Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Research article

Estimation of lipopolysaccharide concentration in the content of ruminal fluid and feces of dairy cows that suffering from subacute ruminal acidosis

Saad Hashim Al-Husseiny[1] Mohammad Mishjel Zenad[2]

[1]- *Department of Internal Veterinary Medicine, College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.*
[2]- *Department of Internal Veterinary Medicine, College of Veterinary Medicine, University of Baghdad, Iraq.*

Corresponding Author Email: saad.ghmeis@qu.edu.iq

(Received 10/8/2017, Accepted 20/12/2017)

Abstract

Subacute *ruminal acidosis (SARA) is a common health and production problem in dairy cattle and described as repeated incidences of drop pH of the rumen. The aim of the study was to estimate the lipopolysaccharide concentration in the content of ruminal fluid and feces of dairy cows as a diagnostic tool for subacute ruminal acidosis. Four hundred and twenty-seven cows examined in this study in Al-Qadisiyah Province, from these cows, Ninety lactating cows, were have affected with subacute ruminal acidosis. Moreover, fifty healthy lactating cows were considered as a control. Both diseased and control cows were exposed to additional tests of ruminal fluid and fecal samples. The difference observed in the lipopolysaccharide (LPS) concentration in the ruminal fluid and fecal samples 97.772 and 108.823 Eu/ml respectively, of SARA group which were significantly (P≤0.05) higher than the control group. The ruminal and fecal LPS can be used as diagnostic tools to identify the SARA.*

Key words: Dairy cows, Lipopolysaccharides, Ruminal fluid, SARA.

Introduction:

The subclinical form must be considered herd or stock rather than a husbandry problem, contrary to acute lactic ruminal acidosis. SARA is possibly a consequence to non-adaptation of the environment of rumen to the acceptance of diet rich in concentrated grains (2). SARA is characterized by daily periods of low rumen pH and threshold for SARA range between 5.5 and 5.0 ; This digestive syndrome is the result of feeding high grain diet to dairy cows, which are adapting to digests predominantly forage diet (3, 4, 5, 6). Microbial deviations in SARA represent those detected through adaptation to grain and have not been well recognized. The reduction in population of ciliated protozoa is a common sign of both types of acidosis and may be a valuable indicator of an acidic rumen (7). In the pathogenesis of acidosis and participate the ruminal bacterial lipopolysaccharides, it must be absorbed from the gastrointestinal tract into the blood circulation. Whether LPS can be absorbed or translocate from the rumen or somewhere else in the gut into the blood remains unsolved (8). Additionally، endotoxins distributed into the abomasum or small intestine may be deactivated by enzymes or acid (9). A recurrent low rumen pH is accompanied by increased concentrations of endotoxins (10, 11) and it has been suggested by (12) that these endotoxins show a significant role in the development of laminitis.

Materials and Methods:

Ethical approval

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: **313**

Animals and area: Ninety crossbreed, lactating cows, were affected with subacute ruminal acidosis (SARA), and fifty healthy cows used as controls and all examined cows were (427) early lactation stage. Animals were selected from different herds in Al-Qadisiyah Province in the period started from May 2013 to April 2014 and this period including both summer and winter.

Animal diet: Most of diets that were provided to the lactating cows in this areas were fed nearly the same food including green fodder (alfalfa, clover), grains (barely, wheat brans), dates, and dry breads especially in the winter and autumn.

Rumenocentesis: Rumenocentesis was also performed to obtain the ruminal fluid. Ten-cm2 area about 12 to 15 cm caudo-ventral to the costochondral junction of the last rib on a line parallel with the top of the stifle was recognized, shaved, and disinfected, (tincture iodine, and wiped with 70 % isopropyl alcohol). Cows were sedated by intravenous administration of xylazine (0.01 mg/kg B.W.) and restrained in standing position by ligature hocks joints together. An incision of 1 cm on the skin of the middle prepared area done, followed by insertion of special stainless steel needle (1.6 × 130 mm) into the ventral rumen, and 20 ml aspirated by disposable syringe. Fifteen to twenty ml were collected by each method (13).

