

## Subacute ruminal acidosis: prevalence and risk factors in Greek dairy herds

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### Summary

Subacute ruminal acidosis (SARA) represents one of the most important metabolic subclinical disorders of high producing dairy cows, having serious impact in both animal health and herd profitability. The aim of this study was to confirm the presence of SARA in Greek dairy herds and record its prevalence and risk factors. Ruminal fluid samples, via rumenocentesis, were obtained from a total of 153 Holstein dairy cows, from 12 herds ( $\geq 12$  cows per herd). Rumen pH was measured on-site with a portable pH-meter in order to establish a SARA diagnosis. Almost sixteen percent (24/153) of the sampled cows were found with rumen pH  $\leq 5.5$ , which is indicative of SARA. Thirty three percent (4/12) of the herds were SARA-positive, 8.33% (1/12) of the herds were SARA-marginal, and 58.33% (7/12) were SARA-negative. Number of lactating cow groups, order in which the feeds were added into the mixing wagon, particle length size, ration composition, housing type (free stall or bedded pack) and stocking density significantly influenced the presence of SARA.

**Key words:** Subacute ruminal acidosis, Prevalence, Risk factors, Dairy cows, Greece

### Introduction

Subacute ruminal acidosis (SARA) is characterized by a ruminal fluid pH between 5.5 and 5.0, in the absence of obvious clinical signs (Kleen *et al.*, 2003). In total mixed rations (TMR)-fed dairy cows this usually happens 5 to 8 h post-feeding (Oetzel, 2004). SARA is most commonly developed under intensive livestock production systems (Krause and Oetzel, 2006). The drop in pH of the rumen fluid is caused by excessive concentration of volatile fatty acids due to ingestion of diets rich in readily fermentable carbohydrates or to their slow absorption by the rumen wall due to maladjustment in high energy diets (Kleen *et al.*, 2003). Finely chopped feeds do not adequately stimulate mastication and rumination and decreased saliva production, which acts as a buffer also seems to play an important role in the etiology of the disease

(Nordlund *et al.*, 1995). Other well-known risk factors for SARA are errors in ration formulation and preparation (false dry matter calculation), errors in TMR mixing and managerial factors like feeding time-schedule and feed bunk space per cow (Kleen *et al.*, 2003).

Although SARA is considered as one of the most common and important metabolic disorders of dairy cows, its prevalence has been studied only in a few countries, ranging between 14-30% (Garrett *et al.*, 1997; Kleen *et al.*, 2009; Tajik *et al.*, 2009). Consequences of SARA in dairy cows include, amongst others, a decrease in dry matter intake, milk production and milk fat content, an increase in laminitis and lameness incidence, liver abscesses formation and, therefore, caudal vena cava syndrome, and an increase in culling rate without any obvious causes (Plaizier *et al.*, 2009). These consequences are not disease-

specific and SARA is very often under-diagnosed.

Definite diagnosis of SARA in clinical practice is only established by determining the pH of rumen fluid either at a specific time-point after feeding (collected by stomach tubing or, more credibly, by rumenocentesis) (Duffield *et al.*, 2004) or continuously (using electronic rumen boluses).

In Greece, although clinical suspicions arise quite often, SARA had never been confirmed before. The main aim of this study was to investigate the presence of SARA in Greek dairy herds and, secondarily, to evaluate the effect of well-known risk factors on its prevalence.

## Materials and Methods

The research was conducted between April and June 2010. Twelve dairy herds keeping Holsteins (range 60-500 cows), in central Macedonia region, northern Greece, feeding TMR diet, were randomly selected. In order to evaluate the prevalence of SARA during the early lactation period, sampled cows were between 10 and 90 days in milk (DIM) and fed the same TMR within each herd. Cows were randomly chosen as long as they met the DIM criteria and were clinically healthy, according to recent history and a detailed clinical examination, always by the same veterinarian. Rumen fluid samples were collected via rumenocentesis from at least 12 cows of each herd. In total, 153 samples of rumen fluid were collected. This protocol (75% confidence interval) is recommended for SARA diagnosis in clinical practice (Oetzel, 2004). The study was performed in compliance with institutional guidelines of the Department of Animal Health, Veterinary Directorate of Thessaloniki, Ministry of Rural Development and Food, Thessaloniki, Greece. All owners gave informed consent for the cows to be included in the study and to undergo the testing procedures.

