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## SUBACUTE RUMEN ACIDOSIS IN ITALIAN DAIRY HERDS: OCCURRENCE AND DIAGNOSTICS TOOLS

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# INDEX

## RIASSUNTO

L'acidosi ruminale subacuta (SARA dall'Anglosassone: subacute ruminal acidosis) rappresenta una delle principali problematiche nell'allevamento della bovina da latte, in grado di provocare notevoli ripercussioni economiche, sia dirette che indirette. Sull'insorgenza di tale patologia influiscono numerosi fattori: tipologia di alimentazione, modalità di alimentazione, capacità del rumine di assorbire gli acidi grassi volatili, periodo dell'anno, frequenza d'ingestione, periodo di transizione asciutta-lattazione e tutta un'altra serie di condizioni che possono contribuire all'insorgenza di tale patologia. L'acidosi ruminale subacuta viene spesso sottovalutata perché non si evidenzia con sintomi eclatanti e gli unici segni che possono essere apprezzati sono quelli secondari, che si manifestano a settimane o addirittura a mesi di distanza dall'inizio del problema. Diminuzione dell'ingestione, zoppie, mastiti, dermatiti, ruminiti, ascessi, emboli polmonari, endometriti e scarsa fertilità sono alcuni esempi dei problemi sanitari che possono avere alla base una condizione di SARA. Considerando tutto ciò, non è possibile emettere diagnosi di SARA basandosi su di un solo segno o sintomo, ma l'unico modo per ottenere una diagnosi corretta, è quello di osservare e mettere in associazione vari aspetti: indagine anamnestica e clinica globale dell'allevamento; valutazione dell'alimentazione; esame del liquido ruminale: quest'ultima è senza dubbio la prova più importante perché è l'unica che ci permette di mettere il luce le condizioni del rumine in quel momento. Dalle ricerche effettuate in questi ultimi anni, si può affermare che la tecnica più idonea e sicura per il prelievo del liquido ruminale al fine di una diagnosi presuntiva di SARA è la rumino centesi: in un'azienda si prende in effettua la ruminocentesi in un gruppo di 12 bovine nelle prime fasi di lattazione e si emette diagnosi di SARA quando almeno il 30 % delle bovine in esame presenta un pH ruminale inferiore a 5.5. Nonostante sia ampliamente riportata in bibliografia l'efficacia, la validità e l'assenza di pericolosità di tale tecnica, la ruminocentesi rimane un mezzo diagnostico moderatamente invasivo per cui risulta difficilmente impiegata dal veterinario libero professionista, oltre ad essere scarsamente accettata dall'allevatore. A tale scopo, una concreta

esigenza di Veterinari ed Alimentaristi, è quella di disporre di un metodo più semplice ed economico di diagnosi delle condizioni di SARA, per poter applicare in maniera tempestiva gli opportuni correttivi dietetici, eliminare le cause dell'insorgenza della dismetabolia e scongiurare il pericolo del progredire della stessa in forme più acute. In risposta a queste richieste, lo scopo della presente Tesi di Dottorato è quello di effettuare 3 diversi studi, ma tra loro correlati, al fine di: definire l'incidenza della SARA negli allevamenti intensivi della bovina da latte presenti in Italia e determinare l'eventuale relazione esistente tra composizione della dieta, pH ruminale ed acidi grassi volatili; valutare l'effetto dell'acidosi ruminale subacuta su alcuni parametri ematologici ed ematobiochimici ed infine valutare l'effetto della ruminocentesi sullo stato di salute e benessere della bovina da latte, mediante la rilevazione dello stato clinico dell'animale ed il monitoraggio di alcuni parametri ematici, produttivi e della temperatura corporea superficiale (rilevata mediante termografia ad infrarossi) evidenziata nella zona di esecuzione della ruminocentesi.

**Lavoro 1:** Scopo del presente studio è stato quello di definire l'incidenza dell'acidosi ruminale subacuta negli allevamenti intensivi della bovina da latte presenti in Italia e determinare l'eventuale relazione esistente tra composizione della dieta, pH ruminale ed acidi grassi volatili. Per la prova sono stati presi in considerazione 10 allevamenti intensivi ed all'interno di ogni azienda sono state selezionate in modo casuale 12 bovine prive di segni clinici di malattia, in buono stato corporeo e comprese tra i 5 ed i 60 giorni di lattazione. Il prelievo del liquido ruminale è stato effettuato mediante ruminocentesi per l'immediata determinazione del pH ruminale mediante pHmetro portatile e la successive analisi di laboratorio degli acidi grassi volatili (AGV). Non sono stati riscontrati problemi nell'esecuzione della ruminocentesi e le bovine non hanno manifestato alcun problema clinico ne durante ne successivamente al prelievo: tale riscontro conferma l'estrema validità di questa metodica quale tecnica d'elezione per il prelievo del liquido ruminale. I dati sono stati oggetto di elaborazione statistica utilizzando il programma SIGMA STAT 2.03. I risultati hanno indicato la presenza di SARA in 3 allevamenti (oltre il 33% degli animali con pH ruminale <5,5), una situazione a rischio di acidosi ruminale subacuta in 5 aziende (oltre il 33% degli animali

con pH ruminale <5,8) e 2 allevamenti indenni da SARA, con una normale condizione di pH ruminale. In particolare, nelle aziende con pH ruminale basso, è stato riscontrato un aumento dei valori assoluti degli AGV: 123 mmol/L nelle aziende classificate come indenni, 145 nelle aziende a rischio e 150 nelle aziende classificate come in acidosi. Per quanto concerne la composizione chimica della dieta non sono state riscontrate differenze statisticamente significative tra i 3 gruppi di aziende, anche se le mandrie con SARA hanno mostrato una discordanza tra composizione chimica della dieta iniziale e quella del residuo, il che, tra le ipotesi, impone di suggerire una maggior attenzione nella preparazione del carro miscelatore. Inoltre, da tale indagine, si può affermare che all'insorgenza di tale condizione concorrono non solo cause alimentari ma anche altri fattori, tra i quali sicuramente il più importante sembra essere un non adeguato management aziendale.

**Lavoro 2:** Scopo del presente studio è stato quello di valutare l'eventuale effetto dell'acidosi ruminale subacuta della bovina da latte su alcuni parametri ematologici ed ematobiochimici. Per la prova sono stati presi in considerazione 10 allevamenti intensivi bovini stazionati in diverse aree dell'Italia settentrionale ed all'interno di ogni azienda sono state selezionate in modo casuale 12 bovine in fase di prima lattazione, prive di segni clinici di malattia ed in buono stato corporeo. Il prelievo del liquido ruminale è stato effettuato mediante ruminocentesi per l'immediata determinazione del pH ruminale mediante pHmetro portatile. Dalle stesse bovine alle quali è stato prelevato il liquido ruminale sono stati effettuati prelievi di sangue dalla vena giugulare al fine di determinare alcuni parametri ematologici ed ematobiochimici. Sulla base dei pH ruminali riscontrati le aziende sono state suddivise in 3 gruppi: gruppo A con pH ruminale medio > 5,8 (normale), gruppo B con pH ruminale medio compreso tra 5,6 e 5,8 (rischio) e gruppo C con pH ruminale medio <5.6 (acidosi). I dati sono stati analizzati statisticamente mediante analisi della varianza per verificare l'effetto del gruppo ed è stata applicato il test di Bonferroni per determinare le differenze statisticamente significative tra i tre gruppi presi in considerazione. Differenze statisticamente significative ($p < 0.05$) sono state osservate per vari parametri ematologi ed ematobiochimici determinati. Sulla base dei risultati ottenuti si è potuto concludere che in corso di acidosi ruminale

subacuta delle variazioni significative di alcuni parametri ematologici ed ematobiochimici possono essere osservate.

**Lavoro 3:** Scopo del presente studio è stato quello di valutare l'effetto della ruminocentesi sullo stato di salute e benessere della bovina da latte, mediante la rilevazione dello stato clinico dell'animale ed il monitoraggio di alcuni parametri ematici, produttivi e della temperatura corporea superficiale (rilevata mediante termografia ad infrarossi) evidenziata nella zona di esecuzione della ruminocentesi. Per la prova sono stati presi in considerazione 2 gruppi di animali composti da 6 bovine ciascuno: gruppo A (GA) al quale è stata effettuata la ruminocentesi e gruppo B controllo (GB), al quale è stata effettuata solo la tosatura e disinfezione nell'area di esecuzione della ruminocentesi. Nel GA le bovine non hanno manifestato alcun problema clinico ne durante ne successivamente al prelievo. I dati sono stati analizzati statisticamente mediante analisi della varianza per verificare l'effetto del gruppo. Nessun effetto gruppo è stato osservato sulla conta leocucitaria, aptoglobina, proteine totali e frazione elettroforetica come pure non sono state riscontrate differenze per quanto concerne la produzione di latte tra i 2 gruppi di animali presi in considerazione. Nel gruppo A, nell'area dove è stata effettuata la ruminocentesi si è rilevato un aumento della temperatura corporea superficiale di 1,0° C subito dopo il prelievo del liquido ruminale, innalzamento che è tornato ai valori basali già dopo le 48 h dal prelievo e che poi è rimasto costante per tutto il periodo di studio. Sulla base dei risultati ottenuti si è potuto confermare l'estrema validità della ruminocentesi quale tecnica d'elezione per il prelievo del liquido ruminale in quanto non provoca alcun effetto negativo sullo stato di salute e benessere della bovina da latte.

## SUMMARY

Subacute rumen acidosis (SARA) represent one of the most important metabolic disorders in intensive dairy farms that affects rumen fermentations, animal welfare, productivity and profitability. Many are the factors that can increase the possible occurrence of SARA in dairy farms and in particular in intensive dairy farms: food type, food administration modality, rumen ability in absorbing short chain fatty acids (SCFA), year period, ingestion frequency, dry-lactation transition period and others conditions able to contribute to the SARA onset. SARA is possibly under diagnosed because of lack of pathognomonic signs, diurnal fluctuations in rumen metabolism, and problems in obtaining representative rumen fluid samples. Clinical signs of SARA include decreased dry matter intake (DMI), laminitis, rumenitis, liver abscesses, pulmonary bacterial emboli and, furthermore, displacement of the abomasums, mastitis and metritis, low fertility. These problems are linked to rumen pathology because of the exceptional osmotic capacity shown by the rumen and the increase in the adsorption rate of ruminal products, which may have toxic and vasomotor effects. Considering the above statement it is impractical to base the diagnosis of SARA only on the outcome of a clinical sign, but the only way to obtain an early and correct diagnosis is to observe and correlate multiple aspects of the condition: a thorough collection of the history in the farm; evaluation of the diet; evaluation of the ruminal fluid. The determination of ruminal pH is a key factor for the diagnosis of SARA. Different methods are available for the collection of rumen fluid for pH analysis. According several researcher rumenocentesis may be useful for the collection of rumen fluid for pH determination. A group of at least 12 cows (early or middle lactation) is defined as having SARA when more than 30% of them show a ruminal pH lower than 5.5. Anyway rumenocentesis remains a mildly invasive diagnostic method so it results hardly used by freelancer veterinarian and poorly accepted by farmer. A practical need for Veterinarians and Food practitioner, is to have a simple and economic method of diagnosis of SARA, in order to implement in a timely manner the appropriate corrective diet, eliminate the causes of arrival of this disorder

and avert the danger of progress in the same very high levels. In response to these requests, the aim of this PhD thesis, was to lead three different studies related to each other but, in order to evaluate the occurrence of subacute rumen acidosis in intensive Italian dairy herds to determine the relationship among diet composition, ruminal pH and short chain fatty acids (SCFA) concentration, to evaluate the effect of the subacute ruminal acidosis on some blood parameters in lactating dairy cows and finally to evaluate the effects of rumenocentesis on the health and welfare status of lactating dairy cows, evaluated by physical examination, blood analysis, milk production and superficial temperature on the area of rumenocentesis using infrared thermography.

**Trial 1:** The aim of the present study was to study the occurrence of subacute rumen acidosis in intensive Italian dairy herds and to determine the relationship among diet composition, ruminal pH and SCFA concentration. Ten commercial dairy herds were investigated; twelve cows in good body condition, between 5 and 60 DIM and without clinical signs of disease were selected randomly from each herd, to perform rumenocentesis and obtain rumen fluid. Ruminal pH was determined immediately after sampling and concentration of SCFA in ruminal fluid was determined on samples after storage. We further studied the effects of rumenocentesis on animal health. Our data confirm the extreme validity of this technique as ruminal sampling. Results were subject to ANOVA and correlation analysis using SIGMA STAT 2.03. The results indicated the presence of SARA in 3 herds (more than 33% cows with rumen pH < 5.5), a critical situation (more than 33% cows with rumen pH < 5.8) in 5 farms and a normal rumen pH condition in 2 herds. In particular dairy herds show an average SCFA concentration of 150, 145, 123 mmol/L for low pH, critical pH and normal pH herds, respectively. The differences among diet composition were not significant even if herds with SARA showed a light discordance between initial composition and residual feed. In the affected herds it was not possible to understand the exact causes of SARA. Animal management seems to be one of the most important factor in developing SARA, including total mixed ration preparation.

**Trial 2:** The aim of this study was to evaluate the effect of subacute ruminal acidosis on some blood parameters in lactating dairy cows. The study was carried out on ten highly productive farms, stationed in different zones throughout northern Italy. In all farms, ruminal fluid was collected through rumenocentesis from 12 dairy cows in early lactation, and ruminal pH was measured with a pH meter. Blood samples for hematochemical and hematological profiles were obtained from the same cows that had rumenocentesis performed by jugular venipuncture. The herds were divided into three groups depending on the mean rumen pH: group A counted farms with average ruminal pH > 5.8 (normal), group B included farms with average ruminal pH between 5.6 and 5.8 (risk), and in group C, dairy farms presented an average ruminal pH < 5.6 (acidosis). Data were statistically analyzed by analysis of variance to verify the effect of the group. The Bonferroni test was applied to determine statistical significances between the three groups. Statistically significant differences ($p < 0.05$) were observed for various parameters. It was concluded that during subacute ruminal acidosis in dairy cows, modifications of the concentrations of some blood parameters can be observed.

**Trial 3:** The aim of this study was to evaluate the effects of rumenocentesis on the health and welfare status of lactating dairy cows, evaluated by physical examination, blood analysis, milk production and superficial temperature on the area of rumenocentesis using infrared thermography. Two groups of 6 cows either underwent ruminocentesis (GA) or sham (GB) procedures. Data were statistically analyzed by analysis of variance to verify the effect of the group. No effect was observed on white blood cell count, haptoglobin, total protein, and electrophoretic fractions between GA and GB groups. Milk yield was not affected by rumenocentesis. On the region of rumenocentesis in group GA, skin temperature increased 1.0°C immediately following rumen fluid collection, returned to the baseline after 48 h, and remained constant till the end of the study. This study suggested that ruminocentesis used to diagnose subacute ruminal acidosis has no negative impact on animal health and welfare of lactating cows.

# INTRODUCTION

Subclinical rumen acidosis, also known as subacute rumen acidosis (SARA), represents one of the most important metabolic disorders in intensive dairy farms that affects rumen fermentations, animal welfare, productivity and eventually farm profitability. The onset of SARA is linked to the intake of low fiber high energy diets, often combined with a ruminal environment not yet adapted to highly fermentable feeds. Under these conditions, rumen papillae are not fully developed with consequent slower absorption of SCFA. The concentration of these latter thus increases, leading to a decrease in ruminal pH below physiological limits (Kleen et al., 2003). According to Nordlund and Garrett (1994), SARA can be defined as a condition characterized by rumen pH below 5.8, increased total concentration of SCFA, ratio between acetic, propionic and butyric acid shifted towards propionic and butyric acid, and elevated concentration of lactic acid in the rumen fluid not exceeding 5-10 mmol/l (Hibbard et al., 1995).

## Terminology

Acute and subacute ruminal acidosis share a similar aetiology but are very different clinical diseases. The definitions are made for both feedlots and dairy cattle (Nordlund et al., 1995; Garrett et al., 1999). In acute ruminal acidosis, an excessive intake of rapidly fermentable carbohydrates results in a sudden and uncompensated drop in ruminal pH. As ruminal pH drops, ruminal lactic acid concentrations rise (Owens et al., 1998). This cascade of often fatal consequences begins when ruminal pH drops below about 5.0. The pathophysiological progression during acute ruminal acidosis includes high concentrations of ruminal lactic acid, peracute rumenitis, ruminal hyperosmolality, dehydration and systemic acidemia (Owens et al., 1998; Radostits et al., 1994). Clinical signs include complete anorexia, abdominal pain, tachycardia, tachypnea, diarrhoea, lethargy, staggering, recumbency and death. Beside acute clinical ruminal acidosis, which is thoroughly described elsewhere (Dirksen, 1970; Rossow, 1984; Underwood, 1992), non-acute, non-

clinical forms have been described; different terms have been chosen to characterize these forms of acidosis. In the literature are used: subacute ruminal acidosis (Garrett, 1996; Nordlund et al., 1995; Stock, 2000) or SARA (Garrett et al., 1999), as well as chronic rumen acidosis (Slyter, 1976; Garry, 2002; Ivany et al., 2002), subclinical rumen acidosis (Møller, 1993; Nocek, 1997), chronic-latent acidosis (Dirksen, 1985; Gäbler, 1990) and latent acidotic stress (Rossow, 1984). Moreover, a differentiation between chronic/subclinical acidosis and a subliminal acidosis is made (Owens et al., 1998). In the following it will be dealt with the term 'subacute ruminal acidosis' or 'SARA' as recently described (Nordlund et al., 1995; Garrett, 1996; Garrett et al., 1999; Stock, 2000). It will be shown that this form of ruminal acidosis has consequences, which are clinically detectable. Therefore, the term 'subclinical' seems not to be suitable. Also the term 'chronic' seems to be inappropriate in dairy cattle because the ruminal pH is usually low just within defined periods, either after feeding or during a certain risk-period, e.g. after calving, whereas in beef cattle the feeding regime leads to a continuously acidotic ruminal environment (Oetzel, 2000). The onset of SARA is marked by the intake of a diet low in structure and high in energy, while the ruminal environment is not yet adapted to ferment and later absorb the arising SCFA adequately enough to keep the ruminal pH within physiological borders. The ruminal wall and its papillae herein play an important role. The adaptational growth has been described (Dirksen et al., 1984). The ruminal papillae are of crucial importance in the absorption of SCFA; the proliferation of the papillae is promoted by SCFA arising from the fermentation. If the ruminal mucosa is not adapted, which especially is the case at the shift from a dry-period to an early lactation diet, the papillae are too short and the resorbing surface too small to deal with the sudden increase of SCFA-levels (Nordlund et al., 1995). Also the microbial population, which has to metabolize the lactic acid arising from the fermentation of abundant carbohydrates of *Streptococcus bovis* or *Dasytricha* spp., is insufficiently developed (Slyter, 1976; Dawson and Allison, 1988; Nordlund et al., 1995). Thus, in cases of SARA, these mechanisms cannot prevent a transient fall of ruminal pH to ranges below pH 5.5. Therefore, some hours after intake of a concentrate-rich diet the ruminal pH first reaches

non-physiological acidic levels before returning to a higher, physiological level. The balance between production and absorption of fermentation end products is disturbed (Garrett, 1996). Generally spoken, SARA therefore has to be defined as an intermittent fall of ruminal pH to non-physiological levels after uptake of a certain concentrate based diet because of a non-adaptation of the ruminal environment in terms of flora and ruminal mucosa. SARA will have clinically detectable consequences, which will become manifest after a certain delay to the initial insult. The critical threshold of the ruminal pH can be identified at 5.5 some hours after concentrate feeding (Nordlund et al., 1995; Garrett et al., 1999; Oetzel, 2000). Probably SARA has to be understood as a maladaptation of the reticulo-ruminal environment.

