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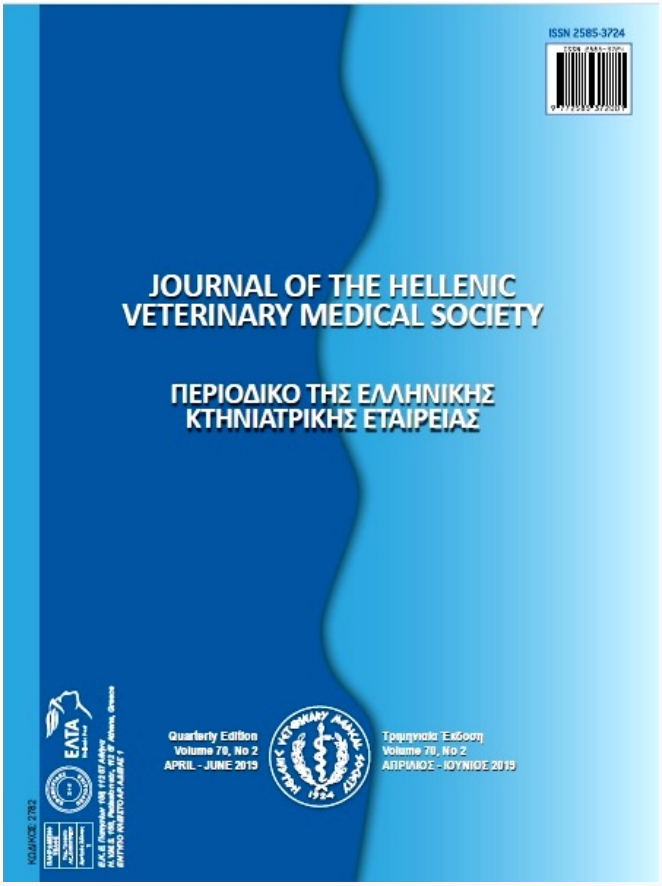

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G. C. KITKAS, G. E. VALERGAKIS, M. KRITSEPI-KONSTANTINOU, A. I. GELASAKIS, G. ARSENOS, E. KALAITZAKIS, N. PANOUSIS

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Research article
Ερευνητικό άρθρο

Effects of ruminal pH and subacute ruminal acidosis on milk yield and composition of Holstein cows in different stages of lactation

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Επίδραση του pH του περιεχομένου της μεγάλης κοιλίας και της υποξείας δυσπεπτικής οξέωσης στα ποσοτικά και ποιοτικά χαρακτηριστικά του γάλακτος αγελάδων Holstein σε διαφορετικά στάδια της γαλακτικής περιόδου

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ABSTRACT. Subacute ruminal acidosis (SARA; implying a rumen fluid pH between 5.5 and 5.0), is one of the most important metabolic diseases of dairy cows. In this study, the effect of SARA and rumen fluid pH on milk yield and composition was assessed in dairy cows under field conditions, with repeated measurements in the same cows, at different stages of lactation. Rumenocentesis was performed in 83 Holstein cows of a commercial herd at 30, 90, and 150 days in milk (DIM). Rumen fluid pH was measured on-site using a portable pH-meter. Milk yield was also recorded at the same days. Milk samples were analyzed for fat, protein, lactose and total solids content. For the statistical analysis, mixed linear regression models were used. Prevalence of SARA was 48.2%, 53.8% and 65.3% at 30, 90 and 150 DIM, respectively. There was a significant negative effect of SARA and decreased rumen fluid pH on milk fat content; SARA was associated with a decrease of milk fat content by 0.22%, while a one-unit increase of rumen fluid pH, even within the normal range, was associated with a 0.28% increase of milk fat content and 0.44% increase of milk total solids content. There was no effect of SARA on milk yield or protein, lactose and total solids content. In conclusion, under field conditions, SARA and decreased rumen fluid pH reduce milk fat content.

Keywords: Subacute ruminal acidosis, dairy cow, milk composition, milk fat.

