Changes of Intestinal Oxidative Stress, Inflammation, and Gene Expression in Neonatal Goats Suffering from a Cold

yan cheng

Institute of Subtropical Agriculture Chinese Academy of Sciences

Chao Yang

Institute of Subtropical Agriculture Chinese Academy of Sciences

ZhiLiang Tan

Institute of Subtropical Agriculture Chinese Academy of Sciences

ZhiXiong He (✉ zxhe@isa.ac.cn)

Research

Keywords: intestinal, oxidative stress, inflammation, neonatal goat kids, cold, diagnostic biomarkers

Posted Date: July 7th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-39361/v1>

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Abstract

Background

Disease and disorders in young goats are serious threats to the animals' health, and they influence the profitability of the goat industry. There is a need to better understand the potential biomarkers that can reflect the mortality and morbidity in young goats. Since gastrointestinal infection is probably the first ailment in young goats, the objective of this study was to explore the changes in oxidative stress, inflammation, and gene expression in the gastrointestinal tract of neonatal goats suffering from a cold.

Results

The activity of glutathione peroxidase (GSH-Px) was less ($P < 0.05$) in the jejunum in neonatal goats suffering from a cold compared with healthy neonatal goats. However, the malondialdehyde (MDA) activities in the jejunum and ileum were higher ($P < 0.05$) in neonatal goats suffering from a cold compared with healthy neonatal goats. There was no significant difference in the super-oxide dismutase (SOD) and catalase (CAT) activity observed between the two groups ($P > 0.05$). For the concentrations of intestinal interleukin-2 (IL2) and interleukin-6 (IL6), only the IL-2 in ileum in the neonatal goats suffering from a cold was higher than that from healthy neonatal goats. Real-time PCR results showed that the expression of most toll-like receptor-4-(TLR4) pathway-related genes was similar in the two groups. The transcriptomic analysis of the jejunum showed a total of 364 differential expression genes (DEGs) identified in neonatal goats suffering from a cold compared with healthy neonatal goats. The Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis of up-regulated DEG was mainly related to the ECM–receptor interaction, pathways in cancer and axon guidance, and the down-regulated DEG mainly related to the arachidonic acid metabolism, complement, and coagulation cascades, and the Ras signaling pathway.

Conclusions

These results suggest that neonatal goats suffering from a cold experienced a higher intestinal oxidative stress compared with healthy neonatal goats. Thus, it is possible that the antioxidant capacity of young ruminants acts as an indicator of health status and the measurements of oxidation stress may be useful as diagnostic biomarkers, reflecting the mortality and morbidity in young goats.

Background

The morbidity and mortality of newborn animals in animal husbandry is relevant to animal health and welfare as well as to economic development and increased productivity. Dwyer et al. have reported that the published average mortality rates of sheep in 1970–2014 from many countries and systems have been remaining stable at 15% [1]. The overall mortality rate of lambs is often ranged from 10–25% [2, 3], and the published estimates of goat kids mortality is between 11.5% and 37% [4]. The mortality figures of newborn calves are over 30% in farms located in Tulare County, California [5, 6]. Numerous studies have clarified the causes, prevention, and treatment of neonatal disease and provided practical means (such as improving management) to reduce mortality rates. There is considerable scientific knowledge about neonatal small ruminant livestock morbidity and mortality, but it

has not exerted significant effects on improving the survival. The reason may be that a substantial amount of research has been focusing on seeking and assessing solutions to the problems due to economic consideration, not on the nature of neonatal morbidity. As such, there is an urgent need to search for more effective potential biomarkers for neonatal disease diagnosis to improve animal health.

