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Effects of activated charcoal and zeolite on serum lipopolysaccharides and some inflammatory biomarkers levels in experimentally induced subacute ruminal acidosis in lambs

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Abstract: Bacterial lipopolysaccharides (LPS) play a key role in the pathogenesis of subacute ruminal acidosis (SARA). Limited attempts have been made to inhibit LPS absorption during SARA. Therefore, this study aimed to evaluate the impacts of oral usage of activated charcoal and zeolite on serum LPS and acute phase proteins (APPs) levels in lambs with SARA. Fifteen lambs were randomly assigned to 3 groups, each containing 5 lambs: control, activated charcoal, and zeolite. In all groups, SARA was induced for 7 days through increasing the crushed corn ration to 52.8% and reducing the rumen pH to the range of 5.7–5.6. In the control group, no additive was added to the ration while the other 2 groups received 1 gr/kg BW activated charcoal and 1 gr/kg BW zeolite daily, respectively. Immediately prior to induction (0 day) and during SARA, blood samples were taken to analyze the lactate, bicarbonate, LPS, and APPs values. During SARA, activated charcoal and zeolite groups showed significant decreasing trends in serum LPS levels ($P < 0.01$). However, no significant changes were observed in serum CRP value in all SARA groups ($P > 0.05$). Moreover, plasma fibrinogen level significantly increased on days 1, 2, and 3 of SARA compared to day 0 in all groups ($P < 0.01$). In conclusion the oral consumption of activated charcoal and zeolite in the lambs with SARA resulted in a significant reduction in serum LPS values during SARA. Factors other than LPS might play a role in inducing inflammatory responses.

Key words: Activated charcoal, lipopolysaccharide, subacute acidosis, sheep, zeolite

1. Introduction

Ruminal acidosis is a major recurring nutritional disorder in ruminants. This condition increases herd mortality, dramatically reduces weight gain in livestock, and complicates nutritional strategies for cattle and sheep. Remarkable studies have been performed on the epidemiology and pathophysiology of the disease in cattle [1–3], but few relevant studies are available in sheep. Ruminal acidosis is classified into 2, as acute and subacute forms. Subacute ruminal acidosis (SARA) is economically more important than its acute form, often impacting a significant proportion of the herd [4]. SARA is defined as a reversible rumen pH depression during extended times per day [5,6]. While rumen pH above 5.9 is considered as normal, a pH range of 5.6–5.8 is suggested as the marginal range for opening ruminal acidosis [7]. In sheep and cattle, the diagnosis of SARA is established when rumen pH remains between 5.6–5.2 for a minimum of 3 h per day [8,9]. In several previous studies, SARA has further been determined as a ruminal pH below 5.8 [10,11].

It has been reported that SARA induction caused by feeding high-grain rations is associated with elevated levels of free bacterial LPS in the ruminal and intestinal digesta in dairy cows [12]. LPS or endotoxin could be translocated into the blood, resulting in many complications such as inflammatory responses, ruminal stasis, tissue poor blood supply, and weakness [13]. Various studies have shown the adverse sequels of increased rumen and blood LPS, including lameness [9], liver changes [14], and induced inflammatory responses [12] in SARA. Inflammatory responses have been surveyed in SARA [15–17]. It has been stated that upon reaching the liver, LPS and biogenic amines, including histamine and ethanolamine, stimulate inflammatory responses, thereby producing acute phase proteins (APPs) [5]. APPs including serum amyloid A, haptoglobin, fibrinogen, and C reactive protein (CRP) are useful indicators for detecting inflammatory processes in ruminants [4,18,19]. Contrary to the acute ruminal lactic acidosis whose pathogenicity is mainly related to increased blood D and L-lactic acid values [4], and also oxidative stress [20], numerous investigations of SARA have shown

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that a major part of SARA pathogenicity is attributed to LPS [12–14]. On the other hand, few attempts have been made to inhibit LPS absorption in SARA or acute ruminal acidosis [21,22].

Given the properties of activated charcoal and certain commercial toxin binders such as bentonite and zeolite to absorb bacterial toxins, mycotoxins, and other known toxins [23], it is hypothesized that these compounds might be able to trap bacterial rumen-derived LPS and to prevent its translocation into the systemic circulation in SARA-affected animals. Accordingly, in this research, the absorbents of activated charcoal and zeolite were expected to potentially trap rumen bacteria originated LPS so as to prevent SARA sequels. However, no *in vivo* study has been performed on inhibiting the absorption of rumen bacteria derived LPS during SARA in farm animals. Thus, the objective of the present study was to investigate the effect of activated charcoal and zeolite on serum LPS levels and its relationship with serum APPs concentrations following SARA induction in sheep.