ΠΕΡΙΛΗΨΗ. Η υποξεία δυσπεπτική οξέωση (ΥΔΟ) αποτελεί μία από τις πλέον σημαντικές μεταβολικές νόσους των γαλακτοπαραγωγών αγελάδων. Ορίζεται ως η πτώση του pH της μεγάλης κοιλίας (ΜΚ) κάτω από 5,5 (έως 5,0) και ο κύριος τρόπος διάγνωσής της στην κλινική πράξη μέχρι σήμερα είναι η παρακέντηση της ΜΚ. Η ΥΔΟ επηρεάζει την ομαλή παραγωγή λιπαρών οξέων στη ΜΚ και, μεταξύ άλλων, προκαλεί μείωση της λιποπεριεκτικότητας και της παραγόμενης ποσότητας γάλακτος. Κύριος σκοπός της έρευνας ήταν η διερεύνηση της σχέσης της ΥΔΟ και του pH του περιεχομένου της ΜΚ με την ποσότητα και την ποιότητα του γάλακτος, σε διαφορετικά στάδια της γαλακτικής περιόδου, υπό συνθήκες εκτροφής. Χρησιμοποιήθηκαν 83 αγελάδες φυλής Holstein μιας εμπορικής εκτροφής, από τις οποίες ελήφθησαν δείγματα περιεχομένου της ΜΚ με παρακέντηση για τον προσδιορισμό του pH, τις ημέρες 30, 90 και 150 της γαλακτικής περιόδου. Η εκτροφή διέθετε αυτόματη ατομική γαλακτομέτρηση και τις ημέρες των παρακεντήσεων γινόταν δειγματοληψία γάλακτος για προσδιορισμό της χημικής του σύνθεσης (περιεκτικότητα σε λίπος, πρωτεΐνες, λακτόζη και ολικά στερεά). Η ανάλυση των δεδομένων έγινε με μια σειρά γραμμικών μοντέλων μικτών επιδράσεων. Ο επιπολασμός της ΥΔΟ ήταν 48,2%, 53,8% και 65,3% τις ημέρες 30, 90 και 150 της γαλακτικής περιόδου, αντίστοιχα. Βρέθηκε ότι η ΥΔΟ σχετίζεται με μείωση της λιποπεριεκτικότητας του γάλακτος κατά 0,22%. Η αύξηση του pH της ΜΚ κατά 1 μονάδα, ακόμη κι εντός των φυσιολογικών ορίων, σχετίστηκε με αύξηση της λιποπεριεκτικότητας του γάλακτος κατά 0,28% και της συγκέντρωσης των ολικών στερεών του γάλακτος κατά 0,44%. Η ημερήσια γαλακτοπαραγωγή, δεν επηρεάστηκε από την ΥΔΟ, καθώς και η συγκέντρωση της πρωτεΐνης, της λακτόζης και των ολικών στερεών του γάλακτος.

Λέξεις ευρετηρίασης: Υποξεία δυσπεπτική οξέωση, αγελάδες γαλακτοπαραγωγής, χημική σύνθεση γάλακτος, λιποπεριεκτικότητα

INTRODUCTION

Subacute ruminal acidosis (SARA) is an issue of major concern in dairy cattle, with significant physiological and economic impacts (Nocek, 1997, Kleen et al., 2003, Enemark, 2008). It is commonly observed in intensive farming systems (Krause and Oetzel, 2006) and is characterized by rumen fluid pH between 5.0 and 5.5, without any characteristic clinical signs (Kleen et al., 2003). This pH reduction is caused by the excessive accumulation of short chain fatty acids (SCFA), associated with the ingestion of diets rich in readily fermentable carbohydrates and their slow absorption by the rumen wall (Kleen et al., 2003). Given the lack of characteristic clinical signs, a definite diagnosis of SARA in clinical practice is only established by measuring the rumen fluid pH, either at a specific time-point after feeding (rumen fluid collection by stomach tubing or, more credibly, by rumenocentesis (Duffield et al., 2004) or continuously (using rumen boluses and pH monitoring systems) (Villot et al, 2017).

Consequences of SARA in dairy cows may include a decrease in milk yield and in milk fat and protein content, due to changes in rumen fermentation and fatty acids' profile (Stone, 1999; Plaizier et al., 2009). Low ruminal pH alters rumen bacteria populations and fermentation patterns, favouring the production of specific long chain fatty acids (LCFA), which, after absorption, inhibit milk fat synthesis in the udder (Kennely et al., 1999).

Studies regarding the effects of SARA on milk yield and composition (Stone, 1999; Fairfield et al.,

2007; Gozho et al., 2007), as well as on rumen fluid fatty acids content (Kennely et al., 1999; Murphy et al., 2000) have produced controversial results. Moreover, these studies deal with experimentally induced SARA, mostly in early lactation cows. Considering the importance of SARA, it is questionable the lack of relevant field studies in the available literature.

