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Full Length Article

High Grain Diet Triggers Inflammation in the Goat Uterus: A Comprehensive Regulation Diet Modulates the Immune Response

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Abstract

The aim of the present study was to explore the causal association between the increase in circulating subacute ruminal acidosis (SARA)-induced lipopolysaccharide (LPS) levels and uterine inflammation; moreover this study sought to evaluate the role of a comprehensive regulation diet in combating the inflammatory effects of a high grain diet. Twelve dairy goats were randomly assigned to two groups. One group was provided a high-concentrate buffered diet as the comprehensive regulation group (RG) control while, only a high concentrate powdered diet was fed to the treatment group (TG). The LPS concentrations in the rumen fluid were 428702±34505.6 and 255027±39756.2 EU/mL, in the TG and RG goats, respectively, while, the LPS levels in the peripheral plasma were 0.032±0.003 and 0.012±0.003 EU/mL in the TG and RG goats, respectively. The concentration of TNF-α, IL-1β and IL-6 was significantly higher, and there was enhanced expression of the inflammatory genes *TLR4, MyD88, TRAF-6, NF-κB, IL-1β* and *IL-6* in the uteri of TG goats. SARA increased the NF-*κ*B expression and level of its phosphorylated form in TG goats. The uteri of TG goats showed higher mRNA levels of pro-inflammatory genes than the RG goats, indicating the presence of inflammation. Thus, SARA-induced LPS derived from a high concentrate diet triggers inflammation in the goat uterus by reinforcing *TLR4* signaling. © 2017 Friends Science Publishers

Keywords: Comprehensive regulation diet; High concentrate diet; Lipopolysaccharide; Sub-acute ruminal acidosis; Uterus

Introduction

In the dairy sector, a high concentrate diet is mostly fed to dairy animals to increase milk production (Jing *et al.*, 2014). The prolonged feeding of a high grain diet leads to sub-acute ruminal acidosis (SARA) (Dong *et al.*, 2011), which is described as moderately decreased ruminal pH that varies from 5.2 to 5.6 (Gozho *et al*., 2005). Moreover, the high concentrate diet, due to a reduction in the ruminal pH, results in change in the microbial population from cellulose-digesting to starch-digesting (Metzler-Zebeli *et al*., 2013). Many of the starch digesting microorganisms are gram-negative bacteria (Bryant, 1970). The concentration of lipopolysaccharide (LPS) in the rumen is altered due to this change in population (Dehority, 2003). Previous experiments with grain-based SARA revealed that LPS is liberated in large quantities in the gastrointestinal tract (GIT) of the affected animals due to the collapse of gram-negative bacteria (Khafipour *et al*., 2009; Dong *et al*., 2011). The ruminal epithelium works as a defensive barrier between the ruminal environment and portal circulation (Graham and Simmons, 2005). The high acidity of the ruminal environment causes damage and sloughing of the epithelial cells in the main gut (Plaizier *et al*., 2008) and the permeability of the gut might increase as a result of the rise in LPS levels (Khafipour *et al*., 2009). Moreover, it was observed that high concentrate feeding increased the free LPS level in the rumen; LPS subsequently translocated into the blood stream with the possible major site of LPS translocation into the blood stream being the intestines (Khafipour *et al*., 2009). Recently, Chang *et al.* (2015) reported that a harmful feature of high grain diet-induced SARA is the induction of liver inflammation. The high rise in circulating LPS triggers inflammation that in turn stimulates extensive *TLR4* signaling and ultimately elevates the expressions of innate immune genes and enhances the secretion of pro-inflammatory cytokines.

The uterus, especially the endometrial lining, has a major role in normal reproductive cycles, the implantation of embryos and the support of a healthy foetus (Turner *et al*., 2012). Gram-negative bacteria are the primary initiators of bovine endometrial infection, causing uterine disease (Cronin *et al*., 2012) that results in tissue damage to the endometrium (Sheldon *et al*., 2009). Endometrial epithelial cells (EECs) exclusively participate in clearing the attacking microorganisms after the identification of pathogen-