2. Materials and methods

The research protocols of this study were approved by the Ethics Committee of the Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran (approval no. EE/97.24.3. 70203/scu.ac.ir). This interventional case control study was performed on 15 6-month-old healthy lambs (12 males and 3 females) with an average live weight of 32.5 kg in the Veterinary Teaching Hospital of Shahid Chamran University of Ahvaz, Khuzestan province, southwest Iran, in September 2018.

2.1. Study period and animal grouping

The study period comprised a week of adaptation and 1 week of SARA induction followed by a 3-d course of SARA challenge. In the beginning of the adaptation period, all lambs were checked for health status and treated with albendazole (7.5 mg/kg) and cypermethrin (1 mL/L of water for spray) for possible internal and external parasites infestation, respectively. After determining the dry matter (DM) content of dietary components (grinded corn 89.4%, alfalfa 86.2%, and wheat straw 92.2%), the lambs were individually fed, during a week, with a basal diet comprised of 3% BW of DM ration with 1:4 ratio of corn and forages (equal portions straw and alfalfa), respectively. Water access was also free. The diurnal rations were offered in 3 servings at 8:00, 14:00, and 19:00 h, such that the corn was fed in the morning (8:00) and afternoon (14:00) meals [24]. A total of 15 lambs were randomly divided into 3 groups including 5 lambs each: as control, activated charcoal, and zeolite groups. Because 3 out of 15 lambs were females, a female lamb was randomly assigned to each group for group matching.

2.2. SARA induction

After a week of adaptation (feeding with basal diet) and immediately before induction (day 0), blood and rumen fluid samples were taken from the lambs of all 3 groups. On a daily basis, 4% of the ration weight (DM basis) (42 ± 8 g), crushed corn was replaced with the same amount of straw in the 3 groups. Of note, each lamb was individually fed with crushed corn. In all groups, on the 7th day of SARA induction, when the amount of crushed corn was increased to 52.8% (566 ± 92 g) of ration, the ruminal pH falling to the range of 5.7–5.6 was observed between 4–7 h after the morning feeding, which represented SARA [10,25]. Immediately after rumen content sampling via orogastric intubation, pH was determined by a pen type digital pH meter (AZ Instrument Corp., Taiwan). To avoid saliva contamination of the rumen samples, a large-bore stomach tube (1.5 cm internal diameter and 40 cm length) was primarily indwelled to the upper esophagus. Afterwards, a small-bore stomach tube (1 cm internal diameter) was passed through the larger tube into the rumen. The first amounts of liquid samples were further discarded to limit saliva contamination as described by Lee et al. [26] and Jasmin et al. in 2019 [27].

In the control group, no additive was added to the diet. During the induction (7 days) and SARA (3 days) phases, 1 g/kg BW activated charcoal (Zoophar Parnian Pars Co. Tehran, Iran) powder and 1 g/kg BW zeolite powder (aluminum silicate) (Zoophar Parnian Pars Co., Tehran, Iran) were daily added to the diet of the activated charcoal and zeolite groups, respectively. Following SARA induction, ruminal pH was determined during 3 consecutive days, and 3 venous blood samples were taken from the jugular vein including a heparinized blood sample by a 2 mL sterile syringe, which manually soaked with 0.05 mL of sodium heparin (125 IU/mL blood) (Caspian Rasht Co., Iran) (for blood gas analysis) to analyze blood lactate and bicarbonate levels, a citrated blood sample (to get plasma) to measure plasma fibrinogen levels, and a free anticoagulant blood sample to assess the serum LPS and C reactive protein (CRP) values.

2.3. Blood biochemical analyses

Heparinized venous blood samples were taken and biochemical variables such as lactate and bicarbonate concentrations, anion gap, and blood pH were measured immediately after sampling, using a blood gas analyzer (EDAN, Edan Instruments Inc, Shenzhen, China). For serum separation, after clotting, anticoagulant free blood samples were centrifuged at 3,000 rpm for 10 min and the harvested sera were stored in 1.5 mL sterile microtubes and frozen at -70 °C prior to testing.

A commercial bacterial LPS ELISA kit (Shanghai Crystal day Biotech, China) was employed to measure serum LPS concentration according to the manufacturer's

instructions with the help of an ELISA reader set (Model AFB0612L, Taiwan). Plasma fibrinogen concentration as an APP was further specified with the help of a Mahsa Yaran kit (Tehran, Iran) according to the manufacturer's instructions. As another APP, serum CRP level was measured by an ELISA kit (Monobind Inc, Lake Forest, CA, USA) according to the manufacturer's recommendations with a detection range of 0–30 mg/dL.