Therefore, the objective here was to assess the effect of ruminal pH and SARA on milk yield and composition in cows of a commercial herd, repeatedly, in three different time points during lactation.

MATERIALS AND METHODS

This study was conducted following the approval of the ethics and research committee of the Faculty of Veterinary Medicine, Aristotle University of Thessaloniki. The farmer gave informed consent for the cows to be included in the study and the sampling procedures. For the purposes of the study, the farm was visited three times weekly for 15 consecutive months, for sample collection and clinical examinations.

Animals and Management

A total of 83 lactating Holstein cows (44 primiparous and 39 multiparous) from a commercial dairy farm, located in the region of Thessaloniki, Greece, were included in the study. Farm selection was based on historical data about SARA prevalence (farm #11, Kitkas et al., 2013). Dry cows were housed in a bedded pack shed as a single group (no far-off/close-up groups) and lactating ones in a two-row free-stall barn, again as a single group. The feed bunk for lactating cows was equipped with headlocks (65 cm center to center) and its length was 50 m, which was appropriate for 77 instead of 83 cows.

Lactating cows were offered a total mixed ration set to meet the National Research Council's recommendations (NRC, 2001) regarding net energy and metabolizable protein requirements, according to milk production level (Table 1). Dry cows' ration is presented in Table 1. Transition from the dry cow ration to the lactation one was abrupt (no close-up and far-off group).

Cows were milked twice daily. Daily milk yield (DMY) was automatically recorded for each individual cow using an automatic milk yield recording system (AfiFarm Herd Management Software®, Afimilk Ltd., Kibbutz Afikim, Israel).

Table 1. Composition of the diets fed to the cows of the study.

Ingredients	Lactation period ration (kg, as fed)	Dry period ration (kg, as fed)
Corn sillage	30.00	14.00
Alfalfa hay	3.50	--
Wheat straw	1.00	5.00
Corn grain	5.00	--
Wheat bran	2.00	1.00
Soybean meal	3.50	1.50
Mineral/vitamin supplement	0.30	0.15
Calcium carbonate	0.10	0.10
Sodium bicarbonate	0.18	--

Body Condition Scoring and Clinical Examination

Body condition score (BCS) was recorded for all cows at 30, 90 and 150 days in milk (DIM), always by the first author, using a five-point scale with increments of 0.25 (Ferguson et al., 1994). Clinical examination was performed on all cows routinely at the above time points and every time the farmer reported a sudden milk drop or clinical illness.

Rumen Fluid Sampling and Analyses

Rumen fluid was sampled via rumenocentesis, at the predetermined time-points (30, 90 and 150 DIM). The puncture site was selected and prepared as described by Garrett et al. (1999). The cows were restrained without sedation. Local anaesthesia was performed prior to each rumenocentesis, injecting 4 mL of 2% Xylocaine (AstraZeneka, Athens, Greece), at the puncture site (2 mL subcutaneously and 2 mL intramuscularly). Afterwards, a 16-G and 13 cm long stainless-steel needle (H. Hauptner & Richard Herberholz GmbH & Co. KG, Solingen, Germany) was used to aspirate 2 to 3 mL of rumen fluid, within 20 sec, into a 5 mL disposable plastic syringe. Rumenocenteses were consistently performed between 12:00-14:00, in order to be within the suggested time-frame of 5-8 hours after the morning feeding.

All cows were monitored for 10 days after each rumenocentesis for the presence of complications like peritonitis, hematoma or abscess formation at the puncture site. Minor complications were recorded only in 3 cases; namely, a small abscess (<3 cm) in

two cows and a larger one (approximately 10 cm) in one cow, all after the 1st rumenocentesis (at 30 DIM). All abscesses resolved spontaneously within two weeks, whereas during this period DMY of the three cows was not affected. From the 83 cows that were initially enrolled, 8 were culled before the end of the study (5 of them before 90 DIM and 3 between 90 and 150 DIM), due to mastitis and/or lameness. Therefore, 236 rumenocenteses were totally performed.

Rumen fluid pH was measured on-site immediately after collection, in room conditions, using a portable pH meter (Horiba, B-213, Kyoto, Japan). The pH cut-off value to define SARA was set at 5.5 (Garrett et al., 1999).