Rumenocentesis was consistently performed 5-8 h after the morning feeding, as described by Nordlund *et al.* (1995). The puncture site was located 15-20 cm, according to cow size, behind the last left rib

on the horizontal line passing through the stifle. A small square area (10 × 10 cm) was shaved and disinfected with 7.5% iodine povidone scrub solution. The cows were restrained, without sedation, in feed bunk headlocks and 4 mL of 2% Xylocaine (AstraZeneca, Athens, Greece), containing 20 mg/mL lignocaine hydrochloride, were injected at the puncture site (2 mL subcutaneously and 2 mL intramuscularly) to provide local anaesthesia. During the procedure, an assistant was raised the cow's tail vertically to her body for better restraint, while in extremely stressed animals a nose holder was additionally applied. Then, a 16-G and 13 cm long stainless steel needle (H. Hauptner and Richard Herberholz GmbH and Co. KG, Solingen, Germany) was inserted through the skin into the rumen. At least 2 mL of rumen fluid was carefully aspirated into a 5 mL disposable plastic syringe, so as not to create excess vacuum in the syringe; excess vacuum forces CO<sub>2</sub> to escape from the sample, thus elevating the pH value. Presence of blood in the sample resulted in it being discarded; a new sample was collected instead, from a different cow.

Rumen fluid pH was measured on-site, at room temperature, right after collection of all samples, by using a portable pH-meter (Horiba, B-213, Kyoto, Japan). Cows having pH measurement of 5.5 or lower were considered as SARA-positive, whereas those with pH >5.5 and ≤5.8 were considered SARA-marginal and those with a pH >5.8 SARA-negative. A herd was considered as SARA-positive if at least 25% of the sampled cows were SARA-positive (Garrett *et al.*, 1997). As SARA-marginal were considered those herds having at least 33% of the sampled cows with rumen fluid pH ≤5.8, but were not concurrently classified as SARA-positive. The rest of the herds were considered as SARA-negative (Oetzel, 2004).

On the day rumenocentesis was performed, two samples of the TMR offered (approximately 2 kg) were also collected immediately after the morning feeding, in order to evaluate particle size. Both were obtained from two random sites of the feed bunk, they were initially placed in two plastic bags and later they were mixed in a paper box. Particle size was evaluated using

the New Penn State Particle Separator (Nasco Ltd., USA), with the 3 sieves having holes of different diameter and a solid pan, according to the technique described by Oetzel (2007). The content of each sieve and the solid pan was weighted and recorded. High risk for SARA were those TMR having: a) less than 7% longer particles remaining in the upper sieve (Krause and Oetzel, 2006), b) more than 50% of particles remaining in the middle sieves, and c) more than 20% of particles remaining at the solid pan (Heinrichs and Kononoff, 2002).

Furthermore, a purpose-built questionnaire was created in order to evaluate possible risk factors for SARA development. The following data were collected from each herd:

- a) Rations offered to the groups sampled, for individual feeds, the NRC (2001) feed value tables were used. Ration formulation was then evaluated based on: i) the theoretical concentrations of neutral detergent fiber (NDF) (min 28% of DM), forage NDF (FNDF, min 21% of DM), acid detergent fiber (ADF) (min 19% of DM) and non-fiber carbohydrates (NFC) (max 38% of DM), ii) minimum forage: concentrate ratio, set at 40:60 of DM and iii) inclusion of sodium bicarbonate (none, <150 g/cow/day, ≥150 g/cow/day). This resulted in a “ration evaluation score” and a three-level herd classification (1: within recommendations, 2: marginal, and 3: at-risk for SARA).
- b) Number of lactating cow groups (1 or 2 groups)
- c) Feed bunk space per cow (0: inadequate; <0.75 m/cow, 1: adequate; ≥0.75 m/cow)
- d) Number of waterers (0: inadequate; one waterer/group, 1: acceptable; two waterers/group, 2: adequate; > two waterers/group)
- e) Timing of ration distribution (1: immediately after milking, 2: later)
- f) Sequence of feed addition into the mixer-feeder wagon (0: wrong, 1: correct; hay/straw first, then concentrates and, finally, silage) (Oelberg, 2009)
- g) Housing (1: free stalls, 2: bedded pack).