The ruminant placenta is epitheliochorial and does not allow the transfer of immune components from the mother to the young [7]. Newborn goat kids are dependent on suckling for the transfer of immunoglobulin via colostrum from the ewe to obtain effective passive immunity. Until newborn lambs acquire passive immunity via colostrum, they are susceptible to infectious disease [8]. The overall consensus is that the direct cause of newborn mortality is infectious diseases, such as neonatal diarrhea and respiratory disease, caused by intestinal pathogens. It has been reported that newborn deaths are frequently caused by diarrhea due to pathogenic agents, such as *Escherichia coli*, accounting for more than 50% of the total neonatal mortality, while respiratory disorders, such as pneumonia, accounting for 15% [9]. Researchers believe that enteropathogenic bacteria can influence pulmonary immunity through the gut–lung axis [10–12]. There is a close relationship between the lung and the large intestine. Intestinal diseases can also affect the lungs (and vice versa) according to a theory in Chinese medicine [13]. Thus, it has been hypothesized that a runny nose associated with cold should be a typical morbidity characteristic of an intestinal pathogen infection. To date, neonatal animals classified as clinically diseased are mainly diagnosed empirically based on a range of clinical features, such as a runny nose and diarrhea. However, it is still unclear which causes will likely induce rapid clinical manifestations of diseases in newborn animals in a short time. There are few descriptions of the physiological and biochemical characteristics of young ruminants under pathological conditions.

The intestine represents the largest component of the immune system. It contains the largest number of immune cells of any tissue in the body (more than 70% of the cells of the immune system are located in the gastrointestinal tract), and it reflects the health of young animals [14, 15]. Redox has emerged as an important modality in the chemical signaling that occurs in the intestine [16]. When the intracellular concentrations of reactive oxygen (ROS) are above the physiological values, it leads to oxidative stress [17, 18], initiating oxidative injury to the gut. Intestinal enteritis (a common occurrence) induces diarrhea that leads to a series of harmful effects on animal health. In the intestine, immune cells boost immunological function via regulating pro-inflammatory effector cells to reduce the secretion of pro-inflammatory cytokines, such as interleukin-2 (IL-2) and interleukin-6 (IL-6), and inhibiting pro-inflammatory pathways, such as the toll-like receptor 4 (TLR4) signaling pathway [19, 20]. In this study, we hypothesized that pathogenic microorganisms induce intestinal physiological dysfunction, which leads to a cold in newborn animals.

The transcriptome refers to the whole-gene transcripts transcribed in specific physiological and pathological states with a high throughput and reliable accuracy [21]. It has been instrumental in the discovery of new diagnostic or therapeutic targets [22] and is widely applied to immune monitoring in inflammatory diseases to unravel pathogenic, diagnostic, and prognostic signatures [23].

The objectives of this study were to evaluate the changes in the intestinal antioxidant status, inflammation state, and gene expression when neonatal goats suffered from a cold. We investigated the changes of the redox state and immune toxicity in diseased goats compared with healthy goats through determining the activities of antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px); the content of malondialdehyde (MDA); secretion of IL-2 and IL-6, and the transcriptional levels of immune genes.

These molecular changes may shed light on the diagnosis of a cold in neonatal goats and provide valuable clues regarding the relationship between the intestinal physiology, inflammation, and immunity.

Materials And Methods

Animals and experimental design

All of the procedures used in this study were approved by the Institutional Animal Care and the Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, China. The experiment was carried out in a small-scale farm based on 200 ewes (the Xiangdong black goat, a local meat breed) in Pingxiang, Jiangxi Province, China. Twin kid goats from the same ewes (one healthy and the other with a diagnosed cold) with the age under 14 days after birth were used in this study, and a total of 10 pairs were successfully matched. The cold was diagnosed using a cold diagnostic indicator with nasal scores. The animals were then allocated to two groups (one with colds and the other for the healthy control experiment; $n = 10/\text{group}$). The goats were maintained in our animal research station at Pingxiang, Jiangxi province, China, and given free access to standard food and water. Once the animals with the age under 14 days after birth were diagnosed as a cold, we started the slaughter test. Nasal scores were categorized as 0: no discharge; 1: a small amount of cloudy discharge from one nostril; 2: cloudy discharge from both nostrils; and 3: excessive thick cloudy discharge from both nostrils, with a nasal score ≥ 1 considered as a cold.

Sample Collection

Once the animals were diagnosed as having a cold, the gut tissues (jejunum, ileum, and colon) were quickly dissected and washed with 0.9% sodium chloride solution. The samples were subsequently divided into three portions in an ice bath, and then immediately frozen in liquid N₂ and stored at -80°C . One portion was used for the analyses of the oxidative index, one portion for the analyses of the changes in inflammatory factors, and the final portion was used for the analyses of the transcriptional levels of mRNA.