2.4. Data analysis

The data was assessed for normality via the Shapiro-Wilk test; all data had normal distributions. One-way ANOVA and post hoc Tukey tests were used to compare the mean of the data at each time among the different groups. Multivariate repeated measures ANOVA and Bonferroni post hoc tests were also employed to evaluate the trend of data changes in each group at different times of the study. Pearson test was used to calculate the correlations between serum LSP and APPs values. The level of significance was set at $P < 0.05$. The analyses were performed using SPSS statistic software (IBM SPSS statistics for Windows, version 21.0) (IBM Corp., Armonk, NY, USA).

3. Results

3.1. SARA induction and ruminal pH variations

Table 1 shows the trends of rumen pH changes during the induction and SARA periods in the studied lambs. According to the results, on the 7th day of SARA induction, rumen pH of all 3 groups decreased to a range of 5.7–5.6, implying SARA induction (Figure 1). Daily ruminal pH values were compared among the 3 study groups using one-way ANOVA. It was shown that the ruminal pH changes in the activated charcoal and zeolite groups matched with the control group during SARA phase, indicating the successful induction of SARA in the 3 study groups.

3.2. Serum LPS variations

Analysis with repeated measures ANOVA showed that experimentally induced SARA was not associated with significant changes in the overall trend of serum LPS level on days 0 (just before induction), 1, 2, and 3 of the SARA

phase in the control group ($P > 0.05$). Serum LPS levels of activated charcoal and zeolite groups had the significant falling trends on days 0, 1, 2 and 3 of SARA ($P < 0.01$), (Table 2, Figure 2). One-way ANOVA analysis showed no significant differences among the 3 groups regarding the serum LPS values on day 0 (immediately before SARA induction). Serum LPS level was significantly lower in the activated charcoal and zeolite groups compared to the control group on days 1, 2, and 3 of SARA phase (Table 2).

3.3. Variations in blood inflammatory biomarkers

In all SARA groups, plasma fibrinogen level significantly increased on days 1, 2, and 3 of SARA compared to day 0 ($P < 0.01$), (Table 3). Analysis with repeated measures ANOVA showed that the overall trend of changes in serum CRP values in the control and zeolite groups underwent no significant changes on days 0 (just before induction), 1, 2, and 3 of SARA ($P > 0.05$) (Table 4). In the activated charcoal group, change in CRP was only significant on day 1 of SARA. Based on the one-way ANOVA analysis, no significant difference was observed in serum CRP concentrations among the groups on days 0, 1, 2, and 3 of SARA ($P > 0.05$) (Table 4). No significant correlation was found between the serum LPS and APPs values in the activated charcoal and zeolite groups.

3.4. Changes in acid-base biomarkers

No significant changes were seen among the study groups in blood lactate, bicarbonate, anion gap, and pH values during SARA (Table 5).

4. Discussion

SARA is an increasingly encountered high-grain ration-related disorder in ruminants; economically, it is more important than its acute form. Ruminal pH range in SARA varied over the course of 24 h, with the lowest value recorded from 2–4 h post concentrate feeding [28]. SARA diagnosis was considered once the rumen pH value reached below 5.8 in dairy cattle [10,11] and goats [29]. In affected cows, degradation of gram-negative bacteria is associated with the release and absorption of large amounts of LPS from the gastrointestinal tract [4].

Table 1. Ruminal pH values (Mean ± SD) during induction and SARA phases in the study groups.

Group	Induction phase (day)						SARA phase (day)		
	0	2	3	4	5	6	1	2	3
Control	7.04 ± 0.3 ^a	6.35 ± 0.2	6.2 ± 0.2	6.05 ± 0.1	5.9 ± 0.0	5.8 ± 0.1	5.6 ± 0.2 ^b	5.67 ± 0.1 ^b	5.6 ± 0.1 ^b
Activated Charcoal	7.12 ± 0.1 ^a	6.4 ± 0.1	6.3 ± 0.2	6.0 ± 0.1	6.0 ± 0.1	5.9 ± 0.08	5.6 ± 0.1 ^b	5.8 ± 0.1 ^b	5.6 ± 0.1 ^b
Zeolite	7.1 ± 0.2 ^a	6.3 ± 0.14	6.2 ± 0.1	5.9 ± 0.1	5.8 ± 0.2	5.8 ± 0.1	5.62 ± 0.1 ^b	5.75 ± 0.2 ^b	5.7 ± 0.2 ^b

Different superscript letters in each row (a, b, c) denote significant difference between values in comparison with the 0 day.