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associated molecular patterns (PAMPs) by pattern recognition receptors (PRR). The LPS from gram-negative bacteria is recognized by toll-like receptor 4 (*TLR4*), which initiates innate immune responses (Fu *et al*., 2013). *TLR4* recognizes LPS with the help of LPS-binding protein (*LBP*) and cluster of differentiation antigen 14 (*CD14*) (Bannerman *et al*., 2004; Sohn *et al*., 2008). LPS triggers the activation of myeloid differentiating factor 88 (*MyD88*) after binding to *TLR4* on the cell surface of the host (Ju *et al*., 2014). *MyD88* activates TNF receptor-associated factor 6 (*TRAF6*), which stimulates the IκB kinase (*IκK*) complex; consequently, phosphorylated nuclear transcription factor (*NF-κB*) translocates from the cytoplasm to the nucleus (Turner *et al*., 2012). The NF-κB-dependent signaling pathway leads to the production of pro-inflammatory cytokines, including interleukins *IL-1β*, *IL-6*, chemokines (*IL-8*) and other inflammatory mediators (Cronin *et al*., 2012; Fernández *et al.*, 2016).

Sodium bicarbonate is normally supplemented in the diets of the ruminants to neutralize ruminal pH (Phy and Provenza, 1998). Magnesium oxide, sodium sesquicarbonate, and sodium bicarbonate together increase the pH of the ruminal fluid and increase its buffering ability, stabilizing the acid-base status of the rumen (Le Ruyet and Tucker, 1992). Phy and Provenza (1998) illustrated that small ruminants preferred pelletized fermentable concentrate diets supplemented with sodium bicarbonate. Dietary sodium butyrate decreased digestive tract permeability and facilitated considerable alterations in intestinal microbial population (Huang *et al*., 2015) in addition to reducing colonic inflammation (Wirtz and Neurath, 2007).

Therefore, the present study focused on the following objectives: (I) to evaluate changes in the expression of inflammation associated genes in the goat uterus due to LPS derived from the digestive tract after extended of a high concentrate diet and (II) to study whether the comprehensive regulation buffered diet can reduce the negative effect of a high concentrate diet in ruminants.

Materials and Methods

Ethical Approval

The sampling procedures and experimental design were approved by the Animal Ethics Committee of Nanjing Agricultural University, China before the start of this experiment.

Animals, Diets and Experimental Design

Twelve multiparous mid-lactation dairy goats exhibiting an average body weight of 39 ± 6 kg were selected for the current study. They were randomly divided into two groups, the comprehensive regulation group (RG) and the treatment group (TG), with each group containing 6 goats. The RG goats were fed a high concentrate palletized diet (6:4 concentrate/roughage ratio) with the addition of uniformly mixed buffer agents, while the TG goats were offered only a high concentrate powdered diet (6:4 concentrate/roughage ratio) for the 24-week experimental period. All the dairy goats were ruminally cannulated at 14th week. The animals were housed in individual tie stalls. Feed was offered two times daily at 08:00 and at 18:00 h, while fresh water was available to them at all times throughout the entire experimental period. The experiment was started after the animals made a full recovery, and no goat showed any clinical signs of infection during the experiment.

The ingredient details and buffer contents used in the diets are presented in Tables 1 and 2.

Sample Collection and Analysis

Blood samples and rumen fluid were collected at the 23rd week. Blood samples from all the goats were taken using 5-mL sterile Vacutainer tubes containing sodium heparin. The plasma was separated from the blood samples by centrifugation at 3000 × g at 4ºC for 15 min and stored at -20°C to determine the pro-inflammatory cytokines in the peripheral blood. The rumen fluid was collected in a depyrogenated, sterilized glass tube (previously heated at 250°C for 2 h). The pH of the ruminal fluid was immediately measured with a pH-metre (Sartorius, Basic pH Meter PB-10, PB-21, Goettingen, Germany) at 0 h, 1 h, 2 h, 4 h, 6 h, 8 h and 10 h after feeding after filtration through four layers of sterilized cheesecloth. At the end of the experiment, the goats were slaughtered after overnight fasting and uterine tissues from all the goats were carefully removed immediately after slaughter, flash frozen in liquid nitrogen and stored at -70ºC.

Determination of Ruminal pH

The ruminal pH was measured with a pH-metre.