**Epidemiology and economic importance of subacute ruminal acidosis**

Very few studies have examined the epidemiology of acute and subacute ruminal acidosis in dairy cows (Krause and Oetzel, 2006). A screening of 15 Holstein herds in the US revealed the presence of SARA in 19% of the early lactation cows and in 26% of the mid-lactation cows. In one-third of the herds observed more than 40% of the total number of cows within the herd were found to have SARA (Garrett et al., 1997). So it seems likely that a subclinical form of acidosis is present in a large number of dairy herds and that the cost because of this disease, arising from health and production problems, is sufficient to justify a closer inspection. Gröhn and Bruss (1990) evaluated the incidence of acute ruminal acidosis in 61,124 Finnish Ayrshire cows. The data were based on veterinary diagnoses collected from 2 days before calving until the subsequent calving. The incidence of acute ruminal acidosis was 0.3% throughout lactation, but was highest during the 1st month post-calving and relatively non-existent within 3 months. SARA is estimated to cost the U.S. dairy industry between US$ 500 million to US$ 1 billion a year (Donovan, 1997). Oetzel et al. (1999) had little difficulty finding herds with SARA; 20% of animals evaluated in 14 Wisconsin dairy herds were diagnosed by rumenocentesis as having SARA. The importance of reducing SARA was demonstrated in a 500-cow dairy diagnosed with SARA by Stone (1999), who replaced

high-moisture corn with corn meal. In an apparent response to increased ruminal pH, milk production increased by 2.7 kg/d, and milk fat and protein increased by 0.3 and 0.1 percentage points, respectively. In this study the author calculated US$ 400 to US$ 475 lost income per cow per year due to SARA. The production and component increases resulted in an increased monthly income of $20,000 for the dairy, presumably in large part from a reduction in the prevalence of SARA and an increase in rumen microbial growth. The financial impact of associated disorders, such as lameness and its deleterious effect on reproduction was not estimated, but was probably higher than the cost of lost milk production. Although SARA is commonly expected to negatively affect cow health, there are very few studies investigating this relationship as the primary objective.

**Occurrence**

Many are the factors that can increase the possible occurrence of SARA in dairy farms and in particular in intensive dairy farms. These conditions can be summarized in an increased use of concentrate and in particular in the amount of starch in the ration and an increased in genetic potential, which affects the demand for energy to support milk yield production.

In the period around calving, dairy cattle suffer a considerable stress. Calving, the onset of the lactation, and housing changes, all lead to a situation of depressed feed intake and eventually to a condition of negative energy balance (NEB) with loss of body condition, which may turn into ketosis and higher susceptibility to disease. Diet changes according to housing and different group of animals, and, furthermore, between the dry and milking periods, when the risk for developing SARA increases (Brand and Warner, 1996; Nocek, 1996). Other factors that show an interrelationship are neuroendocrine conditions, management and environment. Several neuro-peptides controlling voluntary feed intake and eating behavior must be considered among neuroendocrine factors; their effect may result in sorting out feeds from the diet.

**SARA in the early post-partum period**

Transition animals have been considered to be more prone to developing SARA if their rumen bacterial populations and papillae have not been gradually acclimated to a higher starch ration prior to freshening. Rumen papillae significantly increased in size and ability to absorb SCFA when animals were switched from a diet mainly of hay and straw (□70% neutral detergent fiber, NDF, converted from crude fiber according to Mertens, 1992) to a higher energy diet containing a mixture of grass hay and grain 2 wk prior to freshening (Dirksen, 1985; Dirksen, 1989). Starch was gradually increased and fiber reduced during the postcalving period. Rumen papillae appeared to reach their maximum length 4 to 5 wk postcalving. In vivo SCFA absorption rates performed 14 wk postcalving were substantially greater at this time compared with when cows were fed the hay-straw diet. Andersen et al. (1999) changed the diet fed to dry cows from a grass-silage based diet (approximately 64% NDF, converted from crude fiber according to Mertens, 1992) to one supplemented with a small (ration approximating 55% NDF, 11% non-fibre carbohydrates; NFC) or large (ration approximating 38% NDF, 38% NFC) amount of barley grain and concentrate at 4 wk precalving. Allowable intakes were relatively low, ranging from 6.5 to 9.4 kg DM during this period. Postcalving, cows in both treatments were offered grass silage ad libitum and 8.8 kg of grain. In contrast to the results seen by Dirksen (1989), increased carbohydrate levels did not result in any macroscopic or histologic differences in rumen papillae between treatments. Eight days postcalving, cows fed the additional grain during the prefresh period had lower DMI and a more rapid decline in ruminal pH following the morning feeding than control cows. The authors concluded that the lack of rumen papillae response may have been because grain levels were not increased enough during the prefreshening period. Stone et al. (2003) evaluated papillae size and their ability to absorb valerate in 4 Holstein heifers during the prefresh period and for the first 5 wk of lactation. Substantial variability occurred in papillae size and in ruminal absorption of valerate within individual heifers between consecutive weeks during the first 5 wk postcalving. Much of this

variation appeared to be related to postfreshening health disorders. A common recommendation based largely on the papillae results from the Dirksen studies (1985, 1989) is to gradually increase NFC levels over a 5-wk period during the pre- and postcalving time periods. However, cows started these studies with poorly developed papillae, having been fed a 70% NDF diet composed of grass hay and straw. Less time is probably needed to develop papillae when animals are fed rations that contain higher levels of rumen-fermentable carbohydrates during the early part of the dry period. At freshening, slightly more (□1 to 3 percentage units) forage NDF and physically effective NDF (peNDF) (□1 percentage unit) than what is contained in the lactating cow total mixed ration (TMR) is commonly fed to fresh cows in an attempt to minimize ruminal health disorders (SARA and displaced abomasum). More research is needed to better define the relationships between DMI, carbohydrate amount and ruminal fermentability, ruminal mat formation, and rumen papillae development in transition cows. Fresh animals are also at an increased risk of developing SARA if component-fed compared with being fed a TMR. Due to the increased rate of consumption, less saliva is produced per unit of feed consumed when grain is fed separately from forages. Additionally, animals fed ingredients separately may consume all of the allotted grain and leave some of the forage (Maekawa et al., 2002). The net result is the consumption of a diet containing less forage than intended, increasing the risk of SARA. Cows rarely ruminate for more than 9 h, with 10 h proposed as the physiologic limit (Welch, 1982). Salivary flow rates are highest during rumination (1.8 times resting rate), followed by eating (0.18 to 0.22 L/min; Cassida and Stokes, 1986; Maekawa et al., 2002), and then resting (0.10 to 0.15 L/min; Cassida and Stokes, 1986; Maekawa et al., 2002). Although the total flow of salivary buffers reaches an apparent maximum, the intake of rumen fermentable carbohydrates, and hence ruminal acid production, increases with increasing DMI (Beauchemin, 1991; Oetzel, 2000). Firkins (2002) used regression equations evaluating relationships between DMI, carbohydrate digestibility, and chewing time (Firkins et al., 2001) to predict that an increase in DMI would still result in an increase in ruminal degradable

starch, despite a reduction in the percentage of ruminally degraded starch caused by the increase in passage rate. Thus, high-producing cows may be at an increased risk of SARA due simply to higher DMI.

**SARA in mid-lactation**

In mid-lactation the development of SARA is linked to managerial factors-like feeding frequency, processing of feed, e.g. pelleting, and housing and similar influences. The term 'ration formulation and delivery acidosis' is therefore used (Nordlund et al., 1995; Oetzel, 2000). Also here, SARA may occur as the intake of easily fermentable concentrate feedstuffs meets a non-adapted ruminal environment. Because the rumen in mid-lactational cows is usually well adapted to the uptake of concentrates as included in the diet, other factors are contributing to the occurrence. Mistakes in automatic feeding or incorrect preparation of total mixed rations are some of the documented issues accidentally leading to this problem. In herds fed on a component-based diet, the rations not only may be too high in the concentrate-component, but on the other hand also fibre-deficient (Nordlund et al., 1995; Garrett, 1996). Several reasons can lead to this undesired situation. The proportion of components may be miscalculated. There is chance that so the real weight of the forage, if only estimated by volume, does not meet the requirements as a result of falsely calculated dry-matter base. This may result in an insufficient fibre-uptake or, inversely, a concentrate-intake, which is relatively too high (Garrett, 1996). The time-schedule of feeding has a proven impact on ruminal pH (Yun and Han, 1989). Therefore, the decision to feed smaller concentrate portions more frequently will have a considerable impact on the arising of an acidotic ruminal environment. Also the time difference between concentrate and roughage feeding is of importance for regulation of the ruminal pH (Nordlund et al., 1995). The animals themselves may behave in such a way that they take up an imbalanced ration: in larger groups, with only limited access to the feedstuffs, the socially higher cows will eat first and longer, and, in case of component-feeding, take up relatively

more concentrates and less fibre. Greediness of the animals housed in groups and sometimes even weather changes are known to exacerbate this problem (Underwood, 1992; Nordlund et al., 1995). In TMR-fed herds an over-mixing of the ration is documented: instead of letting the ration being mixed for some minutes, the mixing wagon may work up to an hour. This inevitably produces a diet very low in structure with high palatability. It will easily be taken up by the animals, thus not providing the necessary buffering saliva flow, giving less buffering capacity of the feedstuffs within the reticulo-ruminal compartment (Nordlund et al., 1995; Garrett, 1996). The ability and tendency of cattle to sort smaller roughage particles out of TMR is documented (Leonardi and Armentano, 2003). Under certain circumstances this could contribute to an arising problem of SARA because of the mechanisms mentioned above. Therefore it can be stated that SARA might occur as well in the mid-lactation, thus in the phase of highest DMI. More than in the early post-partum period, it is almost exclusively related to management errors. Especially in high-producing dairy herds, where there is a narrow fibre to concentrate ratio, the impact of reduction of the remaining forage proportion may have severe consequences. These factors may cause just a transient SARA-situation in the herd, which usually will pass unnoticed. The depression of feed-intake going along with SARA, however, may cause clinically detectable acidosis, too (Garrett, 1996). Especially in larger herds, a considerable factor is the personnel responsible for the feeding of the cows. Changes in the responsibility for feeding may lead to differences in feeding schedule, proportion of roughage and concentrate, having a considerable impact on an unstable ruminal environment. Although mainly described in feedlots (Elam, 1976), this also could apply to high-producing dairy herds and should therefore be regarded as a hazard in the herd health management.

**Clinical signs of SARA**

Clinical signs of SARA include decreased DMI, laminitis, rumenitis, liver abscesses, pulmonary bacterial emboli (Nordlund, 1995) and, furthermore, displacement of the abomasum (Sarashina et

al., 1990), mastitis and metritis (Enemark et al., 2002), low fertility (Britt, 1995). These problems are linked to rumen pathology because of the exceptional osmotic capacity shown by the rumen and the increase in the adsorption rate of ruminal products, which may have toxic and vasomotor effects. However, the factors associated with SARA vary, and provide ambiguous clinical signs, thus defying definitive diagnosis based only on clinical signs (Garrett et al., 1999).

***Decreased DMI, loss of condition***

Often, decrease of DMI is given as a consistent clinical sign, as a sensitive indicator of ruminal acidosis (Garrett, 1996; Stock, 2000; Garry, 2002). A Swedish study showed a lower feed intake in dairy cows post-calving fed on a ration higher in concentrate, compared with cows fed on a low-concentrate diet (Olsson et al., 1998). A recent study revealed a 25% decrease in the intake of a TMR during SARA-periods induced, compared with normal. Moreover, the digestion of feedstuffs was impaired in general (Krajcarski-Hunt et al., 2002). The reasons for the lower DMI have to be seen in weaker rumen motility, inhibited by certain mechanisms arising during low pH phases within the reticulo-ruminal environment. It has been proposed that the high production of SCFA in ruminants fed on high-concentrate diets leads to a reduction of rumen motility (Slyter, 1976; Fürll et al., 1993). Bacterial endotoxins have been related to the decrease of rumen motility. The principles of rumen hypomotility involving toxaemia in cases of coliform mastitis have been documented (Verheiden et al., 1981; Hoeben et al., 2000). Also histamine has been reported to inhibit rumen motility in sheep after intravenous infusion in a Polish study (Kania et al., 1994), confirming the results of other studies (Underwood, 1992). Another explanation for the decrease of DMI has been given by Owens et al. (1998). The described increase of osmolarity of ruminal content as a result of reduced absorption and increase of osmolarity active substances-like glucose, SCFA or lactate could lead to a flow of fluid into the rumen, which in turn reduces the feed intake of the animal affected further. The rise of butyric acid in the blood will lead to a further decrease of DMI, a situation that has also been reported for the pathogenesis of abomasal displacement (Van

Winden et al., 2003). Subacute ruminal acidosis is often named as one reason for a low body condition (Nordlund et al., 1995; Nocek, 1997; Oetzel, 2000). On the contrary, some authors characterize animals affected by 'chronic ruminal acidosis' as becoming obese because of a narrow C2/C3 relation in ruminal fermentation and associate it with fat-cow syndrome (Dirksen, 1985; Gäbler, 1990). In beef-cattle, the narrow C2/C3 relation is to a certain extent desired in order to maintain the necessary body weight gain, but it has to be managed carefully, because it is an unstable situation which may affect the DMI, negatively influencing the body weight (Stock, 2000). The same situation may arise in dairy cattle, in which, however, the narrow C2/C3 pattern leads to a milk-fat depression (MFD) and an increase of body condition (Dirksen, 1985). A deterioration in body condition often occurs during NEB. This is reported as the more dramatic the more the animals tend to become overfed in dry-period and therefore are in more severe NEB in early lactation (Rukkwamsuk, 1999). Therefore, a certain interaction seems to be possible. Both complexes, NEB and SARA are associated with decrease of DMI and related to the post-partum period. An interaction via the DMI exaggerating the one or the other problem could be hypothesized. Obviously, the described increase of body condition in dairy cows experiencing subacute acidosis only applies to the late-lactation and dry-period. An increase of body condition will not be achieved in an early lactational stadium. This overcondition of the animals, however, may later increase NEB and its effects and eventually lead to the fat-cow syndrome. It appears also likely that those animals may develop a subacute acidosis, possibly by selection of concentrates from their diet. This would lead to a further decrease in body condition as found at reduction of DMI. Therefore, it appears to be useful differentiating a type of subacute acidosis, may be better characterized as chronic, mainly in beef-cattle and late-lactation cows promoting a gain in body condition from the SARA leading to the described loss in body condition. Reasons for poor body condition mentioned may be chronic inflammation, which may presumably antagonize growth by releasing cytokines generally opposing anabolism as well as decreasing DMI in a situation of impaired general health (Webel et al., 1997; Oetzel, 2000). A farmer may try to correct the possibly

occurring loss of body condition, originating from SARA, by increasing the energy level of the ration, thus exaggerating the basic acidosis problem (Nordlund et al., 1995).

***Laminitis***

Lameness is very prevalent throughout the dairy industry. Clarkson et al. (1996) assessed the incidence and prevalence of lameness in 37 dairy herds in the United Kingdom. The mean annual incidence of lameness was 54.6%, while the prevalence was 20.6%. Prevalence rates were higher in the winter (25%) than in the summer (18.6%). Lameness prevalence averaged 15.2% in 17 Minnesota and Wisconsin dairy herds, 2.5 times higher than the rate estimated by herd managers (Wells et al., 1993). Warnick et al. (2001) found the incidence of lameness in 2 New York herds to be 52 and 40% over a one and a half year period. Cook (2002) reported that lameness prevalence averaged 24.8% during the winter and 21.8% during the summer in 30 Wisconsin dairy herds. Laminitis-related hoof problems (sole ulcer, white line abscess, and solar hemorrhage) are typically one of the leading causes of lameness (Clarkson et al., 1996; Smilie et al., 1996; Warnick et al., 2001). For example, in a study involving 13 Ohio dairy herds, all of the herds and at least 62% of the evaluated cattle had hoof lesions associated with laminitis (Smilie et al., 1996). Laminitis has been associated with nutrition, specifically with acute and subacute ruminal acidosis (Nocek, 1997; Vermunt, 2000). Although the exact relationship between SARA and laminitis is not known, one of the theories relates SARA-induced damage to the ruminal epithelium, allowing for the absorption of histamine and endotoxins. These and possibly other compounds disrupt normal circulation and cause inflammation within the hoof, leading to the condition commonly referred to as laminitis (Vermunt, 1992). There is a certain relation between the body condition of dairy cows and development of non-infectious laminitis. Gearhart et al. (1990) found cows being overconditioned at drying off at greater risk for foot problems. However, no differentiation of the type of foot-problems was made. Laminitis in SARA-affected cows is described as having a subacute and sometimes chronic character depending on the duration of disease. Discoloration of the hoof,

haemorrhages in the sole, ulcers and abscesses, misshapen hooves or double-walled soles have been reported to occur in SARA-confirmed patients (Nordlund et al., 1995; Oetzel, 2000). The presence of SARA should therefore be suspected in herds with a high incidence of animals showing clinical lameness or the lesions described. It has to be taken into account, however, that the visible lesions occur in delay to the insulting period. Other factors related to the pathogenesis have also to be examined, for the pathogenesis of the laminitis still remains uncertain (Garrett, 1996). Because published data pertaining to the prevalence of SARA, lameness in general, and specific causes of lameness, along with losses caused by each of these disorders, are scant, it is difficult to estimate the cost of these disorders to the US dairy industry. Warnick et al. (2001) explained that studies investigating the cost of lameness have yielded inconsistent results, probably due to variations among trials in culling bias, lameness measurements, herd management, methods used to estimate milk loss, and statistical methods.

***Parakeratosis–rumenitis–liver abscess complex, culling rate***

Subacute ruminal acidosis is associated with inflammations of different organs and tissues in dairy cows. A physical examination may reveal the presence of subcutaneous abscesses, which are not related to injections (Nordlund et al., 1995). Non-acute forms of ruminal acidosis have been reported to be associated with liver abscesses (Rossow, 1984; Dirksen, 1985; Nordlund et al., 1995; Garry, 2002; Oetzel, 2000). Abscesses or inflammational processes may also be found in kidneys (Rossow, 1984; Oetzel, 2000), the lungs (Nordlund et al., 1995), and the heart and kidneys (Oetzel, 2000). Moreover, haemoptysis and epistaxis are reported to occur in herds diagnosed as affected by SARA. These have to be related to either bacterial pneumonia or caudal vena cava syndrome (Nordlund et al., 1995; Oetzel, 2000). This complex can manifest itself either in an affect of the general condition of the animals, thus contributing to the described negative body condition in SARA-herds, or in a high culling rate. In the US dairy herds, annual culling rates up to 31% and

annual turnover greater than 45% have been observed. Reasons were indistinct, non-responsive pathological conditions and loss of body condition (Nordlund et al., 1995; Garrett, 1996). The reason for the bacterial-induced, multifocal pathology has to be seen in relation to the changes in the ruminal mucosa, referred to as the rumenitis–liver abscess complex (Dirksen, 1985; Gäbler, 1990; Nagaraja, 2000). The pathogenesis of this disease complex has been well researched and described, mainly in beef cattle and veal calves. The term should therefore be extended to 'Parakeratosis–rumenitis–liver abscess complex' for the initial insult on the ruminal mucosa is a parakeratosis of the ruminal epithelium (Scanlan and Hathcock, 1983; Szazados and Takacs, 1978; Tamate et al., 1978; Szemeredy and Raul, 1978). It was shown that the growth of ruminal epithelium is directly linked to the SCFA-presence in the tissue. Propionic and butyric acid promote the growth of the ruminal papillae thus providing a higher absorption from the rumen by the mucosa (Dirksen et al., 1984). During phases of acidosis, however, those SCFA in the rumen are present in larger amounts. This is believed to lead to a parakeratosis of the ruminal epithelium, exaggerated by the presence of processed, e.g. pelleted, feedstuffs provoking the mucosa (Gäbler, 1990). The parakeratosis eventually leads to rumenitis, particularly to the presence of microabscesses within the ruminal mucosa (Szemeredy and Raul, 1978). After a period of increased SCFA-absorption, the pathological alterations of the rumen epithelium eventually hinder the resorptional activity thus exaggerating the acidity of the medium (Dirksen et al., 1984). The function of the ruminal mucosa as a barrier between ruminal environment and bloodstream is impaired, enabling bacteria to translocate via the ruminal mucosa into the portal blood flow, colonizing the liver tissue and from there spreading to other tissues in the body-like heart, lungs and kidneys (Nordlund et al., 1995; Nocek, 1997). Microbiological studies have shown that the bacteria recovered from those abscesses resemble those from the rumen and the microabscesses within the ruminal mucosa, respectively. Mainly present are *Fusobacterium necrophorum* and *Arcanobacterium pyogenes* (Scanlan and Hathcock, 1983; Szazados and Takacs, 1978; Nagaraja, 2000). It may therefore be stated that SARA predisposes to bacterial colonization, first of the liver tissue. From there, other organs may

be colonized, leading to a generalizing abscessation within the ruminant body. The economic consequences are milk yield loss, high rates of culling and annual turnover within the dairy herd. The loss may be exaggerated due to condemnation of the carcasses at meat inspection. The fact that non-acute acidosis by some authors is characterized to fatten the animals, whereas other authors tend to characterize it as leading to decreased body condition has again to be mentioned here. It seems possible that developing parakeratosis, hindering the SCFA-resorption, plays a decisive role. Because it is also due to the high levels of SCFA in the ruminal compartment, it seems possible, to expect a gain of condition in a situation with high SCFA-levels and low parakeratosis. Once the parakeratosis is severe enough to hinder the resorption, it may influence the body condition in a negative way (Dirksen et al., 1984; Dirksen, 1985; Gäbler, 1990).

