and an interaction network of significant GO terms was assembled into a GO map to depict the relationship among prominent functional categories (Fig. 1b). The GO map directly and systematically showed the subordinate relationship between GO terms (Fig. 1b). There were 16 subnetworks clustered into five functions as follows: (1) signalling pathway: cytokine-mediated signalling pathway, pattern recognition receptor, and fibroblast growth factor receptor; (2) cell signalling: cellular lipid metabolic process, protein N-linked glycosylation, and small nucleotide catabolic process; (3) cellular process: cell migration and cycle; (4) apoptosis: apoptotic process; and (5) gene expression: mRNA processing, response to DNA damage stimulus, and transcription from RNA polymerase II promoter (Fig. 1c).

The differential pathways were analyzed according to the functions and interactions of the differential genes. As shown in Fig. 2a, the p53 signalling pathway was involved in both groups but was more important in the CS group than in the MF group. Interestingly, cytokine-related pathways including the chemokine signalling pathway, Toll-like receptor signalling pathway, Janus kinase/signal transducer and activator of transcription (JAK-STAT) signalling pathway, and TGF- β signalling pathway, as well as cytokine-cytokine receptor interaction were also up-regulated (Fig. 2a). Pathways with a P -value of ≤ 0.05 and an absolute value of $\log_2$ ratio of ≥ 1 were considered to be significantly affected. Using these

criteria, we identified a total of 268 probes that were differentially expressed in the CS group compared with the MF group. The pathway-pathway interaction map was drawn to explore the synergy between pathways based on those 268 genes. As shown in Fig. 2b, our analysis yielded 47 pathways, including mitogen-activated protein kinase (MAPK) signaling pathway, cell cycle and several pathways associated with immune response. The importance of immune response to the intake of the CS-based diet is highlighted by the identification of several immune-related signalling pathways that could be considered to be important to different forages, such as cytokine-cytokine receptor interaction, JAK-STAT signalling pathway, TGF- β signalling pathway, and T cell receptor signalling pathway.

Microarray-based GO and KEGG pathway analyses showed that there were important functional genes involved in the immune response (especially cytokines, chemokines, and their receptors). Cytokines that were up- or down-regulated in the CS group compared to the MF group are shown in Table 4. These were cytokines that activate the inflammatory response including IFN- γ (up, 7.14) and TNF (up, 4.35), but also those which have a role in the anti-inflammatory effect such as IL-10 (down, 0.5). Overall, we came to the conclusion that the CS-based diets affected the expression of cytokines in PBMCs to a greater extent than the MF-based diets.

3.3 Confirmation of array results by qPCR

To confirm the microarray results, qPCR was performed on the mRNA samples from dairy cows which were used in microarray assay. As shown in Fig. 3, the general profiles for all of these genes were similar to those observed with the microarray arrays. Moreover, the intensity of fold changes was of the same order of magnitude for most genes. This confirms the data from the microarray analysis.

3.4 Serum levels of hormones and cytokines

To confirm the expression changes of cytokine-coding genes in PBMCs in response to the CS-based diets, we compared the gene changes to the secreted cytokine levels in serum. A panel of ten representative cytokines (IL-2, IL-6, IL-10, IL-12, TNF- α , ADPN, INS, IFN- γ , TGF- β 1, and LEP) from cow peripheral blood was assessed. Corresponding to the

Fig. 1 Histogram of partial GO categories that were significantly different in the two groups of cows (a) Top 15 up-regulated GO categories; (b) Top 15 down-regulated GO categories; (c) The interaction network of significant GO terms. The node represents one GO term. Blue, GO category for down-regulated genes; Red, GO category for up-regulated genes; Yellow, GO category for both up-regulated and down-regulated genes. The line between two nodes represents the subordinate relationship between GO terms (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

Fig. 2 Histogram of partial pathways that were significantly different in the two groups of cows

(a) Top 15 up-regulated pathways; (b) Top 15 down-regulated pathways; (c) The interaction network of significant pathways. The node represents one pathway term. Blue, pathway for down-regulated genes; Red, pathway for up-regulated genes; Yellow, pathway for both up-regulated and down-regulated genes. The line between two nodes represents the subordinate relationship between pathways (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

Table 4 Differentially expressed cytokine-related genes between the CS and MF groups