Determination of endotoxins or lipopolysaccharides (LPS):

Using the microplate Method to determine the LPS in ruminal fluid samples and fecal samples. The performance features of positive microplate readers are optimal with volume sample less than 300 µl. Calculation of Endotoxins concentration under the absorbance at 405–410 nm is linear in the concentration range of 0.1 to 1.0 EU/ml endotoxin There are several methods to determine the endotoxin concentration of samples. According to (1), and in the fecal samples according to method adapted by (14).

Statistical Analysis: Data were analyzed by t- test, moreover, results were offered as mean values and standard deviations (SD).

Results

The ruminal fluid of lactating cows that suffering from SARA contain total free LPS (97.772 ± 1.32 EU/ml) which was significantly increased P≤0.05 from that in control group (27.213 ±1.782 Eu/ml). Moreover, the endotoxins in fecal samples of SARA group was (108.823 ± 1.835 Eu/ml) which was significantly increased P≤0.05 from that of control group (29.325 ± 1.236 Eu /ml). Our study showed differences between ruminal fluids and fecal samples and increased lipopolysaccharides in fecal samples compared with the ruminal fluids and there was significant difference (P≤0.05) between them. Figure (1) and Table (1).

Table (1) Concentrations of LPS (Eu /ml) in the ruminal fluid and fecal samples in the control and SARA groups

Endotoxins concentrations	Control group M ± SD	SARA group M ± SD
Ruminal LPS	27.213 ± 1.782	97.772 ± 1.32
Fecal LPS	29.325 ± 1.236	108.823 ± 1.835

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

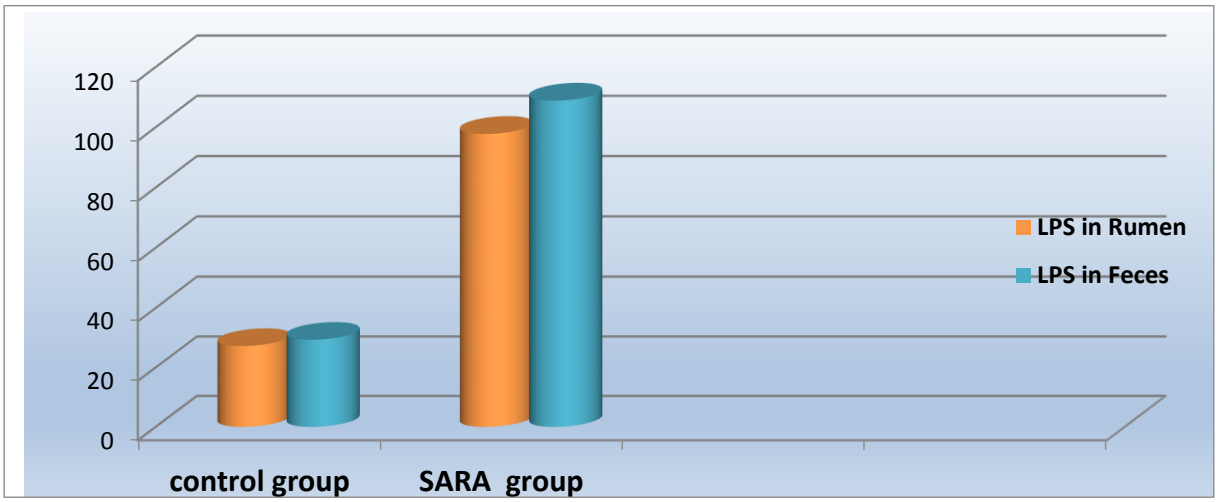

Figure (1) Mean values of LPS in the rumen and feces in both control and SARA groups

Discussion:

The high concentration of free LPS with low pH of the ruminal fluid may be due to increased lysis of dead bacterial cells or shedding of free LPS from quickly growing gram-negative bacteria (6, 15). Because of decreasing of pH in the rumen in SARA lead to decline the quantity of the ruminal bacterial species, while the metabolic activity of the remained bacteria may be persist high which can able to be alive and active (17). The theory that describe frees ruminal LPS concentrations rises subsequent high grain diet feeding (11, 18) especially during experimentally induced acute acidosis (18, 19) or SARA (1, 10, 20) has been obviously recognized. However, the described ranges of free LPS in ruminal fluid different considerably in these previous papers. Both SARA in present study and grain-induced SARA in earlier studies increased free ruminal endotoxins (11, 20, 21, 23). Similar to our results from 27.213 ± 1.782 EU/ml in the control to 97.772 ± 1.32 EU/ml in SARA in ruminal fluid and from 29.325 ± 1.236 EU/ml in control to 108.823 ± 1.835 EU/ml in SARA) in fecal samples. (21) Described LPSs ranges of (42.122 to 145.593 EU/mL) in "Holstein dairy cattle" through stages of and grain induced SARA control, respectively. Another studies by (11) described an LPS range starting 790 ng/mL in control to 5,021 and 8,870 ng/mL when 30% and 45% barley was supplemented in the diet of Holstein dairy cows, respectively. (1, 10) reported a lesser range of LPSs in Jersey steers. In contrary (19) prompted acute acidosis in adult Jersey cattle and recorded both ruminal pH and ruminal LPS concentrations who were found that the LPS concentration decreased with decreasing rumen pH after grain engorgement to prompt acidosis only in cows that earlier have adaptation to a concentrated grains. However, do not explain why? The reason of decline LPS in earlier trainings such as (15, 19) on ruminal LPS in dairy cattle were semi-quantitative and were created on extractions and purifications of LPS from the ruminal fluid. For that reason, the comparison by quantitation methods cannot be done among the previous studies with ones that were more modern. The difference between our study and those of (15, 19) was perhaps due to the variances in the technique of LPS estimation. Moreover, decreases in ruminal pH lower 5.0 that happened when induction of the acute acidosis probably resulted of bacteriological changes that differed from our study on SARA that because of continuous addition of

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

dates and some concentrates in the nutrition that causes rumen and fecal LPS to elevated. In the current study, also there was an elevation in fecal LPS although; there was a rareness of documents on the SARA effects on the fecal LPS quantities in the literatures. Previous studies showed that abnormal increase in Ruminal LPS interrupts the barrier function of the epithelia and increases gut permeability through production of nitric oxide (22). On the other hand, the variances among the composition of the ruminal epithelium and of the small intestine and large intestine might cause alterations in the effect of LPS on barrier impairment, and therefore, on the rate of LPS translocation from these parts of the gastrointestinal tract. As a result, more content of dietary starch during SARA could have triggered the shedding of LPS from gram-negative bacteria such as *E coli* in the large intestine (21).

References:

1-Gozho GN, Plaizier JC, Krause DO, Kennedy AD, Wittenberg KM. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. (2005). J. Dairy Sci. 88:1399–1403.

2-Kleen JL1, Hooijer GA, Rehage J, Noordhuizen JP. Rumenocentesis (rumen puncture): a viable instrument in herd health diagnosis. (2004) Dtsch Tierarztl Wochenschr. 11 (12):458-62.

3-Krause KM, Dhuyvetter DV, Oetzel GR. Effect of a low-moisture buffer block on ruminal pH in lactating dairy cattle induced with subacute ruminal acidosis. (2006). Dairy Sci. 92:352–364.

4-Grove-White D. Rumen healthcare in the dairy cow. In Prac. (2004). 26: 88-95.

5-Tajik J, Mohammad G, Nadalian MG, Raoo A, Afshin Raoofi; Mohammadi GR, Gholam R, Bahonar AR. Evaluation of rumenocentesis practicability as a routine diagnostic Evaluation of rumenocentesis practicability as a routine diagnostic technique in veterinary practice. (2011). Veterinarski Arhiv 81(5): 557-561.