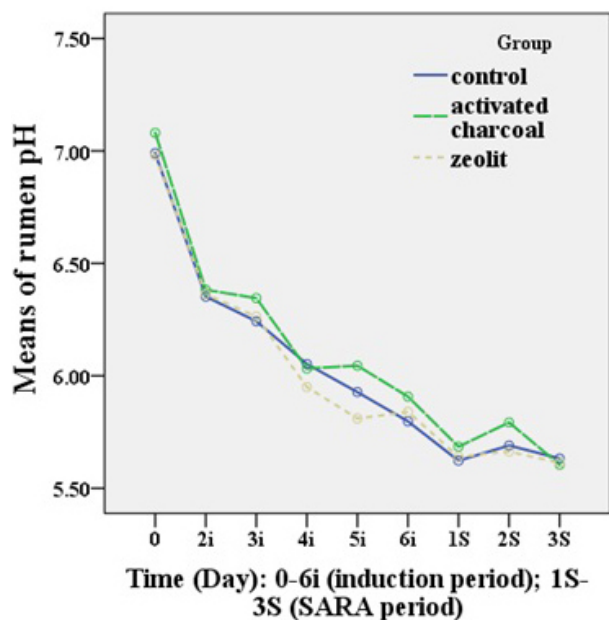


Figure 1. Ruminal pH variations during induction and SARA periods in the 3 study groups.

LPS can be associated with many complications including inflammatory responses, ruminal stasis, tissue poor blood supply, and weakness [13]. Rumen inflammation, lameness, liver abscess, pneumonia, and death have been documented as the consequences of ruminal acidosis [30]. It has further been proven that high-grain diet-related LPS is involved in postacidosis complications [12,15].

On the 7th day of SARA induction in the current study, when the amount of crushed corn increased to 52.8% of ration, the rumen pH of the studied lambs decreased to 5.7–5.6, which indicated SARA. Lettat et al. [25] were able to induce SARA at a ruminal pH of 5.6 in rams during a 3-day challenge by feeding with 1.2% BW of crushed corn. Consistent with our study, Dong et al. [29] showed that

when the concentrate ratio increased to 60% DM of the whole diet, the rumen pH of the study goats decreased to less than 5.8, which is considered the beginning of SARA. SARA diagnosis was established when rumen paracentesis samples in cattle had a pH below 5.8 within 2–4 h after feeding [10,11]. SARA has also been defined via lowering the rumen pH to below 6 through the use of a stomach tube in dairy cattle [31]. In agreement with the foregoing studies, the decreased rumen pH in the 3 groups of the present study indicated a successful SARA induction. In the current study, SARA induction did not significantly change the serum LPS levels in the control group. Similarly, after feeding 2 diets containing 50% and 25% concentrate, serum LPS was not detected in 6 out of the 9 goats [32]. Also, in agreement with the present study, Dong et al. [29] reported that when the rumen pH of goats decreased to less than 5.8, plasma LPS value in the high-grain group was not significantly different from the low-grain groups. Elevated serum LPS levels as a result of SARA induction in cattle [9,14,33] are also expected in sheep. A possible reason for this discrepancy might be the insufficient sensitivity of LPS measurement methods in the studies on sheep and goats [12].

Serum LPS levels of the activated charcoal and zeolite groups on days 0 (just before induction), 1, 2 and 3 of SARA had significant falling trends in comparison with the control group. In other words, oral consumption of activated charcoal and zeolite in lambs with SARA resulted in significant reductions in serum LPS concentrations. There are enough understandings on pivotal role of LPS in causing multiple adverse sequels following SARA in ruminants [14,15,33]. It has been shown that as toxin binders, certain clay minerals such as bentonite and zeolite are able to trap free rumen bacterial toxins [23] and mycotoxins [34,35] in cattle. An in vitro experiment further showed the ability of activated charcoal and montmorillonite to bind to LPS [21]. In a study performed on 16 crossbred Simmental cattle to prevent rumen LPS

Table 2. Changes in the mean (\pm SD) of serum LPS values (pg/mL) of lambs in the study groups during SARA.

Group	Before induction	Days of SARA		
	0	1	2	3
Control	5.54 \pm 1.8 ^{Aa}	5.13 \pm 3.6 ^{Aa}	5.75 \pm 1.8 ^{Aa}	4.75 \pm 3.0 ^{Aa}
Activated charcoal	6.3 \pm 2.3 ^{Aa}	2.5 \pm 1.3 ^{Bb}	2.0 \pm 1.9 ^{Bb}	1.9 \pm 1.1 ^{Bb}
Zeolite	5.13 \pm 1.4 ^{Aa}	2.36 \pm 1.1 ^{Bb}	1.9 \pm 0.8 ^{Bb}	1.1 \pm 0.7 ^{Cc}

Different uppercase superscript letters in each column (A, B, C) denote significant differences among the groups ($P < 0.01$). Different lowercase superscript letters in each row (a, b, c) denote significant differences in LPS values among times in each group ($P < 0.01$).