Gene	Gene description	FDR	P-value	Fold change
<i>CCR7</i>	Chemokine (C-C motif) receptor 7	0.0598	0.003105	0.33
<i>CCR3</i>	Chemokine (C-C motif) receptor 3	0.1250	0.022816	0.16
<i>CCL2</i>	Chemokine (C-C motif) ligand 2	0.1150	0.019325	0.05
<i>CXCL5</i>	Chemokine (C-X-C motif) ligand 5	0.0996	0.013812	0.04
<i>CXCR2</i>	Chemokine (C-X-C motif) receptor 2	0.0461	0.001252	0.05
<i>CXCR5</i>	Chemokine (C-X-C motif) receptor 5	0.1130	0.018549	0.34
<i>CXCL16</i>	Chemokine (C-X-C motif) ligand 16	0.0777	0.006541	0.15
<i>IFNG</i>	Interferon γ	0.0925	0.011361	7.14
<i>IFNGR2</i>	Interferon γ receptor 2	0.0622	0.003328	0.31
<i>IFNAR2</i>	Interferon α receptor 2	0.0495	0.001661	0.20
<i>TNF</i>	Tumour necrosis factor	0.0924	0.010985	4.35
<i>CSF3</i>	Colony stimulating factor 3	0.1490	0.033462	0.09
<i>IL-10</i>	Interleukin 10	0.1400	0.029571	0.50
<i>IL-4R</i>	Interleukin 4 receptor	0.1390	0.029443	0.22
<i>IL-1RN</i>	Interleukin 1 receptor antagonist	0.1400	0.029568	0.09

CS, corn straw; MF, mixed forage; FDR, false discovery rate

Fig. 3 mRNA expression levels of target genes in PBMCs

Expression level for each gene was normalized to β -actin. All data represent the mean $\pm$ SEM of ten independent replicates

gene expression data, the CS-fed cows had significant increases in serum IFN- γ (85.2%; $P<0.05$) and TGF- β 1 (74.6%; $P<0.05$) levels compared with the MF-fed cows (Table 5). These data indicated that a CS-based diet triggers a higher expression of inflammatory cytokines in experimental animals, including IFN- γ and TGF- β 1.

4 Discussion

In the present study, our data supported the proposition that the protein content in milk of the MF group was higher than that of the CS group, and the CS-based diet induced the release of cytokines more than the MF-based diet in dairy cows' PBMCs,

Table 5 Cytokine levels in serum

Group	LEP ($\mu\text{g/L}$)	IL-2 (ng/L)	IL-12 (ng/L)	IFN- γ (ng/L)	ADPN ($\mu\text{g/L}$)	TGF- β 1 (ng/L)	IL-6 (ng/L)	IL-10 (ng/L)	TNF- α (fmol/ml)	INS (mU/L)
MF	3.39	421.19	52.99	410.61	81.22	77.46	16.30	32.32	170.47	23.21
CS	3.53	509.04	56.54	481.86	87.26	103.90	15.38	35.80	242.96	7.18
SEM	0.11	21.74	1.97	16.53	1.45	6.78	0.65	2.48	19.46	3.39
<i>P</i> -value	0.511	0.112	0.381	<0.05	<0.05	<0.05	0.496	0.499	0.398	0.067

MF, mixed forage; CS, corn straw; SEM, standard error of the mean

implying that milk protein content may be affected by cytokines. Most of milk contents, for example, fat, lactose percentages, SNF, total solids, and fat/protein ratio, were not significantly different between the two groups (Table 3). To gain insight into the effects of those diets on animal health, we detected that whole-genome gene expression in PBMCs, and GO analysis, pathway analysis and functional grouping of DEGs suggested a sum of important functional genes involved in the immune response. A CS-based diet increased pro-inflammatory cytokines (IL-2, IL-6, IL-10, IL-12, TNF- α , ADPN, INS, IFN- γ , TGF- β 1, and LEP) in the blood after confirmation by ELISA and radioimmunoassay. This finding suggests a new research field concerning the relation between milk protein synthesis and innate immunity, and also provides us a novel consideration for mammary disease control and milk quality promotion.

High-throughput gene transcript profiling technologies, such as microarray hybridization and serial analysis of gene expression (SAGE), are powerful approaches for evaluating global gene expression in tissues or cells of interest in response to experimental treatments or changes in physiological state. This information can provide insight into mechanisms contributing to observed physiological responses. For instance, the commercially available Affymetrix Bovine Genome Array has recently been applied to reproductive studies in cattle, including characterization of changes in gene expression that may be related to female fertility, embryonic development, and sperm development (Misirlioglu et al., 2006; Schmidt et al., 2007; Connor et al., 2008; Zhou et al., 2014). Similarly, microarray hybridization has been used in production performance studies of cattle to identify the effects of increased milking frequency on gene expression in the bovine mammary gland (Wathes et al., 2007). Díaz-Rúa et al. (2014) performed a microarray analysis and reported that a high-protein (HP; containing 45% of energy from proteins) diet affected cell

communication/signal transduction, gene expression, and immune responses. We compared the molecular effects of two diets that comprised the major nutrient compositions reflecting large-scale farm breeding patterns (MF diet) and extensive cow-raising patterns (CS diet), respectively. We used the Affymetrix Bovine Genome Array to compare the global gene expression in PBMCs between the CS and MF groups. Integrated results of GO analysis and pathway analysis suggested that immune responses (especially cytokine-mediated signalling pathway), cellular processes (cell proliferation, cell cycle, and cell adhesion), stimulus responses and signalling processes were the most critical GO terms related to different types of roughage. The conclusions were confirmed by GO map analysis. This is a method that can systemically construct the interaction network of the significant GO terms (Schmidt et al., 2007). The GO map analysis supported the notion that the process most affected by the diet macronutrient composition was the immune response.