Measurement Of The Intestinal Oxidative Indexes And Inflammatory Cytokines

The gut tissues were homogenized (1:9 w/v) with a glass Teflon homogenizer (Potter-Elvehjem 64792-10) in a 0.9% normal saline buffer. Subsequently, the samples were centrifuged at 3000 g for 10 min at 4°C , and the supernatant was collected to detect the activities of oxidative index and the secretion of IL-6 and IL-2; they were then stored at 4°C .

The activity of the SOD, CAT, GSH-Px, and MDA concentration in the gut tissues was measured by a spectrophotometric method following the instructions of the SOD, CAT, GSH-Px, and MDA detection kits, respectively (Nanjing Jiancheng Bioengineering Institute, China). The level of pro-inflammatory cytokines IL-2 and IL-6 in the gut tissues was measured by the Goat IL-2 ELISA Kit and Goat IL-6 ELISA Kit according to the manufacturer's instructions. (Jiangsu Yutong Biological Technology Co., Ltd., China). All of the experiments were carried out in triplicate.

Expression of genes related to toll-like receptor 4 (TLR4) signaling pathway-related molecules and intestinal barrier function

The primers of the related genes were obtained from the NCBI website, and all of the primer sequences are summarized in Additional File 1. The expression of the genes related to TLR4 signaling pathway-related molecules, such as the myeloid differentiation factor 88 (*MyD88*), TLR4, TNF receptor-associated factor-6 (*TRAF6*), interferon-beta (*IFN-β*), Interleukin-1 beta (*IL-1β*), TUMOR necrosis factor-α (*TNF-α*), IL-6, pyrin domain-containing 3 (*NLRP3*), Interferon regulatory factor 3 (*IRF3*), TANK-binding kinase-1 (*TBK1*), and NF kappa B p65 (*NF-κB p65*), were analyzed by reverse transcription quantitative real-time PCR (RT-PCR).

For RT-PCR, the RNAs were extracted from the tissue samples. Immediately after the samples were obtained, the total RNA was obtained using Katrimox 14 (Takara Biochemicals, Tokyo, Japan). The reaction mixture in RT-PCR contained 1 µl cDNA and 0.4 µM of each primer (Bioline, Luckenwalde, Germany) in a total volume of 10 µl. The PCR amplification of GAPDH was used as an internal loading control. The fold change in the mRNA expression was calculated using the $\Delta\Delta C_t$ method [24].

Similarly, the expressions of genes related to the intestinal barrier function, such as genes encoding tight junction (TJ) proteins, including the claudin family, such as Claudin 1 (*CLDN1*), Claudin 4 (*CLDN4*), occludin, the zonula occludens family, such as zona occludens 2 (*ZO2*), and the genes encoding mucin, such as Mucin12 (*MUC12*), Mucin13 (*MUC13*), and Mucin20 (*MUC20*), were analyzed by RT-PCR.

Jejunal Transcriptome

RNA-seq analysis was carried out on the total RNA from the jejunum from neonatal goat kids in the healthy state ($n = 4$) and diseased state ($n = 3$). The RNA concentration and its integrity were measured using a NanoDrop 2000 (Thermo) and the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA), respectively. The total amount of 1 µg RNA per sample was prepared as an input material. Sequencing libraries were constructed using the NEBNext Ultra™ RNA Library Prep Kit for Illumina (NEB, USA) and clustered using the TruSeq PE Cluster Kit v4-cBot-HS (Illumina) following the manufacturer's recommendations and sequenced on an Illumina platform Immune-related gene expression. The library quality was assessed on the Agilent Bioanalyzer 2100 system.

RNA-Seq clean reads were mapped to the reference genome sequence using Hisat2 [25] software (<http://ccb.jhu.edu/software/hisat2/index.shtml>). The Principal Component Analysis (PCA) was conducted based on the expressed genes' data using R (R version 3.4.2). The expression levels of the mRNAs in each sample were estimated by fragments per kilobase of the transcript per million fragments mapped (FPKM) [26] by the following formula: $FPKM = \frac{\text{cDNA fragments}}{\text{Mapped fragments (Millions)} \times \text{Transcript Length (kb)}}$. The $FDR < 0.05$ & $|\log_2(\text{foldchange})| \geq 1$ was set as the threshold for significantly differential expression.