Determination of LPS

The LPS concentrations in the plasma and rumen fluid were determined with Chromogenic endpoint Limulus Amebocyte Lysate assay kits, CE64406 and CE80545 (Chinese Horseshoe Crab Reagent Manufactory Co., Ltd., Xiamen, China), respectively, as reported by Dong *et al.* (2013). These assays have a minimum detection limit of 0.01 EU/mL.

Radioimmunoassay

Radioimmunoassay (RIA) kits were used to measure the concentration of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in the peripheral blood (IL-1β, C09DJB; IL-6, C12DJB; TNF-α, C06PJB); Beijing North Institute of Biological Technology, Beijing, China.

Table 1: The ingredients and nutritional composition of the diets

Items	Ingredients	Quantity
Concentrate	Corn (g/100g)	23.1
	Bran (g/100g)	28.0
	Soybean meal (g/100g)	2.00
	Rape seed meal (g/100g)	3.70
	Limes stone meal (g/100g)	1.43
	Calcium hydrophosphate (g/100g)	0.60
	Premix[1] (g/100g)	0.50
	Salt (g/100g)	0.40
Coarse material		
	Oat grass (g/100g)	32.0
	Alfalfa (g/100g)	8.00
Nutritional index		
	Net energy (MJ/kg)	17.6
	Fat (%)	3.60
	Crude protein (%)	16.8
	Neutral detergent fiber (%)	42.6
	Acid detergent fiber (%)	7.22
	Calcium (%)	1.55
	Phosphorus (%)	0.76

[1]Premix consists of following ingredients per Kg diet: 6.00×10^3U vitamin A, 2.5×10^3U vitamin D, 80.0mg vitamin E, 6.25mg Cu, 62.5mg Fe, 62.5mg Zn, 50.0mg Mn, 0.125mg I, 0.125mg Co, 0.125mg Mo

Table 2: Formulation of diets for the treatment group (TG) and the regulation group (RG)

	Concentrate (Powdered) (g)	Concentrate (Pelletized) (g)	Roughage (g)		Buffer agents (g)			
			Alfalfa	Oat grass	Magnesium oxide	Sodium bicarbonate	Sodium acetate	Sodium butyrate
Treatment group (TG)	600	-	133	267	-	-	-	-
Regulation group (RG)	-	600	133	267	6	9	10	10

Table 3: Primer sequences for real-time polymerase chain reaction (qRT-PCR) amplification of mRNA

Gene name	Gene Bank accession	Primer sequences (5′–3′)		Length (bp)
β-actin	JQ409458.1	F:	CTTGATGTCACGGACGATTT	380
		R:	CACGGCATTGTCACCAACT	
NF-KB	XM_00569893.1	F:	AGGTGGCGATCGTTGTTCTA	226
		R:	TTGCCTTTGTTCTTCCTGCC	
MyD88	XM_005695671.1	F:	TGGACACATACAAGCCCACT	171
		R:	AGTGTCCCATCTTGTCCAGG	
TRAF-6	XM_005690091.1	F:	TTTCAGAGACCCACCATCCC	166
		R:	GAAAACCCTCCCTCCGAAGA	
IL-1β	XM_005686747.1	F:	CCGTGATGATGACCTGAGGAG	303
		R:	CAAGACAGGTATAGATTCTTGC	
TLR-4	NM_001285574.1	F:	CTGAGAACCGAGAGCTGGGAC	207
		R:	TTCGCATCTGGATAAATCCAGC	
IL-6	JF432019.1	F:	CGAAGCTCTCATTAAGCACATC	127
		R:	CCAGGTATATCTGATACTCCAG	

RNA Extraction and Quantitative Real-time PCR

RNA extraction: Total RNA from 100 mg of uterine tissue was extracted with the RNA iso Plus ^{TM} reagent (Takara Co., Otsu, Japan) using a Dounce homogenizer (Polytron PT 1200 E, Switzerland). The concentration of RNA in every sample was determined using a spectrophotometer (Eppendorf Biotechnology, Hamburg, Germany) at 260 nm. First-strand cDNA was prepared from 250 ng/µL of total RNA template using the Prime Script RT Master Mix Perfect Real Time kit (Takara Co., Otsu, Japan).