***Alterations in faeces, diarrhea***

The fact that faeces of cattle affected by acute as well as SARA change has been well described (Rossow, 1984; Dirksen, 1985; Nordlund et al., 1995; Garry, 2002; Oetzel, 2000). The structure and consistency of the faeces depend on rumination, activity of the ruminal flora and ruminal passage (Garry, 2002). The changes are described as alterations in colour, which appears brighter and yellowish. The pH of the faeces is lower than normal, usually slightly acidic (Dirksen, 1985). The smell of the faeces is said to be sweet–sour (Oetzel, 2000). The size of ingesta particles may be too large, being around 1–2 cm instead of less than 0.5 cm. Whole cereal grains may be present. The alterations are usually transient in nature (Garry, 2002). One explanation for this phenomenon is post-ruminal fermentation in the intestines because of a massive outflow of fermentable carbohydrates from the rumen (Oetzel, 2000). Another explanation could be the high osmolarity, which is described for the ingesta in SARA-affected animals, which could lead to soft faeces, due to binding of fluid in the intestinal lumen (Garry, 2002). Generally speaking, the impaired ruminal

function as mentioned above in terms of rumination, bacterial breakdown and passage, leads to the alteration in faecal aspects.

***Milk-fat depression***

A depression of milk-fat percentage in cows affected by SARA or generally non-acute forms of ruminal acidosis, respectively, has been documented (Dirksen, 1985; Nordlund et al., 1995; Chalupa et al., 2000; Oetzel, 2000). Because it usually occurs in individuals, the decrease of milk-fat remains undetected in the bulk tank testing (Garrett, 1996; Nocek, 1997). The fact that there are alterations in the ruminal fermentation patterns in SARA, has been hold responsible by some authors for this depression (Rossow, 1984; Dirksen, 1985; Gäbler, 1990). Also transient depression of the daily milk production has been reported in cases of SARA (Oetzel, 2000). The fact that the feeding largely influences the milk-fat content is obvious. The terms 'low-milk fat syndrome' and 'milk-fat depression' (MFD) are frequently used to describe a situation where there is a considerable depression in milk-fat, largely because of mistakes in feeding strategy (Baumann et al., 2001). The following reasons for low-milk fat syndrome have been defined: (1) feeding of a ration high in energy but deficient in roughage, (2) feeding of processed roughage, e.g. by pelleting and (3) supplementation of unsaturated fatty acids (Gürtler and Schweigert, 2000). A number of experiments have shown the depression of milk-fat being a sequel to a change in the ration. The increase of concentrates or the processing of roughage usually reduced milk-fat content. Milk-fat in an experiment producing a depressing situation developed as being low as 1.09–2.19% (Van Beukelen et al., 1986). The MFD was accompanied by a number of changes in the ruminal fermentation pattern. Generally spoken, the amount of acetate appearing in fermentation was reduced, while propionate level increased (Van Beukelen et al., 1985; Murphy et al., 2000; Khorasani and Kennelly, 2001). The levels of butyrate have been documented to rise (Van Beukelen et al., 1985; Murphy et al., 2000), although in some studies the butyrate showed a decreasing level together with the acetate (Storry et al., 1974; Kennelly et al., 1999). In all

experiments the ruminal pH usually dropped. In one study, the drop in milk-fat was accompanied by an increase of milk yield and a body weight gain (Van Beukelen et al., 1985). Adding buffering substances to the high-concentrate diet prevented the milk-fat content from dropping and re-established a higher ruminal pH, respectively (Rogers et al., 1982; Van Beukelen et al., 1985; Khorasani and Kennelly, 2001). It was concluded that the addition of buffering substances prevents the forming of *trans*-C18 : 1 fatty acids, which are suspected to inhibit the synthesis of milk-fat in the mammary gland (Kennelly et al., 1999). There is a certain association between SARA and the reported MFD. Both arise in situations in which a diet high in concentrate, low in fibre or structured fibre, respectively, is fed to the dairy cows. The question remains, however, if MFD can be viewed as a sign of SARA. Although the ruminal pH is reported to drop in experimental situations of inducing low milk-fat, this alone does not justify those situations to be characterized as being SARA. It seems justified to state that SARA may develop in the same situations where low milk-fat syndrome is likely to occur, rather than to interpret MFD as a sequel of SARA itself. A depression of milk-fat is not dependent on the presence of SARA. It seems that MFD also may occur in situations in which the adaptation of the ruminal flora prevents the development of SARA with its clinically detectable consequences.

**SARA diagnosis**

SARA is possibly under diagnosed because of lack of pathognomonic signs, diurnal fluctuations in rumen metabolism, and problems in obtaining representative rumen fluid samples (Jorgenson et al., 1993; Nordlund and Garrett, 1994). The symptomatology of SARA has been described above. Summarized, SARA-affected herds may present a depression in DMI and increase of diarrhea, change in faecal features and higher incidence of laminitis and associated locomotive problems, a depression of milk-fat present rather in individuals than in the herd as a whole, a high-culling rate and turnover because of non-specific causes-like inflammations, poor performance or poor body condition. Any of these problems may be the initial complaint of the farmer, leading to a closer

inspection, which eventually results in diagnosis of SARA. It is not possible diagnosing SARA basing on a single sign or symptom, but the only way to obtain a correct diagnosis is to observe and correlate many aspects (Oetzel, 2000; Morgante et al., 2007): correct collection of the history and clinical analysis of the herd, diet evaluation (NDF, NDF from forages, NFC) and examination of ruminal fluid; this is the most important test because it is the only one that allows us to assess the condition of the rumen in that particular moment (Nordlund and Garrett, 1994).

***Rumen fluid collection***

The determination of ruminal pH is a key factor for the diagnosis of SARA (Enemark et al., 2002). Ruminal pH varies considerably throughout the day (Keunen et al., 2002; Oba and Allen, 2000), with decreases occurring following eating and increases occurring following rumination. The method of measuring the pH of ruminal fluid influences the pH measurement. The pH reading of ruminal fluid varies between in vivo and in vitro measurement locations. In vitro results were 0.10 (McArthur and Miltmore, 1968), 0.11 (Dado and Allen, 1993), and 0.28 units (Smith, 1941) higher than in vivo recordings. Measurements for pH were conducted immediately following ruminal fluid collection from the rumen in each of the studies with the exception of Smith (1941), where the measurement was taken within 30 min of collection. Smith speculated that the difference between in vivo and in vitro measurements was due to loss of CO2 by the in vitro samples prior to recording the pH. Common field techniques for collecting rumen fluid for SARA diagnosis include rumenocentesis, by percutaneous needle aspiration (Nordlund and Garrett, 1994) and oral stomach tube (Nocek, 1997). Rumenocentesis is reported to be superior to the use of an oral stomach tube for the determination of rumen pH as the latter technique is susceptible to saliva contamination (Nordlund and Garrett, 1994). The pH of ruminal fluid collected via rumenocentesis was 0.15 (Nocek, 1997) and 0.28 (Garrett et al., 1999) units higher than that collected through a rumen fistula from the same region of the rumen. These results, taken together with the in vivo/in vitro results previously discussed (McArthur and Miltmore, 1968; Dado and Allen, 1993), indicate that the pH

obtained via rumenocentesis is approximately 0.3 units higher than the in vivo pH at the same location in the rumen. The relationship between the ruminal pH of samples collected via rumenocentesis and samples collected from the same area in the rumen with a jar was significant, but not as strong as ideal (R2 = 0.52). Additionally, the lowest pH in the data set used to derive the relationship was 5.9, a value greater than the pH of SARA (Garrett et al., 1999).

Therefore, according to Garret et al. (1999) and Duffield et al. (2004), rumenocentesis may be useful for the collection of rumen fluid for pH determination. Scientific interest in animal health and welfare is rapidly grown in recent years. Many scientists agree that animal health and welfare involve the subjective feelings of animals, so that welfare will be reduced by negative subjective states such as pain and fear, and will be improved by positive states. Also human handling, like diagnostic investigations, could induce pain and fear. It is then very important to investigate if a diagnostic technique is invasive or dangerous for animal health and welfare. The opinions on the potential risks associated with the procedure of rumen collection fluid are controversial. Many authors (Hollberg, 1984; Aceto et al., 2000; Strabel at al., 2006) consider rumenocentesis as an invasive technique and several practitioner consider it too difficult to be introduced in normal clinical investigations. Nordlund and Garrett (1994) judge rumenocentesis being an invasive procedure, potentially endangering the animal health and welfare by sequelae as peritonitis, abscesses in the abdominal an ruminal wall and injury due to the necessary restraint. They report, however, the incidence of subcutaneous abscesses being 1-2 %, while Kleen et al. (2004) reported this alterations on 5.5 % of the their study population. Duffield et al. (2004) describe nodules at the puncture site after rumenocentesis, 1-2 cm in diameter, in one third of the tested individuals. Other alterations, local or general, have not been observed. However evaluation of animal welfare actually remain a controversial and difficult argument: many scientists agree that measures based on biological functioning and on the animal's ability to cope with the environment (for example by thermoregulation) provide relevant information.

***Rumen pH evaluation for diagnosis of SARA***

In a situation of induced SARA, the most useful cut off point to differentiate animals as SARA-positive has been identified as being pH 5.5, for the difference between SARA-induced animals and a control group was largest here. Therefore, $pH \leq 5.5$ should be considered as SARA-positive, at $pH \geq 5.8$ it is considered negative (Garrett, 1996). In a situation where in which farmers have complaints about certain, herd-related problems and SARA is suspected, a screening of the herd or a defined risk group, e.g. the early lactational group seems to be the action of choice, rather than examination, diagnosis and treatment of the individual. According Nordlund and Garrett (1994), the suitable sample size was evaluated and found to be 12, delivering reliable results at justifiable expense. The prevalence in the herd should be regarded as high and thus the herd as SARA-affected if at least three of the sampled 12 cows are found to have a ruminal pH equal to or lower than 5.5 (Nordlund et al., 1995; Garrett, 1996). The ideal time point of sampling the cows depends on the type of ration fed. To sample, it should be aimed at the time when the ruminal pH has dropped to the lowest level. This is achieved by sampling cows within 2–4 h after the concentrate meal in herds fed separate components, within 5–8 h in TMR-fed herds (Nordlund and Garrett, 1994; Garrett, 1996). The choice of animals depends on the type of risk-group which is suspected. Problems in early lactational stages should lead to selection of animals, which are about 3 weeks fed on the lactational diet, the sampling group in mid-lactation would consist of animals being adapted to the ration and 45–150 days in milk. A combination of those groups in order to deliver a thorough diagnosis is, however, recommended (Nordlund et al., 1995). Another scheme is proposed by Garrett (1996). Here, a sampling of cows between 2 and 180 days in milk is recommended, which would cover both risk-periods.

***Haematochemical and haematological parameters during SARA***

Although in the recent years many studies have been published on this pathology in dairy cattles (Krause et Oetzel, 2005; Cottee et al., 2004; De Frain et al., 2002), few studies have investigated the relationship between haematochemical and haematological parameters and an altered rumen state (Patra et al., 1993). The effect of grain-induced SARA in mid lactation dairy cows promotes lysis of gram-negative bacteria that stimulate the inflammatory response (Gozho et al, 2007).

The rumen microbial flora is characterized by a dominance of Gram-negative bacteria even though the number of Gram-positive bacteria is increasing (Owens et al., 1998); in contrast, in the acute clinical rumen acidosis the rumen microbial flora is dominated by gram-positive bacteria (Enemark et al., 2002). It was reported that several blood parameters are supportive of the ruminal pH findings in dairy herds during subacute ruminal acidosis; however no parameter was specifically indicative of SARA (Enemark et al., 2004). The use of blood parameters-like base excess (BE) or blood pH may be relevant in diagnostic of non-acute ruminal acidosis. Because of an absorption of SCFA by the ruminal wall, the BE may be reduced by neutralizing the acids, therefore, the examination may reveal a compensated metabolic acidosis (Dirksen, 1985; Owens et al., 1998). However, under certain circumstances the blood pH may be decreased as well (Rossow, 1984). It is well known that variables such as breed, stage of growth, age, reproductive status and stage of lactation have an influence on many blood parameters (Doornenbal et al., 1988). The use of certain blood parameters as indicators of the physiological, nutritional, metabolic and clinical status of farm animals is gaining a wider application.

**Prevention of SARA in dairy herds**

Preventing the occurrence of SARA in dairy herds is pending between two philosophies of dairy husbandry. On one hand, the aim of producing high milk yields from dairy herds requires the feeding of high-energy diets to dairy cattle. By constant genetic improvement the modern dairy

cows are able to produce high milk yields, which in turn require an excessively high intake of dry-matter, rich in energy. Therefore, the feeding of rations high in concentrate is necessary. This is, however, predisposing the animals to develop some kind of pathology because of an overfeeding (Oetzel, 2000; Stock, 2000). The development of prevention strategies against ruminal acidosis therefore is highly desirable (Owens et al., 1998; Stock, 2000). On the contrary, it has to be considered that dairy cows as ruminants are designed in evolution to digest roughage, not concentrate as, e.g. grains. The question thus remains if the feeding strategies applied today are in fact desirable and sustainable. In fact, those strategies confront the ruminant with feedstuffs the ruminant's digestive system is not developed (Dirksen, 1985; Garry, 2002). In any case, farmers, veterinarians and nutritionists have to consider the problems involved with modern dairy-nutrition strategies. Also the public, who develops a certain conscience of animal husbandry and the production methods applied today in dairy and any other food-producing section, has to be considered in this problem. The prevention of SARA can be described by two principles: first, to allow a proper adaptation of the ruminal mucosa in the post-partum period and the microflora in order to absorb and metabolize the high levels of SCFA arising. Secondly, to keep the ruminal pH in physiological ranges in spite of high-energy intake. In other words, the mechanisms applied consist either of prevention of a fermentation disorder or in regulation of the fermentation process by direct intervention. The first part can be achieved mainly by management of husbandry and feeding, the second, by maintaining fibre-content of the ration within defined borders or adding certain substances to the ration (Nocek, 1997). It is clear that prevention of SARA is a task involving the whole herd (Oetzel, 2000).

***Feeding management for prevention of subacute ruminal acidosis***

The adaptation of the ruminal mucosa to a concentrate-rich diet takes about 4–6 weeks to develop (Nordlund et al., 1995; Nocek, 1997). The bacteriological changes are said to take place within 3 weeks (Dirksen, 1985; Nordlund et al., 1995). A gradual adaptation of dry cows to the lactational

ration is necessary in order to ensure not to overtax the changes within the forestomach. In relatively small herds, fed on TMR, problems may arise. In order to keep the labour to prepare the rations low, the number of ration types may be reduced to one lactating and one dry cow ration. The periparturial switch from one type to another is likely to overtax the ruminal adaptation resulting in SARA or even acute acidosis (Nordlund et al., 1995). However, there is certain evidence that an adaptation to lactational concentrate diets is difficult to achieve prior to parturition. Experiments conducted by Andersen et al. (1994) and (1999) showed no beneficial effects of concentrate feeding in the dry-period. There are, of course, many other possibilities influencing the complex of adaptation and herd management. In any case, the veterinarian confronted with SARA suspected cows should be aware of this and analyse the herd management to define risks arising from here. In Germany for dairy cows a proportion of at least 18% crude fibre in dry-matter is recommended, with at least two-thirds being structured, not processed. This ensures the performance of a dairy herd, for the C2/C3 relation in ruminal fermentation pattern should at least be 2.0, with a butyrate proportion not greater than 15% (Dirksen, 1985). In the Netherlands and Belgium a system based on the structure value of feedstuffs has been used since the 1990s (De Brabander et al., 2002). In component feeding, care should be taken of the sequence of the feedstuffs. It is recommended to have some roughage be fed before the concentrate component is given. This applies especially in the morning (Nordlund et al., 1995). In a TMR-based herd, care should be taken of especially of the time for preparing the ration. A mixing lasting only several minutes will deliver a ration sufficient in structure, buffering better within the reticulo-ruminal environment (Garrett, 1996). Additionally, a continuous monitoring of moisture and weight of the forage components will guarantee a suitable roughage to concentrate ratio (Nordlund et al., 1995).

Management-related problems

Management-related problems, such as free stall overcrowding, inadequately bedded or otherwise uncomfortable stalls, and excessive parlor holding times may also alter feed intake patterns and

animal behavior. Batchelder (2000) noted that cows kept in a pen with 30% more cows than free stalls ruminated less throughout a 24-h period than cows kept in a pen with one stall per cow. Of course, an attempt should be made to alleviate the environmental or management-related condition that is altering normal bovine behavior. If the problem is not corrected, dietary alterations can be made to increase ruminal pH. The degree of dietary adjustment depends on the severity of the environmental insult. An example of these changes would be to increase NDF and reduce NFC by approximately 2 to 3 percentage points, and to increase forage NDF and peNDF by approximately 1 to 2 percentage points, in herds with management-related cow behavior problems. Substituting some fibrous by-product feeds for higher starch containing feeds would reduce the risk of an increase in ruminal lactic acid levels. Rumen SCFA production could be further reduced by replacing a portion of carbohydrate with added fat.

***Feed additives for prevention of subacute ruminal acidosis***

Dietary buffers cannot eliminate the causes of ruminal acidosis, but they can help manage the problem (Krause and Oetzel, 2006). The most common buffer in dairy cattle rations, sodium bicarbonate, has been shown to increase DMI and milk production/milk fat percentage (Erdman, 1988). However, the response to feeding buffers depends upon the type of forage(s) fed and their physical structure. Buffer supplementation increased milk yield and milk fat yield when corn silage was the main forage, whereas results with grass/legume silages were inconsistent (Erdman, 1988; Staples and Lough, 1989). This difference in response could be explained by increased risk for SARA when corn silage is fed, as mentioned earlier. Also, added buffers are probably more likely to be beneficial in diets containing marginal amounts of effective fibre. An important aspect of maintaining a stable rumen environment is maintaining a balance between lactate production and lactate utilization by bacteria that convert lactate to less dangerous SCFA. Enhancing ruminal lactate utilisers reduces the risk for ruminal acidosis (particularly the acute form of ruminal

acidosis). Supplementation with specific yeast strains may enhance lactate utilisation within the rumen under certain dietary conditions (Dawson, 1995). Adding lactate to the diet or using feed ingredients high in lactate may improve the ability of the rumen to adapt to sudden increases in lactate production (Owens et al., 1998). Direct-fed microbials might also be used to provide a steady source of lactate in the rumen. A mixture of direct-fed microbials added to the rumen of dairy cows at the 1×105 dose increased corn digestibility and increased ruminal pH compared to higher doses of microbials (Nocek et al., 1999). *Selenomonas ruminantium* is one of the bacteria that convert ruminal lactate to VFA. *S. ruminantium* is apparently stimulated to utilise lactate by malate (Martin and Streeter, 1995). Although Martin et al. (1999) did not report any effect on ruminal lactate concentration, they did find that ruminal pH increased linearly with increasing malate supplementation in steers fed high grain diets. Supplementing diets with malate as a feed additive may be cost-prohibitive; however, incorporation of forage varieties high in malate may allow for economical inclusion of malate in dairy diets (Callaway et al., 2000).

Microadditives

Feeding supplemental biotin has resulted in a slight reduction in laminitis-associated lesions of the hoof (Midla et al., 1998; Potzsch et al., 2003). The mode of action of biotin in reducing white line disease, a consequence of laminitis, is thought to result from a strengthening of the extracellular cement of the white line tissues (Muelling, 2000).

The use of antibiotics in order to effectively prevent SARA in dairy cattle has been proposed, probably inspired by experience made in feedlots. Monensin and lasalocid have been used to prevent lactic acidosis (Nagaraja et al., 1981). The ionophores may be beneficial in dairy cattle if there is a poor transition to the lactating cow ration at freshening, or if there is slug-feeding occurring as during heat stress. They are not likely to alleviate SARA in dairy cattle where the reduced ruminal pH levels are from high ruminal SCFA and not lactate (Mutsvangwa et al., 2002).