Gene Ontology (GO) seq R packages [27] and KOBAS [28] software were used to test the statistical enrichment of the differential expression genes (DEGs). The significant GO terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were declared at $FDR < 0.05$.

Data analysis

We used the PROC MIXED model in SAS 9.4 (SAS Institute Inc., Cary, NC, USA), in which the animals' health and intestinal region were used as the fixed effect, while the neonatal goat was the random effect.

The level for significance was set at < 0.05 , and the results were expressed as mean $\pm$ standard error of the mean.

Results

Activities of the antioxidant enzymes and oxidative products in gut tissues

The activities of the antioxidant enzymes (CAT, SOD, and GSH-Px) and contents of the oxidative product (MDA) in the gut tissues of the goat kids are summarized in Fig. 1. In the jejunum tissues, the activities of GSH-Px were significantly higher ($P < 0.05$) in the healthy group compared with that in the diseased group, while there was no significant difference in the SOD and CAT activity observed between the healthy group and diseased group ($P > 0.05$). As shown in Fig. 1, the levels of MDA were significantly higher ($P < 0.05$) in the jejunum tissues and extremely higher ($P < 0.01$) in the ileum tissues from the diseased group compared with the healthy group. As a result, the levels of the antioxidants significantly decreased in the diseased goats compared with the healthy goats. In contrast, the MDA levels in the gut tissues were significantly greater in the diseased goats than in the healthy goats.

The level of IL-2 and IL-6 in the intestinal tissues

As shown in Fig. 2, there was a significant difference in that the level of IL-2 was significantly higher only in the ileum homogenate in the diseased group compared to the healthy group, and there was no significant difference in IL-6 observed between the healthy group and diseased group ($P > 0.05$). In short, there was no significant change in the pro-inflammatory cytokine levels in the diseased goats compared with the healthy goats.

Expression of the genes related to the TLR4 signaling pathway and barrier function

RT-PCR was used to analyze the expression of the genes related to the intestinal barrier function and genes related to the TLR4 signaling pathway-related molecules. The results showed that the genes related to the intestinal TLR4 pathway apart from *MyD88*, such as *TLR4*, *TRAF6*, *IFN- β* , *IL-1 β* , *TNF- α* , *IL-6*, *NLRP3*, *IRF3*, *TBK1*, and *NF- κ B p65* (Table 1), and genes related to the intestinal barrier function apart from *MUC20* and *ZO2*, such as *MUC12*, *MUC13*, *Occludin*, *CLDN1*, and *CLDN4* (Table 2), showed no significant difference between the healthy group and diseased group ($P > 0.05$). The expression levels of *MUC20* in the colon and *ZO2* in the ileum were significantly higher in the healthy group compared with the disease group, while the expression of *MyD88* in the jejunum was significantly higher in the diseased group compared to the healthy group. These results indicated that there was no extraordinary difference in the expression of most of the TLR4 signaling pathway-related genes and barrier function genes.

Table 1
The expression of genes related to TLR4 pathway in the lower gut.

Item	Jejunum		Ileum		Colon		SEM	P-value		
	CONT	DISE	CONT	DISE	CONT	DISE		Gut	Treat	Interaction
MyD88	1.96 ^a	0.17 ^b	1.25 ^b	2.06 ^a	0.20	0.45	0.31	< 0.01	0.18	< 0.01
TLR4	1.43	3.06	1.05	1.72	1.43	0.70	1.04	0.26	0.39	0.28
TRAF6	0.97	1.04	1.03	1.15	1.40	1.61	0.52	0.35	0.67	0.98
IFN- β	2.16	4.59	1.38	1.88	0.63	0.96	1.74	0.11	0.29	0.64
IL-1 β	2.27	4.24	1.37	1.74	0.74	0.45	1.50	0.05	0.43	0.55
TNF- α	0.64	0.94	1.08	1.15	0.49	0.36	0.24	0.00	0.58	0.45
IL-6	0.27	0.45	1.31	0.63	4.80	2.78	1.46	0.00	0.32	0.56
NLRP3	0.53	0.61	1.77	1.63	0.49	1.16	0.42	0.00	0.40	0.37
IRF3	0.90	1.22	1.07	1.12	1.24	1.34	0.31	0.56	0.39	0.81
TBK1	0.85	1.09	1.05	0.84	0.47	0.76	0.23	0.23	0.42	0.27
NF- κ B p65	0.73	0.71	1.05	1.21	0.64	0.63	0.19	0.00	0.68	0.74
^{a,b} Means in the same row with different superscript letter are significantly different ($P < 0.05$).										