Interferons are a group of immunocompetent proteins with many functions, and they are produced mainly by monocytes and macrophages. By binding with its receptor, IFN- γ can activate the JAK/STAT pathway (Basham et al., 2008). The STAT protein, a type of DNA-binding protein mediated by prolactin, exerts its regulation of transcription by combining with tyrosine phosphorylation signal pathways, and STAT5 is an important signalling molecule in the process of milk protein synthesis (Darnell, 1997). In vitro, Xia et al. (2016) demonstrated that IFN- γ significantly inhibited milk synthesis via an autophagy-dependent mechanism in primary bovine mammary epithelial cells, which may partly explain why IFN- γ level was lower in dairy cows that produced milk with higher protein levels than in dairy cows with lower milk protein.

TGF- β 1 is an important antiproliferative and apoptogenic factor for mammary gland epithelial cells, acting in auto/paracrine matter and thus considered an important local regulator of mammary

tissue involution. TGF- β 1 affects the cell cycle through regulation of cyclins, cyclin-dependent kinases, cyclin-dependent kinase inhibitors, and c-Myc (Alexandrow and Moses, 1995). Our previous study showed that TGF- β 1 inhibited proliferation of primary mammary epithelial cells in vitro in a dose-dependent manner. The present study showed that TGF- β 1 significantly down-regulated the expression of β -casein in bovine mammary epithelial cells, and these results were consistent with previous reports (Purup et al., 2000; Musters et al., 2004; Wu et al., 2008). Wu et al. (2008) demonstrated that TGF- β 1 abrogated prolactin-stimulated β -casein gene expression in mammary epithelial cells through, at least in part, a Smad3-dependent mechanism. The mechanisms through which TGF- β 1 affects cell growth and gene expression are not fully understood.

The mechanisms whereby the CS-based diets induce production of inflammatory cytokines are poorly understood. Previous studies suggest that the intestinal flora establishes a complex relationship with the host, modulating the host's metabolic pathways and modifying the tolerance-inflammatory axis between the host and the microbiota (Stecher, 2015; Kataoka, 2016). This mutual relationship depends on the genome, nutrition, and lifestyle of the host, and alterations in diet can cause changes in the intestinal flora and metabolites. Some receptors of nutrient expressed by immune cells have been reported to activate the cells to produce different inflammatory cytokines (Nicholson et al., 2012; Huang and Brumell, 2014). Liu et al. (2014) suggested that diets altered the gut microbiota, resulting in dysbacteriosis, and increased the release of proinflammatory cytokines, which can lead to intestinal inflammation.

Additionally, we have identified a pool of genes whose expression in PBMCs was affected by the intake of CS-based diets, and they are helpful in exploring more mechanisms related to the health of the milk cow. Further study on the specific effect of these factors will help to uncover important regulatory roles of cytokines in the process of lactation in cows and provide a theoretical and experimental basis for safely and effectively improving dairy quality through dietary means. More studies in relevant tissues are needed to understand if PBMCs' gene expression can reflect harmful metabolic effects on cows as a result of the intake of a CS-based diet.

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Compliance with ethics guidelines

Yan-yi CHE, Xiao-jing XIA, Bo-ping HE, Yuan-yuan GAO, Wen-bo REN, Hong-tao LIU, Jian-fang LIU, Ting-hao HUANG, Wen-yu HAN, and Lian-cheng LEI declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要

题目: 饲喂玉米秸秆粗饲料可加剧奶牛外周血单核细胞释放炎性细胞因子

目的: 研究不同类型的粗饲料对奶牛外周血细胞因子的影响。

创新点: 明确饲喂玉米秸秆日粮奶牛外周血单核细胞基因的表达特征，研究日粮对外周血细胞因子的影响。

方法: 分别收集饲喂苜蓿混合粗饲料和玉米秸秆粗饲料的奶牛牛奶、外周血单核细胞及血清，检测牛奶营养含量，应用表达谱基因芯片分析筛选外周血单核细胞中差异表达基因，并采用荧光定量聚合酶链反应（qPCR）验证。最后采用酶联免疫吸附试验（ELISA）检测血清细胞因子的表达量。

结论: 与苜蓿混合粗饲料组相比较，玉米秸秆粗饲料组的奶牛外周血单核细胞炎性细胞因子分泌水平较高，牛奶乳蛋白含量较低（ $P < 0.05$ ）。

关键词: 粗饲料；免疫；炎性细胞因子；表达谱分析