Prevalence of latent acidotic stress in dairy cows in the Şanlıurfa region¹⁾

PELİN FATOŞ POLAT DİNÇER, YUSUF GÜL*

Dokuz Eylül University, Faculty of Veterinary Medicine, Department of Internal Medicine, İzmir, Turkey

*Fırat University, Faculty of Veterinary Medicine, Department of Internal Medicine, Elazığ, Turkey

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Polat Dinçer P. F., Gül Y.

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Summary

This study aimed to determine the incidence of latent acidotic stress (LAS), which is an important problem in dairy cow breeding in Turkey, to investigate net acid-base excretion in urine, and to provide veterinary doctors with information about the early diagnosis and treatment of LAS. Two study groups were formed according to the rumen fluid pH values: LAS group (19 cows) with $5.2 < \text{pH} < 6.0$ (19 cows) and healthy group (81 cows) with $6.0 < \text{pH} < 7.2$. Blood, urine and ruminal fluid samples were taken after a general clinical examination of the animals. The physical properties of the rumen fluid, methylene blue reduction time, total infusoria number, and volatile fatty acid (VFA) amounts were determined. In addition, examinations of blood gases, urine pH, and net acid-base excretion (NABE) were performed. The general physical examination did not reveal any statistically significant difference in the body temperature between the LAS group ($n = 19$) and the control group ($n = 81$) ($p = 0.614$), but a significant increase in the heartbeat, respiratory frequency, and the number of rumen movements was observed in the LAS group ($p = 0.001$). The following findings were observed in the rumen fluid from cows of the LAS group: decreased methylene blue reduction time ($p = 0.001$), decreased pH ($p = 0.001$) and infusoria density ($p = 0.001$), increased sedimentation time ($p = 0.001$) and total VFA amount ($p = 0.001$), no flotation. Compared with the healthy cows, the LAS group showed decreased blood pH ($p = 0.001$) and oxygen pressure (pO_2) ($p = 0.001$), increased carbon dioxide pressure (pCO_2), base excess (BE) and bicarbonate (HCO_3^-) values ($p = 0.001$), and decreased urine pH ($p = 0.001$) and net acid-base excretion ($p = 0.001$) values. The incidence of latent acidotic stress in dairy cows in the Şanlıurfa region was determined as 19%. In addition, it was concluded that net acid-base excretion values in urine can be used as an auxiliary parameter in the diagnosis of LAS and can be easily applied in the field.

Keywords: latent acidotic stress, dairy cow, acidotic overload

Latent acidotic stress is a digestive system disorder resulting from increased VFA, particularly an increase in propionic acid synthesis and resorption, during bacterial fermentation due to a long-term intake of energy-rich feedstuffs in ruminants and reduced salivary secretion (19). Pathological events due to acidotic overload do not develop as rapidly as in acute rumen acidosis. However, during continuous acidotic overloading in ruminants, disturbances may occur in different systems (anterior stomachs and abomasum, mineral matter and skeletal metabolism, immune system, energy metabolism, fertility, and renal functions) because of limited regulation of acid-base balance with other buffer systems.

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The most important symptom of the disease is a reduced feedstuff intake, and the clinical diagnosis of the disease is difficult. Therefore, various diagnostic methods (rumen fluid examinations, determination of urine and acid-base excretion, fecal elimination, determination of fecal lipopolysaccharide amount, blood parameters) are used in the diagnosis of the disease. The best way to obtain information about the status of the rumen is to examine the rumen fluid directly (12). In the treatment of the disease, it is prioritized that the ration contains at least 18% of coarse fiber (19). Latent acidotic stress is also called chronic-latent acidosis (9). LAS is an important problem in dairy cattle breeding in Turkey. The aim of this study was to determine the incidence of LAS in the Şanlıurfa region of Turkey and investigate the acid-base excretion in urine. The study was also meant to provide veterinarians with

information about the early diagnosis and treatment of the disease in the field, and thus to increase their awareness of the problem.

Material and methods

Determination of study groups. In this study, a total of 100 dairy cows aged 2-4 years were used: 46 of them Simmental and 54 Holstein. They were deemed to be healthy on the basis of anamnesis. Twenty-five cows were randomly selected from each of 4 different farms in Haliliye, Eyyübiye, Akçakale and Harran districts in the Şanlıurfa region. Two study groups were formed according to rumen fluid pH values: an LAS group with $5.2 < \text{pH} < 6.0$ (19 cows) and a healthy group with $6.0 < \text{pH} < 7.2$ (81 cows). Before starting the study, the ethics committee approval was obtained from Harran University Animal Experiments Local Ethics Committee Presidency (approval no. 2017/004/01 of 10.07.2017).