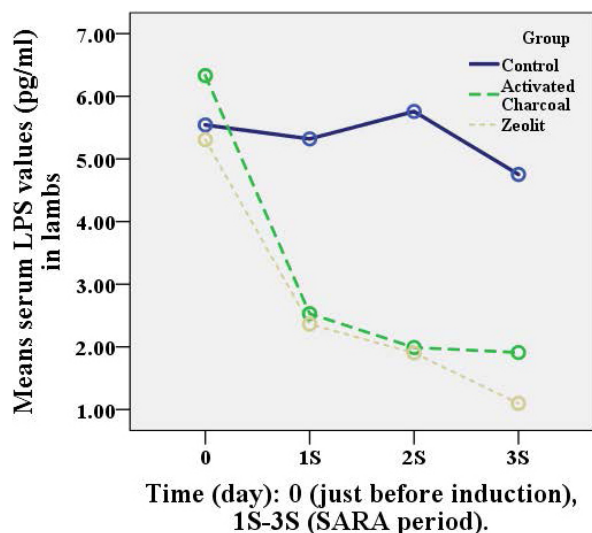


Figure 2. Changes in serum LPS values in the lambs with induced SARA in the study groups.

absorption, the clay mineral of montmorillonite and yeast cell wall with basal diet significantly reduced plasma and fecal LPS concentrations [22]. There is no research on the ruminal LPS entrapment in sheep or lambs with SARA. In the present research, oral administration of activated charcoal and zeolite in lambs with SARA significantly reduced serum LPS concentrations. Therefore, these 2 compounds are proposed for inhibiting ruminal LPS absorption during SARA in lambs.

After entering the bloodstream, LPS is combined with LPS binding protein and transferred to the cell surface. Afterwards, the LPS protein complex binds to CD14 to induce the secretion of proinflammatory cytokines, including TNF- α and interleukins 1 and 6. These cytokines are able to trigger an inflammatory response through activating the expression of receptors in hepatocytes and other target cells [12,36]. APPs such

as serum amyloid A, haptoglobin, fibrinogen, and CRP have been introduced as useful markers of inflammatory processes in ruminants [4,18,19]. In this regard, changes in the inflammatory markers of serum CRP and plasma fibrinogen were assessed in the possible response to serum LPS in the lambs in our study.

During the study period, SARA induction was not associated with significant changes in serum CRP values in all 3 groups, and according to Constable et al. [4] and Ilive and Georgieva, the variations in serum CRP were in the normal range of sheep [37]. In accordance with our study, Ahmed et al. showed that serum CRP value was significantly changed in sheep with acute acidosis [38]. CRP measurement may not be a good approach to evaluate inflammatory responses in lambs with SARA. In the current study, SARA led to a significant increase in blood fibrinogen in all 3 groups. Gonzalez et al. observed an insignificant change in plasma fibrinogen concentration in response to SARA induction [39]. Given the significant decrease in serum LPS levels in the activated charcoal and zeolite groups, no increase was expected in plasma fibrinogen values. Therefore, the increased plasma fibrinogen content in the lambs with SARA might be attributed to factors other than LPS, including the production of histamine, ethanolamine, and other bacterial degradation products [5] as well as the effects of reduced pH and high rumen content osmolality on rumen epithelium during SARA [40]. Kannizzo et al. [41] also claimed that APPs could increase under physical or psychological stresses in dairy cows.

Furthermore, blood biochemical analysis revealed no significant changes in the blood levels of lactate, bicarbonate, anion gap, and pH in the study groups during SARA. Similarly, many researchers have shown that blood lactate, bicarbonate, and pH values are not influenced in cattle with SARA [42–44]. However, no pertaining data was found in sheep or goats with SARA.

In conclusion, oral consumption of activated charcoal and zeolite can inhibit ruminal LPS absorption during

Table 3. Changes in the mean (\pm SD) of plasma fibrinogen values (mg/dL) of lambs in the study groups during SARA.

Group	Before induction	Days of SARA		
	0	1	2	3
Control	404 \pm 24 ^{Aa}	461 \pm 50 ^{Ab}	474 \pm 10 ^{Ab}	484 \pm 0.0 ^{Ab}
Activated charcoal	354 \pm 34 ^{Aa}	461 \pm 50 ^{Ab}	474 \pm 10 ^{Ab}	461 \pm 20 ^{Ab}
Zeolite	339 \pm 72 ^{Aa}	459 \pm 36 ^{Ab}	484 \pm 0.0 ^{Ab}	475 \pm 19 ^{Ab}

Different uppercase superscript letters in each column (A, B, C) denote significant differences among the groups. Different lowercase superscript letters in each row (a, b, c) denote significant differences among times in each group ($P < 0.01$).