Quantitative Real Time PCR (qRT-PCR)

Primers for the *TLR4, MyD88, TRAF6, and NF-κB, IL-1β, IL-6 and β-actin* genes were designed using Premier 6.0 (Premier Biosoft International, USA). The reaction mixture comprised 0.4 µM primers and 2 µL of cDNA in a final volume of 20 µL of supermix. The thermal cycling parameters consisted of an initial denaturation step at 95°C for 15 s, followed by 40 cycles of annealing at 95°C for 5 sec. and primer extension at 60°C for 31 s. All the reactions were run in triplicate. The PCR products were verified by gel electrophoresis with 3.0% agarose gel.

Each cDNA sample was amplified using SYBR Green (Takara Co., Otsu, Japan) on the ABI 7300 Fast Real-Time PCR System (Applied Bio system, USA). The data were normalized to the mean of the internal reference *β-actin* gene to control unpredictability in expression levels and were evaluated using the $R= 2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The primer sequences are presented in Table 3.

Western Blot Analysis

Approximately 100 mg of the frozen uterine tissue was homogenized in 1 mL of ice-cold RIPA protein isolation buffer (Beyotime, Shanghai, China) for 15 s, incubated on ice for 20 min and centrifuged at 12000 rpm for 30 min at 4°C. Care was taken to avoid disturbing the pellet at the bottom when the supernatant was shifted to another tube. The protein concentration was measured using a BCA Protein Assay kit (Thermo Fisher Scientific Inc., USA) and was adjusted to 4 μg/μL. Equal protein amounts were loaded for separation with 10% SDS-polyacrylamide gel electrophoresis (PAGE) before being transferred to a nitrocellulose (NC) membrane (Pall Gelman Laboratory, Ann Arbor, USA) at 4°C. Afterward, the membranes were blocked in 5% skimmed milk (5% BSA for phosphorylated p65) for 2 h at room temperature, followed by incubation together anti-NF-κB (p65) (1:300, SC-109, Santa Cruz Biotechnology, USA), anti-p NF-κB (p-p65) (1:300, SC-33020, Santa Cruz Biotechnology, USA) and GAPDH primary antibodies (1:10000, Bioworld Technology, Inc., Louis Park, USA), at 4°C overnight. The membranes were subsequently washed in tris-buffered saline containing Tween20 (TBST) followed by incubation with horseradish peroxidase (HRP)-conjugated goat-anti-rabbit (1:5000, Santa Cruz Biotechnology, USA) and goat anti-mouse secondary antibodies (1:10000, Sunshine Biotechnology (Nanjing) Co., Ltd, China), at room temperature for 2 h. After washing the membranes 6 times (10 min/wash), the results were developed with an ECL Plus kit (Vazyme, Nanjing, China). The signals were visualized on an imaging system (LAS4000, USA) and evaluated with Quantity One software (Bio-Rad, USA).

Statistical Analyses

All analyses were performed with SPSS statistical software version 16.0 (SPSS Inc., Chicago, IL, USA) and an independent *t*-test was used to evaluate the differences in the mean ± SEM between the groups. The mean differences were considered significant when $P<0.05$.

Results

Ruminal pH and LPS Content of Ruminal Fluid and Blood Plasma

The pH of the rumen fluid is presented in Fig. 1. The ruminal pH in TG goats was less than 5.8 for more than 3 h,

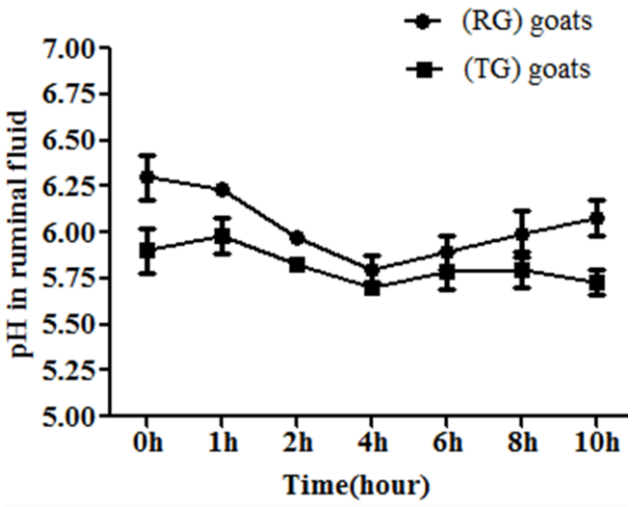

Fig. 1: Comparison of the pH values in the ruminal fluid between regulation group (RG) and treatment group (TG) goats