Collecting samples and analyses. The cows used in the study were taken to paddocks, and clinical examinations were performed according to the Dirksen (7) examination scheme. Two to six hours after the noon feeding, about 300 ml of rumen fluid was collected with a vacuum probe (Kruuse, Denmark), and physical examinations (odor, color, consistency, sedimentation, flotation, pH, infusoria concentration) were performed in the farm environment. The physical properties of the rumen fluid were evaluated according to parameters determined by other researchers (32, 42), and the methylene blue test of the rumen fluid was performed according to Dirksen (7, 8). For the remaining examinations, the rumen fluid was taken to the laboratory in a preheated thermostat, and total infusoria count was performed by the Boyne (4) method. The level of volatile fatty acids in the rumen fluid was determined by a method modified from Leventini et al. (24) and measured by gas chromatography (GC) (Agilent 7890A, USA). For blood acid-base balance measurements, 2 mL of blood was taken from vena jugularis into a blood gas injector. Blood pH, pCO_2 , pO_2 , BE, and HCO_3 levels were determined with a blood gas analyzer (Alere Epoc, Germany) in the farm. Urine samples were collected from each animal with a speculum and catheters into urine containers, pH values were measured with a pH meter (Ohaus

ST20, USA), and the samples were sent to the laboratory in cold chain for determination of NABE (11, 23). NABE was determined by the method of Jorgensen (21) modified by Kutas (23). Net acid-base excretion (excretion) in the urine was expressed in milliequivalents per liter.

Statistical analyses. The conformity of the data to normal distribution was tested by the Shapiro-Wilk test, and the Mann-Whitney U-test was used to compare non-normally distributed data in the two groups. The relationships between categorical variables were tested by the chi-square test. The SPSS 24.0 program was used for statistical analyses, and $P < 0.05$ was considered statistically significant.

Results and discussion

The arithmetic mean values of body temperature, respiration, heartbeat and rumen movements for healthy and LAS animals and the significance of differences between the groups are shown in Table 1. The findings regarding the rumen fluid odor, color, consistency and infusoria density and the significance of differences be-

Tab. 1. Mean values of body temperature, respiration, heartbeat, and rumen movements in healthy and LAS animals and the significance of differences between the groups ($\bar{x} \pm \text{SD}$)

	Healthy (n = 81)	LAS (n = 19)	Z	P
Body temperature (°C)	38.38 ± 0.46	38.32 ± 0.41	-0.504	0.614
Respiratory frequency (time (s)/min)	19.19 ± 4.47	32.74 ± 3.71	-6.605	0.001*
Heartbeat frequency (time (s)/min)	65.91 ± 8.3	91.89 ± 12.42	-6.032	0.001*
Rumen movements (movement/5 mins)	10.47 ± 1.56	13.21 ± 1.23	-5.755	0.001*

Explanation: * – Significant at 0.05

Tab. 2. Rumen fluid parameters of healthy and LAS animals and the significance of differences between the groups

		LAS (n = 19)		Healthy (n = 81)		Chi-square	P
		Number	%	Number	%		
Odor	Aromatic	0	0.0	81	100.0	100.00	0.001*
	Lightly Acidic	19	100.0	0	0.0		
Color	Dirty yellowish	19	100.0	0	0.0	100.00	0.001*
	Olive oil green	0	0.0	81	100.0		
Consistency	Lightly viscose	0	0.0	81	100.0	100.00	0.001*
	Viscose	19	100.0	0	0.0		
Infusoria	++	19	100.0	0	0.0	100.00	0.001*
	+++	0	0.0	81	100.0		

Explanation: As in Tab. 1.

Tab. 3. Arithmetic mean values of rumen fluid pH, methylene blue, and infusoria number, as well as sedimentation and flotation findings for healthy and LAS animals and the significance of differences between the groups ($\bar{x} \pm \text{SD}$)

Variables	Healthy (81) (n = 81)	LAS (n = 19)	Z	P
pH	6.74 ± 0.31	5.52 ± 0.21	-6.796	0.001*
Methylene blue (min)	3.57 ± 0.73	2.24 ± 0.56	-6.309	0.001*
Infusoria number (1 mL) (10^6)	$1.47 \times 10^6 \pm 0.94 \times 10^6$	$0.65 \times 10^6 \pm 0.18 \times 10^6$	-6.774	0.001*
Sedimentation (min)	6.93 ± 1.88	19.79 ± 8.03	-6.650	0.001*
Flotation (min)	26.37 ± 7.39	0 ± 0		

Explanation: As in Tab. 1.

tween the groups are shown in Table 2. The arithmetic mean values of rumen fluid pH, methylene blue, the number of infusoria, sedimentation, flotation findings and the significance of differences between the groups are shown in Table 3. The arithmetic mean values of volatile fatty acid in rumen fluid and the significance of differences between the groups are given in Table 4. The arithmetic mean values of urine pH and NABE and the significance of differences between the groups are given in Table 5. The arithmetic mean values of blood gas and the significance of differences between the groups are given in Table 6.

