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FERMENTATIVE DISTURBS IN DAIRY COW: SUBACUTE RUMINAL ACIDOSIS IN FIELD CONDITIONS AND METABOLIC-INFLAMMATORY EFFECTS OBSERVED

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Abstract

Transition period is defined as 3 weeks prepartum until 3 weeks postpartum and it is a period marked by changes in endocrine status to accommodate parturition and lactogenesis. Most infectious diseases and metabolic disorders occur during this time. Probably nutrition of transition cows is the key for the success of a dairy farm, since cows during the transition period are very sensible and a well fed herd will probably have more opportunity to be in good health status; but it is important at the same time to consider the environment and the management of cows groups within the herd in order to prevent stress which could influence feeding behavior. Forestomach motility of ruminants, especially cattle, is of major concern to the veterinarian, especially during the transition period. Among disturbs of rumen fermentation we can include Subacute Ruminal Acidosis (SARA) that is primarily a variation in rumen fermentation, tipical at the beginning of lactation…but not only. The disease seems to represent one of the most important fermentative/metabolic disorders in intensive dairy farms that affects rumen fermentations, animal welfare, productivity and farm profitability. According to the literature SARA may be caused by formulation of rations that contain excessive amounts of rapidly fermentable carbohydrates but also by a deficency of fiber or errors in delivery of the rations. In fact we can find SARA also in herds with correctly formulated diets in a chemical point of view and in this case the problem is probably related to management and physical treatment of the ration. This is probably the reason why we have SARA not only in early lactation but also in mid lactation: in the lattest situation SARA is not caused by inadequate adaptation of rumen papillae to the lactation diet because SARA appears long time after calving. 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. Many authors studied the acute phase response during SARA. It has been suggested that low rumen pH could result in death and lysis of gram-negative bacteria that are in the rumen and hence increase free endotoxins in the rumen. It has been suggested that the acidic rumen environment, changes in osmotic pressure, and ruminal LPS may render the rumen epithelium susceptible to injury resulting in translocation of rumen endotoxin (which is a strong inducer of acute phase response) into the bloodstream. The role of free ruminal LPS in SARA remains difficult to ascertain because free LPS is detoxified in the liver and hence is not detectable in peripheral blood circulation. Acute phase proteins are the best indicators of an acute phase response but other indicators of inflammation such as fibrinogen and white blood cells can also be used as markers. The use of other blood parameters like base excess

(BE) or blood pH has been cited as diagnostic tool of non-acute ruminal acidosis. Because of an absorption of SCFA by the ruminal wall, the BE may be reduced.

The objective was to investigate, analyze and study changes in blood, urine and faeces parameters in dairy cows affected by SARA in field conditions. The project was a collaboration which involved the Department of Veterinary Clinical Science of the University of Padova, the Istituto Zooprofilattico Sperimentale delle Venezie and the animal feed company Cortal Extrasoy s.p.a. that follow 10 of these farms for the nutrition plan. Twelve farms were investigated; farms where selected with similar characteristics about production, management and structures in the north-east of Italy (Veneto region). Many samples were collected (rumen liquid, urine, faeces and blood) from 132 lactating cows. Rumen liquid, urine and faeces pH was measured in field; ematochemical, ematological and blood gas analysis were then perfomed on blood and urine samples to the IZS delle Venezie and acute phase proteins were measured to the Faculty of Veterinary Medicine of Glasgow. Results showed the absence of strong correlation between SARA and APR leading to think that SARA as described in the literature is not really SARA that we find in field. According to our data we can not say that there is a direct relationship between SARA and metabolism and between SARA and blood gases; some intermediate products like homocysteine or other toxic products like valerate could affect general metabolism but this subject must be investigated more. We found alteration in blood parameters (leukocytosis with stress-like leukogram that included altered neutrophils/lymphocytes ratio, albumin alteration, some alteration in APP that could be related to stress as well): these data suggest that SARA could be related to a general unhealthy status and welfare decrease. It would be interesting to investigate more on causes and effects of SARA to understand if this fermentative disturb is the consequence of stressful situations: neuroendocrine conditions, management and environment could be related to SARA since several neuropeptides control voluntary feed intake and their effect may result in sorting out feeds from the diet. We hypothesize also that at the base of SARA onset in our region there could be a gastro-intestinal motility problem: low rumen pH values detectable during SARA could lead to weaker rumen motility inhibited by certain mechanisms arising during low pH phases within the reticulo-ruminal environment. Our data confirmed that SARA must be considered as a herd problem and even if the transition period is the most delicate in the cow's carrier SARA must be considered in every lactation stage and related not only to feeding management but to the equilibrium between the animals and the environment (general herd management, structures and welfare of cows).

Riassunto

Il periodo di transizione viene definite come il periodo che va da 3 settimane prima del parto a 3 settimane dopo il parto ed è un periodo caratterizzato da profondi cambiamenti a livello endocrine che hanno lo scopo di sopperire alle necessità del parto e della lattogenesi. La maggior parte delle patologie infettive e metaboliche si verificano in questo periodo. Probabilmente la nutrizione delle bovine è la chiave di successo di una azienda da latte, dal momento che le vacche nel periodo di transizione sono molto sensibili e che una mandria ben nutrita probabilmente avrà maggiore opportunità di godere di buona salute. È però importante allo stesso tempo considerare l'ambiente circostante e la gestione dei gruppi all'interno dell'intera mandria con lo scopo di prevenire qualunque elemento di stress che potrebbe a sua volta influenzare il comportamento alimentare degli animali. La motilità dell'apparato gastroenterico dei ruminanti, specialmente per i bovini, è una delle maggiori preoccupazioni per il veterinario, specialmente durante il periodo di transizione. Tra i disturbi fermentativi che colpiscono la vacca da latte possiamo includere l'acidosi ruminale subacuta (SARA) che è primariamente una variazione delle fermentazioni ruminali, e si verifica all'inizio della lattazione…ma non solo. Questa patologia sembra rappresentare uno dei maggiori problemi metabolico-fermentativi delle aziende da latte intensive che interessa le fermentazioni ruminali ma anche il benessere animale, la produzione ed il profitto. Secondo quanto riportato in letteratura SARA potrebbe essere causata dalla formulazione di razioni che contengono un eccesso di carboidrati rapidamente fermentescibili ma anche da una carenza di fibra o errori nella preparazione e distribuzione della razione. Infatti possiamo trovare SARA anche in mandrie con diete correttamente formulate dal punto di vista della composizione chimica e in questo caso probabilmente il problema è collegato al management e ai trattamenti fisici dell'alimento. Questo spiegherebbe perché troviamo SARA non solo a inizio lattazione ma anche a lattazione avanzata: in quest'ultima situazione SARA non può essere causata da un inadeguato adattamento delle papille ruminali alla dieta da lattazione perché compare molto tempo dopo il parto. Durante la lattazione avanzata lo sviluppo di SARA è legato a fattori manageriali come la frequenza di alimentazione, processi di trattamento dell'alimento come ad esempio l'uso del pellet, e i ricoveri o fattori simili. Molti autori hanno studiato la risposta di fase acuta in condizioni di SARA. È stato suggerito che il pH basso del rumine possa risultare nella morte e lisi dei batteri gram-negativi contenuti nel rumine stesso e quindi nell'aumento di endotossine libere (LPS). Si suppone che l'ambiente acido a livello ruminale, i cambi di pressione osmotica e i lipopolisaccaridi (LPS) liberi possano rendere l'epitelio

ruminale suscettibile a insulti che permetterebbero il passaggio delle endotossine (che sono forti induttori della risposta di fase acuta) nel circolo sanguigno. Il ruolo dei LPS in corso di SARA resta difficile da definire perché i LPS liberi vengono detossificati nel fegato e quindi non sono misurabili nel sangue periferico. Le proteine di fase acuta sono i migliori indicatori di una risposta di fase acuta ma altri indicatori di infiammazione come il fibrinogeno e i leucociti possono essere usati come markers. L'uso di altri parametri come il base excess (BE) o il pH sanguigno è stato citato come strumento diagnostico di acidosi ruminale non acuta: a causa dell'assorbimento di acidi grassi volatili a catena corta attraverso la parete ruminale, il BE dovrebbe ridursi.

L'obiettivo di questo lavoro era di approfondire, analizzare e studiare i cambiamenti di parametri a livello ematico, urinario e fecale in vacche da latte con SARA in condizioni di campo. Il progetto è stato realizzato grazie ad una collaborazione tra il Dipartimento di Scienze Cliniche Veterinarie dell'Università di Padova, l'Istituto Zooprofilattico Sperimentale delle Venezie e la ditta mangimistica Cortal Extrasoy s.p.a. che segue 10 delle aziende dal punto di vista nutrizionale. Dodici aziende sono state incluse; sono state scelte nel nord-est dell'Italia (in regione Veneto)con caratteristiche simili relativamente alla produzione, la gestione e le strutture. Diversi campioni sono stati raccolti (liquido ruminale, urine, feci e sangue) da 132 vacche in lattazione. Il pH del liquido ruminale, delle urine e delle feci è stato misurato in campo; sui campioni di sangue e di urine sono stati fatti profili biochimici, oltre all'esame emocromocitometrico, l'emogasanalisi (presso IZS delle Venezie) e la misurazione delle proteine di fase acuta (presso la Facoltà di Medicina Veterinaria di Glasgow). I risultati hanno mostrato l'assenza di correlazioni forti tra SARA e risposta di fase acuta portando a pensare che SARA così come è descritta in letteratura non è realmente la SARA che troviamo in campo. Secondo i nostri risultati non esiste una relazione diretta tra SARA e metabolismo e tra SARA e parametri dell'emogas; alcuni prodotti intermedi come l'omocisteina o altri prodotti tossici come l'acido valerianico potrebbero influire sul metabolismo generale ma questo argomento meriterebbe di essere approfondito ulteriormente. Abbiamo trovato alterazioni in parametri sanguigni (leucocitosi con leucogramma assimilabile a situazione di stress, compreso un alterato rapporto neutrofili/linfociti, alterazione dell'albumina, alterazioni delle APP che potrebbero far pensare anch'esse a stress): questo suggerisce che SARA potrebbe essere legata ad uno stato di cattiva salute generale e mancanza di benessere. Sarebbe interessante approfondire le cause e gli effetti di SARA per capire se questo disturbo fermentativo è la conseguenza di una situazione stressante: condizioni neuroendocrine, management e ambiente potrebbero essere correlati a SARA dal momento che neuro peptidi

controllano l'assunzione volontaria di alimento e il loro effetto potrebbe risultare in alterati comportamenti alimentari. Abbiamo ipotizzato anche che alla base dell'insorgenza di SARA nella nostra regione ci possa essere un problema di motilità gastrointestinale: un basso pH registrabile durante SARA potrebbe portare a diminuita motilità inibita da meccanismi scatenati durante le fasi di abbassamento del pH nell'ambiente reticolo-ruminale. I nostri dati confermano che l'acidosi ruminale subacuta deve essere considerata come problema di mandria e, anche se il periodo di transizione è quello più delicato nella carriera di una vacca, SARA deve essere considerata in ogni stadio di lattazione e correlata non solo all'alimentazione ma anche all'equilibrio tra l'animale e l'ambiente (management, strutture e benessere).

Introduction

I borrow from Drackley (1999) an ancient Chinese curse that states, in effect, "May you always live in interesting times": in this context, the transition period between late pregnancy and early lactation (also called the periparturient period) certainly is the most interesting stage of the lactation cycle!

Over the past several decades dairy cows have undergone intensive genetic selection, which has increased milk yield to a level where the demand for nutrients from the diet and body tissues reserves often results in ill-health and infertility. During the same period systems of dairy production have been significantly developed, with the objective of improving producer profitability as the main driving force (Mulligan and Doherty, 2008).

The transition cow

Transition period is defined as 3 weeks prepartum until 3 weeks postpartum. It is a period marked by changes in endocrine status to accommodate parturition and lactogenesis (Grummer 1995) and that's why nutrition and management of cows during the transition period have received tremendous interest in recent years. Several excellent reviews of aspects of this topic have been published (Bell, 1995; Goff, 1997; Grant, 1995; Grummer, 1995).

The production diseases of the dairy cow, during this period, are a manifestation of the cow's inability to cope with the metabolic demands of high production, and they continue to be a cause of economic loss to the dairy industry and an animal welfare concern (Mulligan and Doherty, 2008). Godden et al. (2003) indicate that approximately 25% of cows that left dairy herds in Minnesota from 1996 to 2001 did so during the first 60 DIM. Changes, which are much more dramatic than at any other time during the gestation-lactation cycle, influence tissue metabolism and nutrient utilization. A reduction in feed intake is initiated during the prepartum transition period, yet nutrient demands for support of conceptus growth and initiation of milk synthesis are increasing (Grummer 1995).

Most infectious diseases and metabolic disorders occur during this time. Milk fever, ketosis, retained fetal membranes, metritis, and displaced abomasum primarily impact cows during the periparturient period. Immunosuppression during the periparturient period (Mallard, 1998) leads to increased susceptibility to mastitis. Indeed, the incidence of environmental mastitis is greatest around parturition (Smith, 1985) Thus, the occurrence of health problems is centered disproportionately on this relatively short period, which certainly contributes to making this an "interesting" time for dairy producers. (Drackley, 1999).

Production diseases may be considered ‘a man-made problem’ resulting in ‘a breakdown of the various metabolic systems of the body under the combined strain of high production and modern intensive husbandry’ (Payne, 1972). But it is important to state that both production and environmental factors are equally implicit in the onset of production diseases (Mulligan-Doherty, 2008). In addition to the metabolic, endocrine, and immune system perturbances experienced by transition dairy cows, they are also likely to experience environmental stressors arising from the normal group changes that are associated with dairy farm management of dry and lactating cows (Mulligan and Doherty, 2008). Moreover during the transition period cows have to cope with dietary changes (more or less sudden but always changes) which could cause digestive disturbances.

Ingvartsen et al. (2003) summarised data from 93000 first parity and 58000 third parity Danish dairy cows which demonstrated that the highest incidence of total diseases (mastitis, ketosis, digestive disorders, and laminitis) occurred in a period from the day of calving until 10 days post-calving.

Many nutritional and management strategies of the pre-calving cow have been reported to alter the degree of negative energy balance, hypocalcaemia, immunosuppression and digestive disorders experienced by the transition cow (Mulligan and Doherty, 2008). Thus it is very important to understand that nutrition of transition cows is the key for the success of a dairy farm, since cows during the transition period are very sensible and a well fed herd will probably have more opportunity to be in good health status; but it is important at the same time to consider the environment and the management of cows groups within the herd in order to prevent stress which could influence feeding behavior.

Another important concept is that digestion and ruminal fermentation disturbances can affect metabolism via direct or indirect effect. In fact altered fermentations can produce metabolic intermediate which can affect metabolic pathway, or they can produce toxic intermediate and then determine tissues damages or inflammatory status, or they can simply alter behaviour and then feed intake leading to inability to satisfy nutrients requirements.

Digestive and ruminal fermentation disturbances

Forestomach motility of ruminants, especially cattle, is of major concern to the veterinarian, especially during the transition period. When a veterinarian is called to visit a transition cow, evaluation of forestomach motility is an integral part of the clinical examination and differentiation of forestomach abnormalities into primary and secondary causes is essential for diagnosis and accurate therapy (Radostis et al., 2007); in fact motility is often symptom of altered ruminal fermentations. Ruminal atony seen in lactic acidosis and endotoxemia, for example, can be attributed to direct depression of the gastric center, usually associated with generalized depression and severe illness (toxemia) or absence of exitatory inputs to the gastric center or increase in excitatory inhibitory inputs to the gastric center or failure of vagal motor pathway (Radostis et al., 2007).

Let's see major disturbs of ruminal fermentations.

Simple indigestion

Simple indiestion is common in dairy cattle and the common causes are dietary abnormalities of minor degree including indigestible roughage, particularly when the protein intake is low, moldy, overheated and frosted feeds, and moderate excesses of grain and concentrate intake.

Cases occur under excellent feeding regimens and are usually attributed to overfeeding with grain. Although the difference between simple indigestion and carbohydrate engorgement (grain overload) is one of degree, their separation can be justified by the marked clinical difference between the two syndromes. Indigestion is more common when heavily fed cows are fed a little more concentrate then they can digest adequately (Radostis et al., 2007).

Indigestible roughage may include straw, bedding or scrub fed during drought periods. It is probable that limitation of the available drinking water may contribute to the occurrence of the disease during dry seasons. Depraved appetite may also contribute to the ingestion of coarse indigestible material. Although good-quality ensilage cannot be considered an indigestible roughage, cases of indigestion can occur in cattle that are allowed unlimited access to it. Prolonged or heavy oral dosing with antimicrobials may cause indigestion due to inhibition of the normal ruminal flora (Radostis et al., 2007).

Rumen alkalosis and putrefaction of rumen ingesta

Rumen alkalosis can occur with the generation of excessive ammonia. Ammonia concentrations rise when high-protein diets are fermented. The pH usually does not increase

above neutral because these diets also contain sufficient readily fermentable carbohydrate to maintain a slightly acidic pH.

More dramatic elevation in ammonia concentration, with rumen fluid pH above 7.5, follow the overfeeding of nonprotein nitrogen sources such as urea, biuret and ammonium phosphate. Accidental ingestion of some common fertilizers that contain ammonium salts can produce the same results.

Putrefaction of rumen ingesta infrequently results from overgrowth of a microflora that decomposes feed material in a putrefactive manner. The existence of a high rumen fluid pH, as occurs with high-protein feeds, and repeated inoculation with abnormal bacteria allow the development of the putrefactive decomposition. This type of abnormal decomposition is normally inhibited by the existence of an active physiologic microflora.

Ruminal tympany

Ruminal tympany is abnormal distension of the rumen and reticulum caused by excessive retention of the gases of fermentation, either on the form of a persistent foam mixed with the rumen contents or as free gas separated from the ingesta. Normally gas bubbles produced in the rumen coalesce, separate from the rumen contents to form pockets of free gas above the level of the contents and finally are eliminated by eructation (Radostis et al., 2007).

According to the etiology we can define two different form of ruminal tympany: a primary ruminal tympany or a secondary ruminal tympany.

Secondary ruminal tympany is caracterised by a physical obstruction to eructation like esophageal obstruction caused by a foreign body, by stenosis of the esophagus, by pressure from enlargement outside the esophagus, such as tubercolous involvement of bronchial lymphnodes, or by obstruction of the cardia (Radostis et al., 2007).