The data were measured during the 23rd week using ruminal fluid samples collected at times ranging from 0 to 10 h (shown on the x-axis). Student's *t*-test was used to evaluate the significance of treatment; significant variations were observed across all of the sampling times ($P<0.05$)

indicating that SARA was elicited effectively. Extended feeding of a high grain diet linearly reduced the dynamic pH curve in the TG goats relative to the RG goats throughout the experimental period. The ruminal pH of the TG goats was significantly less than that in the RG goats ($P<0.05$). The LPS concentration in the rumen fluid of TG goats was 428702 ± 34505.6 EU/mL, while that in RG goats was 255027 ± 39756.2 EU/mL, indicating a significant increase in LPS level in TG goats compared to RG goats ($P<0.02$). Similarly, the LPS level in the peripheral plasma of TG goats 0.032 ± 0.003 EU/mL was significantly higher than that in the RG goats 0.012 ± 0.003 EU/mL ($P<0.002$) (Table 4).

Pro-inflammatory Cytokine Levels in the Peripheral Blood

The concentrations of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α were determined with radioimmunoassay and the results are shown in Fig. 2. The concentration of IL-1β was 0.2167 ± 0.0155 ng/mL in TG goats and 0.1700 ± 0.0041 ng/mL in RG goats ($P<0.02$) (Fig. 2a); the IL-6 concentration was 607.3±4.5 pg/mL in TG goats and 401.5 ± 69.9 pg/mL in RG goats ($P<0.02$) (Fig. 2b) and the TNF-α concentration was 7.603 ± 0.531 fmol/mL in TG goats and 2.628 ± 1.120 fmol/mL in RG goats ($P<0.007$) (Fig. 2c). The concentration of pro-inflammatory cytokines in the blood was significantly higher in the TG goats relative to the RG goats ($P<0.05$).

Table 4: Lipopolysaccharide concentration in the rumen and plasma of treatment group (TG) and regulation group (RG) dairy goats fed a high concentrate diet

LPS Concentration (EU/mL)	Treatment group (TG)		Regulation group (RG)		P-value
	Mean	SEM	Mean	SEM	
Rumen LPS (EU/mL)	428702	34505.6	255027	39756.2	0.02
Plasma LPS (EU/mL)	0.032	0.003	0.012	0.003	0.002

SEM=Standard error of mean between the two groups; The LPS data were compared using Student's *t*-test

Expressions of Genes Involved in Inflammation of the Uterus

As presented in Fig. 3, the high concentrate diet induced SARA altered the expression of inflammation related genes in the uteri of TG goats relative to the RG goats. The gene expression of *TLR4* and *IL6* was significantly higher in the TG goats compared to RG goats (P<0.01). Similarly, the *IL-1β* and *MyD88* levels were significantly higher in the TG goats than the RG goats (P<0.01). Moreover, the expression of *TRAF6* and *NF-κB* was significantly elevated in TG goats relative to RG goats (P<0.01).

Protein expression of NF-κB in the Goat Uterus of the Treatment Group and Regulation Group

Western blot analysis verified that high concentrate diet induced SARA significantly enhanced the levels of NF-κB (p65) (P<0.001) and phosphorylated NF-κB (p-p65) (P<0.003) protein in the uteri of TG goats compared to the RG goats (Fig. 4). Thus, it was confirmed that the *NF-κB* signaling pathway was activated.

Discussion

In the current research, we highlighted the pivotal role of inflammatory genes and the mechanism underlying their expression in the uteri of goats; this change resulted from the higher serum concentration of LPS induced by the extended feeding of a high concentrate diet. It has been documented that the feeding of a high concentrate diet results in digestive disturbance, especially, SARA (Gozho *et al*., 2005), which is described as decreased feed intake, low milk fat and inflammation characterized by a rise in acute phase proteins (Khafipour *et al*., 2009). The key indicator of SARA in many animals is the low pH of the rumen fluids (Khafipour *et al*., 2009). The current study revealed that the dynamic pH curve for the ruminal fluid of TG goats was less than that of the RG goats, depicting the onset of SARA in the TG goats. This is in agreement with the results of Ghorbani *et al*. (2002), who proposed that the an average pH value below 5.8 in TG goats for more than three hours represented confirmation of SARA. However, there is little literature showing that SARA not only depends on pH but on the change in the microbial population that occurs secondarily to the feed type (Calsamiglia *et al*., 2012). In fact low pH values in the rumen resulted in the lysis of gram-negative bacteria, which causes LPS to be released and free to enter the systemic circulation