LAS, which is chronic and latent, is an increasing problem in the dairy industry, causing decreased productivity, often irreversible organ damage, treatment costs, and involuntary culling, which lead to significant losses in the economy (17).

The incidence of latent acidotic stress in dairy cows in this study was 19%, and this finding is consistent with data in the literature (15, 22, 30, 33, 37). Studies on this subject in Turkey are limited (31), and no such study has been carried out in the Şanlıurfa region. It was observed that all clinical parameters (body temperature, respiration, heartbeat, rumen movements) in the group of healthy animal were within physiological limits (3, 7, 20, 34).

Tab. 4. Arithmetic mean values of volatile fatty acids in the rumen fluid from healthy and LAS animals and the significance of differences between the groups ($\bar{x} \pm SD$)

Variables	Healthy (n = 81)	LAS (n = 19)	Z	P
Acetic acid (mmol/L)	64.53 $\pm$ 7.17	104.85 $\pm$ 14.54	-6.709	0.001*
Propionic acid (mmol/L)	22.19 $\pm$ 2.33	43.63 $\pm$ 9.7	-6.691	0.001*
N-Butyric acid (mmol/L)	13.28 $\pm$ 1.77	18.52 $\pm$ 3.39	-6.401	0.001*
Iso-Butyric acid (mmol/L)	0.8 $\pm$ 0.25	1.4 $\pm$ 0.27	-5.985	0.001*
N-Valeric acid (mmol/L)	1.87 $\pm$ 0.25	2.96 $\pm$ 0.57	-6.552	0.001*
N-Valeric acid (mmol/L)	1.39 $\pm$ 0.17	2.39 $\pm$ 0.58	-6.664	0.001*

Explanation: As in Tab. 1.

Tab. 5. Arithmetic mean values of urinary pH and NABE for healthy and LAS animals and the significance of differences between the groups ($\bar{x} \pm SD$)

Variables	Healthy (n = 81)	LAS (n = 19)	Z	P
Urine pH	7.92 $\pm$ 0.29	6.95 $\pm$ 0.5	-6.440	0.001*
NABE (mmol/L)	106.47 $\pm$ 24.99	48.95 $\pm$ 34.91	-0.504	0.001*

Explanation: As in Tab. 1.

Tab. 6. Arithmetic mean values of blood gases for healthy and LAS animals and the significance of differences between the groups ($\bar{x} \pm SD$)

Variables	Healthy (n = 81)	LAS (n = 19)	Z	P
Blood pH	7.42 $\pm$ 0.02	7.41 $\pm$ 0.01	-3.893	0.001*
pCO ₂ (mmHg)	41.82 $\pm$ 2.59	46.98 $\pm$ 1.80	-6.32	0.001*
pO ₂ (mmHg)	39.10 $\pm$ 4.47	34.50 $\pm$ 1.88	-5.011	0.001*
HCO ₃ (mmol/L)	27.89 $\pm$ 1.37	30.85 $\pm$ 1.56	-5.983	0.001*
BE (mmol/L)	5.38 $\pm$ 0.39	7.35 $\pm$ 0.94	-6.040	0.001*

Explanation: As in Tab. 1.

In the present study, a statistically significant increase was found in the mean values of the respiratory rate, heart rate and rumen movements in animals with LAS ($P < 0.05$) when compared to healthy animals, but there was no statistically significant difference between the two groups of animals in mean body temperature ($P > 0.05$). The findings obtained in this study resemble those by Li et al. (25). Tajik et al. (40) reported no changes in their study, while Örtlek et al. (31) reported a decrease in rumen movements in animals with LAS. In the present study, it was determined that rumen movements increased in animals with LAS. It is reported that differences in the results may be caused by strong and frequent rumen movements after feedstuff intake and during rumination (7).

Physical findings regarding the rumen content of healthy animals (color, odor, consistency, sedimentation, flotation) are consistent with those reported in the literature (7, 9, 18, 34). The smell of the rumen fluid of animals with LAS was slightly acidic, the color was dirty yellowish, and the consistency was viscous. These findings are consistent with the literature (7, 13, 18).

The mean times of sedimentation and flotation for the rumen fluid of healthy animals were 6.93 $\pm$ 1.88 min and 26.37 $\pm$ 7.39 min, respectively. These values were within physiological limits (20-35 minutes) given by other researchers (7, 18, 20, 38).