Primary ruminal tympany (also named frothy bloat) is caused by the production of stable foam that traps the normal gases of fermentation in the rumen. The essential feature is that coalescence of the small gas bubbles is inhibited and intraruminal pressure increases because eructation cannot occur (Radostis et al., 2007). Primary ruminal tympay is tipically due to leguminous feeding or pasture: the cause is the foaming qualities of the solluble leaf proteins in bloating forages ingested by cattle on pasture. Alfalfa hay may also cause bloat. Also feeding finely ground grain, which promotes frothiness of rumen contents, can cause frothy bloat but the cause is not clear.

Ruminal parakeratosis

According to the litterature, fattening rations (Jensen et al., 1954; Smith, 1944) and pelleted rations (Jensen et al., 1958) have been associated with a high incidence of ruminal parakeratosis (Hinders and Owen, 1965). The abnormality has been observed most commonly in cattle fed high-concentrate rations of alfalfa pellets that have been subjected to heat treatment, and does not occur in cattle fed on rations containing normal quantities of unpelletted roughage (Radostis et al., 2007). It was shown that the growth of ruminal epithelium is directly linked to the SCFA presence in the tissue. The negative impact of organic acids on the ruminal wall may lead to parakeratosis enabling translocation of pathogens into the bloodstream provoking inflammation and abscessation throughout the ruminant body (Kleen, 2003). The parakeratosis eventually leads to rumenitis, particularly to the presence of microabscesses within the ruminal mucosa (Szemeredy and Raul, 1978). The ruminal parakeratosis could then be considered not a primary disturb of ruminal fermentation but a direct consequence of altered ruminal fermentations.

Vagus indigestion

The etiology of vagus indigestion has been controversial but has been divided into two major subcategories of complications of traumatic reticuloperitonitis: vagal nerve injury and reticular adhesions (Radostis et al., 2007). In addition there are some other causes that are interesting in transition cow context like abomasal impaction (proved expecially in sheep) and indigestion of late pregnancy (expecially in cows). Ruminal distension with atony occurs most commonly in the late pregnancy and may persist after calving. The cow is clinically normal in all respects except that she is anorexic, passes only scant amounts of soft pasty feces, has a distended abdomen and will not respond to tratment with purgatives, lubricants or parasympathetic stimulants. Ruminal movements are seriously reduced or absent and there may be persistent mild bloat. Most cases of abomasal impaction also occure late in pregnancy and are manifested by anorexia and a reduced volume of pasty faeces (Radostis et al., 2007). There may be no abdominal distension and no systemic reaction until the late stages, when the heart rate rises rapidly. Combunation of these types may occur; in particular, distension of the rumen with atony combined with abomasal impaction is the most commonly observed syndrome. Indigestion of late pregnancy in cattle characterized by distension and hypermotility of the rumen with distension of the abomasum has been described (Radostis et al., 2007).

Acute carbohydrate engorgement (Ruminal acidosis)

The sudden ingestion of toxic doses of carbohydrate-rich feed, such as grain, is the most common cause of the acute form of the disease. All types of ruminant are susceptible but this disease occurs most commonly in feedlot cattle and dairy cattle fed on high-level grain diets (Radostis et al., 2007). This problem is particularly linked to sudden changes of the ration, since the type of diet affects the number and species of bacteria and protozoa in the rumen and a change requires a period of microbial adaptation. Less common causes include accidental ingorgement with concentrates or the opportunity for the cow to select a great amount of concentrate from the ration. The ingestion of excessive quantities of highly fermentable feeds by a ruminant is followed within 2-6 hours by a marked change in the microbial population in the rumen. There is an increase in the number of Streptococcus bovis which utilize the carbohydrate to produce large quantities of lactic acid. In the presence of a sufficient amount of carbohydrate the organism will continue to produce lactic acid, which decreases the rumen pH to 5 or less, wich results in the destruction of the cellulolytic bacteria and protozoa. When large amount of starch are added to the diet, growth of S. bovis is no longer restricted by energy source and it multiplies faster than any other species of bacteria.

The concentration of volatile fatty acids increases initially, contributing to the fall in ruminal pH. The low ph allows lactobacilli to use the large quantities of carbohydrate in the rumen to produce excessive quantities of lactic acid, resulting in ruminal lactic acidosis. Both D and L forms of the acid are produced, which markedly increases ruminal osmolarity, and water is drawn in from the systemic circulation, causing hemoconcentration and dehydration. Ruminal osmolarity increases from a normal of 280 mosmol/L to almost 400 mosmol/L. As the ruminal pH declines, the amplitude and frequency of the rumen contractions are decreased and at about a pH of 5 there is ruminal atony. Experimentally, increased molar concentration of butyrate, not the lactic acid, causes ruminal stasis (Owens et al., 1998).

Subacute Ruminal Acidosis (SARA)

Among disturbs of rumen fermentation we can include Subacute Ruminal Acidosis (SARA) that is primarily a variation in rumen fermentation, tipical at the beginning of lactation…but not only. The disease seems to represent one of the most important fermentative/metabolic disorders in intensive dairy farms that affects rumen fermentations, animal welfare, productivity and farm profitability (Morgante et al., 2007).

In the literature different definition are used: subacute ruminal acidosis (Garrett, 1996; Nordlund et al., 1995; Stock, 2000) or SARA (Garrett et al., 1998), 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). The definitions are made for both feedlots and dairy cattle (Kleen et al., 2003).

Since many authors demonstrated that SARA has clinically detectable signs, the world "subclinical" seems inappropriate, and since ruminal pH is low just during defined periods also the world "chronic" seems wrong for this condition; then I choose the name of subacute ruminal acidosis or SARA as described by many autors (Nordlund et al., 1995; Garrett, 1996; Garrett et al., 1998; Stock, 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 shortchain fatty acids (SCFA) adequately enough to keep the ruminal pH within physiological borders (Kleen et al., 2003). 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 (Kleen et al, 2003). Morevover we can observe an increased total concentration of SCFA, ratio between acetic, propionic and butyric acid that has been shifted towards propionic and butyric acid, and an elevated concentration of lactic acid in the rumen fluid that does not exceed 5–10 mmol/l (Hibbard et al., 1995).

But many are the factors that can increase the possible occurrence of SARA in dairy farms and in particular in dairy intensive (Morgante et al., 2007). As I said before, the transition from the pregnant, nonlactating state to the nonpregnant, lactating state is the period during which the majority of fermentative/metabolic diseases occur in the dairy cow. During this period the cow is changed from a high-fiber, low-concentrate diet to a diet that is higher in concentrate feeds and lower in fiber: cows that have not adapted to these high-grain diets are particularly susceptible to ruminal acidosis (Radostis et al., 2007), expecially if we think that this is a stressfull period for the cow (calving and milking onset) who usually undergo to negative energy balance (NEB).

According to the literature SARA may be caused by formulation of rations that contain excessive amounts of rapidly fermentable carbohydrates but also by a deficency of fiber or errors in delivery of the rations.

We can find SARA also in herds with correctly formulated diets in a chemical point of view and in this case the problem is probably related to management and physical treatment of the

ration. This is probably the reason why we have SARA not only in early lactation but also in mid lactation: in the lattest situation SARA is not caused by inadequate adaptation of rumen papillae to the lactation diet because SARA appears long time after calving. 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 (Gianesella, 2008). The term 'ration formulation and delivery acidosis' is therefore used (Nordlund et al., 1995; Oetzel, 2000). Also in mid-lactation, SARA may occur as the intake of easily fermentable concentrate feedstuffs meets a non-adapted ruminal environment; because the rumen in mid-lactation 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 (Gianesella, 2008).

The economic losses associated with SARA have been estimated at $1,12 per cow per day (Keunen et al., 2002).

Metabolic diseases of the transition

All authors agree saying that the hallmark of the transition period of dairy cattle is the dramatic change in nutrient demands (Overton and Waldron, 2004). The primary challenge faced by cows is a sudden and marked increase of nutrient requirements for milk production, at a time when dry matter intake (DMI), and thus nutrient supply, lags far behind (Drackley, 1999).

Estimates of the demand for glucose, AA, fatty acids, and net energy by the gravid uterus at 250 days of gestation and the lactating mammary gland at 4 days postpartum indicate approximately a tripling of demand for glucose, a doubling of demand for AA and approximately a fivefold increase in demand for fatty acids during this timeframe (Bell, 1995). In addition, the requirement for Ca increases approximately fourfold on the day of parturition (Horst et al., 1997).

Why do vastly different nutrition and management programs produce similarly good, or similarly poor, transition success? What controls dry matter intake (DMI) during the transition period? How do nutrition and management during the dry period and transition period impact subsequent reproductive success? (Drackley, 1999).

The fact that many cows are able to meet this challenge without difficulty implies that the metabolic adaptations necessary to support high milk production are a component of the

genetic factors that accompany selection for high milk production (Drackley, 2005). However, the fact that, on average, roughly one in every two to three cows calving succumbs to some health problem during the transition period (e.g., Jordan and Fourdraine, 1993; Duffield et al., 2002) underscores the fragility of the system.

Some authors think that genetic improvement and selection for more and more high milk yield, brought to increased susceptibility to digestive-fermentative-metabolic diseases and to more fragile equilibrium between the animal and the environment leading to increased susceptibility to stress as well.

In particular, during the last 3 weeks of pregnancy, nutrient demands by the fetal calf and placenta reach their maximum (Bell, 1995), yet DMI may decrease by 10 to 30% compared with intake during the early dry period. This in itself may not be cause for alarm, as decreased food or feed intake around parturition is a common finding in many mammalian species (Friggens, 2003). As parturition approaches, concentrations of progesterone in blood decrease and those of estrogen remain high or increase (Grummer, 1995). The high circulating estrogen may be one factor that contributes to decreased DMI around parturition (Grummer, 1993), although regulation of DMI in periparturient cows is complex and far from understood (Ingvartsen and Andersen, 2000; Grummer *et al.*, 2004). After parturition the nutrients demand remain high for the beginning of lactation and especially glucose demand to produce lactose is very high but DMI is still low and a negative energy balance (NEB) is quite common.

Because much of the dietary carbohydrate is fermented in the rumen, little glucose is absorbed directly from the digestive tract (Drackley, 2005).

The first adaptation of a transition cow aim to supply glucose to the mammary gland and then the primary homeorhetic adaptation of glucose metabolism to lactation is the concurrent increase in hepatic gluconeogenesis (Reynolds et al., 2003) and decrease in oxidation of glucose by peripheral tissues (Bennink et al., 1972).

The major substrates for hepatic gluconeogenesis in ruminants are propionate from ruminal fermentation, lactate from Cori cycling, AA from protein catabolism or net portaldrained visceral absorption, and glycerol released during lipolysis in adipose tissue (Seal and Reynolds, 1993). Since propionate production as glucose precursor during the early postpartal period is insufficient to synthesize the total amount of glucose needed (Drackley, 2005), amino acids from the diet or from skeletal muscle breakdown as well as glycerol from mobilized body fat must provide most of the remaining glucose synthesis (Reynolds et al., 2003).

The primary homeorhetic adaptation of lipid metabolism to lactation is then the mobilization of body fat stores to meet the overall energetic requirements of the cow (Overton and Waldron, 2004). The high ratio of growth hormone to insulin in blood of postpartal cows allows mobilization of long-chain fatty acids from adipose tissue triacylglycerol (TG) which are released in blood as non esterified fatty acid (NEFA) and are carried to the liver (Drackley, 2005). The NEFA are utilized to make upwards of 40% of milk fat during the first days of lactation (Bell, 1995). In the liver, NEFA can be completely oxidized to carbon dioxide to provide energy for the liver, partially oxidized to produce ketone bodies that are released into the blood and serve as fuels for other tissues (skeletal muscle uses some NEFA for fuel), or reconverted to TG. Ruminants have an inherently low capacity for synthesis and secretion of very-low density lipoproteins (VLDL) to export TG from the liver (Kleppe et al., 1988; Pullen et al., 1989), then the risk to accumulate them in the liver is very high. This accumulation does not appear to begin appreciably before parturition (Vazquez-Añon et al., 1994; Van den Top et al., 1996), and usually reaches a maximum between 7 and 14 d after parturition (Van den Top et al., 1995, 1996; Rukkwamsuk et al., 1999). If NEFA uptake by the liver becomes excessive, fatty liver may develop (Bobe et al., 2004). Negative energy balance and carbohydrate insufficiency in the liver after calving also lead to increased production of ketone bodies, which can result in clinical or subclinical ketosis (Duffield, 2000; Herdt, 2000; Drackley et al., 2001).

Given that plasma NEFA concentrations increase in response to increased energy needs accompanied by inadequate feed intake, DMI and plasma NEFA concentrations usually are inversely related.

Physiological and pathological changes associated with negative energy balance are important factors related to development of ketosis, displaced abomasum, and retained placenta (Duffield et al., 2002), and may impact the immune system to increase occurrence of infectious diseases such as mastitis and metritis (Dohoo and Martin, 1984; Kremer et al., 1993).

Nutritional strategies to support metabolic demand

Despite the prodigious output of research on the nutrition and physiology of transition cows, the transition period remains a problematic area on many dairy farms (Overton and Waldron, 2004), and metabolic disorders continue to occur at economically important rates on commercial dairy farms (Burhans et al., 2003). Nutritional or management limitations during

this time may impede the ability of the cow to reach maximal milk production (Drackley, 1999). The primary goal of nutritional management strategies of dairy cows during the transition period should be to support the metabolic adaptations described above (Overton and Waldron, 2004) without compromising rumen health and cow's productivity.

BCS control

Usually dairy farm organize the herds in two dry period groups: one for early and mid dry period characterized by low energy diet, and one for the last 2-3 weeks before predicted parturition (close-up group) where ration is more energetic. The primary rationale for feeding a lower energy diet during the early dry period is to minimize BCS gain during the dry period, while data in the literature agree suggesting higher energy diet for the last 2-3 weeks before calving. Many authors showed that this is the correct way to manage dry period, like Domecq et al. (1997), who reported that as BCS of cows at dry off increased, milk yield during the first 120 DIM decreased; furthermore, thinner cows that gained BCS during the dry period yielded more milk during the first 120 DIM.

Collectively, results published in the scientific literature support the concept that cows of moderately lower BCS within a well-managed transition management system are more likely to have positive transition period outcomes than cows of greater BCS due to their propensity to have increased DMI and potentially increased milk yield during early lactation (Overton and Waldron, 2004). Management or environmental circumstances that force cows away from their optimal body condition (i.e., too thin, too fat) may result in increased risk for health problems (Ingvartsen et al., 2003).

Prepartum diet

Many of the metabolic disorders afflicting cows during the periparturient period are interrelated in their occurrence (Overton and Waldron, 2004) and are related to the diet fed during the prepartum period (Curtis et al., 1985). Although it is unlikely that dry period nutrition per se causes metabolic disorders, certain nutritional factors certainly appear to predispose cows to health problems (Drackley, 2005).

Many researches studied effects of different composition of the prepartum diet on cows metabolism and productions, especially effects related on different levels of carbohydrates (NFC). The general concept of diet changes during the transition is that nutrient density is increased gradually from that fed to cows during the first few weeks after dry-off to the higher nutrient density required for postpartal cows (Drackley, 2005). A concept that has been perpetuated through the scientific literature (Rabelo et al., 2003) is that diets higher in NFC

content than traditional dry cow diets must be fed prior to calving to promote development of ruminal papillae for adequate absorption of VFA produced during ruminal fermentation. However, Andersen et al. (1999) reported that cows fed more typical diets during the prepartum period do not have meaningful changes in ruminal epithelium. Research has demonstrated a strong relationship between how much cows eat shortly after parturition and the incidence of metabolic problems. For example, Zamet et al. found that DMI for cows that experienced health problems were 18% lower prepartum and 20% lower postpartum than for healthy cows. While this associations do not prove cause and effect, in many examples cited in the literature, DMI was lower for some period before the health disorders developed. One metabolic connection between DMI and incidence of metabolic disorders may be through propionate supply. Propionate stimulates insulin secretion, which suppresses NEFA mobilization, propionate is antiketogenic in the liver. Propionate supply also is key for glucose production, which in turn would modulate energy balance and favor insulin release (Drackley, 1999). Probably more than the effect on rumen epithelium, feeding diets containing higher proportions of NFC influence ruminal microbial adaptation to NFC levels typical of diets fed during lactation and provide increased amounts of propionate to support hepatic gluconeogenesis and microbial protein (providing the diet contains sufficient ruminally degradable protein) to support protein requirements for maintenance, pregnancy, and mammogenesis. An important concept is that increased NFC means increased energy in the diet: recent evidence suggests that metabolism and performance of transition cows is more sensitive to total energy supplied by carbohydrate than the form of that carbohydrate (i.e., starch versus highly digestible NDF) (Overton and Waldron, 2004).

While implementation of close-up diets has been adopted enthusiastically by the dairy industry in the North America, surprisingly few data are available to support their actual effectiveness in decreasing the incidence of health problems or increasing milk yield. Close-up diets seem to have proven beneficial in the field in many situations.

But NFC is only one factor among all factors that determine a different fermentative and metabolic response in the rumen and the body of dairy cows. Another very important point is the fermentability of NFC in diets and the fiber source and the aspects associated with the improved management of a group of close-up cows that may be more important than the particular diet that is fed.

Overall, research supports 2-group nutritional management schemes for dry cows in order to minimize overfeeding during the early dry period and to increase energy supply to dairy cows during the late prepartum period (Overton and Waldron, 2004), and in order to prevent sudden

changes of substrate for microbial population in the rumen. In addition to dietary aspects, non-nutritional components of a good close-up program may be as important, or more important, than specific nutritional strategies (Drackley, 2005) in order to determine low-stress and comfortable environments for transition cows.

Nutritional management of transition cows: the fiber

Dairy cattle nutrition can be defined broadly as the use of the components of feeds for the processes of maintenance, growth, reproduction, lactation, and health. Applied nutrition is the selection and proportioning of feedstuffs and ingredients to supply the correct amounts and balance of nutrients required for optimal productive and reproductive performance (Drackley-Donkin-Reynolds, 2006).

Farmers, nutritionists and veterinarians in front of a transition cow have to cope with three main problems: negative energy balance, ipocalcemia and rumen health. These three problems are strictly interrelated because to solve the first two you risk to compromise the third.