Table 4. Changes in the mean (\pm SD) of serum CRP values (mg/dL) of lambs in the study groups during SARA.

Group	Before induction	Days of SARA		
	0	1	2	3
Control	3.04 \pm 0.53 ^{Aa}	4.17 \pm 0.59 ^{Aa}	4.72 \pm 1.71 ^{Aa}	4.05 \pm 0.80 ^{Aa}
Activated charcoal	3.83 \pm 0.80 ^{Aa}	5.08 \pm 3.11 ^{Ab}	3.46 \pm 0.52 ^{Aa}	4.28 \pm 1.14 ^{Aa}
Zeolite	3.42 \pm 0.92 ^{Aa}	4.16 \pm 0.78 ^{Aa}	3.69 \pm 0.84 ^{Ab}	4.32 \pm 2.76 ^{Aa}

Different uppercase superscript letters in each column (A, B, C) denote significant differences among the groups. Different lowercase superscript letters in each row (a, b, c) denote significant differences among times in each group.

Table 5. Means (\pm SD) of blood levels of lactate, bicarbonate, anion gap, and pH in lambs of all study groups during SARA.

Parameters	Group	Before induction	Days of SARA		
		0	1	2	3
Lactate (mg/dL)	Control	8.4 \pm 4.5 ^a	4.5 \pm 0.6 ^a	6.2 \pm 2.5 ^a	9.3 \pm 3.3 ^a
	Activated charcoal	10.2 \pm 6.2 ^a	7.2 \pm 4.6 ^a	7.4 \pm 3.1 ^a	12.2 \pm 10 ^a
	Zeolite	11.4 \pm 7.1 ^a	5.8 \pm 1.1 ^a	5.6 \pm 3.1 ^a	11.2 \pm 3.8 ^a
Bicarbonate (meq/L)	Control	25.6 \pm 1.3 ^a	22.5 \pm 0.9 ^a	24.1 \pm 0.2 ^a	22.9 \pm 1.3 ^a
	Activated charcoal	24.9 \pm 2.3 ^a	20.3 \pm 0.9 ^a	24.2 \pm 3 ^a	24.1 \pm 3.7 ^a
	Zeolite	23.7 \pm 2.8 ^a	21.4 \pm 1.7 ^a	23.8 \pm 3.6 ^a	26.3 \pm 4.3 ^a
Anion gap (mmo/L)	Control	8.2 \pm 5.1 ^a	9.2 \pm 3.4 ^a	8.0 \pm 4.5 ^a	11.7 \pm 1.7 ^a
	Activated charcoal	7.6 \pm 2.3 ^a	4 \pm 1.4 ^b	8.2 \pm 3.2 ^a	10.6 \pm 2.9 ^a
	Zeolite	9 \pm 2.3 ^a	4.6 \pm 4.3 ^b	11.8 \pm 5.2 ^a	9 \pm 3.9 ^a
Blood pH	Control	7.42 \pm 0.02 ^a	7.43 \pm 0.04 ^a	7.44 \pm 0.02 ^a	7.43 \pm 0.03 ^a
	Activated charcoal	7.42 \pm 0.07 ^a	7.42 \pm 0.03 ^a	7.43 \pm 0.03 ^a	7.4 \pm 0.06 ^a
	Zeolite	7.41 \pm 0.02 ^a	7.42 \pm 0.01 ^a	7.41 \pm 0.02 ^a	7.42 \pm 0.09 ^a

Different superscript letters in each row (a, b, c) denote significant differences among the times in each group.

SARA in lambs. Because no significant changes were found in LPS in the control group, studies with longer terms of SARA might provide more definite results. The increased plasma fibrinogen content in the lambs with SARA may be ascribed to factors other than LPS, including the production of histamine, ethanolamine, and other bacterial degradation products. CRP measurement is not a good approach for evaluating inflammatory responses in lambs with SARA.

References

1. Bramley E, Lean IJ, Fulkerson WJ, Stevenson MA, Rabiee AR et al. The definition of acidosis in dairy herds predominantly fed on pasture and concentrates. *Journal of Dairy Science* 2008; 91 (1): 308-21.

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Conflict of Interest

The authors declare that they have no conflict of interest.

2. Kleen JL, Hooijer GA, Rehage J, Noordhuizen JPTM. Subacute ruminal acidosis in Dutch dairy herds. *Veterinary Record* 2009; 164 (22): 618-684.