Milk Sampling and Analyses

At the days of rumenocenteses, milk samples were collected from each individual cow at the morning milking using standard sampling protocols, following the recommendations of the International Committee for Animal Recording (ICAR 2016); the samples were maintained at 4°C during transportation to the laboratory. Milk composition (fat, protein, lactose and total solids content) was determined within 24 hours after sample collection, by infrared analysis (FTIR interferometer), using a Milkoscan FT6000 Analyzer (Foss Electric, Denmark).

Statistical Analysis

Data were analyzed using SPSS 21. Initially, the differences regarding milk yield and composition between SARA-positive and SARA-negative cows and among sampling occasions (30, 90 and 150 DIM) were assessed using a one-way ANOVA analysis. For the comparisons among sampling occasions, Bonferroni test was used as *post hoc* test. The differences regarding SARA prevalence among the three sampling occasions were assessed using the χ^2 test.

Afterwards, a series of mixed linear regression models were built to assess the effects of i) SARA and ii) rumen fluid pH on a) daily milk yield and b) milk composition (fat, protein, lactose and total solids content).

The model used to quantify these effects for the g_{th} sampling occasion, of the h_{th} cow (DMY_{gh}), is described as below (Model 1):

$$Y_{gh} = \mu + SARA_{gh} + P_h + G_h + \beta_1 \cdot L + \beta_2 \cdot S + \gamma_h + \delta_h + e_{gh} \text{ (Model 1)}$$

Where:

Y_{gh}= Milk yield, milk composition, μ= intercept, $SARA_{gh}$ = fixed effect of SARA status (2 levels, 0 = no SARA, 1 = SARA), P_h = fixed effect of parity number (2 levels, 1st and ≥ 2nd parity), G_h = fixed effect of days in milk (3 levels; 30, 90 and 150 DIM), β_1= fixed effect of the regression coefficient of the milk lactose or protein or fat content (L) (for the models estimating the effect of SARA on milk yield, milk fat and milk protein content, respectively); for the estimation of the effect of SARA on milk lactose content the specific fixed effect (β_1) was excluded from the model, β_2= fixed effect of the regression coefficient of BCS (S) γ_h= repeated variation of the h_{th} cow, δ_i = random variation of the h_{th} cow and e_{gh} = residual error.

The fixed effect of SARA status was replaced by the fixed effect of the regression coefficient of the rumen fluid pH in all of the aforementioned models, which otherwise were built using the same explanatory variables and setting up, in order to calculate the effects of rumen fluid pH on i) DMY and ii) milk composition.

Among first order autoregressive (ARH1), compound symmetry (CS) and unstructure (UN), the covariance structure with the lowest Akaike's information criteria (AIC) was included, in case a significant improvement of the model was observed ($P<0.05$). The assumptions of homoscedasticity, normal distribution and linearity for the models were checked by visually assessing the plots of standardized residuals against standardized predicted values and histograms, as well as the probability-probability and quantile-quantile plots of standardized residuals.

RESULTS

One hundred and thirty-one SARA cases were recorded throughout the study; 85 in primiparous and 46 in multiparous cows. Prevalence of SARA was 48.2%, 53.8% and 65.3% at 30, 90 and 150 DIM, respectively; the difference in SARA prevalence between 30 and 150 DIM was significant ($P<0.05$). Sixteen cows were SARA-positive (13 primiparous and 3 multiparous) and 13 were SARA-negative (3 primiparous and 10 multiparous) in all 3 sampling occasions. Statistics of all measured parameters (ruminal pH, BCS, DMY and milk composition) for SARA-positive and SARA-negative cows, in each sampling occasion, and partial comparisons between and among them are summarized in Tables 2 and 3.

Rumen fluid pH was significantly lower (mean difference of 0.65) for SARA-positive compared to SARA-negative cows (P<0.001) in all three samplings; interestingly, there were no significant differences either among SARA-positive or among SARA-negative ones at the different sampling days. There was no effect of either SARA status or sampling day on BCS (Table 2).

Mean DMY as well as mean fat, protein, lactose and total solids content were 26.17 L, 3.57%, 2.96%, 4.73% and 11.61%, respectively. Figure 1 presents the box-plots of the rumen pH, milk composition and BCS during the 3 sampling occasions.