6-Kitkas GC, Valergakis GE, Karatzias H, Panousis N. Subacute ruminal acidosis: prevalence and risk factors in reek dairy herds. (2013). Iranian Journal of Veterinary Research, Shiraz University, 14 (3): 183-189.

7-Khafipour ELiS, Plaizier JC, Krause DO. Rumen microbiome analysis during (SARA). (2009[a]). Appl. Environ. Microbiol. doi:10.1128/AEM.00739-09.

8-Kleen JL. Prevalence of subacute ruminal acidosis in Deutch dairy herds – A field study, PhD Thesis, School of Veterinary Medicine (2004). Hanover. PP: 93-104.

9-Nagaraja TG, Bartley EE, Anthony HD, Leipold HW, Fina LR. Endotoxin shock in calves from intravenous injection of rumen bacterial endotoxin. (1979). J. Anim. Sci. 49:567– 582.

10-Gozho GN, Krause DO, Plaizier JC. Rumen lipopolysaccharide and inflammation during grain adaptation and subacute ruminal acidosis in steers. (2006). J. Dairy Sci. 89:4404–4413

11-Emmanuel DGV, Dunn SM, Ametaj BN. Feeding high proportions of barley grain stimulates an inflammatory response in dairy cows. (2008) J.D. Sci. 91, 606–614.

12-Plaizier SLi, Krause DO. Diagnosis of subacute ruminal acidosis (SARA) on-farm by analyzing bacterial toxins in the feces. WCDS Advances in Dairy Technology (2009); 21: 371 (Abstract).

13-Garrett EF, Pereira MN, Nordlund KV, Armentano LE, Goodger WJ, Oetzel GR. Diagnostic methods for the detection of subacute ruminal acidosis in dairy cows. (1999). J. Dairy Sci 82:1170–1178.

14-Zebeli Q, Ametaj BN. Relationships between rumen lipopolysaccharide and mediators of inflammatory response with milk fat production and efficiency in dairy cows. (2009). J. Dairy Sci. 92: 3800–3809.

15-Nagaraja TG, Titgemeye EC. Ruminal Acidosis in Beef Cattle: The Current Microbiological and Nutritional Outlook (2007). J. Dairy Sci. 90(E. Suppl.):E17– E38.

16-Andersen PH. Bovine endotoxicosis: Aspects of relevance to Ruminal acidosis. PhD. Thesis. (2000). The Royal Veterinary and Agricultural University, Copenhagen, Denmark.

17-Garry FB. Indigestion in ruminants. In: B. P. SMITH (ed.): Large animal internal medicine 2nd ed. Mosby, (2002) St.Louis and Baltimore, pp. 722-747.

18-Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Veterinary medicine: a textbook of the diseases of cattle, sheep, pig, goats and horses. (2007). Ed. Elsevier Saunders, New York.

19-Andersen PH, Jarlov N. Investigation of the possible role of endotoxin, TXA2, PGI2 and PGE2 in experimentally induced rumen acidosis in cattle. (1990). Acta Vet. Sca. 31, 27–38.

20-Gozho GN, Krause DO, Plaizier JC. Ruminal lipopolysaccharide concentration and inflammatory response during grain-induced

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

subacute ruminal acidosis in dairy cow. (2007). J. Dairy Sci. 90:856–866.

21-Khafipour E, Krause DO, Plaizier JC. Alfalfa pellet-induced subacute ruminal acidosis in dairy cows increases bacterial endotoxin in the rumen without causing inflammation. (2009) J. Dairy Sci. 92:1712–1724.

22-Chin AC, Flynn AN, Fedwick JP, Buret AG. The role of caspase -3 in lipopolysaccharide-mediated disruption of intestinal epithelial tight junctions. (2006) Can. J. Physiol. Pharmacol 84:1043–1050.

23-Al-Husseiny, SH., Clinical and clinical pathological study of subacute ruminal acidosis in crossbreed lactating cows. PhD. Thesis, (2014) college of Vet. Med., Baghdad University, Iraq.