Also tetracycline-like antibiotics have been proposed (Dirksen, 1985), peptide antibiotics-like thiopeptin proved to be effective in prevention (Dirksen, 1985; Garry, 2002). Owens et al. (1998) give an overview of antibiotic strategies. However, the use of these substances is obsolete, at least in the European Union, and in any case in lactating animals. Generally spoken, the use of feed additives, e.g. in pork production, is about to be banned completely within the EU and there is little acceptance by the consumer for this way of management. Moreover, milk withdrawal and other economic considerations stand against this use. Finally, the idea of using drugs in order to maintain ruminants functioning on a non-ruminant diet can be considered being questionable (Dirksen, 1985).

## TRIAL 1

## SUBACUTE RUMEN ACIDOSIS IN LACTATING COWS: AN INVESTIGATION IN INTENSIVE ITALIAN DAIRY HERDS

### MATERIALS AND METHODS

During winter 2003 – spring 2004 ten intensive Italian dairy herds were visited. The dairy herds were located in Northern Italy. The visit to each herd was organized in cooperation with the local veterinarian and the farmer to obtain the rumen fluid samples just 4-6 hours after feed distribution. In each farm a general investigation was performed, with particular attention to herd management and presence of typical clinical signs of SARA.

#### Farm and nutrition

Farms were selected with these characteristics: high average milk production (about 10000 Kg for year), over 100 lactating cows, housed in free stalls and in the early part of their lactations (first 60 DIM), use of TMR and adoption of steaming-up in the final part of the dry period. Principal characteristic of the ten dairy farms are reported in table 1.

**Table 1**. Characteristic of the ten commercial dairy farms

| Farm | Breed | Lactating Cows (num) | Milk production (Kg/year) | Dry period (d) |
|---|---|---|---|---|
| **1** | Holstein | 120 | 10000 | 40 |
| **2** | Holstein | 450 | 10000 | 60 |
| **3** | Holstein | 230 | 11000 | 60 |
| **4** | Holstein | 180 | 9500 | 40 |
| **5** | Holstein | 600 | 11000 | 60 |
| **6** | Holstein + Jersey | 100 | 9000 | 40 |
| **7** | Holstein | 950 | 10000 | 60 |
| **8** | Holstein | 140 | 11000 | 60 |
| **9** | Holstein | 500 | 8500 | 60 |
| **10** | Holstein | 220 | 10000 | 80 |

**Animals**

Twelve cows without clinical signs of disease, with good body condition and between 5 and 60 DIM, were randomly selected from each herd to perform rumenocentesis. Overall, 67 cows were between 5 and 30 DIM and 53 cows between 31 and 66 DIM. Ruminal fluid was sampled from each cow 4 to 8 hours after TMR distribution, a timeframe chosen because ruminal pH likely reaches its minimum. According the procedure of Edmonson et al. (1989) body condition score (BCS) was recorded for all the cows studied.

**Samples**

Ruminal fluid was obtained by rumenocentesis as described by Nordlund and Garret (1994) without sedation, using a 13G 105 mm needle (figure 1-4). Rumenocentesis was chosen because it is most the used technique, providing the most accurate results (Garrett, 1999; Enemark, 2002; Duffield et al., 2004). An aliquot of 8 ml of rumen fluid was immediately acidified with metaphosphoric acid (25%wt/vol) and stored at 4°C until the samples arrived to the laboratory where they were stored at –80°C until subsequent analysis. Two TMR samples were taken, one right after TMR distribution and one of the residual after 5-8h. Feed samples were stored –20°C until subsequent analysis.

**Figure 1.** Area of rumenocentesis

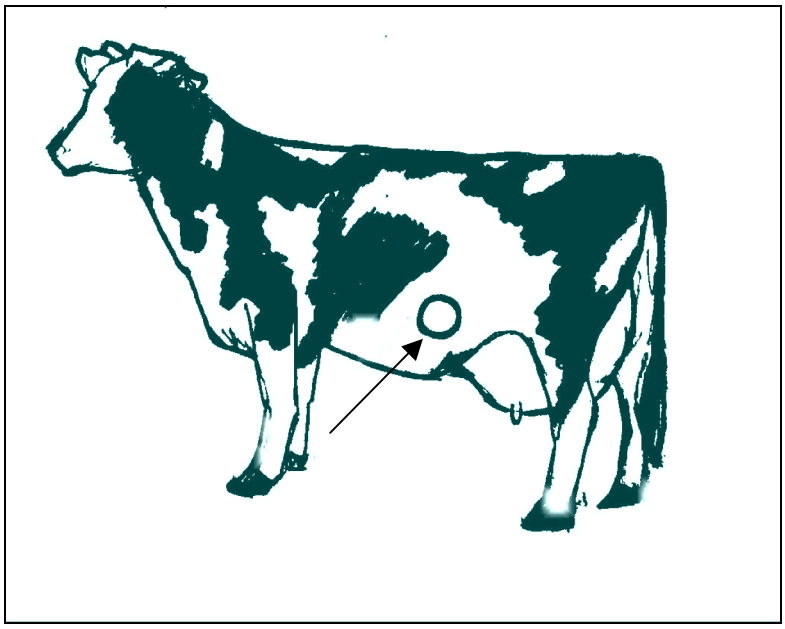


**Figure 2-4.** Principal steps of rumenocentesis

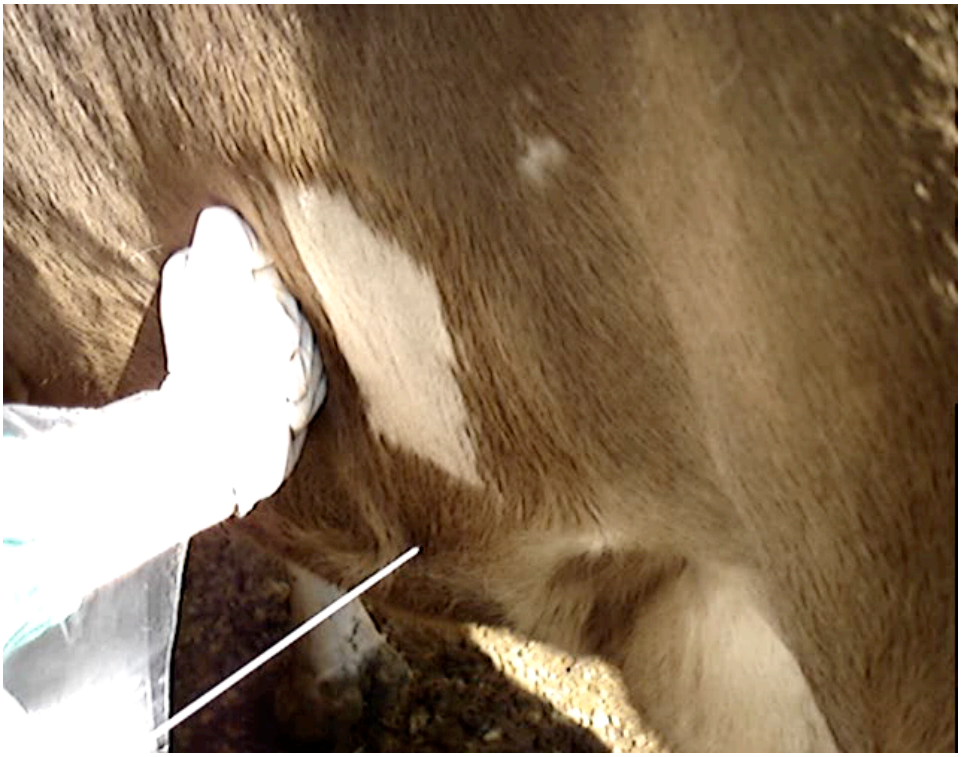


**Figure 2.**

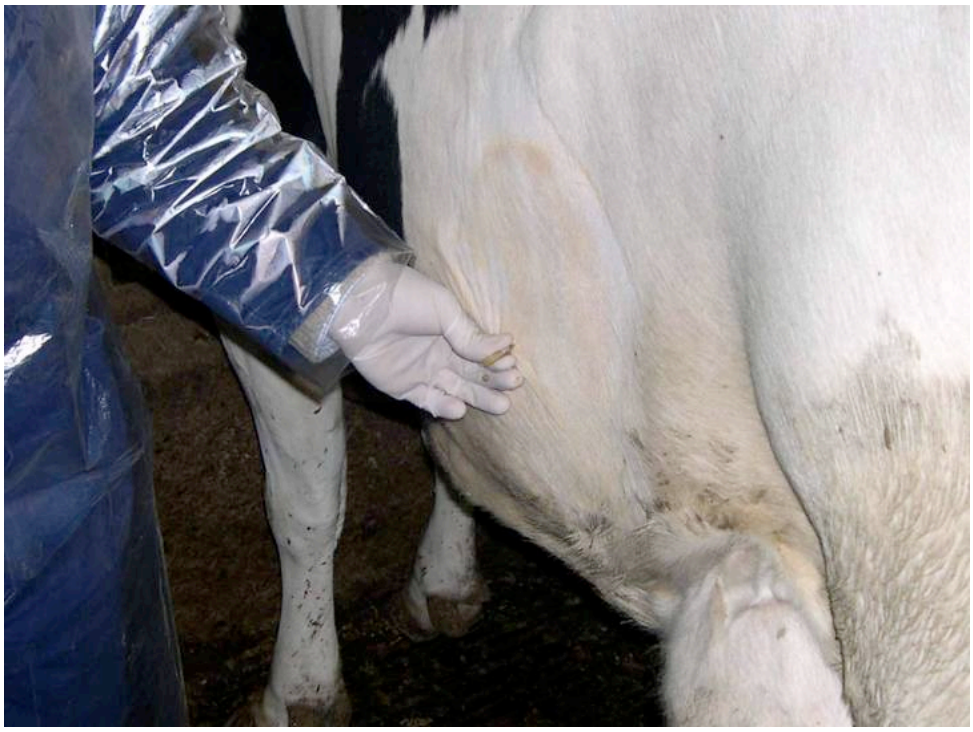


**Figure 3.**

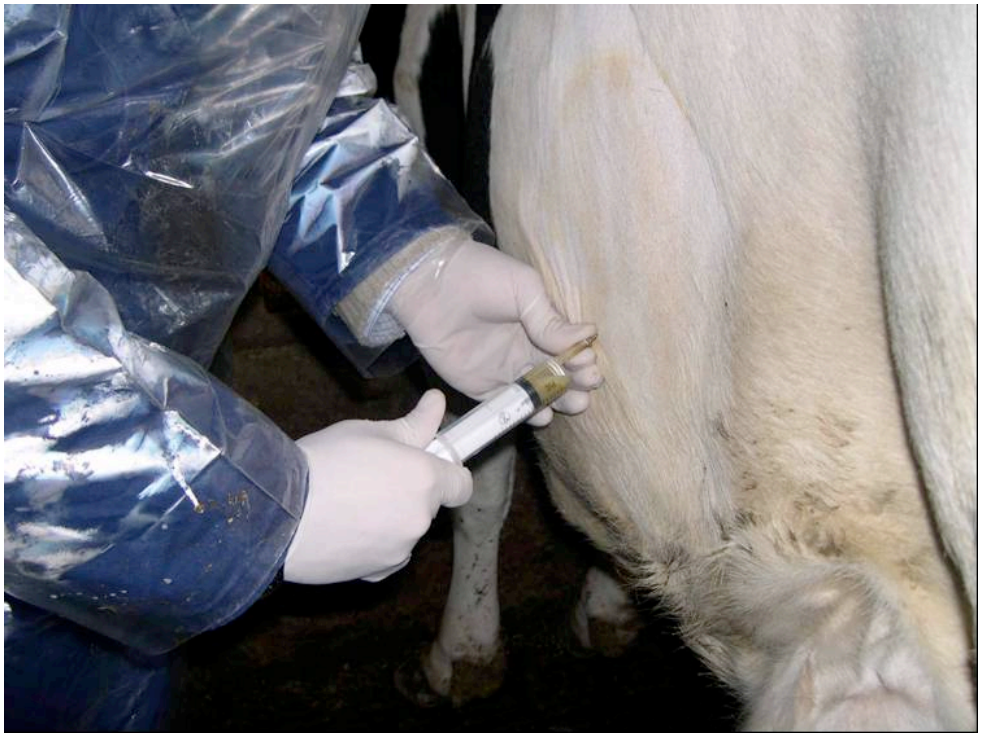


**Figure 4.**

**Field analysis**

Ruminal pH was determined immediately after sampling using a portable pHmeter (Piccolo, Hanna Instruments). A TMR sample taken right after feed distribution was sieved with a modified Penn State method (Lammers et al., 1995) to determine particle size. Table 2 shows the characteristic of the sieve employed.

**Table 2**. Characteristic of fibrometer

| SIEVE | HOLES DIAMETERS | OPTIMUM QUANTITY |
|---|---|---|
| **Sieve I** | 22 mm | 3 % |
| **Sieve II** | 19 mm | 1 % |
| **Sieve III** | 8 mm | > 40 % |
| **Sieve IV** | 5 mm | |
| **Pan** | / | < 40 % |

**Laboratory Analyses**

***Ruminal fluid:*** Concentrations of SCFA in ruminal fluid were determined on the supernatant of stored samples obtained by centrifugation (1300 x g for 15 minutes) by high performance liquid cromatography (HPLC) following the procedure of Martillotti and Puppo (1985) using HPLC Perrkin Elmer Series 10, mobile phase $H_2SO_4$ 0.0025 N, flux 0.6 ml/min, detector Waters 410, column Gecko 2000 at the working temperature of 60°C.

***Feed:*** Feed were analysed by near infrared spettroscopy (NIRS) using NIRS 5000 (Foss NIRSystem) and an in house calibration. Dietary cation/anion balance (DCAB) was calculated based on mineral analysis determined by atomic absorbtion methods.

***Statistical Analysis***

Data obtained were analyzed by ANOVA to verify the effect of farm using the statistical package SIGMA STAT 2.3 according the following model:

$$Y_{ij} = \mu + FARM_i + e_{ij}$$

Where: $Y_{ijk}$ = dependent variable, $\mu$ = overall mean; $FARM_i$ = effect of different farm, and $e_{ij}$ = error term. Pearson linear correlations were also calculated between the parameters.

**RESULTS**

Farm data showed that only one farm (herd n.9) had reproductive, diarrhoea and lameness problems (up to 80% of the cows); this herd had a low rumen pH. Other problems such as abomasal displacement resulted to be not statistically different from the mean values of normal prevalence. Table 3 and 4 show the chemical composition of diets used during steaming-up and subsequent early lactation in the ten farm.

**Table 3**. Chemical composition (%) of diets used during steaming-up in the diet of different herds

| FARM | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| **crude protein** | 12.63 | 13.37 | 14.87 | 13.90 | 12.93 | 14.37 | 13.58 | 13.78 | 13.93 | 13.07 |
| **ethreal extract** | 3.18 | 4..30 | 4.55 | 4.85 | 4.10 | 4.67 | 5.25 | 5.35 | 4.10 | 4.17 |
| **ash** | 7.55 | 7.38 | 8.03 | 7.93 | 9.13 | 7.83 | 7.67 | 7.95 | 8.13 | 11.88 |
| **NDF** | 43.25 | 40.77 | 36.99 | 37.01 | 42.02 | 36.69 | 37.34 | 37.70 | 41.02 | 43.23 |
| **NFC** | 33.39 | 34.17 | 35.56 | 35.22 | 31.82 | 35.16 | 35.27 | 35.23 | 30.27 | 27.65 |
| **dry metter degradable** | 55.58 | 59.02 | 63.68 | 62.62 | 58.97 | 63.73 | 62.19 | 62.59 | 57.88 | 62.20 |
| **ADF** | 27.66 | 24.82 | 21.46 | 22.02 | 24.63 | 21.32 | 23.11 | 23.61 | 24.28 | 30.66 |
| **starch** | 12.71 | 14.74 | 18.49 | 18.31 | 17.31 | 18.29 | 18.27 | 18.38 | 15.31 | 16.57 |
| **dietary cation/anion balance (DCAB)** | 23.93 | 35.49 | 37.52 | 36.04 | 37.07 | 37.50 | 37.90 | 37.06 | 36.07 | 49.92 |

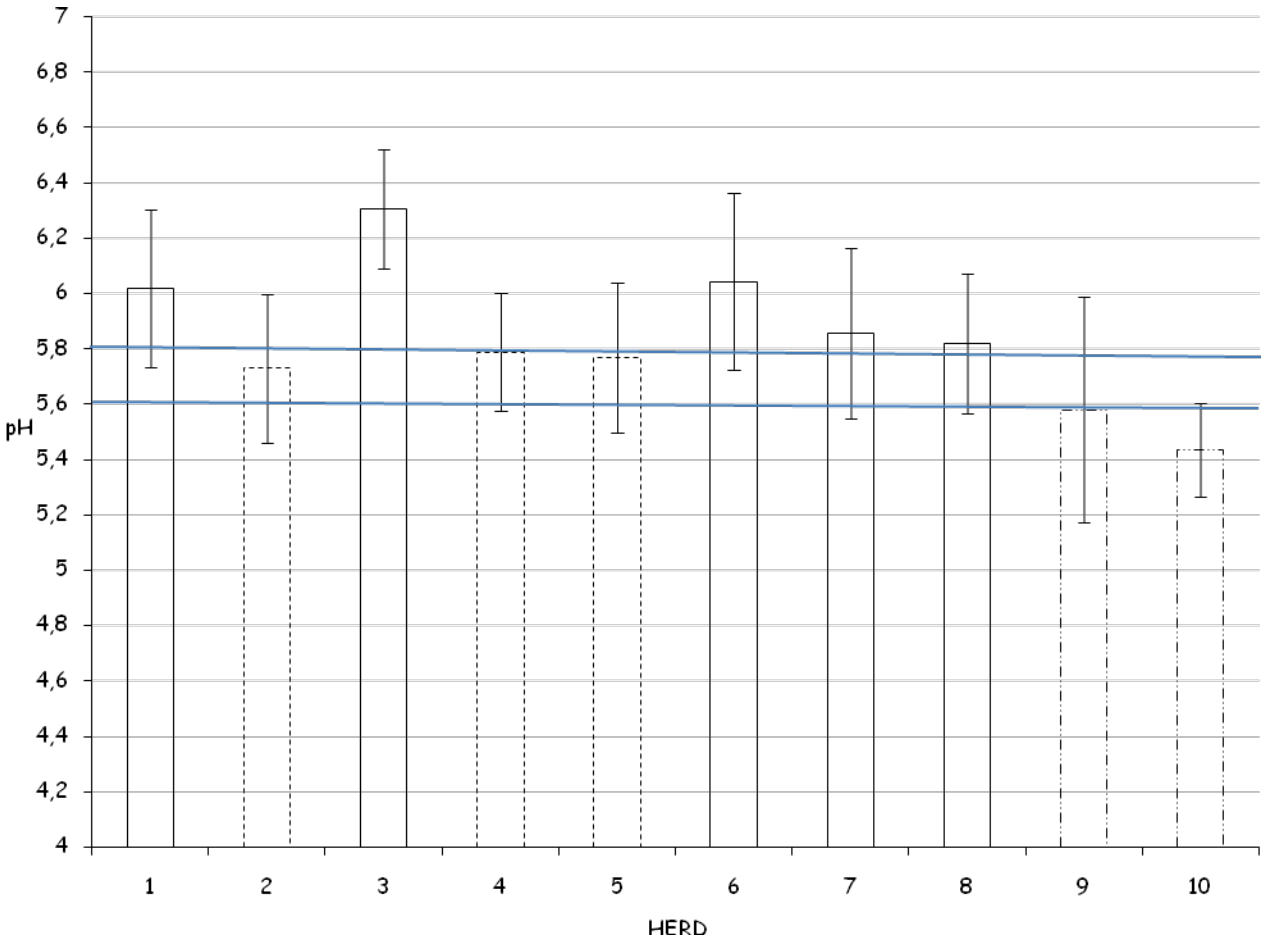
**Table 4**. Chemical composition (%) of diets used during early lactation in the diet of different herds

| FARM | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| **crude protein** | 15.08 | 16.59 | 16.84 | 15.66 | 15.69 | 15.05 | 16.74 | 15.67 | 15.60 | 16.22 |
| **ethreal extract** | 4.86 | 6.01 | 5.56 | 4.32 | 4.63 | 5.70 | 4.76 | 5.19 | 4.66 | 4.81 |
| **ash** | 7.57 | 7.42 | 6.99 | 7.34 | 7.79 | 6.99 | 8.34 | 8.05 | 7.69 | 7.80 |
| **NDF** | 32.56 | 32.17 | 30.00 | 33.41 | 37.53 | 33.40 | 34.11 | 34.10 | 37.33 | 32.07 |
| **NFC** | 39.93 | 37.81 | 40.61 | 39.27 | 34.37 | 38.86 | 35.47 | 36.99 | 33.37 | 39.09 |
| **dry metter degradable** | 68.45 | 68.48 | 70.30 | 68.22 | 63.35 | 66.30 | 66.30 | 66.91 | 64.45 | 69.18 |
| **ADF** | 19.02 | 20.37 | 18.02 | 21.12 | 22.75 | 20.73 | 21.87 | 20.32 | 23.85 | 20.02 |
| **starch** | 25.01 | 25.46 | 25.49 | 24.94 | 25.66 | 22.58 | 20.21 | 23.10 | 25.60 | 23.87 |
| **dietary cation/anion balance (DCAB)** | 35.72 | 49.39 | 40.77 | 39.57 | 36.60 | 56.71 | 16.74 | 40.36 | 36.70 | 41.66 |

The value of NDF (neutral detergent fiber), ADF (acid detergent fiber), NFC (non fiber carbohydrates), starch and crude protein, are similar in all herds. Composition of diet residuals is shown in table 5; it can be noticed large differences across farms compared with the initial chemical composition of diet and same differences between herds, with a situation far from the optimum. In particular, herd 5 showed a substantial increase of NDF level in the data of residual feed respect to

the initial feed; moreover, the residual feed of all herds showed a decrease of starch level. Results of diets particle size at sampling is reported in table 6. Herd 6 and 8 presented high levels of fibrous feed which remained on the first sieve (10.1 % in herd 6 and 7.6 % in herd 8; the normal value from about 3 % of total diet). Five farms had a large percentage of residual feed on the last sieve (more than 40% is the optimal value). Graphic 1 shows the rumen mean pH for each herd. According to the classification proposed by Nordlund and Garrett (1995) on the basis of ruminal fluid pH, the presence of SARA was detected in 3 herds (herds n.2, 9, 10), which had more than 33% of the cows with rumen pH < 5.5. A critical situation was found in 5 herds (number 1, 4, 5, 7, 8), in which more than 33% cows showed a rumen pH lower than 5.8; normal rumen pH condition was detected in 2 herds (number 3 and 6). Graphic 2 shows rumen pH fluctuations in relation to time from TMR distribution; we observed a (not statistical significant) decrease of rumen pH with the increase of time between rumen sampling and TMR distribution. Tables 7 and 8 show the mean values of rumen fluid (short chain fatty acids). Table 9 reports averages of rumen parameters blocked by occurrence of SARA. In particular, significant differences ($p$<0.01) were identified in relation to the average pH value of ruminal fluid among the three groups of herds (normal herds, critical herds and herds with subacute ruminal acidosis). Moreover, we observed a significant ($p$<0.05) linear increase of absolute values of total SCFA, acetic acid and propionic acid in combination with a decreased rumen pH.