Milk yield was not affected by SARA status (Table 3). Milk fat content was significantly lower (P<0.05) in SARA-positive cows at DIM 30; a tendency for lower fat content for SARA-positive cows was again evident at DIM 150 (3.43 vs. 3.92%, P=0.053). Protein content was significantly higher (P<0.05) at DIM 150 for both SARA-positive and SARA-negative cows compared to DIM 30 and DIM 90. Fat to protein ratio was significantly lower for SARA-positive cows (P<0.05) at DIM 30 and 150. Lactose content of SARA-negative cows was higher (P<0.05) at DIM 30 than at DIM 150.

The use of linear regression models also showed that SARA did not affect DMY, milk protein and total solids concentrations. On the contrary, SARA significantly reduced milk fat content by 0.22% (P<0.05) and fat to protein ratio by 0.08 (P<0.05) (Table 4).

When rumen fluid pH was used into the models as a continuous variable (Table 4), no significant effects on DMY, milk protein, and milk lactose content were observed. A one-unit increase of ruminal pH was associated with 0.28% increase of milk fat content (P<0.05), 0.09 increase of fat to protein ratio (P<0.05) and 0.44% increase of milk total solids content (P<0.05).

Table 2. Descriptive statistics of ruminal pH and Body Condition Score (BCS) by days-in-milk (DIM) in SARA-positive and SARA-negative cows.

		Ruminal pH	BCS
Sampling		Mean (±SD)	Mean (±SD)
30 DIM	Cows with SARA n=40	5.26±0.16[a]	2.79±0.31
	Cows without SARA n=43	5.87±0.33[b]	2.83±0.38
	Total cows n=83	5.58±0.40	2.81±0.35
90 DIM	Cows with SARA n=42	5.19±0.19[a]	2.72±0.25
	Cows without SARA n=36	5.86±0.35[b]	2.82±0.36
	Total cows n=78	5.50±0.43	2.77±0.31
150 DIM	Cows with SARA n=49	5.20±0.18[a]	2.82±0.33
	Cows without SARA n=26	5.80±0.32[b]	2.74±0.36
	Total cows n=75	5.41±0.37	2.79±0.34

[a - b] For each sampling occasion separately, means within a row with different superscripts differ (P < 0.05)