Table 2
The expression of immune genes in the lower gut.

Item ¹	Jejunum		Ileum		Colon		SEM	P-value		
	CONT ²	DISE	CONT	DISE	CONT	DISE		Gut	Treat	Interaction
MUC12	2.66	1.41	3.53	1.91	2.20	2.06	1.03	0.64	0.13	0.61
MUC13	1.26	1.02	1.06	0.82	0.67	0.97	0.32	0.39	0.77	0.44
MUC20	1.21	2.38	0.87	1.61	7.76 ^a	5.45 ^b	1.01	< 0.01	0.83	0.06
Occludin	1.07	1.35	1.08	1.21	0.79	0.79	0.24	0.07	0.36	0.75
ZO2	1.06	1.27	1.69 ^a	1.04 ^b	1.24	1.43	0.33	0.66	0.68	0.14
CLDN4	1.33	1.50	1.42	2.16	1.33	1.49	0.64	0.67	0.37	0.79
CLDN1	1.74	2.49	1.74	2.23	4.47	3.63	1.48	0.12	0.88	0.74
^{a,b} Means in the same row with different superscript letter are significantly different ($P < 0.05$).										

Jejunum Transcriptome

A total of 163 million high-quality 100-bp paired-end reads (clean reads) were obtained from all of the samples (File S2), and among all of the clean reads, 95.82%–96.41% were mapped for each sample (File S3). Moreover, there was a high mapping rate with uniquely mapped reads; a range of 87.82–91.13% of reads aligned to the reference genome. The principal component analysis of the transcriptome profiles indicated that the healthy and diseased groups are highly coincident (Fig. S1). When the DEG between the diseased goats and healthy goats was further explored (the FDR < 0.05 and $|\log_2(\text{foldchange})| \geq 1$), a total of 364 DEGs showed a different expression, in which 197 genes had a higher expression in the diseased goats than the healthy goats, whereas 167 genes had a lower expression in the diseased goats than in the healthy goats (Table 3). The GO analyses showed DEGs were mainly involved in the apoptotic process, cell proliferation and migration, and inflammatory response (Fig. S2). The KEGG functional enrichment analysis of up-regulated DEGs identified pathways modified by the cold, mainly related to the ECM-receptor interaction. Down-regulated DEGs were mainly related to the Arachidonic acid metabolism, complement and coagulation cascades, and alpha-Linolenic acid metabolism. The most important pathways are shown in Figs. 3 and 4.

Table 3
Numbers of differential expression genes in disease kid goats as compared with control kid goats.

DEG Number	up-regulated	down-regulated
364	197	167
Additional files		
Additional file 1: The primers of genes in this study.		
Additional file 2: Sequencing results of all samples.		
Additional file 3: Percentage of reads mapped to reference genome of all samples.		
Additional file 4: List of differential expression genes in disease kid goats compare with healthy kid goats.		
Additional figures		
Additional Fig. 1: The principal component analysis of the transcriptome profiles in jejunum of goat kids suffering from a cold disease as compared with control goat kids.		
Additional Fig. 2: The GO analyses of DEGs in jejunum of goat kids suffering from a cold disease as compared with control goat kids.		

Discussion

Animals experience a variety of environmental stressors throughout their lives, both abiotic, such as ambient temperature and humidity, and biotic, such as bacteria, viruses, and fungi, which affects their survival and subsequent growth and development [29]. Stressors prevail and adaptations fail during a cold, ultimately leading to impaired growth, production and decreased immunity.