### Statistical analysis

Feed particle size results and risk factor data collected through the questionnaire were correlated with rumen pH using a

linear multivariate model at both herd and cow-level. The analysis was performed using the SPSS 17.0 software. In all cases, a significance level of  $P \leq 0.05$  was used.

### Results

The mean ruminal fluid pH value from the 153 cows was  $5.98 \pm 0.44$  (min 5.09, max 7.05). At cow-level, 24 of the 153 cows (15.69%) were SARA-positive, 25 (16.34%) were SARA-marginal and the remaining 104 cows (67.97%) were SARA-negative. At the time of sampling, at least one cow had ruminal pH of  $\leq 5.5$  on eight of the 12 sampled herds (66.66%). Their classification was as follows: 4 herds (33.33%) were SARA-positive, 1 herd (8.33%) was SARA-marginal and 7 herds (58.33%) were SARA-negative (Table 1).

Ration characteristics and ration evaluation scores of each of the 12 dairy herds investigated for SARA, based on farmers' response to the questionnaire, are depicted in Table 2.

On 3 farms, feed particle size was inappropriate because of a larger than recommended percentage of short particles (>50% on the middle sieves, >20% on the solid pan) alone or in combination with a smaller than recommended percentage of long particles (<7% in the upper sieve). Ration formulation was evaluated as “within recommendations”, “marginal” and “at-risk for SARA” on 3, 6 and 3 farms, respectively. Only 2 farms had more than one lactating group. Six farms had less than 0.75 m of feed bunk space per cow. Two farms had only one waterer per group, 5 had two and 5 had three waterers. Six farms delivered the TMR immediately after milking and 6 did not. On 7 farms feeds addition in the mixing wagon was done in correct order. Seven farms used freestalls and 5 used a bedded pack.

Statistical analysis showed that rumen fluid pH was significantly higher in cows from herds that had: correct TMR particle size ( $P < 0.001$ ), correct ration formulation ( $P < 0.01$ ), two lactating cow groups instead of one ( $P < 0.001$ ), correct adding order of feeds in the mixing wagon ( $P < 0.05$ ), and free stalls instead of bedded packs ( $P < 0.001$ ). Surprisingly, adequate feed bunk

**Table 1: Rumen pH values arisen from rumenocenteses of the 153 sampled cows and classification of each of the 12 visited herds as SARA-positive, SARA-marginal and SARA-negative**

Herds	Number of sampled cows (1-15) per herd/Rumen pH values of each individual sampled cow															% of animals per herd with rumen pH ≤5.5	Classification of herds
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
1	5.82	5.79	5.56	5.59	5.38	6.09	6.07	5.84	5.90	5.39	6.12	5.82				16.66%	SARA-marginal
2	6.25	6.50	6.00	6.10	6.50	5.81	6.07	6.48	6.02	5.59	5.79	5.79	6.47	6.33		0.00%	SARA-negative
3	7.00	6.80	6.97	6.16	6.13	6.32	6.32	6.16	6.28	6.38	6.36	6.52				0.00%	SARA-negative
4	6.61	5.54	5.69	5.94	6.07	5.91	5.83	6.50	5.98	5.70	5.57	6.12	6.07	6.19	6.33	0.00%	SARA-negative
5	6.05	6.75	6.02	5.80	5.99	6.07	5.99	6.22	6.27	6.49	6.08	6.98				0.00%	SARA-negative
6	5.56	6.78	6.55	6.78	5.85	5.83	6.49	5.56	6.07	6.78	6.70	5.37				8.33%	SARA-negative
7	5.57	5.82	5.53	6.05	6.09	6.03	5.54	6.09	6.47	6.26	5.09	6.70	6.32			7.69%	SARA-negative
8	5.82	6.34	6.05	6.08	6.77	6.08	6.28	5.84	5.38	6.08	5.81	5.38	5.79			15.38%	SARA-negative
9	5.47	5.94	6.37	5.93	5.53	5.91	5.66	5.45	5.19	5.88	5.22	5.62				33.33%	SARA-positive
10	5.36	6.11	6.05	7.05	6.51	5.91	5.82	5.17	5.88	6.74	5.42	5.66				25.00%	SARA-positive
11	6.62	5.28	5.52	6.18	5.44	5.66	5.49	5.31	5.72	5.23	5.44	5.29	5.67	5.91		50.00%	SARA-positive
12	5.91	5.48	5.88	5.83	6.05	5.27	6.07	5.62	5.91	5.25	5.49	6.13				33.33%	SARA-positive