Correct fiber level in the diet are needed in order to maintain good rumen health, but what really is fiber and which is the correct level?

Mertens defined fiber as the portion of feeds that is bulky and difficult to digest (Mertens, 2002), and this explain why if fiber increase in the ration, dry matter intake (DMI) will decrease; but at the same time, during the transition, we want to promote DMI and energy supply (that means more concentrate and less forages) to support milk production and avoid negative energy balance (NEB).

Ruminants require roughage in their diets to maximize production and to maintain health by sustaining a stable environment in the rumen (Allen, 1997). The ability of roughages to stimulate chewing has been investigated extensively because of the relationship between chewing and the flow of salivary buffers (Bailey and Balch, 1961., Emery et al., 1960) into the rumen, which are required to neutralize fermentation acids (Allen, 1997).

Mertens (1997) defined effective NDF (eNDF) as the overall effectiveness of NDF for maintaining milk fat test, and physically-effective NDF (peNDF) as the specific effectiveness of NDF for stimulating chewing activity in relationship to particle size.

Ruminal pH is very responsive to meals and chewing behavior; ruminal pH decreases following meals and increases during bouts of rumination. The rate of ruminal pH decline is faster following a meal as meal size increases and as dietary NDF concentration decreases. Dietary NDF concentration alone is not related to ruminal pH (Allen, 1997). Increased ruminal degradation is desirable to maximize microbial protein production and energy intake,

but the increase in fermentation acids must be compensated by increasing either NDF content of the diet or by increasing the physical effectiveness of the NDF to maintain pH by stimulating salivary buffer secretion via chewing activity. Increasing the physical effectiveness of NDF to increase salivary buffer flow might be a more desirable alternative to maintain ruminal pH because this increase would result in greater ruminal fermentation and production of microbial protein (Allen, 1997).

Relationship between subacute ruminal acidosis and general health status

SARA and acute phase response

Many authors studied the acute phase response during SARA. It has been suggested that low rumen pH could result in death and lysis of gram-negative bacteria that are in the rumen and hence increase free endotoxins in the rumen (Nagaraja et al., 1978; Andersen et al., 1994). Endotoxin, also known as lypopolisaccaride (LPS), is a cellular component of gram-negative bacteria and is an extremely potent toxin. 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) like in lactation. It has been suggested that the acidic rumen environment, changes in osmotic pressure, and ruminal LPS may render the rumen epithelium susceptible to injury (Brent, 1976; Enemark et al., 2002; Kleen et al., 2003), resulting in translocation of rumen endotoxin into the bloodstream (Kleen et al., 2003). The role of free ruminal LPS in the etiology of diseases related to grain engorgement such as ruminal acidosis, rumenitis, sudden death syndrome, and laminitis is not clearly understood but it has been postulated that increased translocation of free ruminal LPS into blood circulation may be an important causative factor (Dougherty et al., 1975; Nagaraja et al., 1978, Andersen et al., 1994). Ruminal endotoxin was implicated in the etiology of multiple metabolic disorders like acidosis, fatty liver, laminitis, and sudden death syndrome (Andersen, 2003; Ametaj et al., 2005).

Endotoxin is a strong inducer of acute phase response, which is a nonspecific immune mechanism aimed at restoring disturbed homeostasis. The presence of LPS in the bloodstream results in the production of multiple proinflammatory cytokines, reactive oxygen and nitrogen intermediates, and bioactive lipids, which affect the host's metabolic response to inflammation (Baumann and Gauldie, 1994). When released in large quantities, these mediators lead to an acute phase response (Kushner and Rzewnicki, 1994). During acute

phase response, there is alteration in the biosynthetic profile of the liver, resulting in production of proteins known as acute phase proteins (APP) such as haptoglobin, serum amyloid A (SAA), and albumin (Boosman et al., 1989; Werling et al., 1996). The main stimulators of APP production are the inflammation-associated cytokines IL-1, IL-6, tumor necrosis factor (TNF)-α, and IFN-γ which are released during inflammatory processes (Gabay and Kushner, 1999). The acute phase response is characterized by leukocytosis, fever, alterations in the metabolism of many organs, as well as changes in the plasma concentrations of various APP (Emmanuel et al., 2008).

Systemic exposure to LPS has been linked to a number of common diseases in the bovine, for example, coliform mastitis, neonatal coliform septicemia, ruminal acidosis, laminitis, and displaced abomasum (Boosman et al., 1991; Andersen, 1994; Blum et al., 2000). Many studies have noted that the ability to withstand LPS exposure or Gram-negative infection varies considerably between individuals (Michaels et al., 1988; Deignan et al., 2000) and that this ability seems to be an innate characteristic of the individual cow (Hirvonen et al., 1999).

Jacobsen (2004) induced APR by LPS infusion in 3 groups at 3 different doses: clinical signs were induced, significant increases in SAA and haptoglobin concentrations, and significant decreases in albumin concentrations in all cows were seen. This was the first study to show induction of an acute phase response by a very low intravenous LPS dose (10 ng/kg), and this finding confirmed that cattle are highly sensitive to LPS (Berczi et al., 1966). In cows with severe coliform mastitis and experimentally induced ruminal acidosis, LPS was detected in the systemic circulation in amounts roughly corresponding to those resulting from the doses used in this study (Katholm and Andersen, 1992; Andersen et al., 1994). In this trial, the serum concentrations of SAA and haptoglobin started to increase within 6 and 36 h after the challenge, respectively, and concentrations remained elevated for up to 144 h. In contrast concentration of albumin, that is a negative APP, after all 3 intravenous LPS challenges, decreased in a biphasic manner: between 1 and 6 h after LPS injection (and was probably a result of an inflammation-induced increase in vascular permeability that allowed efflux of serum proteins to the perivascular tissue) and 36 h postchallenge onwards (this was probably a reflection of decreased hepatic albumin synthesis resulting from a cytokine-induced reduction in gene transcription) (Aldred and Schreiber, 1993). Jacobsen concluded that the APP responses were dose dependent and demonstrated that, in addition to the dose–response relationship with LPS, the APP concentrations achieved during an acute phase response are highly dependent on the individual animal. The individual variation in APP response to LPS may be related both to the genotype of the cow and to the metabolism and disease status of

the cow, as hepatic protein synthesizing capacity may be influenced by a number of pathological conditions or physiological states.

The significance of the individual differences in the APP response remains to be elucidated. In cattle, SAA is generally perceived as an indicator of acute inflammation, whereas haptoglobin is more slowly reacting and thus reflects the presence of chronic inflammatory conditions (Alsemgeest et al., 1994; Horadagoda et al., 1999).

Also Gozho (2005, 2006, 2007) studied APR in cows affected by diseases that are related to feeding high concentrate diets. There is a paucity of literature on changes in rumen fluid LPS concentration due to feeding high concentrate diets. Gozho et all. (2005) shown that haptoglobin concentration in blood serum was not different in animals fed different diets. However, after abrupt induction of SARA they found increased concentrations of Hp from 0.43±0.14 (when only hay was fed) to 0.79±0.14 mg/mL on day 5 of the treatment period. Other researchers have reported that Hp concentrations are undetectable in healthy cattle, and concentrations only becoming detectable when there is an inflammatory response (Deignan et al., 2000). Serum amyloid-A concentrations were not significantly different among the different diets in this study but concentrations in blood plasma increased from 33.6±36.53 (when only hay was fed) to 170.7±36.53 μg/mL on day 5, when concentrate was offered in addition to hay. The acute phase protein profiles obtained in this study indicate that as time with pH below 5.6 increased, the intensity of the acute phase response increased. Gozho hypothesized for the first time that this could be due to formation of extensive microlesions on the ruminal epithelium leading to increased LPS translocation across the ruminal epithelium into the systemic circulation, and was the first study that examined an APR due to SARA.

One year later Gozho et al. (2006) published a subsequent study that showed that after gradual adaptations to a 60% concentrate diet over a 4 weeks period followed by grain-induced SARA (by feeding a mixture of wheat and barley grain), an APR could be seen. The Hp concentration increased when the 61 and 76% concentrate diets were fed. Serum amyloid-A concentrations increased when steers were on the 76% concentrate diet. These data confirmed that a relationship between the occurrence of SARA and changes in the SAA concentration may be developed.

The quantification of acute phase proteins in these two studied indicated that SARA initiates an inflammatory response.

The role of free ruminal LPS in SARA remains difficult to ascertain because free LPS is detoxified in the liver and hence is not detectable in peripheral blood circulation (Andersen,

2000). Other indicators of inflammation such as fibrinogen and white blood cells can also be used as markers of inflammation (Arthington et al., 1996; Horadagoda et al., 1999). Fibrinogen shows only a minor increase in response to inflammation in cattle (Hirvonen, 2000) and could play an ancillary role to augment the diagnostic value of acute phase proteins to SARA under field conditions. In 2007 Gozho went deeper into this subject and published another paper that had the objective to determine whether inducing SARA in dairy cows in mid lactation affects free ruminal LPS, the concentration of LPS in peripheral blood, SAA, Hp, fibrinogen, serum copper, and white blood cell profiles. Averaged across periods, white blood cell counts did not differ between treatments in this trial and were within normal range. Haptoglobin, fibrinogen, and platelet counts did not differ as well. Serum copper concentrations did not differ between treatments but inducing SARA tended to depress its serum concentration. Serum amyloid A increased when SARA was induced.

As shown before, free ruminal LPS that are translocated into the hepatic portal circulation can be detoxified by the liver before they reach the peripheral blood circulation (Andersen, 2000), however, the majority of cytokine receptors are found in the Kupffer cells in the liver (Bode and Heinrich, 2001) and therefore the first wave of proinflammatory cytokines may be initiated before detoxification. The disparity in the concentrations of Hp and SAA that Gozho have seen in cows with inducted-SARA could be due to a difference in the cytokines involved in initiating the synthesis of these acute phase proteins (Jacobsen et al., 2004). Serum amyloid A synthesis can be induced by the release of either IL-6 or tumor necrosis factor-α but both these cytokines are required for haptoglobin to be synthesized (Alsemgeest et al., 1996). Therefore, the combination of cytokines required for SAA synthesis may be different from the combination that activates haptoglobin synthesis (Jacobsen et al., 2004).

In 2007 Emmanuel et al. confirmed that there was translocation of LPS from the mucosal to the serosal side of a Ussing chamber in all rumen tissues treated with LPS. But exposure of rumen tissues to different acidic pH did not affect translocation of LPS and there was no difference in the quantity of LPS translocated between the different pH groups. Furthermore, no interaction between pH and LPS was obtained. The most important finding of this study was that in presence of LPS at acidic pH values of the perfusate similar with acute ruminal acidosis there was an increase of more than 6-fold in the permeability of rumen tissues to 3H-mannitol. Conversely, pH values similar with SARA and normal pH values of the perfusate did not affect permeability of rumen mucosal tissues to 3H-mannitol. Whereas LPS and acidic pH are known to increase the permeability of the mucosa separately (Chin et al., 2006), the increased permeability observed in this study at both acidic pH of the perfusate and presence

of LPS suggests that the 2 factors combine to further enhance the permeability of mucosa to 3H-mannitol. Results of a study from Emmanel (2008) confirmed previous studies demonstrating that concentration of SAA increased in plasma of cows fed higher amounts of barley grain (30 and 45%) compared with cows fed lower amounts (0 or 15%) of barley grain agreeing with the idea that an APR is supposed to be during SARA.

SARA and stress, dry matter intake, body condition score

In addition to the metabolic challenges and potential for "nutritional stress" if periparturient nutrition is sub-optimal, cows may face additional stressors from the natural environment and from deficiencies in management (Grant and Albright, 2001). These may include heat stress, overcrowding, infectious challenge, poor ventilation, poor footing, uncomfortable stalls, poor management of grouping and cow movement, and rough handling (Drackley, 2005).

It is now well established that, without exhibiting any clinical signs of disease, animals reared in an environment in which they are exposed to environmental antigens, or those affected by chronic subclinical disease or intestinal parasites, show reduced appetite and growth compared to healthy animals (Le Floc'h, 2003). These modifications are usually attributed to immune system stimulation and to a moderate inflammatory response and require an adaptation of the metabolic response by the animal. (Le Floc'h, 2003). This explains that animals submitted to such a stress use nutrients less efficiently for production than healthy animals (Williams et al., 1997).

In a study from Emmanuel et al. (2008), feed intake decreased, whereas DMI increased as the amount of barley grain in the diet was increased. The authors of this trial suggested that two potential mechanisms might explain the feed intake responses of cows to different amounts of grain in the diet: 1) the amount of VFA released in the rumen and 2) translocation of endotoxin from ruminal fluid into blood circulation. Feeding dairy cows high-grain diets rich in rapidly fermentable carbohydrates is associated with increased release of propionate in the rumen fluid and its absorption into the bloodstream (Sutton et al., 2003). Intraruminal or i.v. infusion of propionate solutions before or after a scheduled meal was associated with depression of feed intake in cattle (Shepherd and Combs, 1998). Therefore, it is possible that absorption of propionate into the blood circulation or its effect on rumen receptors may have contributed to decreased feed intake in cows fed high-grain diets (Emmauel et al., 2008). For these reasons we can suppose that cows experiencing SARA, as the acetic-propionic acid ratio in the rumen decrease in favor to propionic, could exhibit decrease in DMI.

According to Beauchemin (2007) ruminal acidosis can cause erratic fluctuations in feed intake because low ruminal pH causes the cow to go "off-feed," (thanks to the propionate

effect as described above) which reduces the production of VFA, allowing the pH to recover. The cow then resumes a high feed intake that causes excessive production of acids, and the cycle is repeated.

A decreased DMI in cows suffering from SARA can lead to a negative energy status (NES) resulting in body condition loss, immune suppression and suboptimal productive and reproductive performances. Immune suppression is kept responsible for the vague health problems and accompanying higher incidences of many infectious diseases; the lowered DMI may lead to a wrong utilization of nutrients and a shortage of (micro)minerals and vitamins as well. At the same time ruminal acidosis decreases the digestibility of fibre in the rumen, which decreases feed conversion efficiency and increases feed costs. In a study with ruminally and duodenally cannulated cows, Beauchemin (2007) observed that NDF digestion in the rumen declined from 52% for cows with a mean ruminal pH of 6.4 to 44% for cows experiencing repeated episodes of acidosis with a mean ruminal pH of 5.8. This reduction in potential fibre digestion is equivalent to a loss of 2.5 kg/d of milk.

Stressors (of physiological or psychological nature), infection, or endotoxin released from the rumen because of feeding practices all could stimulate inflammatory processes, with the resulting cytokine release contributing to decreased DMI again. In turn, the decreased DMI could increase body fat mobilization, resulting in elevated NEFA concentrations and increased hepatic lipid accumulation (Drackley,, 2005). In such a situation, nutrients may be diverted away from the critical functions of fetal growth, lactogenesis, and preparation of support functions for lactation to support the stress response (Moberg, 2000). Moreover, activation of the sympathetic nervous system and release of stress hormones, such as glucocorticoids and epinephrine, generally are antagonistic to milk production (Nbibualonji et al., 1995; Hopster et al., 1998; Waldron et al., 2003). Hormones and cytokines associated with the stress response may alter secretion of hormones important for lactogenesis, such as growth hormone (Munksgaard and Lovendahl, 1993; Kushibiki et al., 2003), insulin and glucagon (Waldron et al., 2003), and the thyroid hormones (Kushibiki et al., 2003). Moreover, factors secreted in response to infection, stress, or trauma, most likely the cytokines, result in increased lipid synthesis in liver even in the face of similar NEFA concentrations in blood (Herdt et al., 1983). The mechanism of this putative hepatic effect of cytokines remains unclear, however, because administration of endotoxin to mid lactation cows did not greatly alter metabolism of palmitate by liver slices (Waldron et al., 2003). Fat accumulation in liver appears to decrease endotoxin clearance (Andersen et al., 1996), which could result in a futile cycle that sustains or worsens the negative effects of endotoxin in the cow.

One more mechanism by which environmental or behavioral stressors may impact periparturient cows involves greater suppression of immune function via increased cortisol concentrations (Kushibiki et al., 2003; Waldron et al., 2003). As a result, cows under stress could become more susceptible to diseases (Hopster et al., 1998), including infectious and metabolic diseases (like SARA). Research implicates endotoxins or cytokines even in the etiology of hypocalcemia (Waldron et al., 2003b), which potentially could lead to milk fever or the decreased smooth muscle function that may underlie displaced abomasum or other disorders. Finally, the pro-inflammatory cytokines are involved in disruption of the normal metabolic adaptations to lactation and result in wasting of muscle tissue, increased fat mobilization, increased fat deposition in the liver, and induction of the acute phase response in liver. Pro-inflammatory cytokines decrease synthesis of some proteins (albumin, retinol binding protein, apolipoproteins) while synthesis of others (fibrinogen, globulins, haptoglobin, ceruloplasmin, c-reactive protein, serum amyloid A, calcitonin-generelated peptide, lipopolysaccharide binding protein) is increased (Johnson et al., 1997; Ingvartsen and Andersen, 2000; Schroedl et al., 2001). In such a situation, the farmer will be tented to increase energy supply of the diet in order to increase body condition of the cows and avoid downer cow syndrome and other typical diseases of the transition, but in this way he will increase also the risk of SARA. All of the systems invoked to deal with stress produce changes in biological function, and these changes may directly affect the animal's wellbeing and productivity. For many day-to-day stressors, the biological cost of the response is inconsequential. However, in the face of prolonged, severe, or multiple stressors, the biological cost of the response may become significant to the animal (Moberg, 2000), thereby diverting enough resources to place the cow (as stated above) at greater risk for developing various pathologies, such as infectious diseases (Drackely 2005) or metabolic diseases.

SARA and other systemic/metabolic disturbs

Forestomach motility

Low rumen pH values lead to 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).