The intestine, the largest immune organ and a multifunctional organ central *in vivo*, is mainly involved in nutrient uptake and absorption, pathogen recognition, and resisting intestinal microorganisms from invading the body [30]. It is known that the intestine is the main site for the production of pro-oxidants, such as ROS, mainly because

of the presence of a large number of microorganisms, nutrients, and interactions between immune cells [31]. When the intracellular concentrations of ROS are greater than the physiological values, oxidative stress results [17, 18], producing cellular component damage (such as that experienced by lipids, proteins, and DNA) [32–34]. Oxidative stress has been observed in many infectious diseases of farm animals [35], and it was reported that damaged tissues undergo more free radical reactions than healthy ones [36–39], and ROS can contribute to the pathogenesis of a variety of diseases [40]. Numerous studies have reported that animals had a significant increase in their immune response for the increased production of proinflammatory cytokines in response to stress, leading to intestinal dysfunction and disease [41, 42]. Consequently, there is a prevalent hypothesis that oxidative stress and inflammatory processes play a role in cold susceptibility. Our data shed light on the molecular changes reflected in the intestinal oxidative stress, inflammation, and gene expression, which could potentially yield critical diagnostic markers for neonatal goats catching a cold.

Antioxidant enzymes *in vivo*, including SOD, GSH-Px, and CAT, are regarded as the first line of the antioxidant defense system preventing biological macromolecules from damage during oxidative stress [43, 44]. GSH-Px helps strengthen the oxidative defense system by catalyzing the reduction of harmful peroxides into harmless compounds and protecting the cell membrane structure and function [45]; it plays an important role in the protection of cells against ROS by eliminating free radicals and is considered as an indicator of the oxidative stress (as well as SOD and CAT). MDA is a typical marker for the degree of oxidative stress and cell injury [46], and the increased level of MDA suggested an enhanced peroxidation of the membrane lipids under the attack of ROS, indicating damaged membranes [47]. Earlier studies reported that Crohn's disease patients showed decreased main cellular antioxidant enzyme (SOD and GSH-Px) activities in the intestinal mucosa [48, 49] and trinitrobenzenesulfonic acid (TNBS)-induced colitis in mice, which was widely used as Crohn's disease (CD) models, showed the same result – that the enzyme activity of GSH-Px was significantly decreased, while MDA was significantly increased in the TNBS group compared with the blank group [50]. In addition, the bacteria-infected model in which lipopolysaccharide (LPS) was used to induce intestinal inflammation showed significantly decreased activities of GSH-Px and increased MDA levels [51]. In this study, we showed that goats with colds exhibited an oxidative stress status in the intestine as evidenced by an increase in the MDA level and a decrease in GSH-Px level, which are also in accordance with the previous studies. The reason may be that the invasion of pathogens easily causes excessive stress, which induces immune reactions to cope with the attack of pathogens by activating the activity of neutrophils and macrophages, resulting in excessive ROS production and accumulation, eventually resulting in oxidative stress [52–54].

The cells of the immune system secrete cytokines to combat infection and then present modulatory effects on inflammatory reactions [55]. Pro-inflammatory and anti-inflammatory cytokines coordinate the immune response to maintain homeostasis *in vivo* [56]. IL-2, IL-6, and TNF- α are considered pro-inflammatory because they attenuate the immune response to inflammation by chemoattracting leukocytes to inflammatory sites and inducing inflammatory cell proliferation [57]. To date, the most frequently studied cytokines in inflammation have been IL-2 and IL-6. IL-2 is now known to have a wide range of immunoregulatory effects. Binding of IL2 to its receptor was first demonstrated to be critical for inducing the proliferation of T cells *in vitro*, and its IL2R complex subsequently leads to the increase of proliferation, cytokine secretion, and cytolytic activity [58]. For example, the IL2R complex leads to the activation of many genes associated with cell proliferation, such as c-myc and fos [59]. IL-2 can also boost the cytolytic activity of natural killer (NK) or lymphokine activated killer cells, increase the cytolytic activity of tumor-infiltrating lymphocytes (TILs), augment immunoglobulin production by activated B cells, maintain the homeostatic proliferation of Treg cells, act on innate lymphoid cells, and modulate effector T-