**Graphic 1.** Rumen pH mean and standard deviation for each herd




**Graphic 2**. Rumen pH fluctuations (mean and standard deviation) in relation to time past from TMR distribution

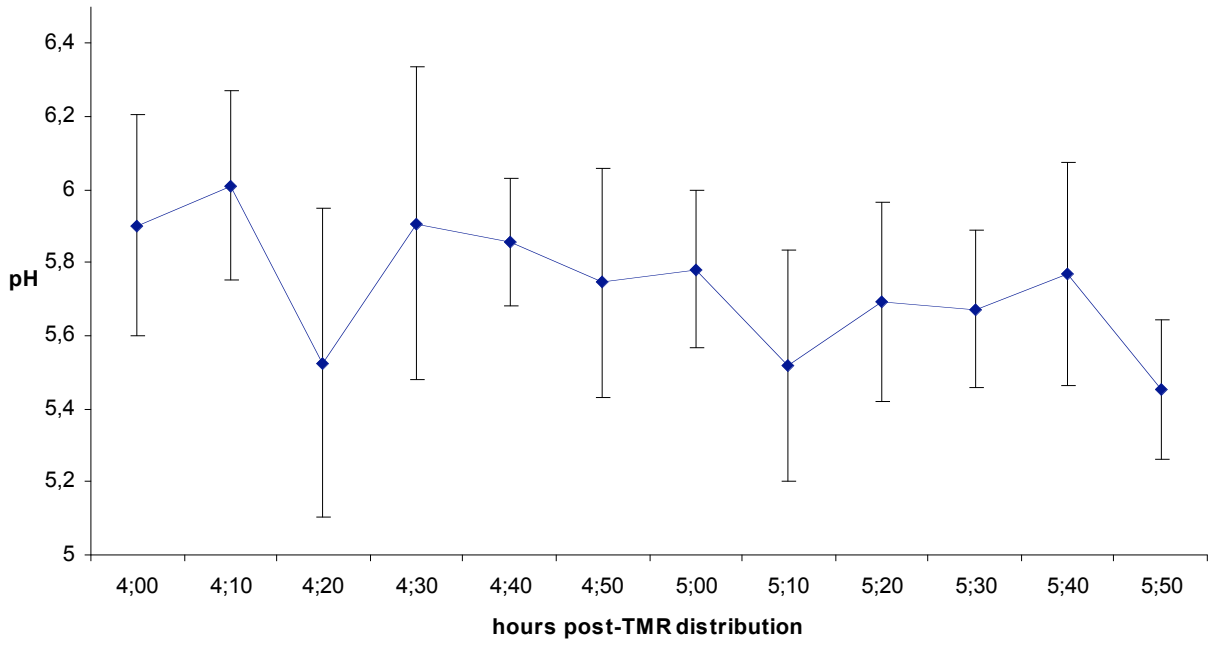


**Table 5.** Chemical composition (% DM) of residual feed at day of sampling

| FARM | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| **DM (%)** | 47.38 | 50.16 | 46.05 | 47.20 | 49.30 | 46.56 | 48.62 | 46.49 | 49.58 | 49.89 |
| **Ash** | 6.93 | 6.92 | 6.82 | 6.90 | 8.15 | 7.44 | 7.62 | 7.10 | 7.01 | 7.08 |
| **CP** | 15.03 | 15.77 | 15.15 | 14.97 | 13.84 | 16.6 | 16.29 | 16.03 | 15.88 | 15.93 |
| **EE** | 4.80 | 5.25 | 5.06 | 4.10 | 4.07 | 5.40 | 4.26 | 5.50 | 5.29 | 5.40 |
| **NDF** | 34.05 | 34.73 | 34.00 | 35.33 | 41.24 | 32.88 | 33.17 | 34.08 | 36.20 | 38.87 |
| **ADF** | 22.02 | 21.66 | 21.31 | 22.75 | 25.38 | 19.41 | 21.99 | 21.11 | 22.20 | 23.48 |
| **Starch** | 22.99 | 21.48 | 22.75 | 23.33 | 19.36 | 23.14 | 22.93 | 22.33 | 21.66 | 20.66 |

**Table 6.** Results (%) of particle size of sampling

| FARM | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| **SIEVE 22 MM** | 1.4 | 3.5 | 2.4 | 0.6 | 0.8 | 10.1 | 2 | 7.6 | 2.8 | 2.6 |
| **SIEVE 19 MM** | 2.5 | 1.7 | 1.6 | 0.8 | 0.8 | 1.6 | 2 | 2 | 4 | 2 |
| **SIEVE 8 MM** | 19.5 | 45.8 | 30.4 | 18.5 | 16 | 18.2 | 17.1 | 17 | 13 | 17.1 |
| **SIEVE 5 MM** | 55.1 | 29.4 | 26.4 | 34.3 | 31.3 | 24 | 30.6 | 35.1 | 35.2 | 35.3 |
| **PAN** | 21.4 | 19.6 | 39.2 | 45.8 | 51 | 46.1 | 48.4 | 39.7 | 45 | 43 |

**Table 7**. Mean values of short chain fatty acids (mmol/L) ± SEM of rumen fluid for each herd

| FARM | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| **ACETATE (mmol/L)** | 80.53 ± 4.66 | 90.14 ± 5.67 | 71.50 ± 4.09 | 95.20 ± 2.67 | 101.26 ± 3.51 | 73.62 ± 2.53 | 86.03 ± 3.45 | 83.60 ± 5.27 | 97.34 ± 5.67 | 87.01 ± 3.63 |
| **PROPIONATE (mmol/L)** | 30.62 ± 2.84 | 34.07 ± 2.37 | 26.72 ± 1.79 | 33.15 ± 1.95 | 32.59 ± 1.65 | 32.21 ± 3.50 | 34.70 ± 2.04 | 28.89 ± 2.01 | 50.38 ± 5.67 | 33.30 ± 1.58 |
| **ISO-BUTYRATE (mmol/L)** | 0.93 ± 0.03 | 0.91 ± 0.06 | 1.04 ± 0.07 | 1.06 ± 0.06 | 0.85 ± 0.03 | 0.77 ± 0.04 | 1.15 ± 0.03 | 0.90 ± 0.05 | 1.07 ± 0.07 | 0.98 ± 0.04 |
| **N-BUTYRATE (mmol/L)** | 15.22 ± 1.28 | 15.90 ± 1.12 | 11.93 ± 0.72 | 18.53 ± 0.45 | 16.85 ± 0.80 | 13.96 ± 0.84 | 13.67 ± 0.66 | 14.79 ± 0.87 | 16.11 ± 1.93 | 14.11 ± 0.58 |
| **ISO-VALERATE (mmol/L)** | 1.59 ± 0.11 | 2.31 ± 0.23 | 2.08 ± 0.18 | 1.82 ± 0.07 | 1.47 ± 0.07 | 1.92 ± 0.22 | 2.26 ± 0.12 | 1.75 ± 0.12 | 1.37 ± 0.13 | 1.65 ± 0.09 |
| **N-VALERATE (mmol/L)** | 1.19 ± 0.14 | 2.48 ± 0.11 | 1.91 ± 0.15 | 2.10 ± 0.08 | 2.07 ± 0.09 | 2.00 ± 0.19 | 2.19 ± 0.12 | 2.11 ± 0.13 | 2.37 ± 0.36 | 1.96 ± 0.07 |
| **TOT AGV (mmol/L)** | 130.83 ± 8.72 | 145.84 ± 8.75 | 115.21 ± 5.75 | 151.88 ± 3.42 | 155.11 ± 5.27 | 124.51 ± 6.29 | 140.02 ± 6.09 | 132.06 ± 8.29 | 168.66 ± 12.87 | 139.04 ± 4.55 |
| **C2 / C3** | 2.68 ± 0.11 | 2.68 ± 0.14 | 2.75 ± 0.17 | 2.96 ± 0.18 | 3.16 ± 0.12 | 2.54 ± 0.23 | 2.49 ± 0.07 | 2.90 ± 0.07 | 2.02 ± 0.29 | 2.58 ± 0.18 |
| **NH3N (mg / L)** | 110.82 ± 11.61 | 102.80 ± 14.79 | 94.00 ± 11.12 | 131.36 ± 17.63 | 66.30 ± 9.21 | 35.75 ± 6.50 | 148.54 ± 16.45 | 60.97 ± 9.22 | 98.28 ± 15.57 | 78.19 ± 15.45 |
| **LACTATE (mmol/L)** | 0.36 ± 1.28 | 2.19 ± 0.11 | 0 | 1.80 ± 0.23 | 0.82 ± 2.20 | 2.56 ± 0.77 | 0 | 0 | 3.67 ± 4.32 | 0.38 ± 0.28 |

**Table 8**. Mean values of short chain fatty acids (%) ± SEM for each herd

| FARM | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| **ACETATE** | 61.96 ± 0.75 | 61.69 ± 0.89 | 62.00 ± 1.23 | 62.67 ± 1.05 | 65.31 ± 0.62 | 59.81 ± 1.67 | 61.53 ± 0.34 | 63.31 ± 0.37 | 58.78 ± 1.96 | 62.43 ± 1.03 |
| **PROPIONATE** | 23.07 ± 0.72 | 23.47 ± 0.91 | 23.24 ± 1.14 | 21.81 ± 1.18 | 20.98 ± 0.76 | 25.20 ± 1.66 | 24.63 ± 0.56 | 21.79 ± 0.42 | 29.06 ± 0.76 | 24.11 ± 1.20 |
| **ISO-BUTYRATE** | 0.73 ± 0.03 | 0.63 ± 0.03 | 0.91 ± 0.05 | 0.69 ± 0.04 | 0.55 ± 0.01 | 0.63 ± 0.03 | 0.83 ± 0.04 | 0.68 ± 0.01 | 0.66 ± 0.04 | 0.71 ± 0.02 |
| **N-BUTYRATE** | 11.52 ± 0.37 | 10.86 ± 0.28 | 10.38 ± 0.39 | 12.22 ± 0.24 | 10.84 ± 0.32 | 11.24 ± 0.45 | 9.79 ± 0.32 | 11.23 ± 0.13 | 9.29 ± 0.44 | 10.12 ± 0.18 |
| **ISO-VALERATE** | 1.24 ± 0.08 | 1.60 ± 0.13 | 1.80 ± 0.11 | 1.20 ± 0.04 | 0.96 ± 0.05 | 1.50 ± 0.10 | 1.63 ± 0.08 | 1.34 ± 0.08 | 0.85 ± 0.08 | 1.19 ± 0.05 |
| **N-VALERATE** | 1.45 ± 0.05 | 1.73 ± 0.07 | 1.64 ± 0.08 | 1.38 ± 0.03 | 1.33 ± 0.04 | 1.58 ± 0.10 | 1.55 ± 0.04 | 1.61 ± 0.06 | 1.34 ± 0.12 | 1.41 ± 0.03 |

**Table 9.** Significant different between rumen pH and volatile fatty acids concentrations on three class of herd

| PARAMETERS | NORMAL FARMS | CRITICAL FARMS | FARMS WITH ACIDOSIS |
|---|---|---|---|
| **rumen pH** | 6.16A | 5.86B | 5.68C |
| **total SCFA (mmo/L)** | 123.02a | 135.43ab | 150.68b |
| **Acetic Acid (mmo/L)** | 76.02Aa | 87.93ABb | 91.33B |
| **Propionic Acid (mmol/L)** | 28.67A | 32.32AB | 38.94B |
| **C2/C3 ratio** | 2.74 | 2.82 | 2.53 |
| **Lactic Acid (mmol/L)** | 0.36 | 2.18 | 2.89 |
| **N-Butyric Acid** | 13.58 | 15.56 | 15.35 |
| **N-Valerate Acid (mmol/L)** | 1.92 | 2.1 | 2.27 |

A, B, C: same letters on the same line to exclude statistic significance ($P < 0.01$)
a, b: same letters on the same line to exclude statistic significance ($P < 0.05$)

## DISCUSSION

The aim of this study was to survey the incidents of SARA syndrome in dairy herds in Northern Italy. Therefore the farms were chosen based on their correspondence to the established parameters and not in relation to previous suspect of SARA. Herds were homogeneous for several characteristics: Holstein cows (except herd 5, where we had also Jersey cows), high production, presence of more than 100 lactating cows, free stalling, a feeding group in early lactation (first 60 days), utilization of TMR and utilization of steaming-up at the end of the dry period. History recorded showed reproductive, diarrhea and lameness problems (up to 80% of the cows) in one farm only (herd n.9) with low rumen pH. Other health problems such as outbreaks and displacement of the abomasum were within normal prevalence range. However we also stress that information given by farmers does not always correspond to the real situation. Several herds showed an unfit flooring and a poor system of effluents ejection; this situation can induce limping or influence the welfare of animals, a key factor for the onset of numerous diseases, including those studied here.

Herd 1, 4 and 6 adopted a short dry period, of 40 days, while the farm 10 prolonged the dry period up to 80 days. In this study we didn't find any relationship between the length of dry period and

subacute rumen acidosis and we suggest that an appropriate steaming-up at the end of the dry period is important to prevent the development of the syndrome.

The data of the initial chemical analysis of diets (table 3 and 4), showed that cows were fed correctly; the value of NDF, ADF, NFC, starch and crude protein, resulted in all the herds similar to the values reported in the literature (Nordlund, 2001). However, data of residual feed (table 5) showed large differences with the initial chemical composition of diet. In particular, herd 5 (with a condition of subacute ruminal acidosis), had an increase of NDF level in the data of residual feed; moreover, a marked decrease of starch level was observed in residual feed of all the herds.

The particle size of diet at sampling (table 6) showed a large portion of residual feed on the last sieve (more than 40% is the optimal value) in five farms. This indicates the presence of large quantities of small particles (especially concentrates) in feed, or excessive grinding of TMR. A large quantity of concentrates promotes the development of amylolitic bacteria and decreases chewing, with consequent curtailed salivary secretion, a situation that can facilitate the occurrence of SARA. We observed smaller than optimum quantities of NDF from forage (essential portion, because able to chewing stimulation) in several herds. In fact, previous studies (Mertens, 1997; Stone, 2004) showed that the best value of peNDF (physically effective NDF) should be about 22% of ration DM to maintain an average ruminal pH of 6.0. According to Lammers et al. (1995), physically effective NDF could be measured as a proportion of DM retained by the 19- and 8-mm Penn State Particle Separator (PSPS) screens multiplied by dietary NDF content ($peNDF_{>8}$). Mertens (1997) determined peNDF as the proportion of DM retained by a 1.18-mm screen multiplied by dietary NDF ($peNDF_{>1.18}$), using a dry-sieving technique. The choice of the most reliable measure of peNDF to provide the most accurate estimate of chewing, saliva production, and rumen buffering (Einarson et al., 2004) is still debated. A number of studies (Lammers et al., 1995; Mertens, 1997; Einarson et al., 2004; Stone, 2004) investigated the effects of peNDF on rumen fermentation, feed intake, milk production, chewing activity, and nutrient digestibility in high-yielding, early lactation dairy cows, with the results being far from conclusive.

The dietary cation-anion balance (DCAB) was positive in all herds during the transition period and at the beginning of lactation. The lower and the higher values during the transition period were detected in herd n.1 (23.93 meq/Kg of dry matter) and in herd n. 10 (49.92) respectively. Data from the literature (Delaquis and Block, 1995) report that small variations on the positive anion-cation balance can influence the acid-bases status. In fact, a high anion-cation balance can increase water absorption and urinary volume; a low anion-cation balance increases faecal excretion and both absorption and excretion of sulphur, whereas it decreases urinary $H^+$ and $HCO_3^-$ concentration. Anionic salts may eventually decrease rumen pH, with an average of 0.12 units, possibly due to the influence of salts on rumen fluid (Vagnoni and Oetzel, 1998). Nevertheless, the difference of anion-cation balance in our experimental herds was not sufficient to influence the acid-bases status, and there was no relationship between DCAB and rumen acidosis.

Ruminal fluid was sampled from each cow 4 to 8 hours after TMR distribution, when ruminal pH is expected to be at the lowest. Analyzing pH data by sampling time (relative to feeding), we observed a decrease of rumen pH with the increment of the sampling time after TMR distribution (graphics n.2).

To date, rumenocentesis is considered the best technique for rumen fluid sampling (Garrett, 1999; Enemark, 2002; Duffield et al., 2004). An objective of this research was to study in detail both positive and negative aspects of rumenocentesis and our results confirm the reliability of this method. We had no sampling problems with the 120 cows of our experimental series, and no animal developed any health problem during or after the procedure, including peritonitises, local abscesses or even decrease in milk production.

According to the classification proposed by Nordlund and Garrett (1995) on the basis of ruminal fluid pH, we detected the presence of SARA in 3 herds, a critical situation in 5 herds and a normal rumen pH condition in 2 herds. An analysis of SCFA determination reported in tables 7 and 8 shows that the concentration of acetate in rumen fluid resulted higher than reference values reported

by Murphy et al. (2000), except for farms 4 and 5. Propionate values varied between 33 and 53 mmol/l, thus resulting higher than the concentration of 25 mmol/l reported by Murphy et al. (2000). In all farms, butyrate concentration seemed to be slightly lower than range values (18-20 mmol/l) reported by Murphy et al., 2000. Valerate and isovalerate showed values higher than those reported in the literature where traces of these SCFA are taken into account (Kristensen et al., 2000; Allen et al., 2004). From our data total valerate concentration ranged between a minimum mean value of 3.73 mmol/l (farm 4) and a maximun value of 5.25 (farm 2). Ratio of C2:C3 resulted low in all farm if compared to the data reported by (Hutjens, 1996) for healthy cows. N-valerate is a toxic acid, derived from proteins (rather than from carbohydrates) that is absorbed by the rumen wall (Kristensen et al., 2000; Allen et al., 2004). Little is known about its metabolism. N-valerate reaches the liver (where it may induce damage) or the mammary gland when it is released into the blood stream. A previous study (Morgante et al., 2004) showed a significant correlation between N-valerate levels in the rumen and the presence of somatic cells in the milk. Although our study didn't show a statistic correlation between herds with low ruminal pH and high levels of valerate concentration, we think it is important to extend the investigation and define the role of valerate in respect to SARA.