When pH decreases to 5.0 during acidosis, ionization of acids increases slightly, with an acidotic pH osmotic pressure is increased by greater ionization of acids and presence of free glucose. Compared with normal concentrations, the change during acidosis is much greater in osmolality than in the hydrogen ion concentration (Owens et al., 1998). Absorption from the

rumen normally prevents acid accumulation but when ruminal osmolality is markedly greater than blood osmolality, water from blood is drawn rapidly inward through the rumen wall leading to damage to the wall of the rumen or small intestine due to high osmotic pressure. Subsequently, repaired tissues of the digestive tract will be thickened (hyperkeratosis or parakeratosis); this may inhibit rate of VFA absorption for months or years after the damage has occurred (Krehbiel et al., 1995). Passage of VFA postruminally for absorption is possible, but abomasal presence of VFA hinders acidification and protein and mineral digestion; this may reduce postruminal starch digestion. Consequently, a single bout of non- fatal acidosis may have prolonged effects (Owens et al., 1998). Although ruminal hypertonicity usually but not always reduces the frequency of ruminal contractions (Carter and Grovum, 1990), inhibited gut motility or hypertonicity at the abomasum may halt flow and exacerbate ruminal acidification; altered motility or tonicity also may cause feed intake to fluctuate with chronic acidosis.

An increase of valerianic acid was seen by Gianesella (2008): in his work 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 Gianesella's data, total valerate concentration ranged between a minimum value of 3.73 mmol/l and a maximun value of 5.25. 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 Nvalerate levels in the rumen and the presence of somatic cells in the milk. More investigation to define the role of valerate in the organism should be done, and, since we don't really know the metabolic pathway of this VFA, should be very interesting to understand its role in the metabolism and the forestomach health especially related to SARA. The reduced rumen motility together with the low rumen pH negatively affect protozoa population in the rumen (Slyter 1976; Underwood, 1992). As a result cows could respond by reducing DMI and milk yield (Underwood, 1992). Bacterial endotoxins have been related to the decrease of rumen motility as well: 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).

Hemoconcentration and blood gas parameters

Among the effect of SARA on the hole organism, an increase in the effective osmotic pressure in the rumen resulting in a flow of water from blood to gastro-intestinal tract and then in hemoconcentration, is often cited.

The use of blood parameters like base excess (BE) or blood pH has been cited as diagnostic tool of non-acute ruminal acidosis. Because of an absorption of SCFA by the ruminal wall, the BE may be reduced. A base excess is normally seen in healthy cows but an overload of VFA absorbed by the gastroenteric tract or L-lactate absorption from muscles metabolism, could decrease the BE especially because at the same time bicarbonate is used to buffer ruminal pH. Since the main blood buffer system is the bicarbonate, blood pH could varies during rumen acidosis condition, but usually acids are successfully neutralized thanks to lung and kidney compensatory mechanisms leading to a compensated metabolic acidosis (Dirksen, 1985; Owens et al., 1998). The result depends on compensation system efficacy of these organs; however, under certain circumstances the blood pH may be decreased as well (Rossow, 1984).

Milk fat depression

Also the milk fat depression (MFD) is often related to rumen acidosis. To explain the mechanism, research has established that diet-induced MFD is related to altered rumen biohydrogenation of mono and polyunsatured (PUFA) fatty acids. PUFA, that are linolenic (C18:3) linoleic (C18:2) and oleic (C18:1) acid, are toxic to the rumen microbes and therefore biohydrogenated to satured stearic acid by bacterial enzymes in the rumen; biohydrogenation is often accompanied by positional isomerization of H atoms of the double bond under attack, for example changing H atoms from cis to trans position. However biohydrogenation is not always complete leading to accumulation of intermediated of fatty acids like trans C:18 and its isomers trans-11-C18:1 and trans 10-C18:1 octadecenoic acid. To have MFD dietary factors and ruminal metabolic factors must exist together: a low dietary forage to concentrate ratio, very fine chopped or pelletted forages, presence of PUFA (soybean, sunflower, …) or high amount of corn in the diet and shift in bacterial population together with a low rumen pH. The low rumen pH interferes with ruminal biohydrogenation of PUFA by reducing the final step from C18:1 to C18:0 and consequently causing accumulation of trans-C18:1 isomers in ruminal fluid; it was then found that under certain conditions pathways of biohydrogenation of C18:2 (octadecenoic acid) are alterd to produce unique fatty acids intermediates (conjugated linoleic acids) that are more potent inhibitors of milk fat synthesis (Bauman et al., 2001). MFD occurs then by depressing de novo synthesis of short-chain fatty

acids (< C16) and esterification of those FA into triglycerides in milk fat in the mammary gland.

Heat stress

SARA is often related also to heat stress. When cows start panting to get rid of hit, CO2 concentration increases leading to a respiratory alcalosis. Since the buffer capacity of blood is based on ratio CO2/HCO3, an extra loss of CO2 causes more excretion of HCO3 by the kidneys in order to balance the ratio between CO2 and HCO3; then after end of heat stress panting decreases and CO2 concentration is normalized but kidneys excreted high amounts of bicarbonate leading to compensatory metabolic acidosis. In this situation the buffering ability of the rumen is decreased because of the decreased availability of bicarbonate to be excreted by salivary gland and the rumen pH could decrease causing DMI and rumination decrease, stasis in the rumen and higher risk for rumen acidosis.

Vitamin B12 and homocysteine

Talking about systemic effect of SARA is important to analyze vitamin B12 involvement. Vitamin B12 is synthesized exclusively by bacteria in the rumen and absorbed in the intestine; cobalt is essential for synthesis and many non-active analogues are produced; vitamin B12 is stored in the liver (60%) and in muscles (30%) then signs of deficiency are delayed due to relatively high depot reserves. Synthesis of vitamin B12 is influenced by many factors; the first is the cobalt availability but also the rumen pH since an acidic environment may cause lysis of B12 synthesizing bacteria, the concentrate gift is important as well because the demand of vitamin B12 increases when propionate production is increased (Girard, 1998). This vitamin is a coenzyme for several enzymes involved in gluconeogenesis, metabolism of odd numbered fatty acid like propionate and biosynthesis of methionine from homocysteine. In particular the methyl cobalamin form is used by the enzyme methionine synthase to turn homocysteine into methionine, while the desoxyadenosylcobalamin form assists trough the enzyme methylmalonyl-CoA mutase a mitochondrial enzyme involved in the metabolism of odd numbered fatty acids (like propionate) and several amino acids (valine, isoleucine, methionine, threonine). The effects of shortages of vitamin B12 and biotin in the body are primarily related to a decreased conversion of odd numbered fatty acid (propionate) to succinyl CoA with a subsequent accumulation of intermediary metabolites such as methylmalonyl acid, homocysteine and propionate and other odd numbered fatty acid.

Then the cow could show a progressive loss of appetite and live weight loss due to impairment of propionate metabolism, neurological symptoms due to incorporation of odd

numbered fatty acids into membranes; moreover there will be a restricted entry of succinyl CoA from odd numbered fatty acid into the Krebs cycle.

APR and protein metabolism

Cytokines and hormones appear to be the most frequently suggested mediators involved in the modifications of protein and amino acid metabolism. IL-6 is the primary initiator of the APP synthesis by the liver (Marinkovic et al., 1989). Among involved hormones, glucocorticoids are known to have a catabolic effect on muscle but an anabolic effect on the liver. In the liver, glucocorticoids act as permissive or synergetic factors to the action of cytokines (Marinkovic et al., 1989). Hyperinsulinaemia and hyperglycaemia are commonly observed during inflammation and are a characteristic sign of insulinoresistance (Beisel, 1984). Nevertheless, this insulinoresistance seems to preserve protein synthesis whereas the capacity of insulin to decrease protein breakdown is reduced at least during the first days of infection (Vary et al., 1998). It is supposed to have an acute phase response during SARA, then protein synthesis rate is increased in liver and immune tissues and cells (Klasing and Austic 1984; Papet et al. 2002). In the liver, inflammation increases preferentially the synthesis of exported proteins such as acute phase proteins (fibrinogen, C-reactive protein, haptoglobin). APP plasma concentrations increase 2 to 100-fold depending on the protein, the animal species and the challenge, while their synthesis increases in greater proportion (Le Floc'h, 2003).

At the same time, ruminal acidosis lowers the efficiency of microbial protein production in the rumen (i.e., the amount of microbial protein produced per unit of carbohydrate digested in the rumen). A decrease in microbial efficiency will decrease the yield of microbial protein (g/d), unless more fermentable carbohydrate is supplied, which further increases the risk of acidosis. Decreased microbial protein synthesis increases the need for supplemental feed protein in the diet, which in most cases increases feed costs.

MATHERIALS AND METHODS

Farms

During this trial 12 farms were investigated; farms where selected with similar characteristics in the north-east of Italy (Veneto region) especially in the area around Vicenza. The project was a collaboration which involved the Department of Veterinary Clinical Science of the University of Padova, the Istituto Zooprofilattico Sperimentale delle Venezie and the animal feed company Cortal Extrasoy s.p.a. that follow 10 of these farms for the nutrition plan.

The objective was to investigate, analyze and study changes in blood, urine and faeces parameters in dairy cows affected by SARA.

Farms were selected with characteristics about production, management and structures that made them at risk for subacute ruminal acidosis (SARA); in particular farms had these characteristics:

- ✓ Holstein or Bruna breed
- ✓ Medium or high milk production (with average daily production between 30 and 50 kg per cow)
- ✓ Between 50 and 125 lactating cows
- ✓ One feeding group for lactating cows or 2 feeding group (early lactation and mid/late lactation cows) and adoption of steaming-up in late dry period
- ✓ Use of total mixed ration (TMR) as feeding technique with or without silage
- ✓ One time feeding distribution per day
- ✓ Free stalls
- ✓ Dry off period of about 50-60 days

In each farm data about general situation were collected by interviewing the farmer in order to complete an anamnestic table that was prepared; the interview was about structures, management, health status of the herd and health management. In particular we asked to the farmer: the total number of animals and the number of lactating cows, males and calves, the number of pens and criteria to separate animals in the different groups, milking technique and milking equipment, mastitis frequency and etiology for them, presence of pen specific for sick animals and for calving ones, feeding technique and distribution, clinical signs more frequently seen, culling rate and mean lactation number, cow and calves mortality. Only three farms had two different groups for lactating cows (early lactation and mid/late lactation): farm

number 4, number 6 and number 10 (see table 1). Farm number 6 was also the only one characterized by a short dry period (45 days).

Table 1: characteristics of the dairy farms

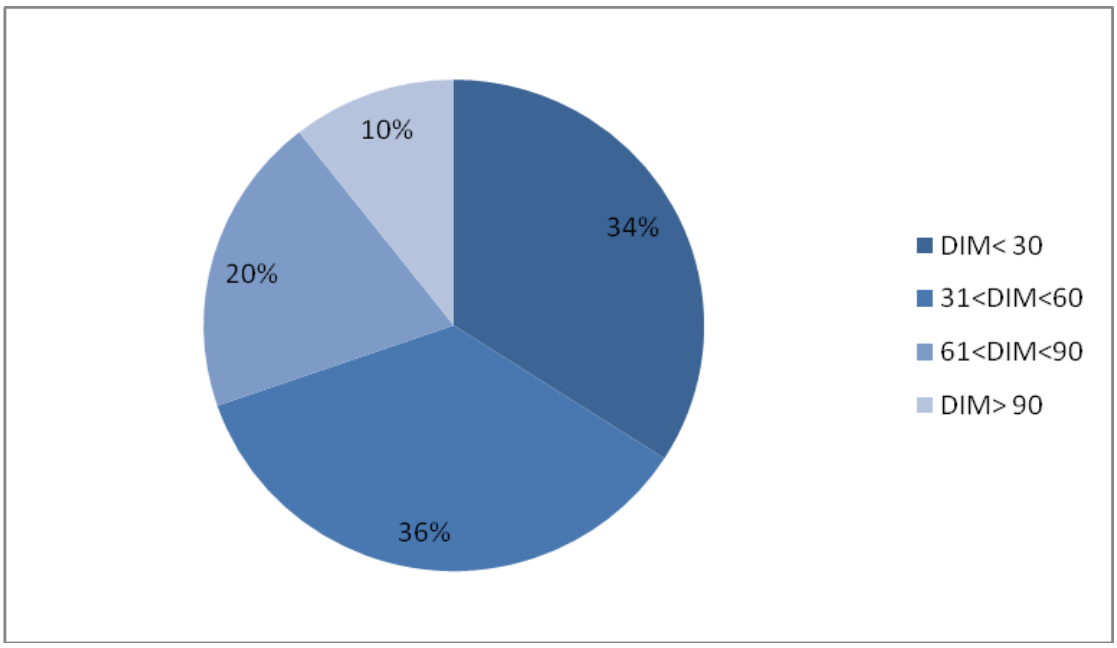
Farm	Breed	Feeding technique	Lactating cows	Groups lactating cows	Dry period (days)
1	Holstein	Unifeed	50	1	60
2	Bruna + Holstein	Unifeed (no silage)	73	1	60
3	Holstein	Unifeed	70	1	60
4	Holstein	Unifeed	70	2	60
5	Holstein	Unifeed	74	1	60
6	Holstein	Unifeed (2/day in summer)	80	2	45
7	Holstein	Unifeed	125	1	60
8	Holstein	Unifeed	65	1	60
9	Bruna + Holstein	Unifeed	94	1	60
10	Bruna	Unifeed	53	2	60
11	Holstein	Unifeed	60	1	60
12	Bruna + Holstein	Unifeed	53	1	60

Animals

In each farm 12 animals were randomly chosen because this is the number demonstrated to be statistically significant to investigate a herd about subacute ruminal acidosis (Nordlund and Garrett, 1994; Nordlund, 2001; Kleen et al., 2003); they were lactating cows without clinical signs of disease, in good general health status and they were not submitted to surgery or treatment in the past month at least. The great majority of these cows were between 7 and 90 days in milk (DIM), in particular we have 45 cows with less then 30 DIM, 47 cows between 31 and 60 DIM, 26 cows between 61 and 90 DIM and 14 cows with more then 90 DIM (see graphic 1). Seventy per cent of cows was under 60 DIM because fresh cows are supposed to

be more at risk for SARA. The final number of cows that entered in the trial is 132. Ruminal fluid, blood, faeces and urine were collected from each cow. In some farms it was not possible to collect 12 samples because there was not enough animals with the characteristics mentioned above, or because after 10 or 11 ruminal pH checked the situation of the farm was clear as normal or in SARA.

Graphic 1: distribution of cows depending on days in milk (DIM)



Samples

Samples were collected between 4 and 6 hours after the TMR distribution since it was demonstrated to be the time when the ruminal pH has dropped to the lowest level in a TMR-fed herd (Morgante et al., 2007; Nordlund et al., 1995); moreover this method lead to a standardization of procedure and allow the comparison and correct interpretation of data.

Total Mixed Ration

A sample from the TMR was taken just after the distribution and it was sieved to determine particle size. Then an aliquot of the same sample was taken to the laboratory of Cortal to determine chemical composition of the diet.

Blood

Blood was collected from jugular vein before any other sample collection trying to be less stressful as possible for the cow in order to avoid modification of hematological and hematochemical parameters. Limiting stress is quite important especially for leukogram interpretation and to avoid hemolysis. The cows were into the rack and blocked with a rope turning their head on their left side. Samples were collected into ethylenediamine tetra-acetic acid (EDTA), lithium heparin, and plain vacutainers (BD Vacutainer Systems®, Preanalytical Solutions, Plymouth, UK).

Rumen liquid

Ruminal fluid was obtained by rumenocentesis as described by Nordlund and Garret (1994) without sedation, using a 13G 105 mm needle (Intranule PP, Vygon, Francia). Rumenocentesis was chosen since it is considered the technique providing the most accurate results (Garrett et al., 1999; Enemark et al., 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.

Urine and faeces

Urine was collected by spontaneous urination or by provoked urination (with perineal massage) or by a vesical catheter if necessary. The sample was divided into 2 aliquots, refrigerated for the transport and then stored at -20°C until the laboratory analysis. Faeces were collected from the rectum and stored at -20°C as well.

In field: data and analysis

Data collection

Data about individual anamnesis were recorded for each cow. We had a work sheet predisposed to record the following data: parity, days in milk, body condition score (according to Edmonson procedure (1989) based on 5 points scale), milk daily production, milk fat and protein content and somatic cells count from the last official control.

Analysis: pH

Ruminal pH, urine pH and faeces pH were determined immediately after sampling using a portable pHmeter (Piccolo, Hanna Instruments). About 1ml of rumen liquid was used to immerge the pHmeter probe and this measurement was done quickly after the sampling in order to avoid errors provoked by contact of the sample with air and VFA evaporation or

alkalinization caused by atmospheric CO2 contact. To determine urine and faeces pH the same pHmeter was used putting the probe into their container and shaking gently in order to assure the hole probe superficy contact with the biological material. Results were recorded on a work sheet.

The animals and 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, each cow was classified as normal (group 1), at risk (group 2) to develop SARA, with SARA (group 3); after this individual classification dairy farms were classified into 3 groups: 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.

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 was analyzed by near infrared spectroscopy (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 absorption methods.

Blood

Blood samples were refrigerated during transport; samples for blood gas analysis were transported in water and ice to the Istituto Zooprofilattico Sperimentale delle Venezie.

Gas analysis and hemochrome were immediately performed while the other samples were centrifuged with a Megafuge 1.0R centrifuge (3000 rpm at 4°C for 15 minutes to obtain plasma from tubes with lithium-heparin and K_3EDTA; 4000 rpm at 20°C for 10 minutes to obtain serum). Many aliquots of serum and plasma were stored at -20°C.

Hemochrome was then performed, within 2 hours from sampling, on hole blood collected in tubes with K_3EDTA: 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; software 6.1).

Hematochemical profile was determined on plasma obtained from blood collected in tubes with lithium-heparin and the following parameters were measured by the mean of the Boehringer Mannheim/Hitachi 911 automated chemistry analyzer (Roche, Basel, Switzerland): creatinine, aspartate transaminase (AST), gamma-glutamyl transferase (γ-GT), glucose, cholesterol, triglycerides, nonesterified fatty acids (NEFA), total - direct and indirect bilirubin, calcium, phosphorus, magnesium, sodium, potassium, chloride, zinc, urea, alkaline phosphatase (ALP), beta-hydroxy-buthirate (BHO); total proteins (PRTO TOT), albumin (ALB) and total globulins (G), as well as albumin/globulin ratio (A/G) were determined on serum samples by the same analyzer.

For acute phase proteins determination samples were brought to the Faculty of Veterinary Medicine of Glasgow and assays were performed at Reactivlab ltd.