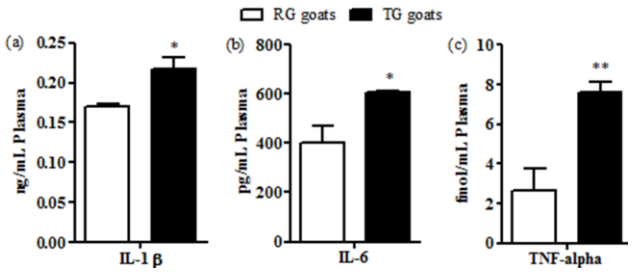

Fig. 2: *IL-1β, IL-6* and *TNF-α* levels in peripheral blood
The data are expressed as the mean ± SEM; asterisks indicate differences between the goats in the regulation group (RG) and treatment group (TG) (*P<0.05, **P<0.01, n=12)

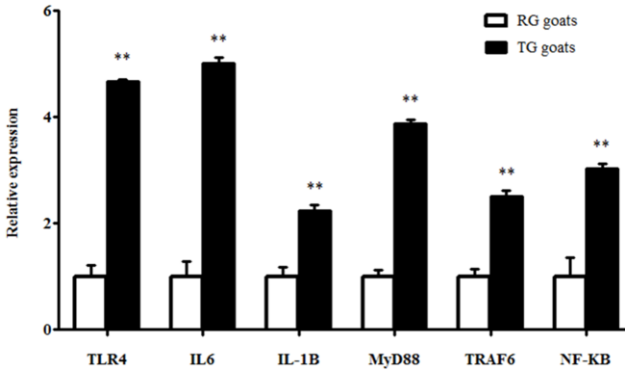

Fig. 3: Uterine gene expression profile analysis with qRT-PCR
The genes involved in transcriptional regulation were measured in the uterine tissue. The error bars indicate the standard error of the mean and ** indicates significance at P<0.01

(Andersen, 2000). Moreover, feeding high concentrate diets to animals places their GIT at a high risk of impairment due to perturbations in the functions of the ruminal epithelium (Tao *et al*., 2014). Due to epithelial damage and alterations in GIT permeability, LPS might also translocate into the circulation where this rise in LPS level triggers an immune response, which is in accord with earlier studies (Dong *et al*., 2011; Khafipour *et al*., 2009). This is the main reason why many studies on dairy animals recommended an appropriate coarse fibre buffered diet for good rumination and enhanced saliva secretions to overcome ruminal acidosis (Mertens, 1997; Calitz, 2009). The clinical evidence indicated that the supplementation of buffering agents, bicarbonates and carbonates including sodium bicarbonate ($NaHCO_3$),

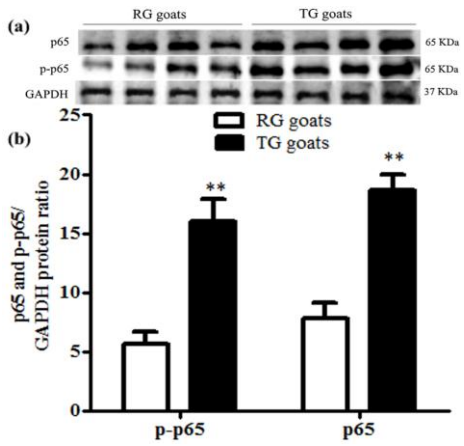

Fig. 4: Western blot for NF-κB (p65) and phosphorylated-p65 protein in the uterus