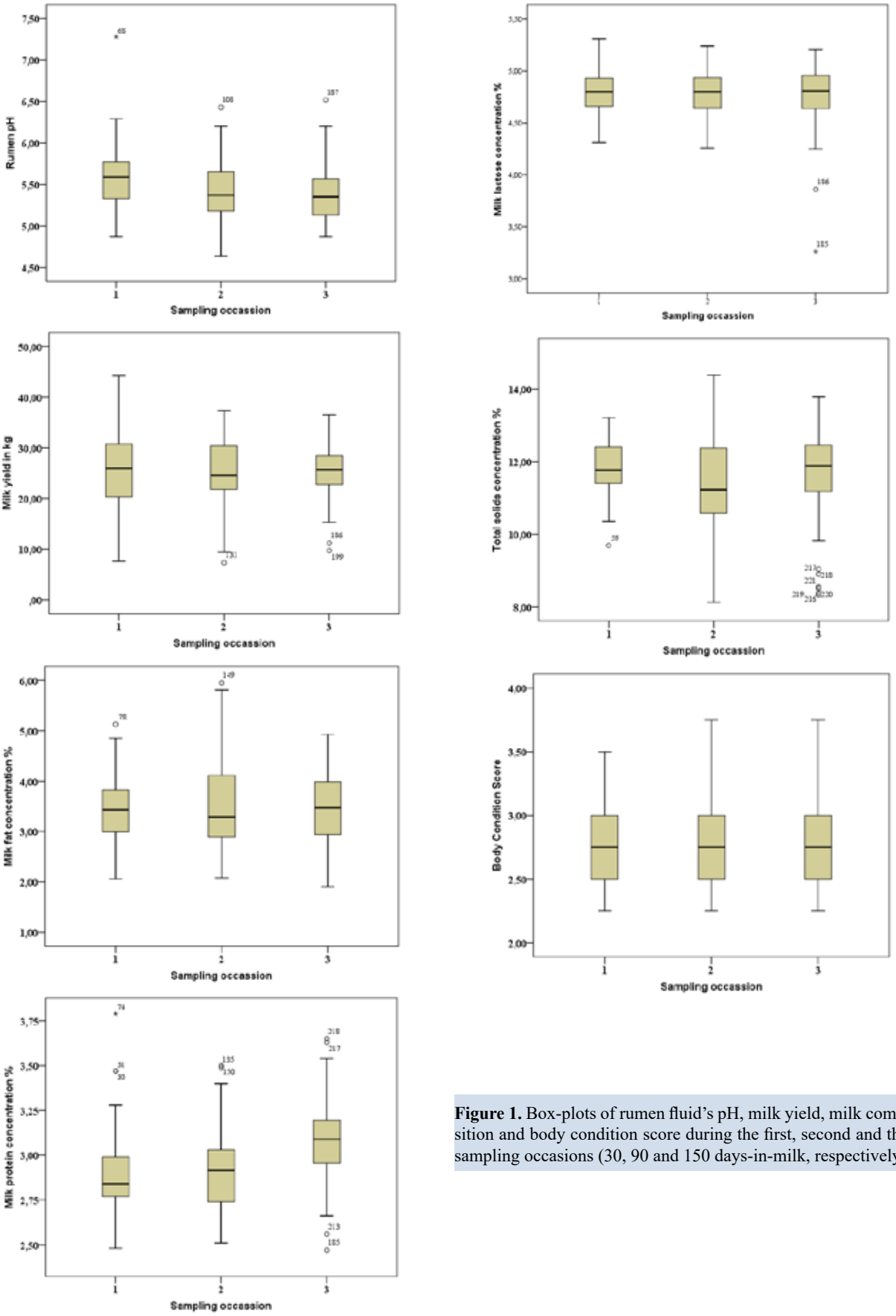

Figure 1. Box-plots of rumen fluid's pH, milk yield, milk composition and body condition score during the first, second and third sampling occasions (30, 90 and 150 days-in-milk, respectively).

Table 3. Descriptive statistics of daily milk yield (DMY) and milk composition by days-in-milk (DIM) in SARA-positive and SARA-negative cows.

		DMY (kg)	Fat content %	Protein content %	Fat:Protein ratio	Lactose content %	Total solids content %
Sampling		Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)
30 DIM	Cows with SARA n=40	26.60±9.58	3.30±0.66^{a}	2.90±0.27^{x}	1.15±0.26^{a}	4.79±0.22	11.67±0.68
	Cows without SARA n=43	27.13±6.77	3.65±0.65^{b}	2.86±0.22^{t}	1.28±0.22^{b}	4.73±0.20^{t}	11.91±0.79
	Total cows n=83	26.87±8.22	3.49±0.67	2.88±0.25	1.22±0.25	4.76±0.21	11.80±0.74
90 DIM	Cows with SARA n=42	25.50±7.18	3.44±1.52	2.89±0.21^{x}	1.20±0.57	4.75±0.0.26	11.21±1.87
	Cows without SARA n=36	25.68±6.13	3.81±0.84	2.94±0.24^{t}	1.30±0.28	4.71±0.27^{t}	11.59±11.31
	Total cows n=78	25.58±6.67	3.61±1.26	2.91±0.23	1.25±0.46	4.73±0.26	11.38±1.64
150 DIM	Cows with SARA n=49	25.95±6.34	3.43±0.86	3.11±0.25^{y}	1.10±0.27^{a}	4.76±0.27	11.55±1.28
	Cows without SARA n=26	26.23±4.87	3.92±1.32	3.07±0.32^{r}	1.27±0.32^{b}	4.61±0.44^{r}	11.80±1.27
	Total cows n=75	26.05±5.83	3.60±1.06	3.09±0.28	1.16±0.30	4.71±0.35	11.64±1.27

$^{a-b}$ Means referring to cows with SARA and cows without SARA, for each sampling occasion separately, with different superscripts differ ($P < 0.05$)
$^{x-y}$ Means referring to cows with SARA during the three sampling occasions with different superscripts differ ($P < 0.05$)
$^{t-r}$ Means referring to cows without SARA during the three sampling occasions with different superscripts differ ($P < 0.05$)

Table 4. Significant effects of subacute ruminal acidosis (SARA) and rumen fluid's pH on milk composition.

					95 % CI	
	Parameter	*B*	SE	P-value	Lower	Upper
SARA	Fat concentration (%)	-0.22	0.101	0.028	-0.42	-0.02
	Fat:protein ratio	-0.08	0.034	0.024	-0.14	-0.01
pH	Fat content (%)	0.28	0.124	0.025	0.03	0.53
	Fat:protein ratio	0.09	0.043	0.033	0.01	0.18
	Total solids content (%)	0.44	0.173	0.012	0.10	0.78

DISCUSSION

As asserted in the Introduction, the novelty of this study is that SARA was not experimentally induced; instead, this was conducted on a commercial dairy herd (field conditions) and each cow was sampled at three different time points at the first half of lactation.