cell differentiation [60]. These compelling findings suggest that IL2 promotes inflammation via an effect on the activation-induced cell death. IL-6 is a pleiotropic cytokine with a variety of biological activities, including the mediation of both the pro-inflammatory responses and cytoprotective functions [61]. IL-6 is involved in the recruitment of neutrophils and promotes the migration and proliferation of T lymphocytes into the affected tissue [62]. In addition, interleukin 6 promotes T-cell differentiation and activation. Under experimental conditions, interleukin 6 and TNF- α co-stimulate naive CD8 T cells, resulting in strong cytolytic activity [63]. TLR4 and its downstream signaling pathways play a pivotal role for inducing the secretion of inflammatory cytokines during bacterial infection [64, 65]. Shi et al. reported that the levels of IL-6 and IL-2 in the gut were increased in piglets orally infected with *C. perfringens* type C [64]. However, the results from our study showed that there was no significant difference in the secretion of pro-inflammatory cytokines in the diseased goats compared with healthy goats, both for IL-2 and IL-6, although the level of IL-2 was significantly higher in the ileum homogenate in the diseased goats. The real-time PCR results showed that the majority of the TLR4 pathway-related genes have no significant differences between the 2 groups apart from MyD88, which mainly mediates the production of pro-inflammatory cytokines by activating a series of toll-like receptor signaling pathways [66, 67], was significantly higher in the ileum of the diseased goats. The expression of genes related to the intestinal barrier function apart from MUC20 and ZO2 was the same in both the diseased and healthy goats. Mucins form the first line of innate immunity in vivo and MUC20 is a part of the membrane-bound mucins and are highly expressed in the colon [68]. Intercellular tight junctions (TJs) are closely related to the integrity of the intestinal barrier, and ZO2 as a scaffolding protein was studied. It was reported that the expression of MUC20 was down-regulated in ulcerative colitis mucosa [69], and our results showed that the expression of MUC20 in the colon and ZO2 in the ileum were significantly lower in the diseased goats, which was consistent with the previous study. These results indicate that diseased goat intestines may experience a slightly higher inflammatory response than healthy goats. The expression of the entire genome of the jejunum transcriptome was determined under the circumstances and the results showed that there was no remarkable difference in the level of most inflammatory cytokines and immune genes. Our transcriptome data also provides plausible evidence, as the PCA analyses showed no clear separation between the healthy group and diseased group, and the GO analysis showed that DEGs were not involved in the inflammatory response while the KEGG pathway analysis proved DEGs were not related to immunity. These results further demonstrate there was no strong inflammatory response occurring in the diseased goats. We can draw the conclusion that goats with colds may have little change of the immune function of the intestine.

Numerous studies have shown that intestinal inflammation was coupled to the increase of oxidative stress [70], but in our study, diseased goats experienced more free radical reactions compared with healthy goats but the change of the immune function was not obvious. The difference may have occurred for several reasons: (1) the overwhelming production of ROS in the intestine may have occurred before the immune cells reached the intestinal mucosa [71] and the "free radical induction theory" suggests the inflammation in intestinal mucosa is triggered by oxidative stress [72] due to the fact that oxidants produced by oxidative stress are activators of NF- κ B, a crucial regulator for the activation of inflammation [73]. In addition, ROS is involved in intermicrobial competition [74] and was proved by a recent study in which the increased concentration of ROS in the intestine accompanied by an expansion of the *E. coli* population in weaned piglets [75], suggesting an important role of the oxidative stress for the onset of infectious diseases; (2) It was reported that *Salmonella enterica* serovar Enteritidis and *S. Typhimurium* caused a strong inflammatory response while *S. Pullorum* induced the systemic infection of chicks without obvious inflammation [76], and the occurrence of inflammation is probably related to the invading pathogenic microorganisms; (3) The experimental animals we chose suffered from a cold, and the

cold was likely caused by the ambient temperature variation. It may not have been related to the invasion of intestinal pathogens.