3. Plaizier JC, Li S, Tun HM, Khafipour E. Nutritional models of experimentally-induced subacute ruminal acidosis (SARA) differ in their impact on rumen and hindgut bacterial communities in dairy cows. *Frontiers in Microbiology* 2017; 7: 21-28.
4. Constable PD, Hinchcliff KW, Done SH, Grunberg W. *Veterinary Medicine, a text book of the disease of cattle, sheep, pigs and horses*. 11th ed. London, UK: W.B. Saunders; 2017.
5. Plaizier JC, Krause DO, Gozho GN, McBride BW. Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. *Veterinary Journal* 2008; 176 (1): 21-31.
6. Kleen JL, Cannizzo C. Incidence, prevalence and impact of SARA in dairy herds. *Animal Feed Science and Technology* 2012; 172 (1-2): 4-8.
7. Olson JD. The relationship between nutrition and management to lameness in dairy cattle. *Bovine Practitioner* 1997; 31: 65-68.
8. Gozho GN, Krause DO, Plaizier JC. Rumen lipopolysaccharide and inflammation during grain adaptation and subacute ruminal acidosis in steers. *Journal of Dairy Science* 2006; 89 (11): 4404-4413.
9. Li S, Gozho GN, Gakhar N, Khafipour E, Krause DO et al. Evaluation of diagnostic measures for subacute ruminal acidosis in dairy cows. *Canadian Journal of Animal Science* 2012; 92 (3): 353-364.
10. Morgante M, Giancesella M, Casella S, Ravarotto L, Stelletta C et al. Blood gas analyses, ruminal and blood pH, urine and fecal pH in dairy cows during subacute ruminal acidosis. *Comparative Clinical Pathology* 2009; 18 (3): 229-232.
11. Sharma R, Munns K, John SJ, Penner G, Oba M et al. Commensal fecal *Escherichia coli* diversity in dairy cows at high and low risk for incurring subacute ruminal acidosis. *Foodborne Pathogens and Disease* 2009; 6 (8): 973-980.
12. Plaizier JC, Khafipour E, Li S, Gozho GN, Krause DO. Subacute ruminal acidosis (SARA), endotoxins and health consequences. *Animal Feed Science and Technology* 2012; 172 (1-2): 9-21.
13. Pulina G. *Dairy sheep nutrition*. King's Lynn, UK: CAB, Biddles Ltd; 2004. pp. 166-170.
14. Guo J, Chang G, Zhang K, Xu L, Jin D et al. Rumen-derived lipopolysaccharide provoked inflammatory injury in the liver of dairy cows fed a high-concentrate diet. *Oncotarget* 2017; 8 (29): 46769-46780.
15. Emmanuel DGV, Dunn SM, Ametaj BN. Feeding high proportions of barley grain stimulates an inflammatory response in dairy cows. *Journal of Dairy Science* 2008; 91 (2): 606-614.
16. Mani V, Weber TE, Baumgard LH, Gabler NK. Growth and development symposium: Endotoxin, inflammation, and intestinal function in livestock. *Journal of Animal Science* 2012; 90 (5): 1452-1465.
17. Kurashima Y, Goto Y, Kiyono H. Mucosal innate immune cells regulate both gut homeostasis and intestinal inflammation. *European Journal of Immunology* 2013; 43 (12): 3108-3115.
18. Gruys E, Obwolo MJ, Toussaint MJ. Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry: a review. *Veterinary Bulletin* 1994; 64 (11): 1009-1018.
19. Kirbas A, Kandemir FM, Celebi D, Hanedan B, Ozkan M. The use of inflammatory markers as a diagnostic and prognostic approach in neonatal calves with septicaemia. *Acta Veterinaria Hungarica* 2019; 67 (3): 360-367.
20. Kirbas A, Apaydin B, Baydar E, Kandemir FM. Status of lipid peroxidation and some antioxidants in sheep with acute ruminal lactic acidosis. *Medycyna Weterynaryjna* 2014; 70 (6): 357-361.
21. Spieker H. Efficacy of clay minerals and activated charcoal to bind endotoxins in rumen fluid. PhD, University of Utrecht, Utrecht, the Netherlands, 2010.
22. Lie CL, Dong GZ, Jin L, Zhou J. Effects of dietary supplementation of montmorillonite and yeast cell wall on lipopolysaccharide adsorption, nutrient digestibility and growth performance in beef cattle. *Livestock Science* 2013; 158 (1): 57-63.
23. Ramos AJ, Fink-Gremmels J, Hernandez E. Prevention of toxic effects of mycotoxins by means of nonnutritive adsorbent compounds. *Journal of Food Protection* 1996; 59 (6): 631-641.
24. Liu H, Xu T, Xu S, Han X, Wang X et al. Effect of dietary concentrate to forage ratio on growth performance, rumen fermentation and bacterial diversity of Tibetan sheep under barn feeding on the Qinghai-Tibetan plateau. *Peer Journal* 2019; 7: e7462.
25. Lettat A, Noziere P, Silberberg M, Morgavi DP, Berger C et al. Experimental feed induction of ruminal lactic, propionic, or butyric acidosis in sheep. *Journal of Animal Science* 2010; 88 (9): 3041-3046.
26. Lee M, Jeong S, Seo J, Seo S. Changes in the ruminal fermentation and bacterial community structure by a sudden change to a high-concentrate diet in Korean domestic ruminants. *Asian-Australasian Journal of Animal Sciences* 2019; 32 (1): 92-102.
27. Jasmin BH, Boston RC, Modesto RB, Schaer TP. Perioperative ruminal pH changes in domestic sheep (*Ovis aries*) housed in a biomedical research setting. *Journal of the American Association for Laboratory Animal Science* 2011; 50 (1): 27-32.
28. Harrison JH, Riley RE, Loney KA. Effect of type and amount of buffer addition to grass silage-based total mixed rations on milk production and composition. *Journal of Dairy Science* 1989; 72 (7): 1824-1830.
29. Dong H, Wang S, Jia Y, Ni Y, Zhang Y et al. Long-term effects of subacute ruminal acidosis (SARA) on milk quality and hepatic gene expression in lactating goats fed a high-concentrate diet. *PLoS One* 2013; 8 (12): e82850.
30. Lean IJ, Wade LK, Curtis MA, Porter J. New approaches to control of ruminal acidosis in dairy cattle. *Asian Australasian Journal of Animal Sciences* 2000; 13: 266-269.
31. Plaizier JC. Replacing chopped alfalfa hay with alfalfa silage in barley grain and alfalfa-based total mixed rations for lactating dairy cows. *Journal of Dairy Science* 2004; 87 (8): 2495-2505.