The NF-κB level in the uteri of regulation group (RG) and treatment group (TG) goats was evaluated using western-blot (a). The protein quantities were measured as arbitrary units relative to GAPDH; fold alterations in NF-κB (p65) and phosphorylated NF-κB (p-p65) are shown (b). The data are expressed as the mean ± SEM; asterisks indicate differences between the treatment group (TG) group and the regulation group (RG) goats (**P<0.01)

magnesium oxide (MgO), potassium bicarbonate ($KHCO_3$) and potassium carbonate (K_2CO_3) are of significant value in increasing the ruminal pH of SARA affected animals (Erdman, 1988). It is well recognized that the cows fed a buffered diet have a higher ruminal pH (Ghorbani *et al*., 1989). Notably, stromal and endometrial epithelial cells have an essential role in inflammatory gene expression in the reproductive tract due to their interactions with LPS (Sheldon and Roberts, 2010). This inflammation is mediated by the release of particular mediators which ultimately enhance the production and release of the immune cell infiltrates that are responsible for marked cellular changes in the epithelial cells (Chapwanya *et al*., 2009).

In the present study, we sought to explore the influence of SARA derived endogenous LPS at the molecular level and observed increased expression of pro-inflammatory cytokines, probably because of a high LPS concentration in the peripheral plasma (Baumann and Gauldie, 1994). The LPS activates the immune system during the stimulation of cytokine release (Netea *et al*., 2002). Our findings showed that the levels of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 were significantly higher in the peripheral blood of the TG goats. This finding was in agreement with the earlier reports of (Lukacs *et al*., 1995; Dörffel *et al*., 1999).

Moreover, RT-qPCR based analysis of inflammatory markers in the goat uterus confirmed that the TG goats suffered from uterine inflammation. TLRs recognize the PAMPs of gram-negative bacteria; this recognition subsequently enhances *TLR* expression, the most important part of the innate immune system (Akira *et al*., 2006). Our data confirmed that the LPS induced by TG goats, showed *TLR4* elevation compared to the RG goats. In agreement with previous studies (Fu *et al*., 2013; Ju *et al*., 2014), our research illustrated that when bovine endometrial epithelial cells are challenged with LPS, *TLR4* molecules are significantly regulated.

Furthermore, a significant increase in the expression of the adapter molecule *MyD88* was observed in TG goats versus RG goats (Fu *et al*., 2013; Ju *et al*., 2014). Many reports have shown that IL-1 receptor-associated kinase (*IRAK*) is recruited by *MyD88* triggering *TRAF6*, which stimulates the IκB kinase (*IκK*) complex (Fu *et al*., 2013). Similar findings were also observed in this study, as higher *TRAF6* expression was found in TG goats relative to RG goats. *TRAF6* triggers inhibitory-κB (*IκB*) phosphorylation, resulting in the degradation of *IκB* proteins; consequently, *NF-κB* translocates from the cytoplasm to the nucleus to regulate the transcription of inflammatory genes (Kawai and Akira, 2007). Substantial evidence has proven that high expression of *NF-κB* in the uterine tissue was due to inflammation of the endometrium (Chapwanya *et al*., 2009). Our results also proved that SARA derived LPS triggers *NF-κB* signaling causing endometrial inflammation.

The expression levels of both NF-κB (p65) and phosphorylated p65 proteins were noticeably elevated in the TG goats compared with the RG goats. Taken together, the distinctive base of above mentioned facts firmly suggests that LPS triggered the activation of *TLR4* signaling, resulting in an inflammatory response in the uterus of goats suffering from SARA. Overwhelmingly, the data in the current study indicate that LPS triggers the *TLR4-NF-κB* signaling pathway, in accord with an earlier study (Chang *et al*., 2015). The present study found that SARA increased the mRNA expression and protein concentrations of *NF-κB* in the uterus of TG goats. It was also observed that *TLR4* exerted a pro-inflammatory effect by activating *NF-κB* and these results confirm that the *TLR4*-dependent activation of the *NF-κB* pathway plays a key role in the endometrial immune response (Ju *et al*., 2014).

Conclusion

It has been unmistakably suggested that the extended feeding of a high concentrate diet alters inflammatory gene expression in the uteri of dairy goats. The feeding of a comprehensive regulation buffered diet will be beneficial in reducing SARA and has been proved to be effective in combating inflammatory effects and ultimately endometritis in ruminants. However, further studies are needed to determine further prophylactic measures to overcome SARA-induced uterine inflammation.

Acknowledgements

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