Conclusion

The early diagnosis of neonatal disease is vital to reducing neonatal morbidity and mortality and remains a major challenge for animal health. Here, we detected the changes of intestinal oxidative stress, inflammation, and gene expression in neonatal goats suffering from a cold. Our data revealed that the predisposition to the cold is closely associated with intestinal oxidative stress in neonatal goats while there was no significant difference in the intestinal inflammatory status. This study revealed oxidative stress may be the potential mechanism underlying the pathophysiology of colds in newborn goats, which suggests that the antioxidative mechanisms in the intestine may be more important for health than we previously appreciated. The antioxidant activity could act as an indicator of health status. The biomarkers of oxidative stress such as GSH-Px and MDA might be used to reflect the mortality and morbidity for young goats.

Abbreviations

Abbreviations

ROS, reactive oxygen; CAT, catalase; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; IL-2, interleukin-2; IL-6, interleukin-6; TBA, Thiobarbituric Acid ; GSH, glutathione; GSSG, oxidized glutathione; TLR4, Toll-like receptor 4; MyD88, myeloid differentiation primary response 88; TRAF6, TNF receptor associated factor 6; IFN- β , interferon-beta; IL-1 β , interleukin 1beta; TNF- α , tumor necrosis factor alpha; NLRP3, pyrin domain-containing 3; IRF3, interferon regulatory factor 3; TBK1, TANK-binding kinase 1; NF- κ B, nuclear factor kappa B; RT-PCR, reverse transcription quantitative real-time PCR; TJs, tight junctions; CLDN1, Claudin1; CLDN4, Claudin4; ZO2, Zona Occludens2; MUC12, Mucin12; MUC13, Mucin13; MUC20, Mucin20; DEG, differential expression gene; NK, nature killer; TILs, tumor-infiltrating lymphocytes.

Declarations

Ethics approval and consent to participate

All animal procedures such as ethical and animal welfare issues were approved by the Institutional Animal Care and the Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, China. The animal trials were conducted in a local farm (Pingxiang, Jiangxi, China) and the owner of the farm agreed to provide eligible kid goats for us to support this experiment.

Consent for publication

Not applicable.

Availability of data and material

The jejunum RNA-seq data from this study have been submitted to the Sequence Read Archive (SRA) database (<http://www.ncbi.nlm.nih.gov/sra>) and the data are accessible through SRA Series accession number PRJNA635255 (<http://www.ncbi.nlm.nih.gov/bioproject/635255>). Other data including activities of antioxidant

enzymes, contents of oxidative product and pro-inflammatory cytokines and relative gene expression generated during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was jointly supported by the National Natural Science Foundation of China (31772631), CAS Science and Technology Service Network Initiative (KFJ-STZ-ZDTP-075), and Innovation Province Project (2019RS3021).

Author's Contributions

The authors' contributions are as follows: YC, ZXH and ZLT contributed to experimental design; ZXH, YC and CY conducted the animal and laboratory experiments, collected samples; CY and ZXH prepared libraries, acquired and analyzed the data, interpreted the results and draft the writing; ZLT, and ZXH revised the manuscript. All authors read and approved the final version of the manuscript and approved publication.

Acknowledgments

Not applicable.

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Figures

Figure 1

The changes of intestinal SOD (a), GSH-Px (b), CAT (c) and MDA (d) in goat kids induced by a cold disease. The data were organized and analyzed with Stat View 9.4 software (SAS Institute Inc., Cary, NC), significance was observed at * $P < 0.05$ and ** $P < 0.01$. Results are expressed as means $\pm$ standard error.

Figure 2

The level of intestinal IL-2 (a) and IL-6 (b) in goat kids induced by a cold disease. The data were organized and analyzed with Stat View 9.4 software (SAS Institute Inc., Cary, NC), significance was observed at *P < 0.05 and **P < 0.01. Results are expressed as mean ± standard error.

Figure 3

KEGG enrichment analyses of the up-regulated DEGs in jejunum of goat kids suffering from a cold disease as compared with control goat kids. The vertical axis represents the pathway category, and the horizontal axis represents Enrichment Factor which means the proportion of DEGs annotated to the pathway on genes annotated to the pathway.

Statistics of Pathway Enrichment

Figure 4

KEGG enrichment analyses of the down-regulated DEGs in jejunum of goat kids suffering from a cold disease as compared with control goat kids. The vertical axis represents the pathway category, and the horizontal axis represents Enrichment Factor which means the proportion of DEGs annotated to the pathway on genes annotated to the pathway.

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