Dairy cows are prone to SARA in early- and mid-lactation (Kleen et al, 2003); however, the majority of studies on SARA prevalence were conducted between 10 and 90 DIM. Prevalence of reported SARA cases can range significantly. Overall, among cows studied it was found to be 11% (O'Grady et al.,

2008), 13.8% (Kleen et al., 2009), 14.0% (Stefańska et al., 2016), 15.7% (Kitkas et al., 2013), 20.0% (Kleen et al., 2013), 20.1% (Oetzel et al., 1999), and 27.6% (Tajik et al., 2009). The notion it that there was a considerable variation among herds, ranging from 0.0% to 38.0% (Kleen et al., 2009). SARA-positive cows were more than 40.0% of the herd in about one third of herds examined by Garrett et al. (1997), more than 33.0% of the total in 30.0% of those examined by Morgante et al. (2007) and more than 25.0%, again, in one third of the herds examined by Kitkas et al. (2013).

The particularly high SARA prevalence recorded in the present study was rather expected, since the main selection criterion was the known history of high SARA prevalence on this specific farm (Kitkas et al., 2013). An adequate number of SARA-positive cows was necessary to detect statistically significant differences in the examined variables and this farm fitted well to the SARA-positive herd profile. Prevalence was high in early- and mid-lactation (48.2%, 53.8% and 65.3% at 30, 90 and 150 DIM, respectively). Relevant prevalence was lower in other studies, which included more farms. Garrett et al. (1997) reported SARA prevalence of 19.0% vs. 26.0%, Kleen (2004) 11.0% vs. 18.0% and Tajik et al. (2009) 29.3% vs. 26.4%, in early- and mid-lactation cows, respectively. In the above studies the differences in SARA prevalence at the various stages of lactation can be attributed to different management practices across herds in different regions and countries over time. The latter was not the case in the present study that refers to a single farm.

Prevalence of SARA at DIM 150 (mid-lactation) was significantly higher than at DIM 30 (early lactation). Generally, the over-accumulation of SCFA that causes SARA in early lactation results from their low absorption rate, associated with the short length of rumen papillae and the low number of bacteria capable of utilizing them, due to inappropriate transition management (Kleen et al., 2003), while in mid-lactation the over-accumulation of SCFA results from the high intake of low buffering capacity (low in effective fiber), high energy rations (Plaizier et al., 2009). Indeed, the inadequate transition management was the culprit for high SARA prevalence for this particular farm in early lactation (DIM 30), but for mid-lactation cows (DIM 90 and DIM 150) feed bunk management (inadequate bunk space for the 83 cows) and not the ration was the plausible cause. Moreover, continuous bouts of acidosis make it more difficult for cows to restore normal ruminal pH (Dohme et al., 2008), which might explain the increased prevalence of SARA as lactation progressed.

The above could also partly explain why 16 cows were SARA-positive throughout the study. An alternative explanation for that and for the fact that 13 cows were SARA-negative at all samplings is that some cows might be genetically susceptible while others resistant to SARA. This could be a research challenge for the future.

Regarding parity, almost twice as much cases of SARA were recorded in primiparous than in multiparous cows. This finding is in agreement with Enemark et al. (2004) and Krause and Oetzel (2005), who stated that primiparous cows are more prone to the disease. Access to feed bunk for primiparous is difficult in the presence of older cows, due to competitive interactions, resulting in the consumption of large meals in short time periods (Oetzel, 2003). This was certainly the case on this farm, where feed bunk length was inadequate.