Subacute ruminal acidosis is certainly related to management errors, especially in large herds where the attitude of the personnel responsible for feeding the cows is of paramount importance. Substitution or replacement of feeding personnel may lead to differences in feeding schedules, proportion of roughage and concentrate, with consequent considerable impact on unstable ruminal environments. These problems, although mainly reported in feedlots (Elam, 1976), may apply also to high-producing dairy herds and should therefore be regarded as a potential hazard for the health management of the herd.

## CONCLUSIONS

Subacute ruminal acidosis is a common and serious health and production problem in many high producing Italian dairy herds. Although rumenocentesis is today the best test used to diagnose subacute ruminal acidosis, a timely diagnosis is difficult and the causes warrant further research. A detailed analysis of rations is certainly a useful diagnostic procedure. In many dairy farms, particular attention was given to the amounts of fiber fractions (ADF, NDF) and NFC. In particular, a strong relationship between ruminal pH and SCFA concentration was noted. More studies would be necessary to further understand the role of valerate acid and in particular its possible correlation with SARA. Differences between initial chemical composition and residual feed were found, a result which suggests more attention in TMR preparation and management. The exact causes of SARA were not determined, but it appears that the synbdrome is not related only to diet formulation. Animal management seems to be one of the most important factors in developing SARA.

## TRIAL 2

## PROFILES OF HEMATOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN LACTATING DAIRY COWS AFFECTED BY SUBACUTE RUMINAL ACIDOSIS

### MATERIALS AND METHODS

The study was carried out in 10 intensive Italian dairy herds, located in different areas throughout northern Italy, visited during the external consultation of the Department of Veterinary Clinical Science of Padua. The farms showed a high production level. Twelve cows were randomly selected for rumen fluid collection by rumenocentesis from animals on the absence of external clinical signs of disease, with body condition score (BCS) between 2.75 and 3.25 according the procedure of Edmonson et al. (1989), and between 5 and 70 days in milk (DIM), because SARA occurs the most frequently during that period (Kleen et al., 2003; Nordlund, 2001; Nordlund and Garrett, 1994).

One hundred and twenty cows with 35 ± 30 DIM were sampled. Rumenocentesis was performed using a 13G 105-mm needle (Intranule PP, Vygon, France) and was chosen because it is the most commonly used technique and provides the most accurate results (Duffield et al., 2004; Garrett et al., 1999). The time of sampling was between 4 and 6 hr post total mixed ration (TMR) distribution as recommended previously (Morgante et al., 2007; Nordlund, 1995). An area in the left flank of 20 × 20 cm, 20 cm caudal to the last costae, and on the level of the top of the stifle joint was prepared with an aseptic technique by disinfection with ethanol and iodine. The farmer was instructed to restrain the dairy cows by means of a tail grip and the needle was introduced into the rumen by one of the veterinary surgeons. The ruminal fluid was collected by gentle aspiration with a 20-ml syringe, and the ruminal pH was immediately determined after sampling using a portable pH meter (Piccolo, Hanna Instruments, Leighton Buzzard, Bedfordshire, UK).

Blood samples were collected from the same cows by jugular venipuncture into ethylenediamine tetra-acetic acid (EDTA), lithium heparin, and plain vacutainers (BD Vacutainer Systems®, Preanalytical Solutions, Plymouth, UK).

Total white blood cell (WBC), differential WBC counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils), red blood cells (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red distribution white (RDW), platelet total count (PLT), and mean platelet volume (MPV) were determined on blood samples collected into EDTA-Vacutainer tubes by mean of the Abbott Cell Dyn 3500 (Abbott Diagnostic Division, CA). The hematology profile was performed within 2 hr of sampling. The blood samples in lithium heparin and plain vacutainers were centrifuged at 2500 × *g* at 4°C for 20 min. Plasma was analyzed for to measured the following hematochemical parameters: creatinine, aspartate transaminase (AST), gamma-glutamyl transferase (αGT), calcium, phosphorus, magnesium, sodium, potassium, chloride, glucose, cholesterol, triglycerides, nonesterified fatty acids (NEFA), and total, direct, and indirect bilirubin.

Serum total proteins were determined by the Boehringer Mannheim/Hitachi 911 automated chemistry analyzer (Roche, Basel, Switzerland). Albumin (A) and total globulins (G), as well as albumin/globulin ratio (A/G) were determined on serum samples. The electrophoretic protein separation in 0.8% agar gel revealed serum concentrations for alpha-1, alpha-2, beta-1, beta-2, and gamma globulins; it was performed by mean of the Hydrasys LC, a semiquantitative and multiparametic biochemical analyzer (SEBIA, Issy-les-Moulineaux, France) provided with specific Phoresis software.

In this work we studied these previous parameters because the influence of ruminal acidosis on ruminal microbiology has received considerable attention, but less is known about systemic manifestations that arise from ruminal acidosis.

The dairy farms were divided into three groups. According to the classification proposed by Nordlund and Garrett (1994) on the basis of ruminal fluid pH, group A included farms with an

average ruminal pH > 5.8 (normal), group B included farms with an average ruminal pH between 5.6 and 5.8 (risk), and farms with an average ruminal pH < 5.6 (acidosis) were included in group C. Results were expressed as mean ± standard deviation (SD). All data was normally distributed and the repeated measures one-way analysis of variance (ANOVA) was applied to evaluate the effect of SARA on hematochemical and hematological parameters. If ANOVA showed an acceptable level of significance ($p < 0.05$), Bonferroni's test was applied for post hoc comparison.

**RESULTS**

Figure 1 shows the rumen mean pH for each herd. On the basis of ruminal fluid pH, normal rumen pH condition (group A) was detected in 4 herds (number 1, 3, 6 and 7), a critical situation (group B) was found in 3 herds (number 4, 5 and 8) and the presence of SARA (group C) was detected in 3 herds (herds n.2, 9, 10).

The three groups were homogeneous for average DIM (32 ± 29, 38 ± 25, and 34 ± 32, respectively), BCS (3.05 ± 0.30, 3.08 ± 0.27, and 3.06 ± 0.29, respectively), and all the animals were on absence of external clinical signs of disease. In effect all cows didn't show a decreased of DMI, laminitis, mastitis, ulcers and displacement of the abomasum and no other metabolic or infectious diseases were observed in cows selected for the study within the study period.

All parameters were expressed as mean concentrations/counts in their conventional units with standard deviations and all blood values were within expected reference ranges (Kaneko et al., 1997).

The statistical differences observed between the three groups (A, B, and C) are shown in Tables 1–3; table 4 shown the statistical significant differences of ANOVA.

The post hoc multiple comparison Bonferroni test showed statistically significant differences ($P < 0.05$) when comparing these parameters between the three groups. There was no significant difference for the concentration of total proteins, beta-2 globulins, AST, αGT, calcium, glucose, cholesterol, and total, direct, and indirect bilirubin. Red blood cell counts, HGB, Hct, RDW, PLT,

MPV, neutrophils, lymphocytes, monocytes, eosinophils, and basophils were not significantly different. Magnesium, sodium, potassium, chloride, triglycerides, and NEFA concentrations were significantly decreased in animals with ruminal acidosis. Whereas, the concentration of phosphorus increased statistically in the three groups.

**Table 1.** Average values of serum proteins, expressed in their conventional units together with the related standard deviations in the dairy cows of Group A (Normal), B (Risk) and C (Acidosis), and statistical significance observed.

| **Parameters** | ***Normal values*** | **Group A** | **Group B** | **Group C** |
|---|---|---|---|---|
| **Total protein (g/l)** | *67 – 75§* | 70.24 ± 5.36 | 69.92 ± 6.86 | 68.92 ± 5.71 |
| **Albumin (g/l)** | *30 – 36§* | 33.32 ± 2.56 | 30.11 ± 2.82* | 30.45 ± 3.05 |
| **Alpha-1 (g/l)** | *2 – 6§* | 4.76 ± 0.75 | 5.83 ± 0.88* | 4.76 ± 0.89† |
| **Alpha-2 (g/l)** | *5 - 9§* | 8.89 ± 0.91 | 7.96 ± 0.89* | 8.67 ± 0.96† |
| **Beta-1 (g/l)** | *2 - 7§* | 6.05 ± 0.82 | 6.37 ± 1.10 | 6.79 ± 0.55* |
| **Beta-2 (g/l)** | *4 - 10§* | 4.89 ± 1.00 | 4.74 ± 1.31 | 4.84 ± 0.93 |
| **Gamma (g/l)** | *18 - 25§* | 18.88 ± 4.16 | 22.79 ± 5.12* | 21.39 ± 4.74 |
| **Albumin/Globulin** | *0.8 – 0.9§* | 0.88 ± 0.14 | 0.85 ± 0.16* | 0.86 ± 0.16 |

Significance: * vs Group A $p < 0.05$; † vs Group B $p < 0.05$

§ Data from Kaneko et al., 1997

**Table 2.** Average values of haematochemical parameters, expressed in their conventional units together with the related standard deviations in the dairy cows of Group A (Normal), B (Risk) and C (Acidosis), and statistical significance observed.

| **Parameters** | ***Normal Values*** | **Group A** | **Group B** | **Group C** |
|---|---|---|---|---|
| **Creatinine (µmol/l)** | *35 - 280§* | 65.46 ± 7.13 | 59.94 ± 6.62* | 62.92 ± 7.87 |
| **AST (I.U./l)** | *43 - 127§* | 88.00 ± 17.35 | 92.23 ± 11.81 | 83.42 ± 13.30 |
| **αGT (I.U./l)** | *15 - 39§* | 25.78 ± 8.76 | 27.27 ± 12.98 | 21.75 ± 6.12 |
| **Calcium (mmol/l)** | *2.43 – 3.10§* | 2.44 ± 0.19 | 2.43 ± 0.22 | 2.44 ± 0.14 |
| **Phosphorus (mmol/l)** | *1.08 – 2.76§* | 1.26 ± 0.37 | 1.57 ± 0.30* | 1.67 ± 0.13* |
| **Magnesium (mmol/l)** | *0.74 – 1.10§* | 1.02 ± 0.11 | 0.89 ± 0.12* | 0.91 ± 0.08* |
| **Sodium (mmol/l)** | *132 – 152§* | 138.4 ± 2.93 | 134.2 ± 5.14* | 133.5 ± 5.31* |
| **Potassium (mmol/l)** | *3.9 – 5.8§* | 4.13 ± 0.49 | 3.99 ± 0.40* | 3.97 ± 0.29* |
| **Chloride (mmol/l)** | *95 - 110§* | 98.53 ± 3.10 | 95.10 ± 3.92* | 95.08 ± 3.35 |
| **Glucose (mmol/l)** | *2.47 – 4.12§* | 3.21 ± 0.34 | 3.26 ± 0.36 | 3.26 ± 0.37 |
| **Cholesterol (mmol/l)** | *2.08 – 3.12§* | 3.06 ± 1.42 | 2.74 ± 1.61 | 3.06 ± 0.98 |
| **Triglycerides (mmol/l)** | *0 - 0.2§* | 0.14 ± 0.04 | 0.11 ± 0.02* | 0.09 ± 0.03* |
| **NEFA (meq/l)** | *0.2 – 2.28§* | 0.58 ± 0.37 | 0.51 ± 0.24 | 0.33 ± 0.21* |
| **Total Bilirubin (µmol/l)** | *0.17 – 8.03§* | 6.00 ± 1.58 | 5.71 ± 1.98 | 4.87 ± 1.31 |
| **Direct Bilirubin (µmol/l)** | *0.68 – 7.52§* | 1.42 ± 0.52 | 1.70 ± 0.45 | 1.49 ± 0.46 |
| **Indirect Bilirubin (µmol/l)** | *0 - 6§* | 4.53 ± 0.97 | 4.13 ± 1.37 | 3.38 ± 0.94 |

Significance: * vs Group A $p < 0.05$; † vs Group B $p < 0.05$

§ Data from Kaneko et al., 1997

**Table 3.** Average values of haematological parameters, expressed in their conventional units together with the related standard deviations in the dairy cows of Group A (Normal), B (Risk) and C (Acidosis), and statistical significance observed.

| **Parameters** | ***Normal Values*** | **Group A** | **Group B** | **Group C** |
|---|---|---|---|---|
| **RBC ($10^6$/µl)** | 5 - 10§ | 5.91 ± 0.60 | 6.00 ± 0.78 | 6.29 ± 0.64 |
| **HGB (g/dl)** | 8 – 15§ | 9.90 ± 0.86 | 10.07 ± 0.83 | 9.71 ± 0.92 |
| **HCT (%)** | 24 – 46§ | 29.37 ± 2.80 | 29.87 ± 2.64 | 29.17 ± 2.71 |
| **MCV (fl)** | 40 – 60§ | 49.81 ± 3.36 | 50.09 ± 3.85 | 46.50 ± 2.80*† |
| **MCH (pg)** | 11 - 17§ | 16.81 ± 1.14 | 16.90 ± 1.30 | 15.58 ± 0.94*† |
| **MCHC (g/dl)** | 30 - 36§ | 31.81 ± 1.13 | 34.12 ± 2.26* | 36.31 ± 0.47*† |
| **RDW (%)** | 16.7 – 23.3§ | 20.15 ± 1.29 | 20.11 ± 1.56 | 19.93 ± 1.23 |
| **PLT ($10^3$/µl)** | 100 - 800§ | 515.7 ± 93.2 | 538.5 ± 105.5 | 618.3 ± 101.8 |
| **MPV (fl)** | 3 - 7§ | 4.05 ± 0.65 | 4.10 ± 0.59 | 4.10 ± 0.20 |
| **WBC ($10^3$/µl)** | 4 - 12§ | 5.58 ± 1.67 | 5.55 ± 2.61 | 8.09 ± 1.56*† |
| **Neutrophils ($10^3$/µl)** | 0.6 - 4§ | 3.35 ± 0.88 | 3.80 ± 1.06 | 4.20 ± 0.80 |
| **Lymphocytes ($10^3$/µl)** | 2.5 – 7.5§ | 2.66 ± 0.95 | 2.55 ± 0.71 | 2.98 ± 1.01 |
| **Monocytes ($10^3$/µl)** | 0.02 – 0.9§ | 0.82 ± 0.24 | 0.88 ± 0.34* | 0.76 ± 0.26 |
| **Eosinophils ($10^3$/µl)** | 0 – 0.24§ | 0.07 ± 0.11 | 0.04 ± 0.08 | 0.0 ± 0.0 |
| **Basophils ($10^3$/µl)** | 0 – 0.2§ | 0.04 ± 0.03 | 0.07 ± 0.03 | 0.0 ± 0.0 |

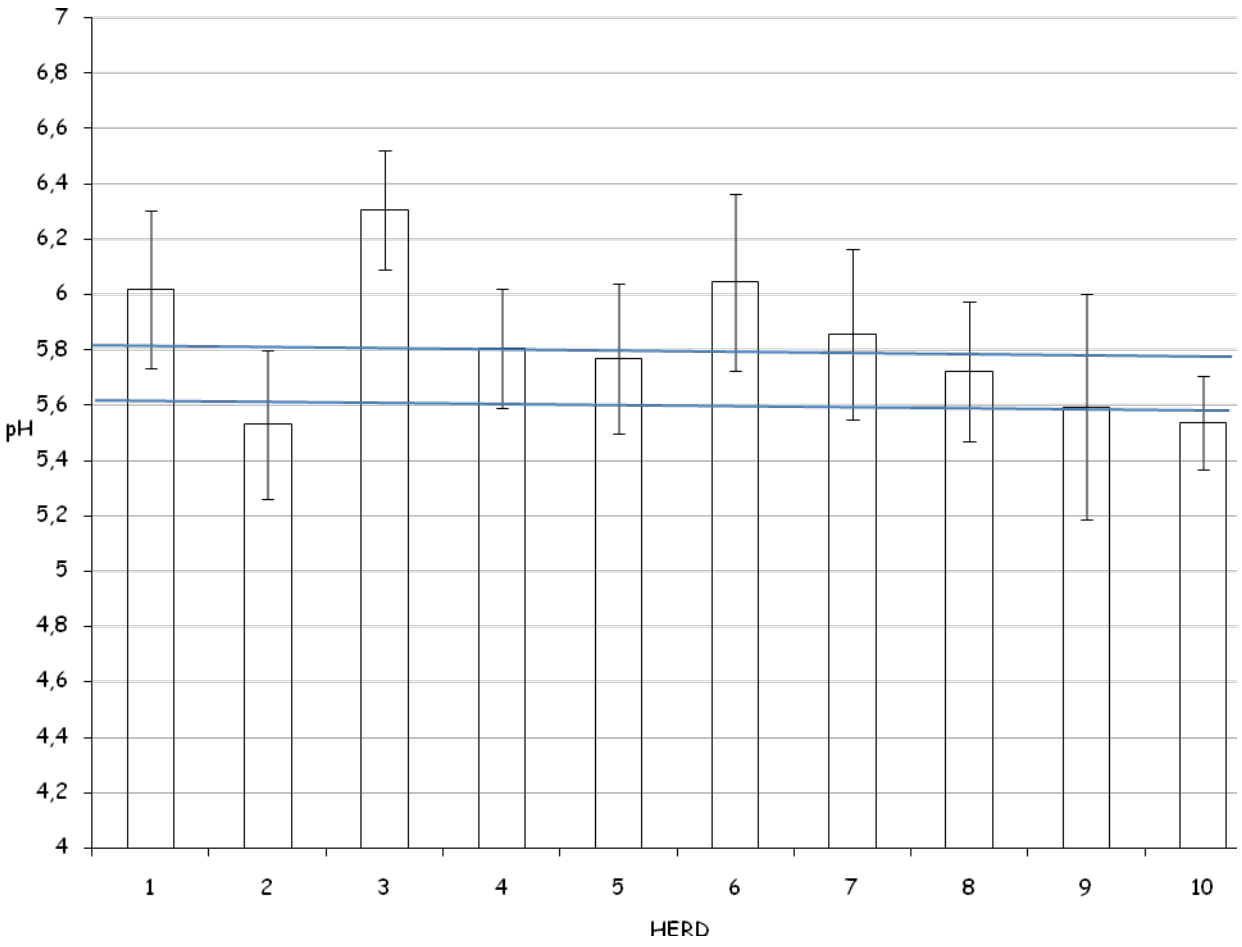
Significance: * vs Group A $p < 0.05$; † vs Group B $p < 0.05$

§ Data from Kaneko et al., 1997

**Table 4.** Statistical differences of group effect on parameters investigated. One-way ANOVA was used. F statistic values, degrees of freedom and probability (P) values for each comparison are given.

| Parameters | $F_{(2,116)}$ | P |
|---|---|---|
| **Albumin (g/l)** | 8.82 | < 0.001 |
| **Alpha-1 (g/l)** | 9.07 | < 0.05 |
| **Alpha-2 (g/l)** | 9.79 | < 0.01 |
| **Beta-1 (g/l)** | 3.82 | < 0.05 |
| **Gamma (g/l)** | 4.08 | < 0.05 |
| **A/G ratio** | 9.41 | < 0.001 |
| **Creatinine (µmol/l)** | 3.77 | < 0.05 |
| **Phosphorus (mmol/l)** | 15.01 | < 0.001 |
| **Magnesium (mmol/l)** | 15.66 | < 0.001 |
| **Sodium (mmol/l)** | 8.23 | < 0.05 |
| **Potassium (mmol/l)** | 9.04 | < 0.05 |
| **Chloride (mmol/l)** | 8.36 | < 0.001 |
| **Triglycerides (mmol/l)** | 17.89 | < 0.001 |
| **NEFA (meq/l)** | 3.28 | < 0.05 |

**Figure 1.** Rumen pH mean and standard deviation for each herd




Normal mean rumen pH condition (group A: pH > 5.8) was detected in 4 herds: herd 1 (6.08±0.28), herd 3 (6.30±0.13) and herd 6 (6.07±0.32). A critical situation (group B: pH between 5.6 and 5.8) was found in 3 herds: number 4 (5.80±0.21), herd 5 (5.77±0.27) and herd 8 (5.73±0.24). The presence of SARA (group C: pH < 5.6) was detected in 3 herds: herds 2 (5.52±0.27), herd 9 (5.59±0.37) and herd 10 (5.56±0.19).