Haptoglobin was determined by the mean of a method that is described and validated by Eckersall et al (1999): an automated biochemical assay for haptoglobin. The principle of this test is that free haemoglobin (Hb) exhibits peroxidase activity, which is inhibited at low pH; haptoglobin (Hp) present in the sample combines with haemoglobin and at low pH preserves the peroxidase activity of the bound haemoglobin. Preservation of the peroxidase activity of haemoglobin is directly proportional to the amount of haptoglobin present in the sample. Conditions described apply to a COBAS MIRA analyser (Roche Diagnostics Ltd), maintained at 37^{o}C. Samples were diluted 1:10 in saline prior to analysis. The increase in absorbance at 600nm over the 50s after substrate addition was used to calculate the standard curve by the MIRA computer programme. Haptoglobin concentration in unknown samples was calculated by comparison with the standard curve. Results from the control samples were assessed and confirmed to be within an acceptable range.

Serum amyloid A was measured using a solid phase sandwich ELISA manufactured by Tridelta Development Ltd. Buffers were prepared as directed in the kit. All serum samples were diluted 1:500 in sample diluent buffer prior to use. The standard was solubilised as directed in the kit instructions and diluted 1:5 in diluent buffer followed by doubling dilutions to provide SAA standards in the range 0-300ng/ml. Biotin anti-SAA was diluted 1:100 in diluent buffer. Streptavidin-peroxidase was diluted to 1:4000 in diluent buffer. 50ul of diluted

biotinylated anti-SAA was added to each well coated with anti-SAA. Standards and samples were added at 50ul/well in duplicate. After incubation at 37^{o}C for 1 hour, any SAA present in the well is both captured on the plate by the immobilized antibody and labelled with the conjugate antibody in a one step procedure. After washing to remove all of the unbound material, 100µl/well Streptavidin-Horse Radish Peroxidase conjugate is added and incubated for 30 minutes. Wells were washed as before and 100ul/well TMB substrate added for 30 minutes. The reaction was stopped by adding 50µl/well of stop solution and the absorbance of each well read at 450nm. The assay was performed and read by a Triturus automatic ELISA processor. SAA concentration in unknown samples was calculated by comparison with the standard curve. Results from the control samples were assessed and confirmed to be within an acceptable range.

Homocysteine (OMOCIST) was measured on plasma by a quantitative immunological test by the mean of IMMULITE analyzer equipped with computer HP Brio RDA IGL 16, producted by Cirrus Diagnostics Inc.

Blood gases were analyzed by the instrument SYNTHESIS 15 and the following parameters were measured: extracellular base excess (BE ECF), bicarbonates (HCO3-), metahemoglobin (MET HB), oxyhemoglobin (O2 HB) partial pressure of CO2 (PCO2), pH, partial pressure of O2 (PO2), calculated and measured oxygen saturation (SO2 C and SO2 M) and total CO2 (TCO2).

Urine

Urine samples were stored at –20°C and then analyzed for the following parameters determination: urea and creatinine (CREA) by the mean of the Boehringer Mannheim/Hitachi 911 automated chemistry analyzer (Roche, Basel, Switzerland) and calcium (CAu), phosphorus (Pu), magnesium (MGu), sodium (NAu), potassium (Ku) and chloride (CLu) by Cobas Mira.

Statistical analysis

The objective of this work was to analyze and verify correlation between subacute ruminal acidosis and many systemic parameters. Field data and laboratory results were analyzed within the “Bovine Class” (based on individual classification into group 1, 2 or 3 as described above) and then in “Herd Class” (based on farm classification into group A, B or C as described above). Results were expressed as mean ± standard deviation (SD) and the repeated measures one-way analysis of variance (ANOVA) was applied (by SGMA STAT 3.05

software) to evaluate the effect of SARA on hematochemical and hematological parameters, blood gases and urine parameters within the “bovine class” and the “herd class”. After that a two-way ANOVA was applied to verify the effect of the DIM class (DIM 1=cows with less than 60 days in milk; DIM 2=cows with more than 60 days in milk) and Herd Class together on the same parameters. Pairwise multiple comparison procedures (Tuckey Test) was applied to determine if differences were statistically significant (P<0,05). Data that were particularly interesting were then analyzed by Pearson correlations.

After that, a logistic regression model was applied: this model use the Bovine Class or the Herd Class as dependent variable and try to explain the probability that a farm (or a cow) has to pass from class A or 1 (normal) to B/2 (risk) or to C/3 (SARA) depending on these variables: BCS, parity, DIM class, SAA, haptoglobin, LYM, NEU, WBC, ALB, BE ECF, HCO3, PCO2, blood pH, PO2, urine pH, CAu, Pu,_MGu,_SIDu, BASO, EOS, HCT, HGB, MCH, MCHC, MCV, RBC, NEFA, BHO, PROT TOT, OMOCIST, VIT B12. Since they were too much, a selection procedure was used on variables in order to keep in the model only significant ones (P<0,05).

Data were analyzed especially within the “herd class” since we think that SARA is more related to a herd problem rather than an individual problem.

Results

Field data

First data available in this trial were obtained by field analysis and in particular the first result was individual cow rumen pH followed by cow classification (named bovine class) as "normal" (group 1), "at risk" (group 2) and with "SARA" (group 3). After 10-12 rumen fluid samples analyzed (since this is the statistically significant number to investigate metabolic problem in a herd) it was then possible to classify the herd (herd class) as "normal" (group A), "at risk" (group B) or with "SARA" (group C). Table 2 shows the situation in the 12 herds examined.

Table 2: distribution of cows in the herds and within the different classes

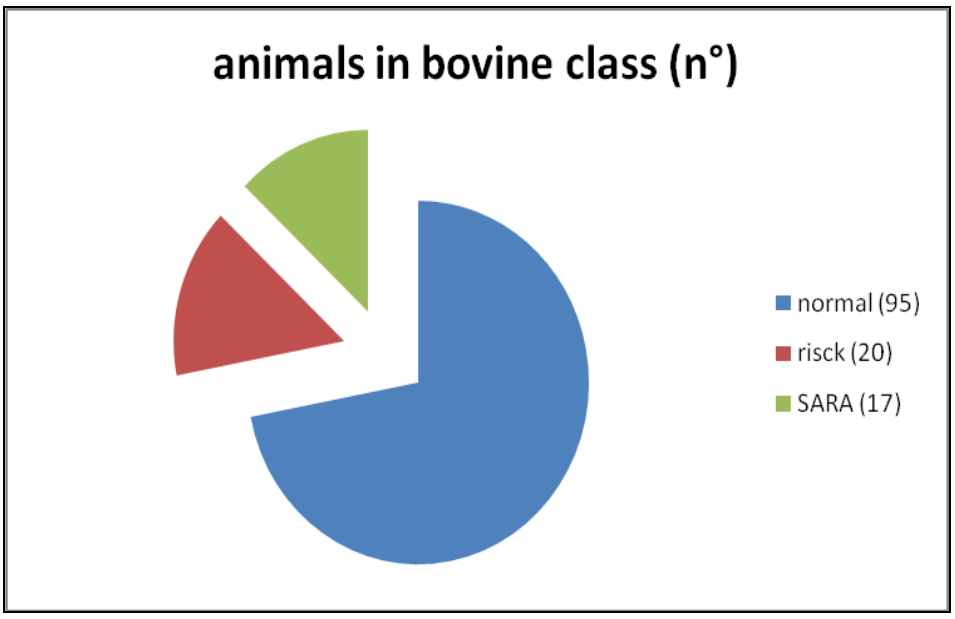
Farm	Normal cows (group 1)	Risk cows (group 2)	SARA cows (group 3)	Herd class	Total cows
1	5	2	3	C	10
2	9	-	-	A	9
3	7	-	-	A	7
4	9	1	1	A	11
5	5	3	3	C	11
6	11	-	1	A	12
7	10	-	2	B	12
8	9	2	1	B	12
9	11	1	-	A	12
10	8	3	1	B	12
11	7	2	3	C	12
12	4	6	2	C	12
Total cows	***95***	***20***	***17***		***132***

In the 12 farms, 5 herds were found in normal rumen pH condition (number 2, 3, 4, 6 and 9) and were classified in group A, 3 herds were defined at risk to develop SARA and then classified as group B (number 7, 8, and 10) and in 4 herds SARA was diagnosed and they were then classified in group C (number 1, 5, 11 and 12).

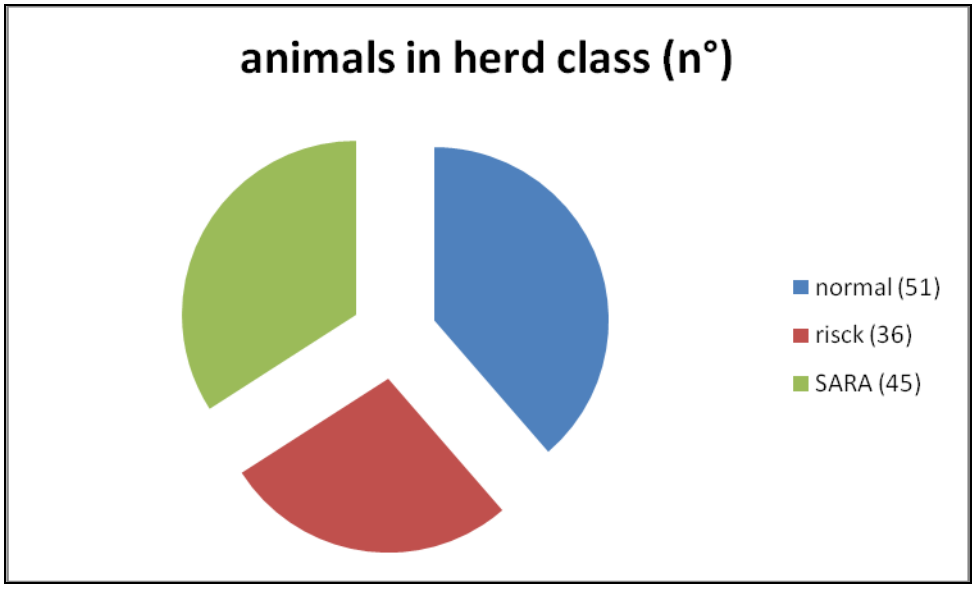
Graphics 2 and 3 show distribution of animals in the bovine classes (groups 1, 2 and 3) and the herd classes (group A, B and C). It is evident that the distribution in groups 1, 2 and 3 is

not equilibrated because 95 cows were normal while 20 were at risk and 17 had SARA, but in groups A, B and C the number of animals is very similar (51 cows in group A, 36 in group B and 45 in group C).

Graphic 2: cows distribution in bovine classes



Graphic 3: cows distribution in herd classes



Tables 3 and 4 show that the three groups in herd and bovine class were homogeneous for mean parity, daily milk yield and BCS. The mean of DIM is higher in group C, but not

statistically significant, because in the farm number 1 cows with high DIM were chosen (because of the veterinary suspect) and this farm was classified in group C.

Table 3: mean and standard deviation of rumen pH, parity, DIM, BCS, daily milk yield and urine and faeces pH measured in field, in herd class (A=normal herds, B=herds at risk for SARA, C=herds with SARA)

	Group A	Group B	Group C
rumen pH	6.19 ± 0.39	6.06 ± 0.34	5.84 ± 0.35
Parity	2.77 ± 1.84	2.44 ± 1.29	2.78 ± 1.52
DIM	43.69 ± 29.05	38.39 ± 22.24	82.73 ± 62.10
BCS	3.04 ± 0.22	3.09 ± 0.22	3.02 ± 0.21
daily milk yield (kg/d)	39.70 ± 6.36	37.24 ± 10.79	40.63 ± 7.11
urine pH	8.67 ± 0.30**	8.34 ± 0.21*	8.46 ± 0.23**
faeces pH	6.87 ± 0.26*	6.64 ± 0.34**	6.79 ± 0.30
N° cows/class	*51*	*36*	*45*

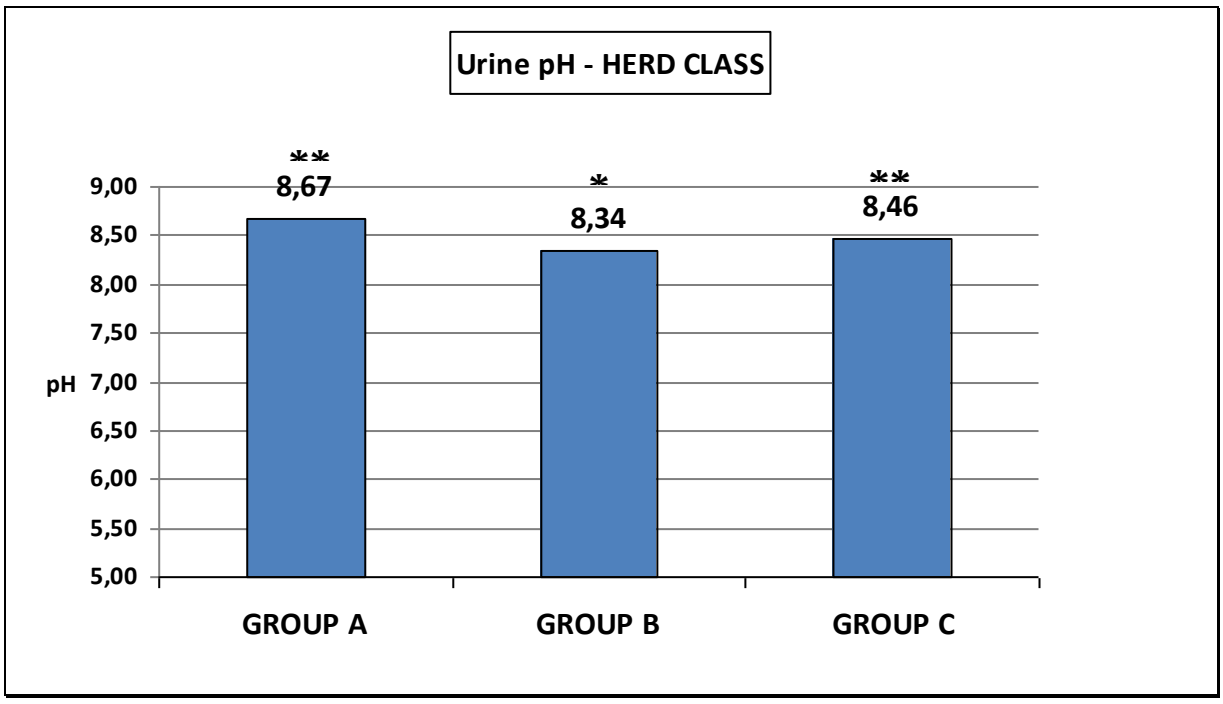
*, **: same number of * on the same line to exclude statistic significance ($P < 0.05$)

It is interesting to note that there are statistical significant differences on urine and faeces pH in groups A, B and C but not in groups 1, 2 and 3. In particular, even if the trend is not linear, urine and faeces pH are quite similar and characterized by a lower pH in classes B (urine pH = 8.34 ± 0.21; faeces pH = 6.64 ± 0.34) and C (urine pH = 8.46 ± 0.23; faeces pH = 6.79 ± 0.30) compared with class A (urine pH = 8.67 ± 0.30; faeces pH = 6.87 ± 0.26) with a statistic significance ($P<0.05$) between A and B and between B and C (graphics 4a and 4b).

Table 4: mean and standard deviation of rumen pH, parity, DIM, BCS, daily milk yield and urine and faeces pH measured in field, in bovine class (1=normal cows, 2=cows at risk for SARA, C=cows with SARA)

	Group 1	Group 2	Group 3
rumen pH	6.21 ± 0.32	5.70 ± 0.06	5.48 ± 0.12
Parity	2.75 ± 1.73	2.60 ± 1.10	2.41 ± 1.33
DIM	50.06 ± 43.82	67.90 ± 43.70	71.71 ± 57.61
BCS	3.04 ± 0.22	3.10 ± 0.13	3.00 ± 0.25
daily milk yield (kg/d)	39.39 ± 8.31	40.73 ± 6.53	37.46 ± 8.61
urine pH	8.53 ± 0.30	8.41 ± 0.21	8.53 ± 0.28
faeces pH	6.78 ± 0.33	6.71 ± 0.26	6.86 ± 0.23
N° cows/class	*95*	*20*	*17*

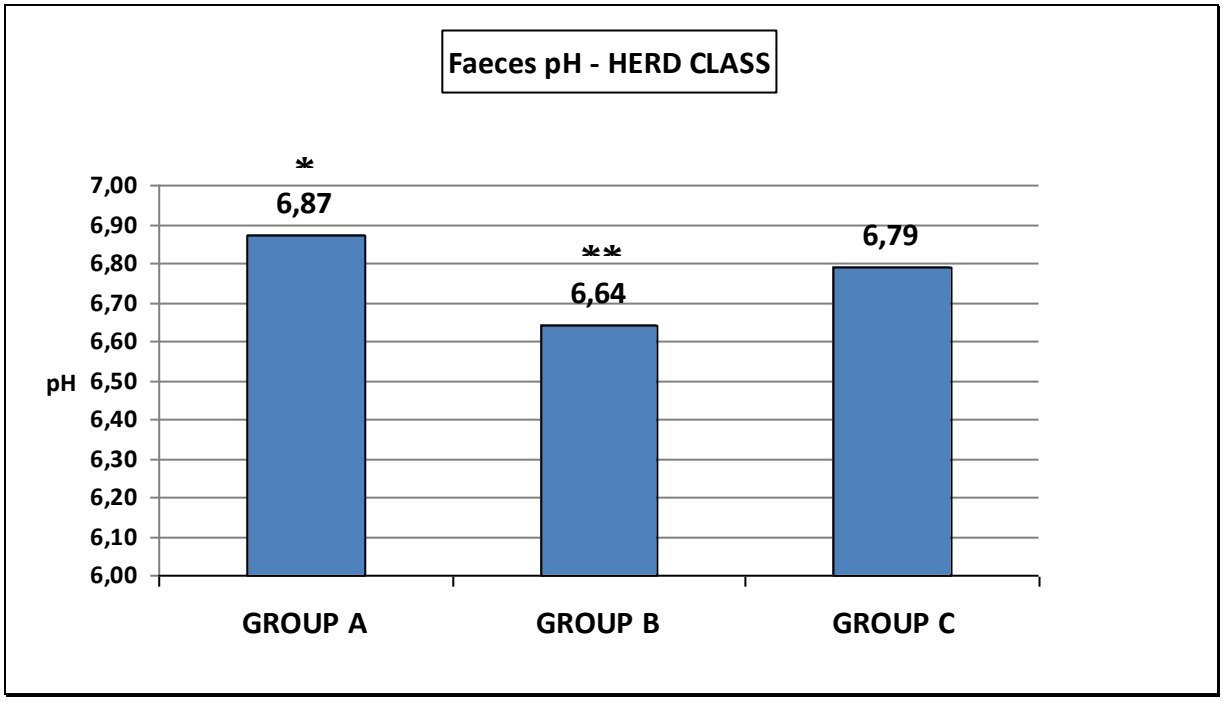
Graphic 4a: mean urine pH in group A, B and C (A=normal herds, B=herds at risk for SARA, C=herds with SARA)



A two way ANOVA was then applied to verify the effect of DIM class on urine and faeces Ph. For urine pH the effect of different level of herd class does not depend on what level of

DIM class is present and there is not a statistically significant interaction between herd class and DIM class (P=0,083); anyway, main significant effect were analyzed by pairwise multiple comparison procedure (Tuckey Test). For faeces pH the effect of different levels of herd class depends on what level of DIM class is present and there is a statistically significant interaction between herd class and DIM class (P=0,006). Results and data are shown table 5 and graphics 5 and 6.