Dark grey highlighted cells: SARA-positive cows (rumen pH = 5.5 or lower) and herds (having at least 25% of SARA-positive cows). Light grey highlighted cells: SARA-marginal cows (rumen pH >5.5 and ≤5.8) and herds (having at least 33% of cows with rumen pH ≤5.8, without concurrently classified as SARA-positive). Non-highlighted cells: SARA-negative cows (rumen pH >5.8) and herds

**Table 2: Ration characteristics and ration evaluation scores on each of the 12 dairy herds investigated for subacute ruminal acidosis, based on farmers' response to questionnaire**

Ration parameters	Herds											
	1	2	3	4	5	6	7	8	9	10	11	12
NDF	34.8	37.6	34.7	32.2	33.0	37.4	37.8	34.4	35.9	33.7	37.4	36.7
F-NDF <sup>1</sup>	30.7	29.3	26.8	25.3	27.3	28.6	30.0	30.2	21.3	24.3	28.1	23.9
% F-NDF	0.88	0.78	0.77	0.79	0.83	0.76	0.79	0.88	0.59 <sup>5</sup>	0.72 <sup>5</sup>	0.75	0.65 <sup>5</sup>
ADF	21.7	24.4	22.6	20.2	21.6	22.7	24.6	21.8	21.6	20.5	24.0	22.1
NFC <sup>2</sup>	42.2 <sup>5</sup>	37.2	40.9 <sup>5</sup>	45.9 <sup>5</sup>	40.3 <sup>5</sup>	41.7 <sup>5</sup>	38.9	46.0 <sup>5</sup>	41.8 <sup>5</sup>	43.0 <sup>5</sup>	36.3	44.3 <sup>5</sup>
Forage to concentrates ratio	63.8	57.6	56.2	50.4	59.9	55.0	60.5	61.3	44.0	51.6	55.3	47.5
Sodium bicarbonate <sup>3</sup>	1	2	0	2	2	0	0	0	2	2	1	0
Ration evaluation score <sup>4</sup>	2	1	2	2	2	2	1	2	3	3	1	3

<sup>1</sup> Forage NDF, <sup>2</sup> Non-fiber carbohydrates, <sup>3</sup> 0: None, 1: Inadequate: <150 g/cow/day and 2: Adequate: ≥150 g/cow/day, <sup>4</sup> 1: Within recommendations, 2: Marginal, 3: At-risk for SARA, and <sup>5</sup>: Deviations after comparison with NRC (2001) feed value tables

space ( $P < 0.01$ ), adequate number of waterers ( $P < 0.01$ ), and ration distribution immediately after milking ( $P < 0.001$ ) were correlated with lower rumen fluid pH (Table 3).

**Table 3: Factors affecting ruminal fluid pH of the 153 sampled cows**

Factor	F-value	P-value
TMR <sup>1</sup> particle size	16.750	0.001
Ration formulation	8.394	0.004
Lactating cow groups	15.159	0.001
Feed bunk space	10.415	0.002
Number of waterers	6.149	0.003
Timing of ration distribution	16.451	0.001
Feed adding sequence in the mixing wagon	3.996	0.021
Housing	13.131	0.001

R<sup>2</sup> squared = 0.296 (adjusted R Squared = 0.241). <sup>1</sup> Total mixed ration

## Discussion

In the present study, rumenocentesis was consistently performed in all sampled cows 5-8 h after the morning feeding in order to coincide with the time of nadir rumen fluid pH (Krause and Oetzel, 2006). In clinical practice, a veterinarian can obtain rumen fluid by either a rumen tube or by performing rumenocentesis. We preferred the latter method because it is easy to perform and more accurate than rumen tubing because rumen fluid samples not "contaminated" (free of saliva) are collected (Duffield *et al.*, 2004). Gianesella *et al.* (2010) also showed that rumenocentesis is a safe procedure with very few adverse effects.