## DISCUSSION

The three groups were homogeneous for average values of DIM (32 ± 29, 38 ± 25, and 34 ± 32, respectively), BCS (3.05 ± 0.30, 3.08 ± 0.27, and 3.06 ± 0.29, respectively), and all the animals were on absence of external clinical signs of disease.

The variations between groups are more likely to be related to different ruminal pH values. The results of the present study suggest that variations of ruminal pH, beyond being useful for the diagnosis of SARA in dairy cows, involved changes of some hematochemical and hematological parameters.

An earlier study by Gozho et al. (2005) showed that abrupt induction of SARA increased free ruminal lipopolysaccharide (LPS), serum amyloid A (SAA), and haptoglobin (Hp) in peripheral blood. A subsequent study from the same group of study (Gozho et al., 2006) showed that gradual adaptations to a 60% concentrate diet over a 4-wk period followed by grain-induced SARA increased free ruminal LPS, SAA, and Hp. It has been suggested that low rumen pH could result in death and lysis of gram-negative bacteria and hence increase free LPS in the rumen (Andersen et al., 1996; Nagaraja et al., 1978). However, there is evidence showing that free ruminal LPS can also result from bacterial cell lysis due to excessive autolytic enzymes that facilitate growth during the rapid bacterial growth phase (Wells and Russell, 1996).

In our work the presence of SARA-influenced albumin, beta 1-globulin and gamma-globulin levels in dairy cow during the pathology regarding group A where the values of ruminal pH higher than 5.8, probably because SARA causes a failure in the acquired immunity system of dairy cows by altering the serum biochemical profile. Moreover, in group C, alpha-2 globulin concentrations were significantly different from group A, but not in group B, in which ruminal pH values were considered as marginal for the same reason. Beta-2 globulin and A/G concentrations did not change in agreement with the results reported by other authors (Fernandez et al., 1997).

Macrominerals calcium, phosphorus, potassium, magnesium, sodium, and chloride are of extreme interest especially in relation to periparturient diseases (e.g., milk fever and SARA). Unfortunately,

most of these minerals are tightly regulated in the body through a variety of homeostatic processes. Their blood concentrations are not reflective of dietary status when these homeostatic systems are functioning properly (Herdt, 2000). Phosphorus, potassium, and magnesium are macrominerals for which blood concentrations are somewhat sensitive to dietary intake (Herdt, 2000). Sodium and chloride concentrations are altered when renal or digestive functions are compromised or in extreme dietary deficient states. Therefore, macromineral blood concentrations need to be carefully interpreted in light of whether the homeostatic systems are functioning properly.

In recent retrospective studies, a number of significant predictive relationships were found between serum mineral concentrations in the four weeks prior to or following calving and periparturient disease (Van Saun et al., 2004; Van Saun et al., 2006). The association between cows with pre- or postpartum calcium concentrations below 2.0 mmol/l was the most significant observation. Pre- or postpartum cows with serum total calcium <2.0 mmol/l were four times more likely to have postpartum disease problems. Pre- and postpartum sodium concentration <139 mmol/l was also highly associated with postpartum disease risk, particularly SARA. Prepartum potassium concentrations >4.7 mmol/l were also suggestive of an increased periparturient disease risk. These were interesting findings, and suggested that macromineral concentrations may be more predictive than previously thought. Considering this previous data, the concentrations of magnesium, sodium, potassium, and chloride, as well as the triglycerides and NEFA concentrations measured in the current study, were significantly decreased in dairy cows suffering from SARA as observed in periparturient cows. We suppose that this higher level of NEFA in herds of group C is not related at the condition of subacute ruminal acidosis but is fortuitous and related at strong differences level of NEFA between the cows on this periparturient period where there are a high risk of ketosis. The same consideration can be possible for the triglycerides, in effect although this study showed statistical differences between the tree group we supposed that this result is fortuitous because the range of values of triglycerides in this work is very narrow. The fall in potassium and magnesium serum concentrations in cows with ruminal acidosis could probably have been the result of an

increased urinary mineral excretion. The concentration of phosphorus increased linearly as the risk of SARA is more important.

The changes in the concentrations of blood parameters are mainly a response to colostrum formation, changes in dry matter intake, and ruminal metabolism around calving, when dairy cows are more exposed to the risk of developing SARA (Meglia et al., 2001). In particular, this significant association between serum electrolytes (sodium, potassium, and chloride) and SARA might suggest that the acid-base status or the fluid dynamics may play a role in the pathogenesis of periparturient diseases. Data from the literature (Delaquis and Block, 1995) report that small variations on the positive anion-cation balance can influence the acid-bases status. In fact, a high anion-cation balance can increase water absorption and urinary volume; a low anion-cation balance increases faecal excretion and both absorption and excretion of sulphur, whereas it decreases urinary $H^+$ and $HCO_3^-$ concentration. Anionic salts may eventually decrease rumen pH, with an average of 0.12 units, possibly due to the influence of salts on rumen fluid (Vagnoni and Oetzel, 1998).

The calculated erythrocytic indices showed that the values obtained for MCV, MCH and MCHC were within expected normal ranges. Although no substantial changes in the level of RBC, MCH, MCHC, and MCV were observed, dairy cows with SARA in the present study exhibited a significant decrease in MCV and MCH, and an increase in MCHC, in contrast to observations made by other authors (Akinbamijo et al., 1998; Omer et al., 2002). It is possible that the slight increase in MCHC observed in the herd with low ruminal pH is a compensatory erythropoietic action in response to the slowly decline in the HCT. The values of these factors were essential for the classification of anemia. Although anemia is an important symptom reported by other authors (Bonfanti et al., 2004; Mbassa et al., 1994), its precise link with subacute ruminal acidosis is still unknown.

White blood cells were significantly higher in the dairy cows belonging to the farms of group C (acidosis). As suggested in previous studies (Jacob et al., 2001), this involves an increase of cortisol levels that are significantly involved in various events during periparturient period including initiation of parturition. In effect, according this previous work (Jacob et al., 2001), were the study was conducted to estimate the serum cortisol concentration in cows and the neonatal calves in order to correlate the effect of cortisol on certain haematological and biochemical parameters such as blood glucose level, total plasma protein, lymphocyte:neutrophil ratio and mitogen induced lymphocyte proliferative response, our study confirmed that the dam at the time of parturition and neonatal calf before taking colostrum are under a high risk of infection and diseases (for example subacute ruminal acidosis) because of the low profile of immune status. The lymphocyte:neutrophil ratio also justified the above suggestion.

## CONCLUSIONS

Based on the results obtained in the current study, it is possible to suggest that, during SARA in dairy cows, beyond the variations of ruminal pH, alterations of some hematochemical and hematological parameters are present.

## TRIAL 3

## EFFECTS OF RUMENOCENTESIS ON HEALTH AND WELFARE STATUS OF DAIRY COWS

### Infrared thermography

Infrared thermography (IRT) is a non-invasive technique capable of detecting thermal radiation from the surface of any object (Eddy et al., 2001). In animals, the body surface temperature is a function of blood flow and metabolic rate of the underlying tissues. Thus, the physiological state of the underlying cells could potentially be assessed by measuring skin temperature using IRT (Eddy et al., 2001). Infrared thermography and potential veterinary applications for this imaging technique have already been described (Purohit and McCoy, 1980; Vaden et al., 1980; Turner, 1991; Marr, 1992; Mogg and Pollit, 1992; Denoix, 1994; Von Schweinitz, 1999; Spire et al., 1999; Eddy et al. 2001; Turner, 2001). These reports mostly describe thermographic imaging of spontaneous disease and attempts to correlate images to disease or injury diagnosed by other means. A study in cattle has revealed the successful utilization of thermography in the detection of localized sepsis in the pinna after contaminated growth stimulant pellets had been administered (Spire et al., 1999). Infrared thermography has been used to predict changes in udder temperature (Berry et al., 2003) and to detect inflammation associated with hot-iron and freeze branding in cattle (Schwartzkopf-Genswein and Stookey, 1997), and bovine viral diarrhea infection in calves (Schaefer et al., 2004). Soles of hooves affected by subclinical laminitis commonly appear soft and warm long before the appearance of yellowish discoloration, lesions, and ulcers (Nocek, 1997).

## MATERIALS AND METHODS

### Animals

Twelve multiparous (third lactation and greater) Holstein dairy cows from a commercial herd in which prevalence of SARA was highly suspected were used in this trial. Selected cows were without clinical signs of disease, had high average milk production (about 10000 Kg per year) and good body condition score (BCS), and were between 31 and 48 days in milk (DIM) at the initiation of the study. All animals object of study were fed the same diet. Days in milk was equally balanced between the two cow groups (38 vs 37.2, $P>.7$). Cows were housed in free stalls, fed a total mixed ration (TMR), and had received a steam-up diet in the final part of the dry period.

### Experimental Design

Selected cows were divided in to 2 groups of 6 cows. One group had rumenocentesis (GA) performed while the second group (GB) received a sham procedure. Both cow groups underwent shearing by electric clipper (Kruuse, ca. n. 247070) and triple manual disinfection of the proposed rumenocentesis site. Rumenocentesis was performed on GA cows as described by Morgante and Vescera (2000), without sedation, using a 13 ga 105 mm needle. None of animals showed any resistance during the sampling and puncture of the rumen.

Animal response to rumenocentesis or sham procedure was monitored from just prior (reference time) to 20 days following the procedure. A physical examination of each cow was performed at 48 h, 96 h, and 20 days after rumenocentesis. Daily milk yield was determined at each time period.

Blood samples were collected at -1 h (reference period), 48 h, 96 h, and 20 days (relative to procedure application) from a jugular vein into EDTA and lithium heparin containing tubes and vacutainer tubes without anticoagulant (BD Vacutainer Systems®, Preanalytical Solutions, Belliver Industrial Estate, Plymouth, UK). Blood samples in the tubes without anticoagulant and with lithium heparin were centrifuged at 2500 x g at 4°C for 20 min, and serum and plasma, respectively, were harvested and frozen (–20°C) until further analysis.

Skin surface temperature at the rumenocentesis site was measured prior to, immediately after, and at 48 h, 96 h, and 20 days after rumenocentesis. All images were scanned using a hand-held portable infrared camera (Flir System, Model ThermaCam P25), which was calibrated to ambient temperature and absorptive conditions on each sampling day. The emissivity value settled on the camera before conducting scanning was 0.93. To reduce the effects of environmental factors on thermal data, all images were scanned within the barn and at the same distance (1 m) from the subject. Temperatures were recovered by processing the thermographic images of a squared area located in the area of rumenocentesis, described by Nordlund and Garret (1994). All thermographic images were analyzed for average and maximal temperature by ThermaCam Researcher Basic Software (Flir System).

**Laboratory Assays**

Within 2h of collection, blood in the EDTA-containing tubes was analyzed for white (WBC) and red (RBC) blood cell counts, differential WBC count (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total platelet count (PLT), and mean platelet volume (MPV) using automated equipment (Abbott Cell Dyn 3500, Abbott Diagnostic Division, CA, USA).

Harvested plasma samples were analyzed for a range of clinical chemistry parameters (creatinine, aspartate transaminase (AST), albumin, total protein, gamma-glutamyl transferase (γGT), calcium, phosphorus, magnesium, sodium, potassium, chloride, glucose, cholesterol, triglycerides, non-esterified fatty acids (NEFA), total bilirubin, direct and indirect bilirubin using an automated analyzer (*BM Hitachi 911,* ROCHE, Basel, Switzerland). Total protein in serum was determined by automated analyzer (BM Hitachi 911, ROCHE, Basel, Switzerland). Albumin and globulin, alpha-1, alpha-2, beta-1, beta-2, and gamma were determined from serum samples by electrophoresis. Albumin to globulin ratio (A/G) was calculated from these data. The electrophoretic protein

separation in 0.8 % agar gel was obtained using a semiquantitative and multiparametic analyzer (Hydrasis LC, SEBIA, Issy-les-Moulineaux, France) provided with specific Phoresis software.

Haptoglobin was determined by manual method measuring the spectrophotometric peroxidase activity of free hemoglobin (Phase$^{TM}$Range, Haptoglobin Assay, Tridelta Development Limited, Bray, County Wicklow, Ireland).

**Statistical Analysis**

Data obtained were analyzed by ANOVA for repeated measures to verify the effect of rumenocentesis using the Proc Mixed procedure of SAS (Littell et al., 1999). All dependent variables measured over time periods were evaluated using the following model:

$$Y_{ijk} = \mu + Treatment_i + Time_j + (Treatment \times Time)_{ij} + e_{ijk}$$

where: $Y_{ijk}$ = dependent variable, $\mu$ = overall mean; $Treatment_i$ = main effect of rumenocentesis or sham procedure, $Time_j$ = main effect of time period, $(Treatment \times Time)_{ij}$ = interaction term of main effects, and $e_{ijk}$ = residual error term. Covariance structures accounting for differing time intervals were used and best model fit was determined by lowest parameter values for covariance structure. Where significant main effects were determined, pairwise mean comparisons were determined by least significant difference method. Significance was determined at $\leq 0.05$, unless otherwise indicated. Data presented are least squared means ± SEM.

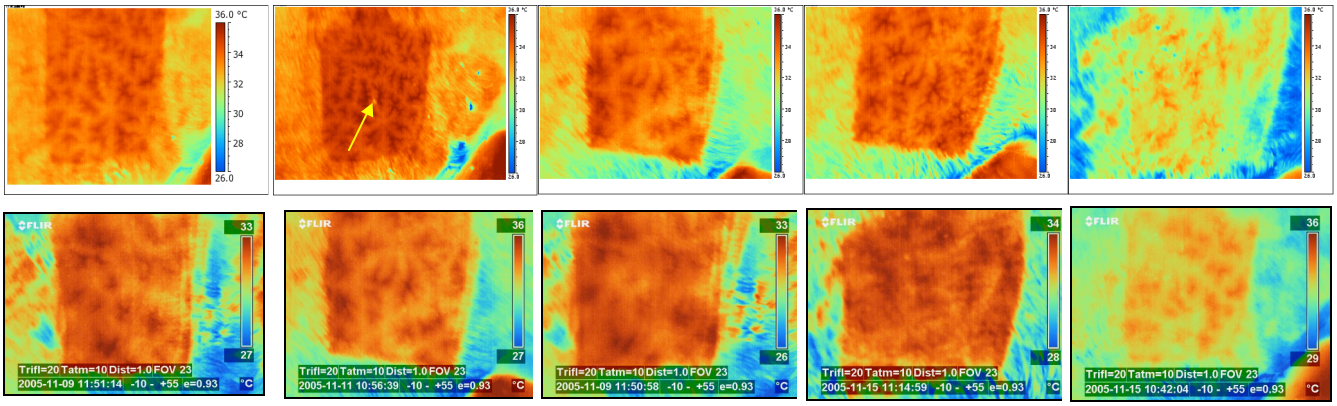
## RESULTS

Physical examinations did not identify any clinical signs associated with the animals of either group. Figure 1 shows a series of thermographic images of the rumenocentesis site from a selected representative cow from both treatment groups. Neither average nor maximal skin temperatures were influenced by procedure group, though there was a group by time interaction ($P < 0.0001$). At the site of rumenocentesis average skin temperature in GA cows showed an increase of nearly 1.0 °C immediately after the rumen collection with a reduction at 48 h and stabilization to a normal

level for the remainder of the study (Figure 2A). In contrast, GB cows maintained a stable normal skin temperature for the duration of the study. A similar response over time was observed for maximal skin temperature (Figure 2B). Milk production was not different between groups, but milk yield was influenced by period during the study (Figure 3). Averaged milk yield for cows in both groups was lower at 48 h (28.5 vs. 28.3 kg/d, $P = 0.03$) and tended to be lower at 96 h (28.5 vs. 27.9 kg/d, $P = 0.1$) compared to initial milk production. Milk yield in GA cows at 96 h was more variable. Although no differences were found in determining milk yield change between time points or from reference point across groups, group GA cows showed a decline and a tendency for a decline in milk from reference time (28.5 kg/d) to 48 h (28.3 kg/d, $P = .04$) and 96 h (27.7 kg/d, $P = 0.08$), respectively. In GA group cows only, milk yield at 20 d (28.1 kg/d) was greater ($P = 0.03$) compared to 96 h (27.7 kg/d).

Of all parameters measured in blood samples, six parameters were influenced by procedure group and nearly half of the parameters were influenced by a treatment by time interaction. Neutrophil count ($P = 0.003$), monocyte percent ($P = 0.04$), total ($P < 0.0001$) and direct ($P = 0.01$) bilirubin, haptoglobin ($P = 0.03$) and magnesium concentration ($P = 0.02$) were all influenced by procedure group. For all comparisons by procedure group, GB cows had higher mean values compared to GA cows with the exception of monocyte percent. All values within each procedure group were considered to be within expected reference values (Kaneko et al., 1997). Absolute neutrophil cell count at -1 and 48 h after rumenocentesis were greater ($P = 0.03$) in GB cows compared to GA cows (Figure 4). Although there were a number of time x treatment interactions that were significant (Table 1), this interaction effect reflected differences in parameter values changing over time within procedure group with minimal significant difference between mean values at specific times.

**Figure 1.** Series of thermographic images from a representative cow in group GA (upper) and GB (bottom).




IRT before rumenocentesis

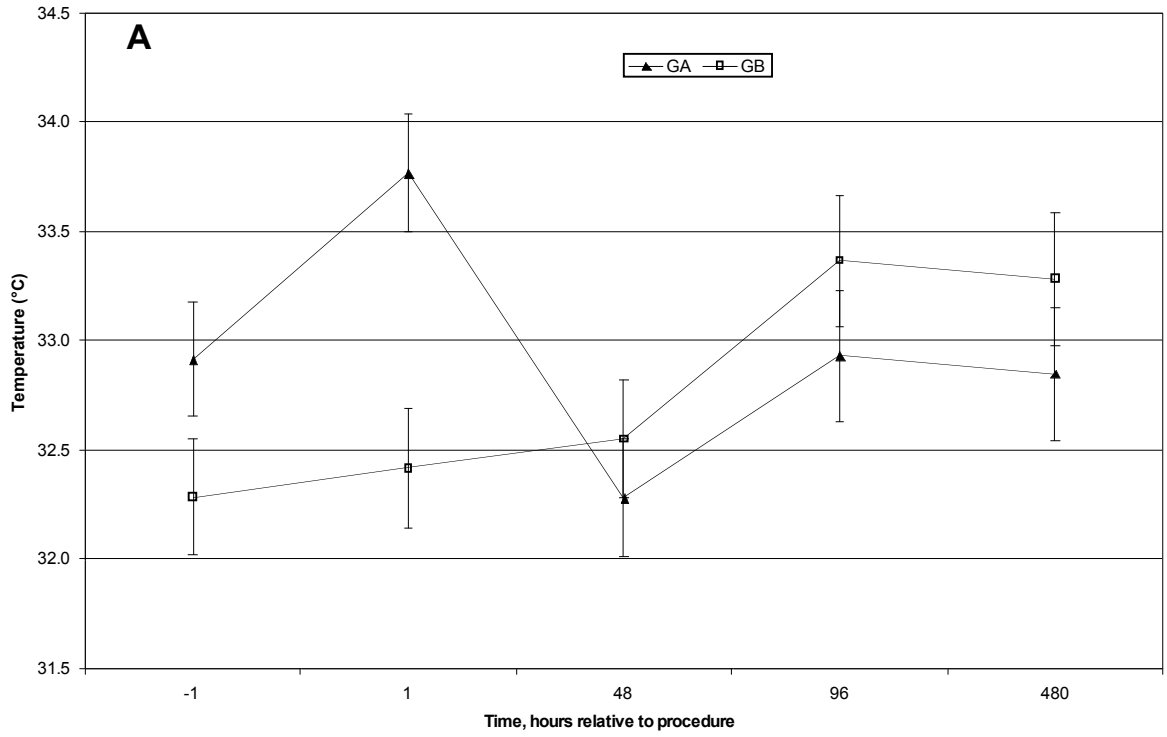
IRT immediately after rumenocentesis

IRT 48 hours after rumenocentesis

IRT 96 hours after rumenocentesis

IRT 20 days after rumenocentesis

**Figure 2.** Least squared means of average (A) and maximal (B) skin temperature (°C) in cows at the site of rumenocentesis (GA) or sham procedure (GB).