Graphic 4b: mean urine pH in group A, B and C (A=normal herds, B=herds at risk for SARA, C=herds with SARA)



The trend is more clear for both urine and faeces pH in the class DIM 2 (more than 60 days in milk) because the normal class (A) has the higher pH compared with class B and C but, even if it is not statistically significant, class C is always higher then B. In the class DIM 1 (graphic 5) we can see a significant difference on faeces pH between the risk group (B) and the other two (A and C).

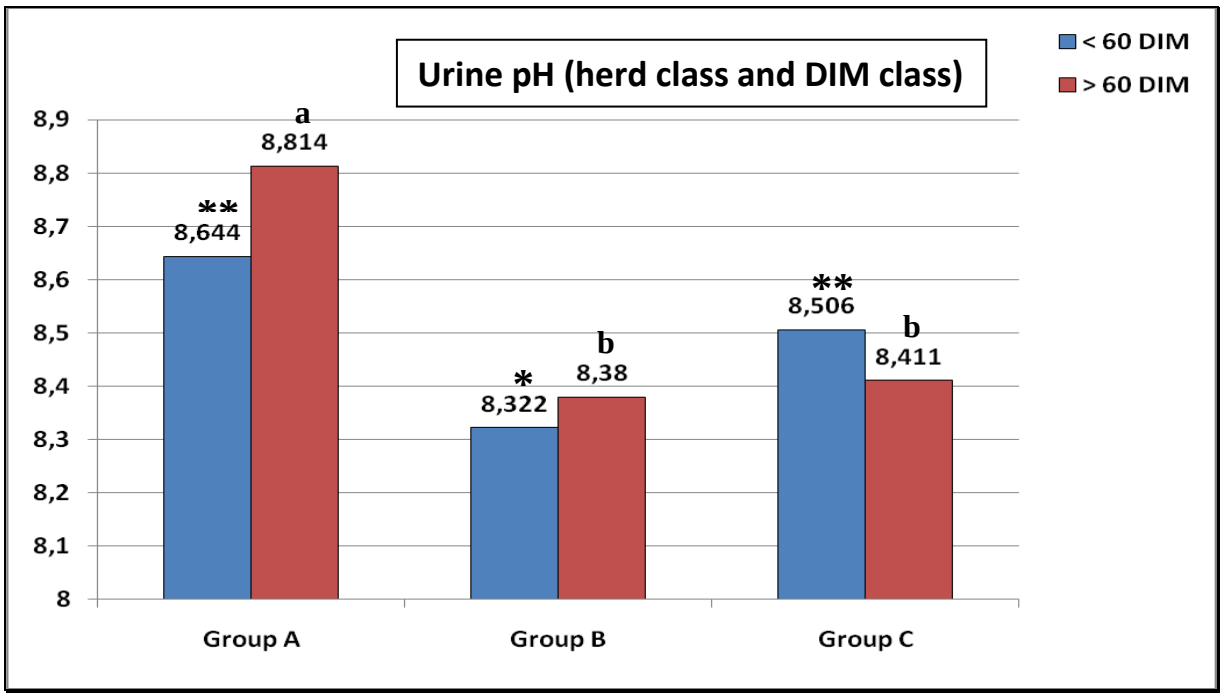
Table 5: least square means for herd class x DIM class, for urine and faeces pH (A=normal herds, B=herds at risk for SARA, C=herds with SARA)

		Group A (mean ± SEM)	Group B (mean ± SEM)	Group C (mean ± SEM)
URINE pH	**< 60 DIM**	8,644** ± 0,038	8,322* ± 0,050	8,506** ± 0,054
	> 60 DIM	8,814[a] ± 0,083	8,380[b] ± 0,088	8,411[b] ± 0,053
FAECES Ph	**< 60 DIM**	6,810 ± 0,045	6,648 ± 0,055	6,830 ± 0,062
	> 60 DIM	7,151* ± 0,097	6,595** ± 0,103	6,750** ± 0,060

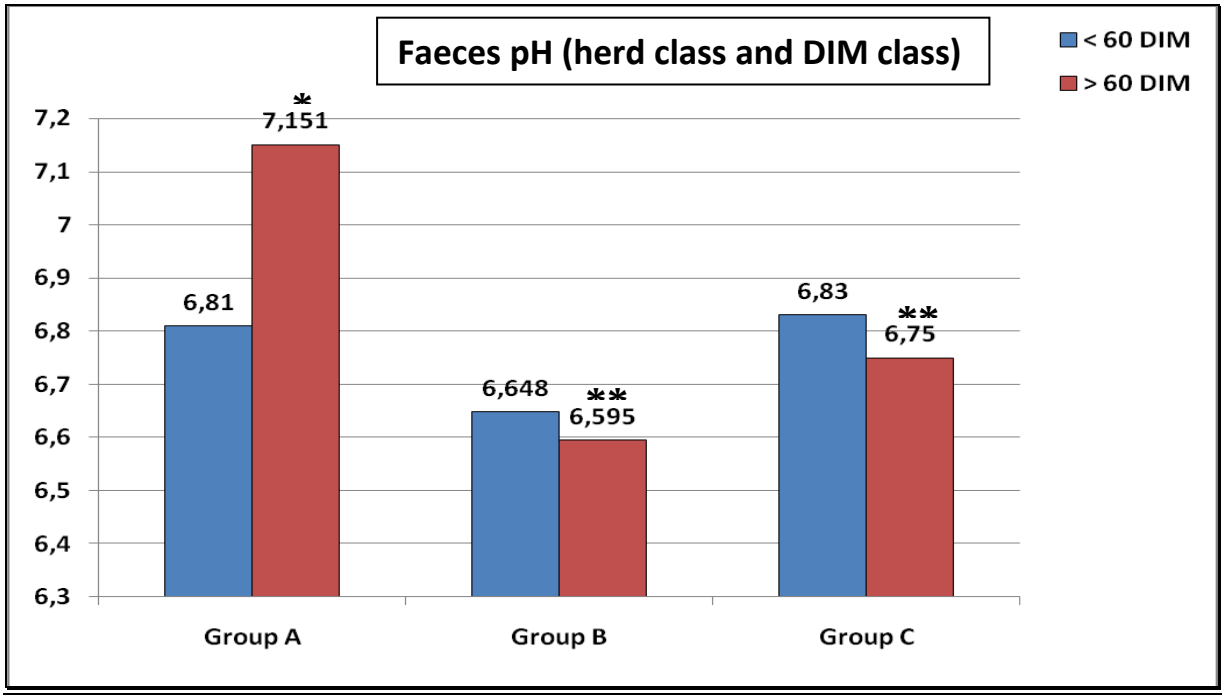
*, **: same number of * on the same line to exclude statistic significance (P < 0.05)

[a], [b]: same letter on the same line to exclude statistic significance (P < 0.05)

Graphic 5: mean urine pH in group A, B and C (A=normal herds, B=herds at risk for SARA, C=herds with SARA)



Graphic 6: mean faeces pH in group A, B and C (herd and DIM classification; A=normal herds, B=herds at risk for SARA, C=herds with SARA)



Laboratory data

Blood analysis

Hemochrome, biochemical analysis, blood gases, homocysteine, acute phase proteins (haptoglobin and serum amyloid A) and vitamin B12 were performed for each cow.

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) except for cholesterol, total proteins and globulins. The statistical differences observed within the three groups are represented in the following graphics and discussed in the next chapter.

Special attention was paid on acute phase parameters, blood gas analysis, vitamin B12 and homocysteine.

Data are shown in the following tables both for bovine class and herd class; graphs represents also results of the two way ANOVA analysis were this was interesting.

Table 6: Average values of haematological parameters, expressed in their conventional units together with the related standard deviations in the dairy cows of Group A (=normal herds), B (=herds at risk for SARA) and C (=herds with SARA), and statistical significance observed.

Parameters	*Normal values*	Group A	Group B	Group C
RBC (10^6/µl)	*5 - 10§*	5.05 ± 2.01**	5.78 ± 0.62*	5.83 ± 0.60*
HGB (g/dl)	*8 - 15§*	8.88 ± 3.49**	10.18 ± 0.92*	10.28 ± 0.88*
HCT (%)	*24 - 46§*	26.12 ± 10.30**	30.04 ± 2.91*	30.43 ± 2.81*
MCV (fl)	*40 - 60§*	45.17 ± 17.74**	52.19 ± 4.22*	52.30 ± 2.88*
MCH (pg)	*11 - 17§*	15.36 ± 6.04**	17.69 ± 1.40*	17.69 ± 1.07*
MCHC (g/dl)	*30 - 36§*	29.54 ± 11.54**	33.91 ± 0.60*	33.83 ± 0.77*
RDW (%)	*16.7 – 23.3§*	17.39 ± 6.80**	19.86 ± 1.09*	19.92 ± 1.21*
PLT (10^3/µl)	*100 – 800§*	434.54 ± 209.88**	548.24 ± 146.89*	447.16 ± 106.29*
MPV (fl)	*3 – 7§*	3.32 ± 1.31**	3.81 ± 0.45	3.99 ± 0.54*
WBC (10^3/µl)	*4 – 12§*	6.06 ± 2.71	6.89 ± 1.84	6.51 ± 1.91
Neutrophils (10^3/µl)	*0.6 – 4§*	2.89 ± 1.69	3.57 ± 1.54	3.06 ± 1.36
Lymphocites (10^3/µl)	*2.5 – 7.5§*	2.46 ± 1.94	2.48 ± 0.59	2.66 ± 0.78
Monocites (10^3/µl)	*0.02 – 0.9§*	0.67 ± 0.33	0.74 ± 0.25	0.73 ± 0.27
Eosinophils (10^3/µl)	*0 – 0.24§*	0.01 ± 0.05	0.06 ± 0.19	0.02 ± 0.05
Basophils (10^3/µl)	*0 – 0.2§*	0.36 ± 0.27**	0.45 ± 0.18	0.56 ± 0.20*
N° animals/class		*51*	*36*	*45*

*, **: same number of * on the same line to exclude statistic significance ($P < 0.05$)

§ Data from Kaneko et al., 1997
Ψ Data from Kramer, 2000

Table 7: Average values of haematological parameters, expressed in their conventional units together with the related standard deviations in the dairy cows of Group 1 (=normal cows), 2 (=cows at risk for SARA) and 3 (=cows with SARA), and statistical significance observed.

Parameters	*Normal values*	Group 1	Group 2	Group 3
RBC (10^6/µl)	*5 - 10§*	5.39 ± 1.58	5.81 ± 0.47	5.84 ± 0.50
HGB (g/dl)	*8 - 15§*	9.50 ± 2.71	10.29 ± 0.83	10.21 ± 0.81
HCT (%)	*24 - 46§*	31.90 ± 3.36	31.85 ± 3.12	31.65 ± 2.78
MCV (fl)	*40 - 60§*	48.48 ± 13.73	52.48 ± 2.46	51.77 ± 2.35
MCH (pg)	*11 - 17§*	16.46 ± 4.67	17.72 ± 0.93	17.48 ± 0.85
MCHC (g/dl)	*30 - 36§*	31.57 ± 8.57	33.78 ± 0.73	33.78 ± 0.80
RDW (%)	*16.7 – 23.3§*	18.64 ± 5.21	19.76 ± 1.12	19.56 ± 0.89
PLT (10^3/µl)	*100 – 800§*	462.73 ± 186.19	465.80 ± 111.33	514.41 ± 119.77
MPV (fl)	*3 – 7§*	3.64 ± 1.08	3.91 ± 0.48	3.68 ± 0.31
WBC (10^3/µl)	*4 – 12§*	6.23 ± 2.31	6.85 ± 1.45	7.13 ± 2.55
Neutrophils (10^3/µl)	*0.6 – 4§*	2.99 ± 1.52	3.38 ± 1.22	3.66 ± 1.97
Lymphocites (10^3/µl)	*2.5 – 7.5§*	2.49 ± 1.00	2.70 ± 0.66	2.61 ± 0.68
Monocites (10^3/µl)	*0.02 – 0.9§*	0.70 ± 0.30	0.67 ± 0.17	0.82 ± 0.35
Eosinophils (10^3/µl)	*0 – 0.24§*	0.02 ± 0.08	0.07 ± 0.22	0.01 ± 0.02
Basophils (10^3/µl)	*0 – 0.2§*	0.02 ± 0.01**	0.03 ± 0.01	0.04 ± 0.02*
N° animals/class		*95*	*20*	*17*

*, **: same number of * on the same line to exclude statistic significance ($P < 0.05$)

§ Data from Kaneko et al., 1997
Ψ Data from Kramer, 2000

These data (table 6) show that neutrophils increase in risk class (B) and SARA class (C): 3.57 ± 1.54 and 3.06 ± 1.36 respectively. Among leukocytes, basophils is the only parameter that increase with a linear trend both in bovine class and in herd class.

WBC count is higher in groups B and C (graphic 7) but there is not statistically significant difference.

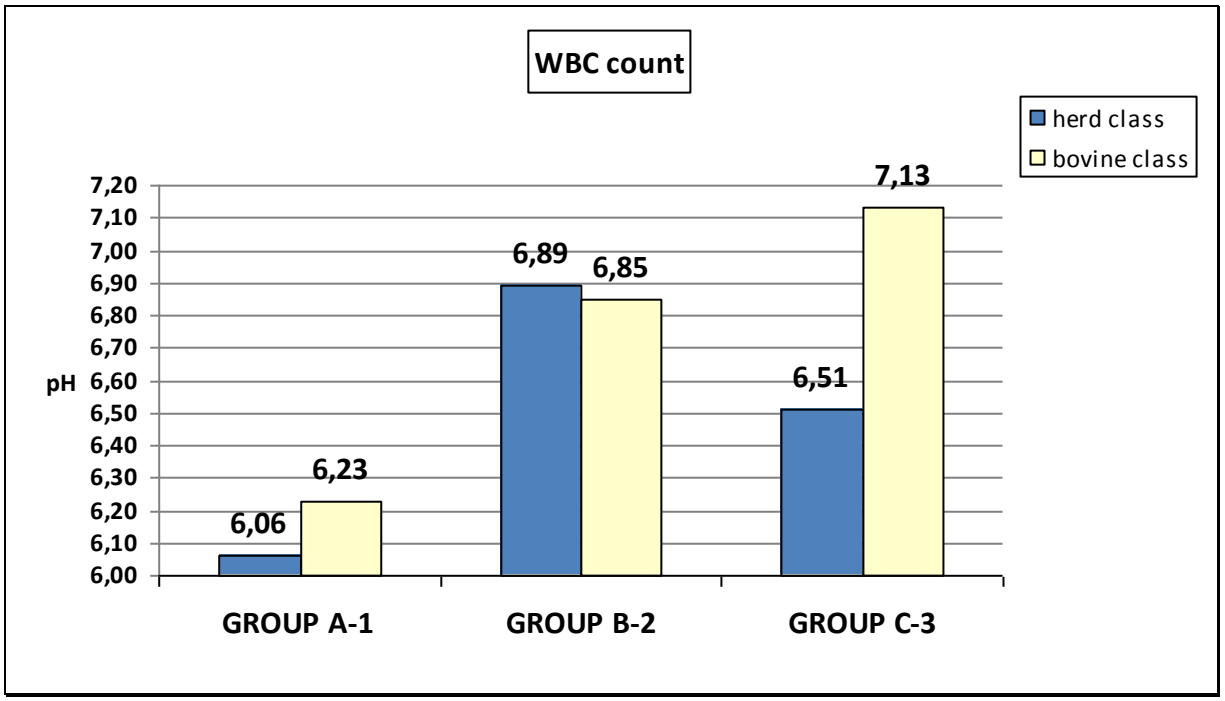
This parameter was then analyzed with two way ANOVA for herd class and DIM class: the effect of different level of herd class does not depend on what level of DIM class is present and there is not a statistically significant difference between herd class and DIM class.

A pairwise multiple comparison procedure (Tuckey Test) was done and a significant difference (P<0,05) was found between group A and group C within class DIM 2 (graphic 8).