32. Huo W, Zhu W, Mao S. Effects of feeding increasing proportions of corn grain on concentration of lipopolysaccharide in the rumen fluid and the subsequent alterations in immune responses in goats. *Asian Australasian Journal of Animal Sciences* 2013; 26 (10): 1437-1445.
33. Khafipour E, Krause DO, Plaizier JC. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. *Journal of Dairy Science* 2009; 92 (3): 1060-1070.
34. Diaz DE, Hagler WM JR, Hopkins BA, Whitlow LW. Aflatoxin binders I: In vitro binding assay for aflatoxin B1 by several potential sequestering agents. *Mycopathologia* 2003; 156 (3): 223-226.
35. Spotti M, Fracchiolla M, Arioli F, Caloni F, Pompa G. Aflatoxin B1 binding to sorbents in bovine rumen fluid. *Veterinary Research Communication* 2005; 29 (6): 507-515.
36. Guha M, Mackman N. LPS induction of gene expression in human monocytes. *Cellular Signaling* 2001; 13 (2): 85-94.
37. Iliev PT, Georgieva TM. Acute phase proteins in sheep and goats-function, reference ranges and assessment methods: an overview. *Bulgarian Journal of Veterinary Medicine* 2018; 21 (1): 1-16.
38. Ahmed MK, Hany YH, Mahmoud AA, Mohammad AN, Ahmed ME et al. The clinical significance of acute phase proteins and biochemical changes in sheep with acute ruminal acidosis. *Kufa Journal of Veterinary Medical Sciences* 2017; 8 (2): 221-230.
39. Gonzalez FHD, Ruiperez FH, Sanchez JM, Souza JC, Martinez-Subiela S et al. Haptoglobin and serum amyloid A in subacute ruminal acidosis in goats. *Revista de la Facultad de Medicina Veterinaria y de Zootecnia* 2010; 57: 159-167.
40. Kleen JL, Hooijer GA, Rehage J, Noordhuizen JPTM. Subacute ruminal acidosis (SARA): a review. *Journal of Veterinary Medicine Series* 2003; 50: 406-414.
41. Cannizzo C, Ganesella M, Giudice E, Messina V, Piccione G et al. Serum acute phase proteins in cows with SARA (Subacute Ruminal Acidosis) suspect. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia* 2012; 64: 15-22.
42. Brown MS, Krehbiel CR, Galyean ML, Remmenga MD, Peters JP et al. Evaluation of models of acute and subacute acidosis on dry matter intake, ruminal fermentation, blood chemistry, and endocrine profiles of beef steers. *Journal of Animal Science* 2000; 78: 3155-3168.
43. Ganesella M, Morgante M, Cannizzo C, Stefani A, Dalvit P et al. Subacute ruminal acidosis and evaluation of blood gas analysis in dairy cow. *Veterinary Medicine International* 2010, 1-4.
44. Danscher AM, Li S, Andersen PH, Khafipour E, Kristensen NB et al. Indicators of induced subacute ruminal acidosis (SARA) in Danish Holstein cows. *Acta Veterinaria Scandinavica* 2015; 57: 39-53.