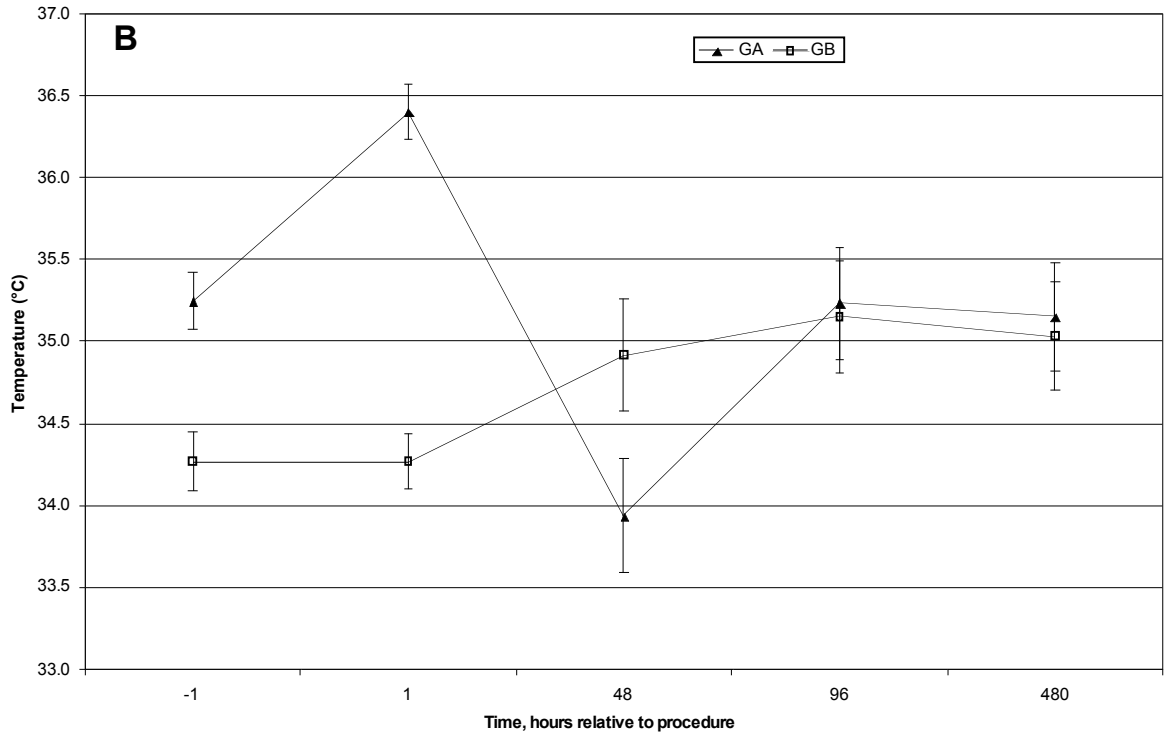



**Figure 3.** Least squared means for milk yield in cows either experiencing a ruminocentesis (GA) or sham (GB) procedure.

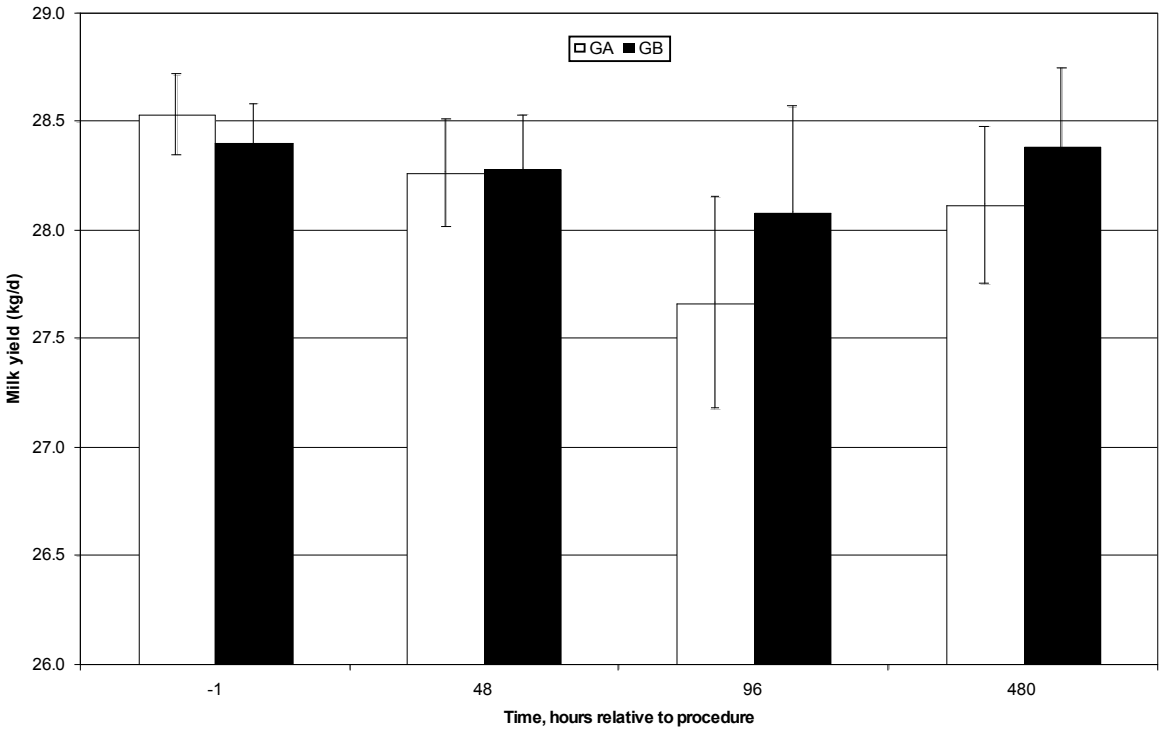



**Figure 4.** Least squared means for neutrophil count in cows either experiencing a ruminocentesis (GA) or sham (GB) procedure.

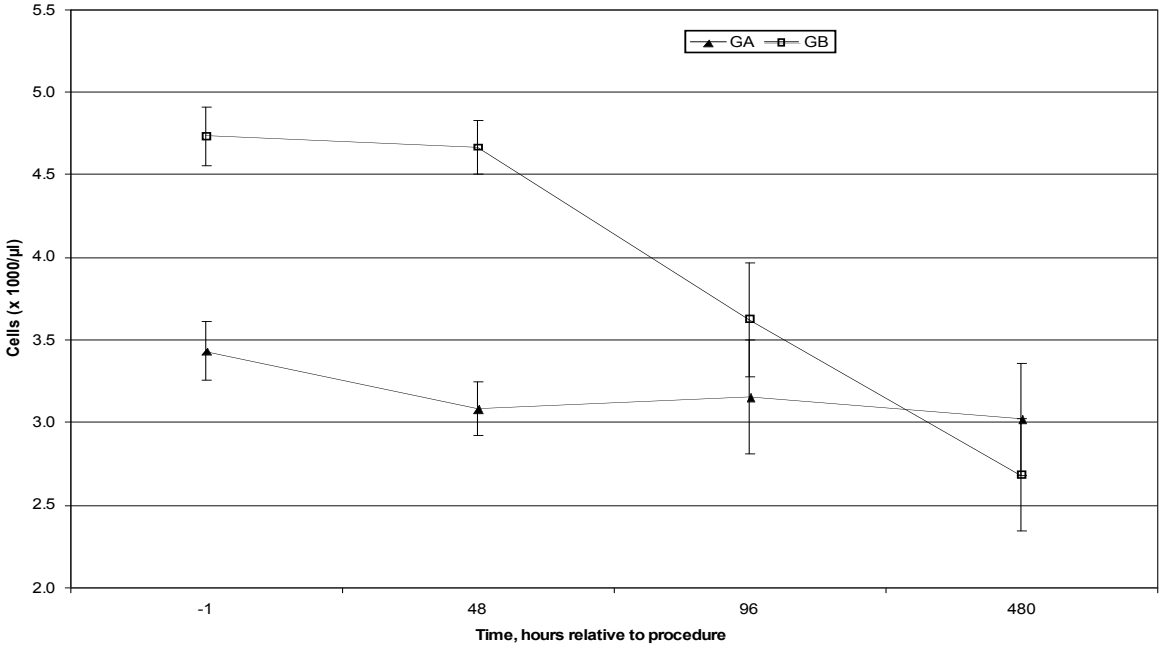


**Table 1**. Least squared means and pooled standard error (PSE) for selected blood parameters (significant treatment (Trmt) or treatment by time (Trmt x Time) interaction effects) measured in cows either experiencing a ruminocentesis (GA) or sham (GB) procedure.[1]

| Parameter[2] | Units | 1 hour before | | 48 h post-collection | | 96 h post-collection | | 20 days post-collection | | PSE | Main Effects (P<F)[3] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | GA | GB | GA | GB | GA | GB | GA | GB | | Trmt | Time | Trmt x Time |
| **WBC Measures:** | | | | | | | | | | | | | |
| **Neutrophils** | $10^3/\mu l$ | $3.43^{b}$ | $4.73^{a}$ | $3.08^{b}$ | $4.67^{a}$ | 3.16 | 3.62 | 3.02 | 2.68 | 0.15 | 0.003 | 0.02 | 0.09 |
| **Neutrophils** | % | 49.97 | 56.50 | 42.38 | 53.35 | 45.95 | 50.00 | $48.50^{c}$ | $38.27^{d}$ | 10.4 | NS | 0.009 | 0.01 |
| **Monocytes** | % | 9.85 | 8.87 | $10.63^{a}$ | $8.83^{b}$ | 1.42 | 1.33 | $10.97^{a}$ | $7.98^{b}$ | 0.57 | 0.04 | <0.0001 | 0.03 |
| **Lymphocytes** | % | 39.92 | 33.83 | 45.32 | 35.22 | 32.77 | 39.33 | $40.47^{b}$ | $49.83^{a}$ | 9.3 | NS | 0.0001 | 0.02 |
| **Basophils** | % | 0.45 | 0.60 | 0.62 | 0.52 | 0.83 | 0.50 | 0.37 | 0.42 | 0.36 | NS | 0.14 | 0.002 |
| **Hematology Measures:** | | | | | | | | | | | | | |
| **MCV** | fL | 46.9 | 47.0 | 46.8 | 47.5 | 44.6 | 45.1 | 46.4 | 46.9 | 0.33 | NS | <0.0001 | <0.0001 |
| **MCH** | pg | 15.60 | 15.95 | 15.75 | 15.95 | 16.23 | 17.55 | 15.75 | 16.15 | 0.17 | NS | 0.03 | 0.05 |
| **MCHC** | g/dL | $33.3^{d}$ | $33.9^{c}$ | 33.7 | 33.7 | 36.4 | 38.9 | $34.0^{d}$ | $34.4^{c}$ | 0.32 | 0.10 | <0.0001 | 0.0003 |
| **RDW** | % | $18.85^{d}$ | $21.78^{c}$ | 19.98 | 21.72 | 21.25 | 22.70 | $21.80^{a}$ | $20.08^{b}$ | 0.26 | 0.13 | 0.001 | 0.0002 |
| **Protein Measures:** | | | | | | | | | | | | | |
| **Total protein** | g/l | 74.7 | 70.5 | 76.0 | 71.8 | 73.2 | 73.8 | $70.5^{b}$ | $75.7^{a}$ | 0.65 | NS | 0.05 | 0.0008 |
| **Albumin** | g/l | 35.1 | 35.0 | $36.3^{c}$ | $34.6^{d}$ | 33.3 | 36.0 | 34.4 | 35.7 | 0.28 | NS | NS | 0.002 |
| **Alpha-1** | % | 5.25 | 5.48 | 5.13 | 5.40 | 5.60 | 5.38 | 5.90 | 5.53 | 0.09 | NS | 0.0004 | 0.006 |
| **Alpha-2** | g/l | 8.57 | 8.67 | 8.70 | 8.67 | $8.37^{d}$ | $9.43^{c}$ | 9.32 | 9.08 | 0.10 | NS | NS | 0.004 |
| **Alpha-2** | % | 11.47 | 12.23 | 11.47 | 12.07 | $11.42^{b}$ | $12.77^{a}$ | 13.30 | 12.05 | 0.15 | NS | 0.07 | 0.0006 |
| **Beta-1** | g/l | 6.53 | 6.35 | $6.58^{a}$ | $6.23^{b}$ | 6.45 | 6.35 | $6.35^{f}$ | $7.13^{e}$ | 0.12 | NS | 0.008 | 0.0004 |
| **Beta-1** | % | 7.67 | 6.28 | 8.67 | 8.68 | 8.80 | 8.58 | 9.02 | 9.40 | 0.18 | NS | 0.0004 | 0.01 |
| **Beta-2** | % | 6.28 | 6.30 | 5.48 | 6.43 | 6.50 | 6.00 | 5.90 | 6.58 | 0.13 | NS | 0.09 | 0.04 |
| **Gamma** | g/l | 15.88 | 13.22 | 16.48 | 14.40 | 14.92 | 13.90 | 12.27 | 14.75 | 0.51 | NS | <0.0001 | <0.0001 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gamma** | % | 21.10 | 18.47 | 21.52 | 19.67 | 20.12 | 18.52 | 17.22 | 19.37 | 0.54 | NS | <0.0001 | 0.008 |
| **Clinical Chemistry Measures:** | | | | | | | | | | | | | |
| **Bilirubin, Total** | µmol/l | 3.38 | 3.27 | 3.49 | 2.97 | 4.17 | 4.72 | 3.95[f] | 4.80[e] | 0.11 | <0.0001 | <0.0001 | 0.03 |
| **Bilirubin, Direct** | µmol/l | 1.41 | 1.77 | 1.29 | 1.25 | 0.58 | 1.00 | 0.80[b] | 1.11[a] | 0.06 | 0.01 | <0.0001 | 0.11 |
| **Bilirubin,Indirect** | µmol/l | 1.98 | 1.50 | 2.21 | 1.72 | 3.59 | 3.72 | 3.15[d] | 3.69[c] | 0.14 | NS | <0.0001 | 0.001 |
| **CK** | U/l | 166.7 | 135.5 | 110.2 | 110.5 | 112.0 | 113.5 | 99.0 | 120.2 | 4.1 | NS | 0.001 | 0.03 |
| **Haptoglobin** | mg/dl | 17.33 | 20.17 | 18.83 | 27.67 | 21.67[b] | 33.33[a] | 28.67 | 24.50 | 1.44 | 0.03 | 0.01 | 0.04 |
| **GGT** | U/l | 27.83 | 28.50 | 28.17 | 30.00 | 25.00 | 29.00 | 27.83 | 28.50 | 0.66 | NS | <0.0001 | 0.02 |
| **Magnesium** | mmol/l | 1.01 | 1.08 | 1.04 | 1.02 | 1.01[f] | 1.24[e] | 0.99 | 0.99 | 0.013 | 0.02 | <0.0001 | <0.0001 |
| **Urea** | mmol/l | 4.15 | 3.48 | 4.48[e] | 2.78[f] | 5.65[e] | 3.23[f] | 3.28[f] | 6.53[e] | 0.20 | 0.18 | 0.0007 | <0.0001 |

[1]Mean comparisons within rows between treatment groups and within time periods: [ab]P<0.05; [cd]P<0.01; [ef]P<0.001

[2]Abbreviations: WBC = white blood cell; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red distribution while; CK = creatine kinase; GGT = gamma-glutamyltransferase.

[3]NS = not significant, P>0.25

## DISCUSSION

Many authors consider rumenocentesis as an invasive technique and several practitioners consider it too difficult to be used in routine clinical investigations. Rumenocentesis is considered an invasive technique involving surgical preparation of the site as well as physical and chemical restraint, thus placing the animal at risk for localized abscessation or peritonitis.

In the current study none of the animals showed any resistance during sampling and no local or systemic reactions were observed in this study. A previous study (Morgante and Vescera, 2000) also reported no local or general problems. Nevertheless, many authors reported various problems related to the rumenocentesis such as abscesses and peritonitis (Aceto et al., 2000; Hollberg, 1984; Strabel et al., 2006).

In this study superficial temperatures of a squared area located on the rumenocentesis site were measured before rumenocentesis, immediately after, and at 48 h, 96 h and 20 days.

Temperature (average and maximal) on the area of rumenocentesis increased (not significantly) 1.0°C immediately after the rumen collection. Therefore, we can see that this increase of 1.0°C on group GA is related to the manual procedure during rumenocentesis. In effect, already 24 hours after rumenocentesis the temperature returned to the initial level and remained constant for the remaining period of the study.

Moreover, daily milk productions were recorded to verify that rumenocentesis did not decrease production, although there was some suggestion of reduced milk yield at 96 hours in the cows experiencing rumenocentesis. Further study with larger number of animals would be necessary to determine if this temporal change is truly significant.

Complications related to rumenocentesis may include some local inflammation with a subsequent increase in temperature at the site of the rumenocentesis, and some variation of blood parameters (i.e., albumin and globulin, alpha-1, alpha-2, beta, gamma and albumin-globulin ratio). A complete blood count is helpful in supporting a diagnosis of local inflammation. Unless the infection is completely isolated by a fibrous tissue capsule or is small in size relative to the size of the animal

there will be a leukocytosis and an elevation of polymorphonuclear leukocytes in acute lesions or of lymphocytes and monocytes in more chronic ones. A moderate anemia is usual in chronic lesions, and mild proteinuria is common.

In all the blood samples studied (Table 1), there were minimal significant differences between the hematological parameters obtained from the cattle of the two groups and all values were within expected reference ranges.

Observing the differences between group (GA, cow underwent ruminocentesis and GB, sham procedures) we observed a greater value in neutrophil and lymphocyte percents at 20 days after rumenocentesis in GA cows compared to GB cows, which can suggest a minimal evidence of inflammatory response. Nevertheless, all values were within expected reference ranges and absolute counts of neutrophil and lymphocyte were not significantly different.

Through electrophoresis different subpopulations of blood proteins can be distinguished. Modification in serum protein fraction concentrations suggests special significance in relation to the issue of animal welfare. Inflammatory conditions often result in electrophoretic protein separation alterations, especially the α-globulin fractions. Inflammatory situations result in increased production of serum anti-inflammatory proteins such as α1-antitrypsin and α2- macroglobulin to adjust homeostatic environmental conditions. In addition, a high gamma-globulinemia may be due to an active infective environment. For the duration of the current study we observed similar shifts in electrophoretic protein separation, particularly an increase of beta-1 and globulin in GB compared to GA cows at 20 days after rumenocentesis. We hypothesize that these differences were not related to the different treatments, but most likely a result of factors (intensity feed intake, high milk production) that typically occur in early lactation and can influence these parameters. Similar differences in total and direct bilirubin at 20 days following the treatment procedure were observed with higher values in group GB, though all values were within expected reference ranges.

Haptoglobin is an acute phase glycoprotein synthesized by the liver in response to soluble mediators produced by activated white blood cells and macrophages. Under normal

circumstances without an active cellular response it is absent or present at low concentrations in serum. Haptoglobin significantly increases in response to problems of infection, inflammation, and immune disorders. In the current study a significant increase in haptoglobin concentration was not observed in animals where rumenocentesis was performed (GA group). This finding suggests that rumenocentesis, as used to diagnose subacute ruminal acidosis, has no to minimal negative impact on health of lactating cows.

Although nearly half of the measured blood parameters were influenced by a treatment by time interaction in the current study, we suggest that much of these observed responses were related at the period of study relative to calving. These blood parameters are greatly influenced by time relative to calving as evidenced by the highly significant effect of time found with most parameters. Mean differences observed for serum urea concentration are related a feed intake differences over time during the study observational period. The observed higher mean urea values for GA compared to GB cows might suggest greater intake and further substantiate minimal negative effects of the rumenocentesis procedure.

According to previous studies (Duffield et al., 2004; Kleen et al., 2004; Morgante et al., 2007) our data suggest that rumenocentesis is a useful and valuable diagnostic tool in bovine medicine. Our experience suggests that the side effects of rumenocentesis depend on the material and procedure used. A single use sterile needle of 12 cm 13 gauge is adequate. In our experience, good preparation of the area is very important (shaving and disinfection) as well as the handling of the cow during the procedure. The puncture area should also be appropriately desensitized by prior compression from the operator first to avoid a sudden reaction from the cow to the needle introduction. It is also very important to avoid inserting the needle during the contraction of the ventral sac of the rumen and is essential that the duration of the aspiration procedure must not exceed one or two minutes.

## CONCLUSIONS

In conclusion, although some authors described several problems that could be related to use of rumenocentesis to diagnose subacute ruminal acidosis (SARA), this study showed no significant negative impact on animal health, welfare, and milk yield of lactating cattle following this technique. At the Faculty of Veterinary Medicine of the University of Padua, rumenocentesis has since been used as a standard procedure without observed complications for several years.

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