Table 8: least square means for herd class x DIM class, for WBC count (A=normal herds, B=herds at risk for SARA, C=herds with SARA)

		Group A (mean ± SEM)	Group B (mean ± SEM)	Group C (mean ± SEM)
WBC count	< 60 DIM	6,449 ± 0,338	7,098 ± 0,414	6,428 ± 0,467
	> 60 DIM	4,242** ± 0,729	6,176 ± 0,774	6,597* ± 0,456

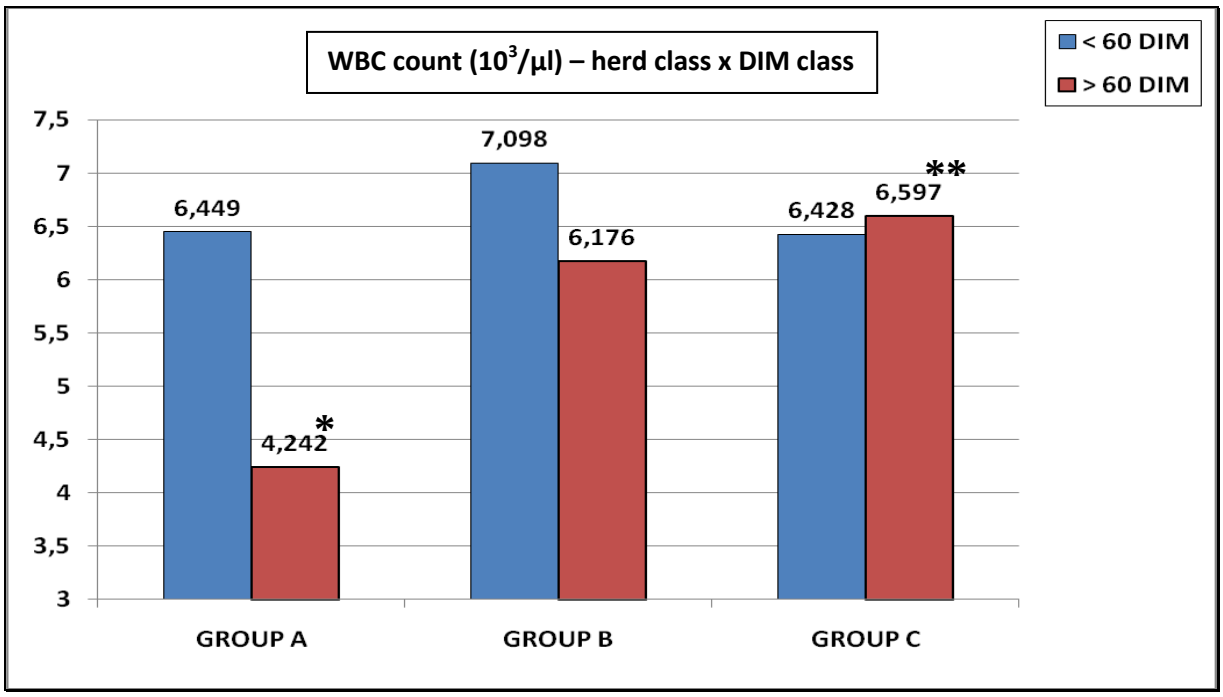
Graphic 7: mean WBC count in group A, B and C and 1, 2 and 3 (A=normal herds, B=herds at risk for SARA, C=herds with SARA; 1=normal cows, 2=cows at risk for SARA, 3=cows with SARA)



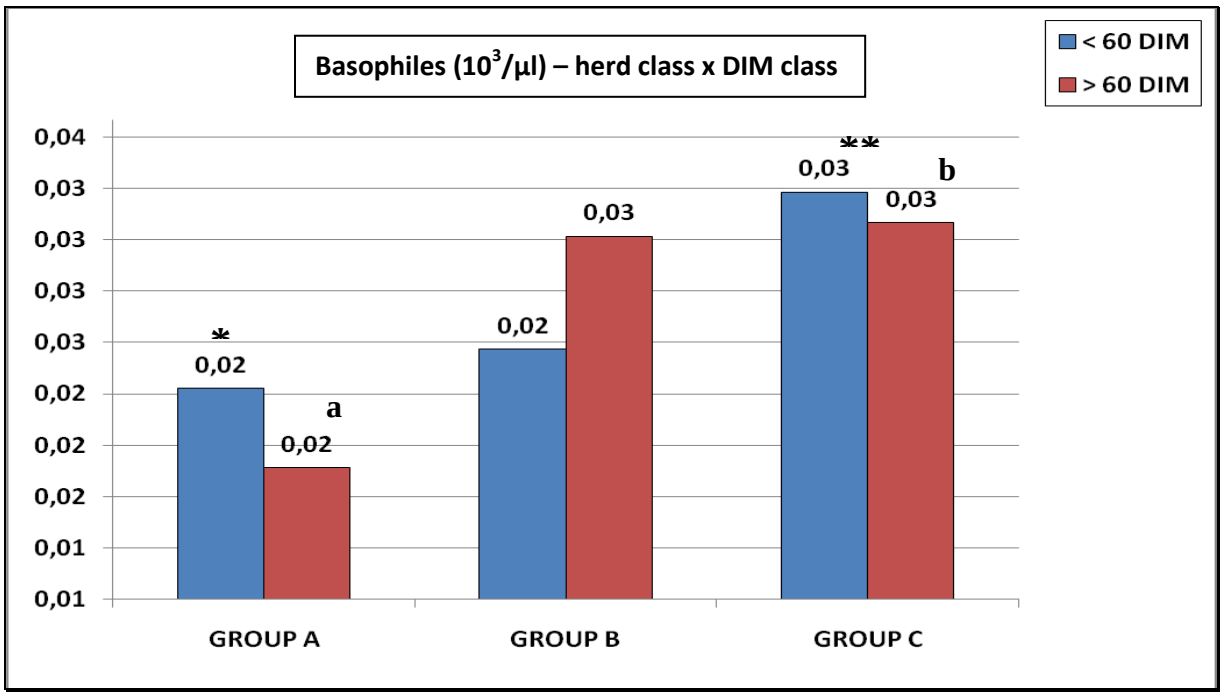
Neutrophils, basophiles and lymphocytes were tested by two way ANOVA as well and even if there were not statistically significant differences, trend became linear for class DIM 2 (more then 60 days in milk) for all them three (graphics 9, 10 and 11).

Hematological parameters in bovine class are characterized by an absence of statistically significant differences except for basophiles. WBC (graphic 7) and neutrophils increase with a linear trend from group 1 to 3, while lymphocytes are a bit higher in group 2 then in 3 (but both higher then group 1). In general parameters in bovine class don't vary so much and could be considered almost constant.

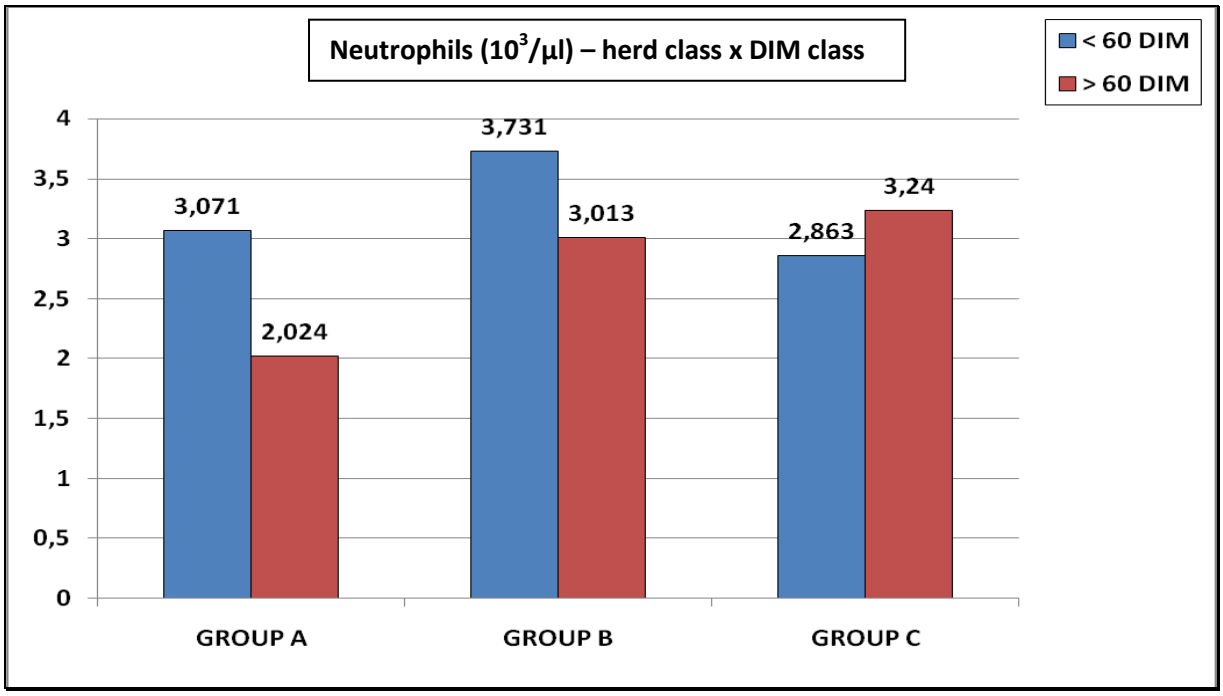
Graphic 8: mean WBC count in group A, B and C (herd and DIM classification; A=normal herds, B=herds at risk for SARA, C=herds with SARA)



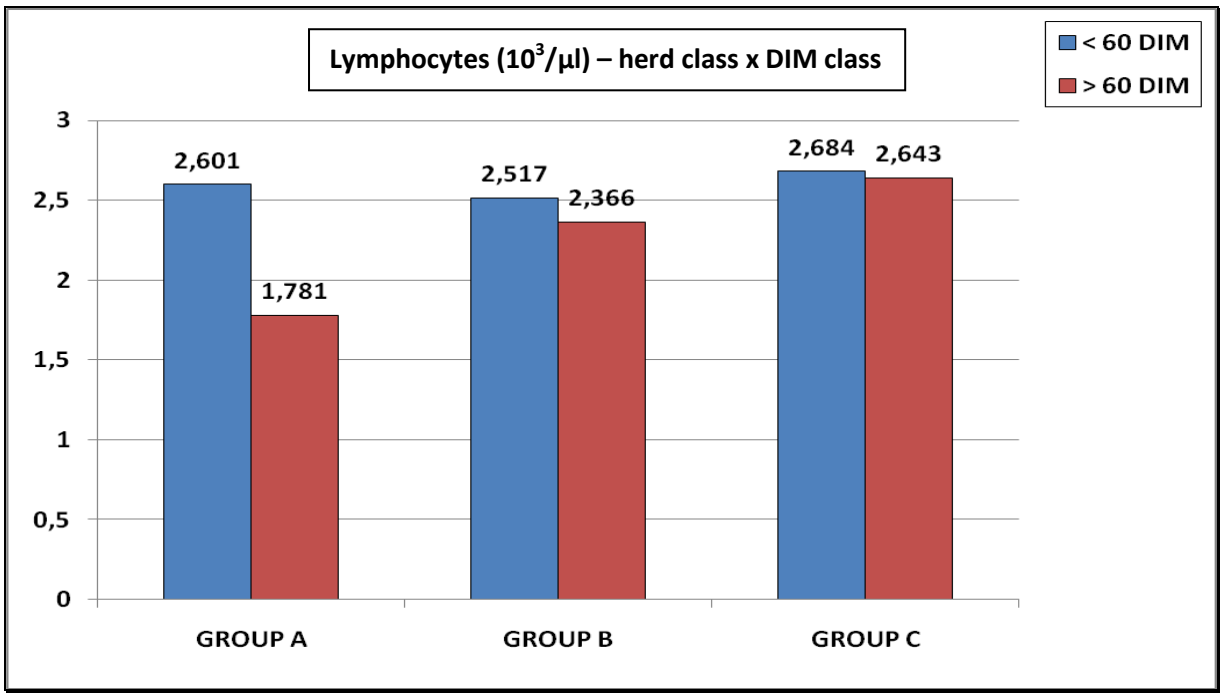
Graphic 9: mean basophiles in group A, B and C (herd and DIM classification; A=normal herds, B=herds at risk for SARA, C=herds with SARA)



Graphic 10: mean neutrophils in group A, B and C (herd and DIM classification; A=normal herds, B=herds at risk for SARA, C=herds with SARA)



Graphic 11: mean lymphocytes in group A, B, C (herd and DIM classification; A=normal herds, B=herds at risk for SARA, C=herds with SARA)



It is interesting to note that both lymphocytes and basophils are sinificative parameters if introduced in a logistic regression model and they affect the probability to pass from herd class A to the herd class B and C.

Table 9: Average values of haematochemical parameters, expressed in their conventional units together with the related standard deviations in the dairy cows of Group A (Normal herds), B (Risk herds) and C (SARA herds), and statistical significance observed.

Parameters	*Normal values*	Group A	Group B	Group C
Creatinine (µmol/l)	*35 – 280§*	93.32 ± 11.03	95.19 ± 10.30*	89.72 ± 8.23**
AST (I.U./l)	*43 - 127§*	96.19 ± 19.02	106.42 ± 27.96*	93.04 ± 21.78**
γ-GT (I.U./l)	*15 - 39§*	26.81 ± 10.35	27.78 ± 14.59	27.12 ± 10.40
Glucose (mmol/l)	*2.47 – 4.12§*	2.90 ± 0.31**	3.03 ± 0.43	3.24 ± 0.56*
Cholesterol (mmol/l)	*2.08 – 3.12§*	3.97 ± 1.21	3.91 ± 1.32	4.07 ± 1.01
Triglycerides (mmol/l)	*0 – 0.2§*	0.13 ± 0.03	0.13 ± 0.03	0.14 ± 0.03
NEFA (meq/l)	*0.2 – 2.28§*	0.41 ± 0.34	0.24 ± 0.17	0.25 ± 0.17
Tot bilirubin (µmol/l)	*0.17 – 8.03§*	2.45 ± 1.90	2.12 ± 1.83	1.62 ± 1.39
β-HO-B (mmol/l)	*0.00 – 0.86§*	0.73 ± 0.76	1.88 ± 7.57	0.61 ± 0.48
Urea (mmol/l)	*2.0 – 7.5* 3	3.95 ± 1.00	4.29 ± 1.11	3.94 ± 1.23
ALP (I.U./l)	*27 - 107§*	46.20 ± 52.14	48.20 ± 19.47	38.12 ± 18.10
Total Proteins (g/l)	*67 - 75§*	79.92 ± 8.22	78.58 ± 9.26	82.09 ± 8.69
Albumine (g/l)	*30 - 36§*	34.29 ± 2.83**	33.94 ± 4.56**	35.98 ± 3.08*
Total Globulins (g/l)	*30 – 34.8§*	45.69 ± 8.50	44.64 ± 9.76	46.11 ± 8.99
Calcium (mmol/l)	*2.43 – 3.10§*	2.57 ± 0.13	2.58 ± 0.12	2.51 ± 0.16
Phosphorus (mmol/l)	*1.08 – 2.76§*	1.79 ± 0.31	1.78 ± 0.34	1.82 ± 0.33
Magnesium (mmol/l)	*0.74 – 1.10§*	1.03 ± 0.08	1.05 ± 0.06	1.02 ± 0.09
Sodium (mmol/l)	*132 - 152§*	144.58 ± 2.36**	146.17 ± 1.77*	144.80 ± 1.85**
Potassium (mmol/l)	*3.9 – 5.8§*	4.12 ± 0.28	4.04 ± 0.22	4.09 ± 0.39
Chloride (mmol/l)	*95 - 110§*	101.78 ± 2.43	102.63 ± 2.41	102.20 ± 2.27
N° cows/class		*51*	*36*	*45*

*, **: same number of * on the same line to exclude statistic significance ($P < 0.05$)

§ Data from Kaneko et al., 1997

3 Data from Radostits et al., 2007

Table 10: Average values of haematochemical parameters, expressed in their conventional units together with the related standard deviations in the dairy cows of Group 1 (Normal cows), 2 (Risk cows) and 3 (SARA cows), and statistical significance observed.

Parameters	*Normal values*	Group 1	Group 2	Group 3
Creatinine (µmol/l)	35 – 280§	93.14 ± 10.51	92.78 ± 10.31	89.39 ± 7.08
AST (I.U./l)	43 - 127§	98.50 ± 22.69	95.61 ± 21.75	97.27 ± 28.09
γ-GT (I.U./l)	15 - 39§	27.21 ± 12.68	26.64 ± 8.60	27.67 ± 8.09
Glucose (mmol/l)	2.47 – 4.12§	3.01 ± 0.47	3.19 ± 0.43	3.09 ± 0.38
Cholesterol (mmol/l)	2.08 – 3.12§	3.97 ± 1.23	4.43 ± 0.99	3.55 ± 0.85
Triglycerides (mmol/l)	0 – 0.2§	0.13 ± 0.03	0.14 ± 0.03	0.12 ± 0.03
NEFA (meq/l)	0.2 – 2.28§	0.34 ± 0.30	0.25 ± 0.10	0.20 ± 0.07
Tot bilirubin (µmol/l)	0.17 – 8.03§	2.30 ± 1.96	1.59 ± 0.90	1.44 ± 0.54
β-HO-B (mmol/l)	0.00 – 0.86§	0.71 ± 0.66**	0.56 ± 0.17**	3.18 ± 11.04*
Urea (mmol/l)	2.0 – 7.5з	3.89 ± 1.03	4.65 ± 1.38	4.18 ± 1.00
ALP (I.U./l)	27 - 107§	44.80 ± 40.63	43.53 ± 18.19	40.02 ± 16.33
Total Proteins (g/l)	67 - 75§	80.03 ± 9.01	82.40 ± 7.82	79.29 ± 8.12
Albumine (g/l)	30 - 36§	34.22 ± 3.20	37.50 ± 2.50	34.65 ± 4.92
Total Globulins (g/l)	30 – 34.8§	45.83 ± 9.18	44.80 ± 8.19	44.82 ± 9.90
Calcium (mmol/l)	2.43 – 3.10§	2.56 ± 0.13	2.58 ± 0.18	2.48 ± 0.13
Phosphorus (mmol/l)	1.08 – 2.76§	1.80 ± 0.33	1.71 ± 0.32	1.90 ± 0.28
Magnesium (mmol/l)	0.74 – 1.10§	1.03 ± 0.08	1.04 ± 0.08	1.04 ± 0.06
Sodium (mmol/l)	132 - 152§	144.90 ± 2.11	145.10 ± 1.61	146.14 ± 2.58
Potassium (mmol/l)	3.9 – 5.8§	4.09 ± 0.29	4.06 ± 0.45	4.09 ± 0.23
Chloride (mmol/l)	95 - 110§	102.02 ± 2.28	102.21 ± 3.02	102.84 ± 2.10
N° cows/class		*95*	*20*	*17*