The prevalence of SARA in northern Greece was found to be similar to that in other countries, both at herd and cow-level. In Italy 3 out of 10 herds were SARA-positive (Morgante *et al.*, 2007), in Ireland in grazing cows 3 out of 12 herds were SARA-positive (O'Grady *et al.*, 2008). In The Netherlands 27 of 197 cows (13.7%) were SARA-positive (Kleen *et al.*, 2009) and in Iran 54 of 196 cows (27.6%) were also SARA-positive (Tajik *et al.*, 2009). In studies where prevalence of SARA is investigated, setting the cut-off point is very important. The rumen pH cut-off point used to define SARA in the present, as well as the rest of the above studies, was 5.5 or lower,

as it is suggested by the literature (Garrett *et al.*, 1999).

It was interesting to note that moving the cut-off point to a pH of 5.6, as advocated by some researchers in the review article of Plaizier *et al.* (2009), significantly altered our results. The prevalence of SARA was considerably increased, both at cow (from 15.68 to 23.52%) and, at herd level (from 33.3 to 58.3%). This finding emphasizes the importance of the accuracy of rumen pH measurements when determining SARA prevalence at herd level. Factors that could influence the measurement accuracy are the method of rumen fluid collection, collection time and the accuracy of the pH-meter used. Of course, it would be very useful to know how long ruminal pH remains below normal levels when defining SARA. In the future, extensive research by the use of electronic rumen boluses that measure rumen pH continuously and are not influenced by the time of sampling would certainly provide valuable data regarding true SARA prevalence and, perhaps, change the perception of the disease.

In our study, most of the SARA-positive herds had either errors in ration formulation or inappropriate TMR particle size. Similar results were found by Morgante *et al.* (2007), where feeds chopped too fine were correlated with SARA in five farms. Ration formulation and mixing errors are well-established risk factors for SARA development (Nordlund, 2003). Consequently, it is very important to regularly evaluate both feed particle size and ration formulation, in the latter case based on feed analysis.

Errors in sequence of feed addition to the mixer-feeder wagon were correlated with lower rumen pH values, as well. The recommended sequence is hay/straw first, then liquid feeds and concentrates; silage should be added last (Oelberg, 2009). Deviations from this rule cause mixing errors that result in irregular concentrate delivery to the feed bunk, which in turn contributes to SARA development as some cows have the opportunity to consume a higher amount of concentrates than originally planned (Oelberg, 2009).

In the present study, farms with more than one group of milking cows were less

likely to develop SARA. It is accepted that grouping of animals implies better management compared to farms with no grouping and, consequently, one could attribute this result to a better overall management of these farms. The significantly lower rumen pH of cows kept on bedded packs compared to free stalls can probably be attributed to inappropriate stocking density. None of the studied farms provided the recommended bedded pack space, which in early lactation is at least 9 m<sup>2</sup> per each mature Holstein cow (Cook, 2007). At the same time, floor conditions on these farms were inadequate. Towards this end, the accumulation of increased amount of manure that is not regularly removed, combined with the lack of bedding, has a negative effect on cow welfare. As a consequence, the cows spend more time standing and the normal resting behavior is disrupted, which results in slug feeding, reduces total rumination time and thus the production of saliva that buffers rumen pH. On the other hand, free stalls had appropriate dimensions, provided adequate cushion and were regularly cleaned.

The positive effect on SARA prevalence of higher number of waterers, adequate feed bunk space per cow and TMR delivery immediately after milking, conditions that generally reduce feeding competition among cows, is puzzling and a sound explanation cannot be provided. Similar findings are not reported in the literature. It is possible that this result is biased by the rather small number of herds included in the study or that any positive impact they have is lesser than the negative influence of other SARA-predisposing factors.

In conclusion, SARA seems to be a common problem in Greek dairy herds. Bovine practitioners should definitively consider its presence when dealing with herd level problems (like lameness, increased culling rate, low milk fat tests etc) and appropriate management measures should be implemented to avoid it.

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