In LAS animals, the rumen fluid sedimentation time was longer than in healthy animals, and flotation did not occur at all. In a study, Enemark et al. (13) reported that no flotation occurred in animals with LAS.

On the basis of the literature, the physiological pH of the rumen content was assumed to be 6.2-7.0 (1, 3, 10, 20), while the ruminal pH value for animals with LAS was accepted as 5.2 $<$ pH $<$ 6.0 (10). The mean pH was 6.74 $\pm$ 0.32 for healthy animals and 5.52 $\pm$ 0.21 for animals with LAS. The findings of the physical examination of the rumen content of healthy animals and LAS animals were similar to those reported by other researchers (7, 13, 17, 20, 28). It has also been suggested that there may be changes in the pH of the rumen content due to differences in the VFA absorption level, saliva production, transmittance of rumen fluids, and VFA metabolism discrepancies (39).

Rumen fluid pH values for LAS animals were lower than those for healthy animals. This is consistent with findings by other researchers (2, 5), who obtained similar results in animals fed highly concentrated feedstuff.

The density of infusoria for healthy animals was similar to that reported by other researchers (7, 18, 20). The infusoria density for LAS animals showed a statistically significant decrease compared to healthy animals, which is consistent with findings by other researchers (7, 10, 34). However, Enemark et al. (13) reported that there was no difference in the density of infusoria between LAS and healthy animals. The methylene blue reduction time was 3.57 ± 0.73 for healthy animals. Dirksen (7) reported that the methylene blue reduction time of rumen content was less than 3 min for cattle in which ruminal fermentation was active. In the present study, the mean value for animals with LAS was 2.24 ± 0.56 . It was within the limits of < 3 min reported by other researchers (7, 13) and shorter than for healthy animals. The infusoria count was performed using the Mc-Master slide, and the mean value for healthy animals was $1.47 \pm 0.94 \times 10^6$ (1 mL), which was consistent with values reported for healthy cattle in the literature (20, 34). The mean number of infusoria in 1 mL of rumen fluid from the 19 animals with LAS was $0.65 \pm 0.18 \times 10^6$, which was approximately half of the infusoria number in 1 mL of rumen fluid from healthy animals. The number of infusoria found by Voia et al. (41) in their study on lambs was in line with our findings, while Enemark et al. (13) reported that the infusoria density did not change when the rumen pH was between 5.3 and 6.2. According to Franzolin and Dehority (Franzolin, 1996), there are many factors that affect the concentration of infusoria (diet, frequency of feeding, nutritional level, individual status) and the number of infusoria decreases when the rumen pH is < 6.

In the present study, the mean values of all measured volatile fatty acids and the amount of total volatile fatty acid (mmol/L) for animals with LAS showed a statistically significant increase compared to healthy animals. Although the differences in the mean values of volatile fatty acids between healthy animals and LAS animals were not consistent with findings by other researchers (29), the increase in total volatile fatty acids in LAS animals compared to healthy animals was similar to results reported elsewhere (25, 27).

In the present study, the mean urine pH value and urine NABE (mmol/L) values for the group of healthy animals were within physiological limits reported by other researchers (36). Urine pH and NABE values for LAS animals were lower than for healthy animals. The findings of this study resemble those reported by Ganesella et al. (16), Danscher et al. (6), and Roby et al. (35). Other researchers (13, 18) also confirm that the determination of NABE in urine is useful in clinical practice.

Blood gas values determined in the group of healthy animal were within reference limits (14, 38). Blood gas values obtained in this study were significantly higher for LAS animals compared to healthy animals, which is consistent with results published by Morgante et al.

(26). The pO_2 values were significantly lower for LAS animals compared to healthy animals. This finding stated that the decrease in pO_2 value was associated with an increase in vascular O_2 consumption and that the decrease in pO_2 value was due to anaerobic metabolism and the increase in O_2 consumption. Ganesella et al. (16) reported high pCO_2 , low pO_2 , and low blood pH values in cows with a high LAS risk, which was consistent with the results of the present study.

The incidence of latent acidotic stress in dairy cows in the Şanlıurfa region was determined as 19%. In addition, it was concluded that the pH of the rumen content and urine, as well as net acid-base excretion values for urine, can be used as auxiliary parameters in the diagnosis of LAS and can be easily applied in the field.

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Corresponding author: Dr. Pelin Fatoş Polat Dinçer, Dokuz Eylül University, Faculty of Veterinary Medicine, Department of Internal Medicine, İzmir, Turkey; e-mail: vtrnr_pelin@hotmail.com