There was no effect of SARA status or rumen fluid pH on milk yield in this study. Stone (1999) found an increase of 2.7 kg in daily milk yield when corn meal replaced high-moisture corn in a commercial herd; actually, this is the only field study with high yielding cows assessed in the literature. In other cases, where milk production was negatively affected by SARA, cows were either low producing (Bipin et al., 2016) or rumen cannulated ones under experimental settings (Enjalbert et al., 2008; Malekkhahi et al., 2016; Xu et al., 2016). However, a negative effect of SARA on milk production is not always observed (Gozho et al., 2007; Danscher et al., 2015) under the same conditions. Stage of lactation may affect the outcome of such comparisons; during early lactation, cows may compensate for a negative energy balance by mobilizing fat reserves. In this case, BCS loss is usually greater for SARA-positive cows (Kleen et al., 2003) but not always (Tajik et al., 2009); this was not observed in our study, either. Comparisons regarding yield traits should account for the effect of genotype. Higher genetic merit cows which are SARA-positive due to higher DM intake (Enemark, 2008; Kleen et al., 2013; Plaizier et al., 2009), produce less milk than their genetic potential dictates and thus, no difference is detected when they are compared with SARA-negative cows. This could explain the present results for DMY; unfortunately, breeding values for milk yield

were not available and, therefore, could not be included in the statistical model. Previous lactation records could be paired but, in our case, as SARA was a permanent problem in this herd (Kitkas et al., 2013), records were not considered representative of true genetic potential; moreover, most SARA cases were observed in primiparous cows.

While the one-way analysis of variance showed a significant negative effect of SARA on milk fat content only at DIM 30, the use of a mixed linear regression model that besides SARA or pH included parity, DIM, BCS and protein content as independent variables, clearly showed a significant effect of both on milk fat content. Stone (1999) reported a reduction of milk fat of 0.30%; the difference in favor of SARA-negative cows was similar (0.22%) in this study. Other researchers have also found a decrease in milk fat content (Enjalbert et al., 2008; Danscher et al., 2015; Bipin et al., 2016; Malekkhahi et al., 2016; Xu et al., 2016) but, again, most were experimental studies using a small number of cows. Individual test-day milk fat records of mid-lactation cows have been proposed as a herd-level screening tool (Enemark, 2008). However, milk fat depression is not a consistent finding. Keunen et al. (2002) and Gozho et al. (2007) found no effect under experimental conditions; neither did Tajik et al. (2009) who sampled a small number of cows under field conditions. To our knowledge, the present study is the only one reporting a significant negative effect of SARA and low ruminal pH on milk fat content, using linear regression models.

There was no effect of SARA status or rumen fluid pH on milk protein content in this study. This is in contrast with Stone (1999) who showed a drop of 0.10%, as well as with the results of Keunen et al. (2002) and Xu et al. (2016); on the other hand, Li et al. (2012) found an increase in milk protein content. Our result is in agreement with most other researchers (Gozho et al., 2007; Enjalbert et al., 2008; Tajik et al., 2009; Danscher et al., 2015; Malekkhahi et al., 2016), who found no statistically significant differences. Either microbial protein yield is not significantly affected or differences are impossible to be detected because the number of cows used is too small.

Both the one-way analysis of variance and the use of mixed linear models detected statistically significant differences in milk fat to protein ratio in this study. This ratio is not commonly reported in the aforementioned studies but, as in most of them milk fat content decreases in SARA-positive cows while milk protein does not, it could be assumed that this is generally the case. The milk fat to protein ratio is not considered useful in investigating SARA-induced milk fat depression cases; besides problems related to analytical procedures, reasons mentioned include different physiologic processes of milk fat and protein synthesis and lack of scientific documentation (Oetzel, 2007). However, a ratio is just a number (fraction) and whatever the reason, when the numerator decreases while the denominator does not, the ratio decreases as well. Nevertheless, there are no published peer-review data so far dealing with the effect of SARA or ruminal pH on this ratio. In light of our findings, more research is warranted on this issue, in our quest to come up with an inexpensive (monthly DHI records), non-invasive method to screen herds for SARA.

Effect of SARA status or rumen pH on milk lactose content is not reported in the literature. There was no such effect in this study. There was no effect of SARA status on milk total solids content either, but a one-unit increase of rumen fluid pH would increase total solids content by 0.44%; fat would represent about 64% of the total. This has obvious economic benefits.

CONCLUSIONS

The present study is the first that was conducted under field conditions, with repeated measurements from the same animals, and denotes a clear negative effect of SARA and low rumen fluid pH on milk fat content. Milk yield and protein content were not affected. The present study also demonstrated a reduction of the milk fat:protein ratio, a number not often mentioned in relative studies but an inexpensive measure for practitioners to suspect SARA and pursue further examination. The fact that a significant number of cows were consistently SARA-positive or SARA-negative throughout the study, under the same conditions, should be further investigated.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest.

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