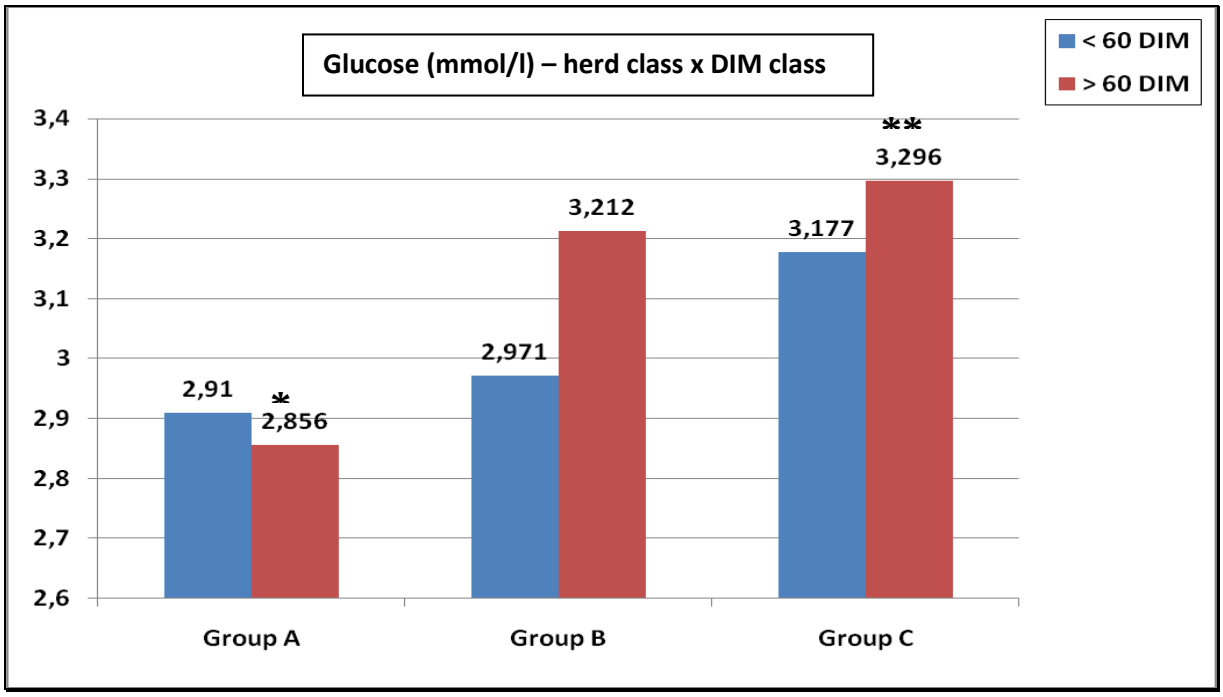
*, **: same number of * on the same line to exclude statistic significance ($P < 0.05$)

§ Data from Kaneko et al., 1997
3 Data from Radostits et al., 2007

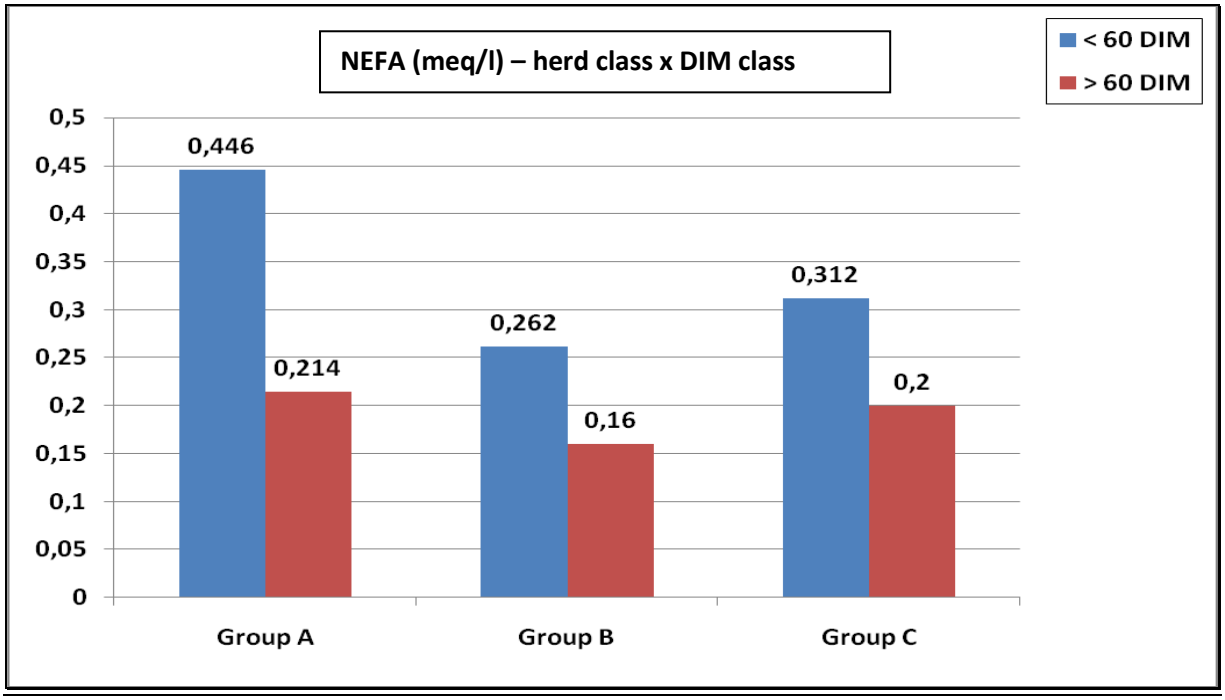
Biochemical profile of blood samples was characterized by greater variations in herd class rather than bovine class again. Parameters related to energetic and lipidic metabolism, in herd class, are generally constant: β-hydroxy-butyrate is higher only in the risk group (B: 1.88 ±

7.57) respect to value of the normal class (A: 0.73 ± 0.76) while triglycerides are constant in the three classes; NEFA lightly decrease from 0,41 ± 0.34 (group A) to 0.25 ± 0.17 (group C), cholesterol increases exclusively in group C (4.07 ± 1.01), glucose increases with a linear trend from group A (2.90 ± 0.31) to group C (3.24 ± 0.56) where there is also a statistically significant difference. In bovine class the only parameter that vary is β-hydroxy-butyrate that is strongly increased in group 3 (3.18 ± 11.04) respect to the group 1 (0.71 ± 0.66). Urea is increased in the risk group (B or 2) but there is not statistically significant difference. About hepatic parameters, creatinine and AST increase in the risk group (group B) but decrease again in the SARA group (C) being always in the interval of normal values; total bilirubin decreases (from 2.45 ± 1.90 in group A to 1.62 ± 1.39 in group C). Glucose, NEFA and B-HOB were analyzed with two way ANOVA to determine the DIM class effect (graphics 12-15). For glucose there is not a statistically significant interaction between herd class and DIM class (P=0,456) and even if the trend is linear (graphic 10) the only statistically significant difference was found within class DIM2 (more than 60 days in milk) between normal (A) and SARA cows (C). Also for NEFA (graphic 13) there is not a statistically significant interaction between herd class and DIM class (P=0,519) and among the different classes.

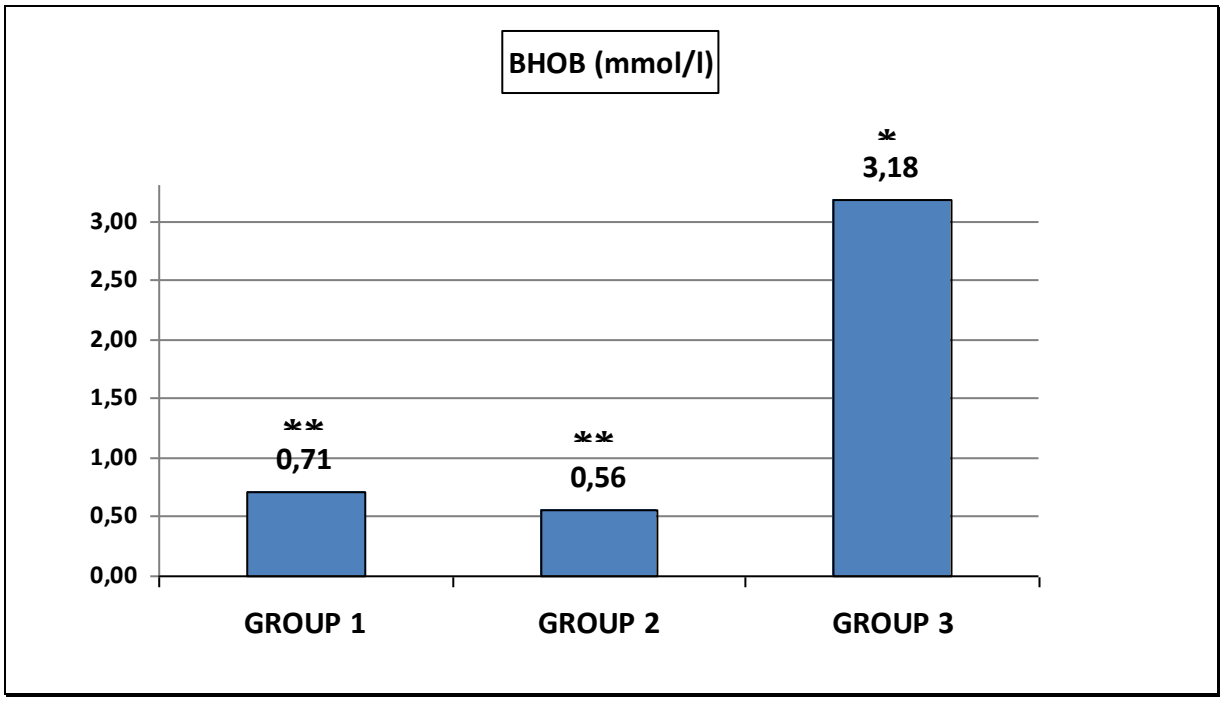
Graphic 12: mean glucose in group A, B and C (herd and DIM classification; A=normal herds, B=herds at risk for SARA, C=herds with SARA)



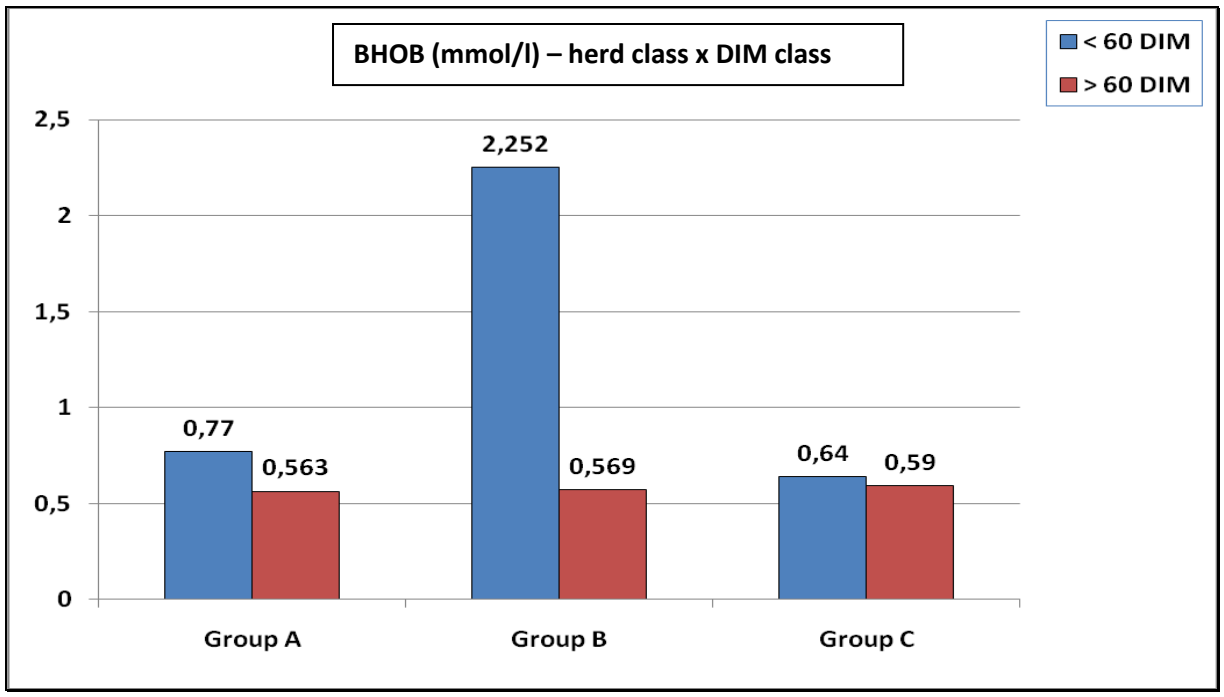
Graphic 13: mean NEFA in group A, B and C (herd and DIM classification; A=normal herds, B=herds at risk for SARA, C=herds with SARA)



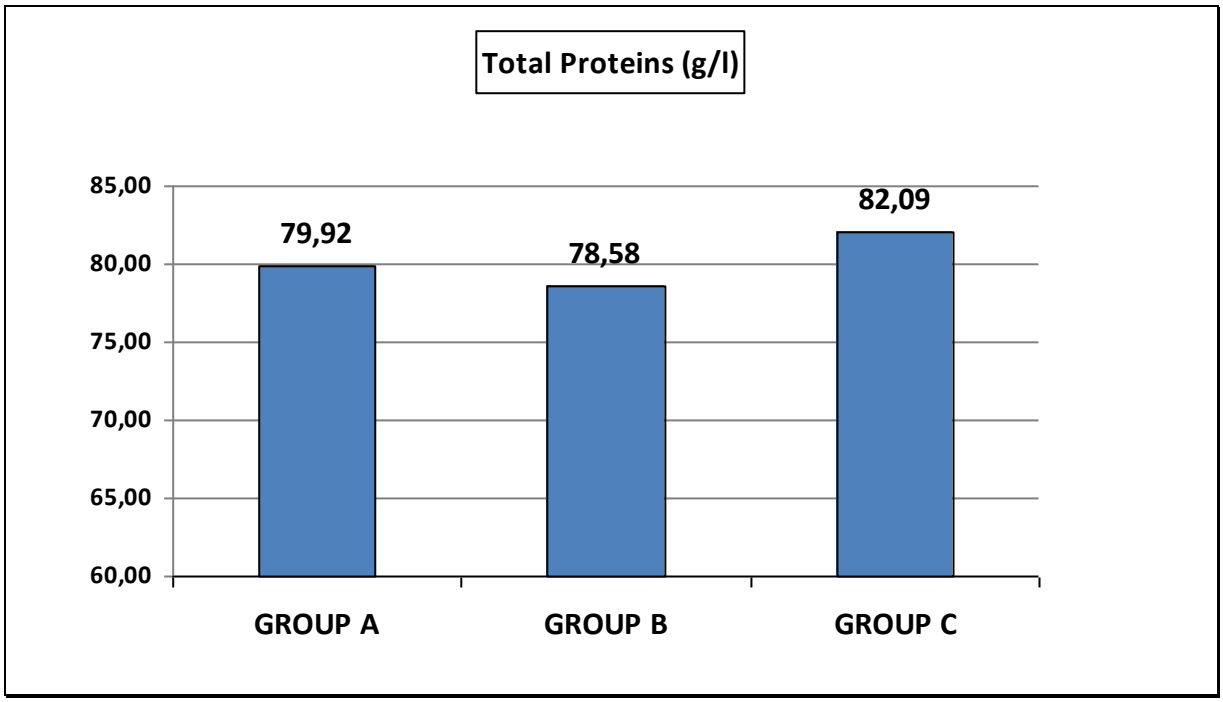
Graphic 14: mean BOHB in bovine class (1=normal cows, 2=cows at risk for SARA, 3=cows with SARA)



Graphic 15: mean BHOB in group A, B and C (herd and DIM classification; A=normal herds, B=herds at risk for SARA, C=herds with SARA)



Graphic 16: total serum proteins in herd class (A=normal herds, B=herds at risk for SARA, C=herds with SARA)

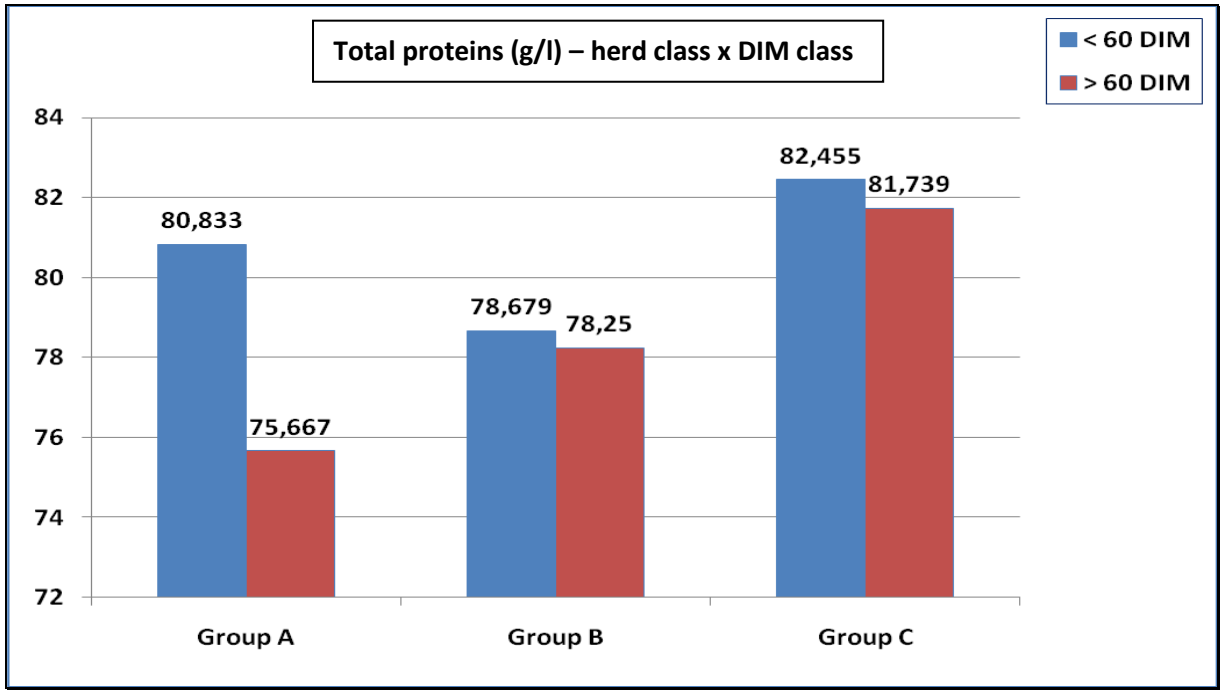


In the bovine class, the only parameters that show a statistically significant difference is BHOB that is strongly increased in group 3 (3.18 ± 11.04) where the difference is statistically

significant (P<0,05). This parameter was then analyzed in a two way ANOVA to evaluate the effect of DIM class on the herd class: it was seen (graphic 15) that there is not a statistically significant interaction between herd class and DIM class (P=0,437).

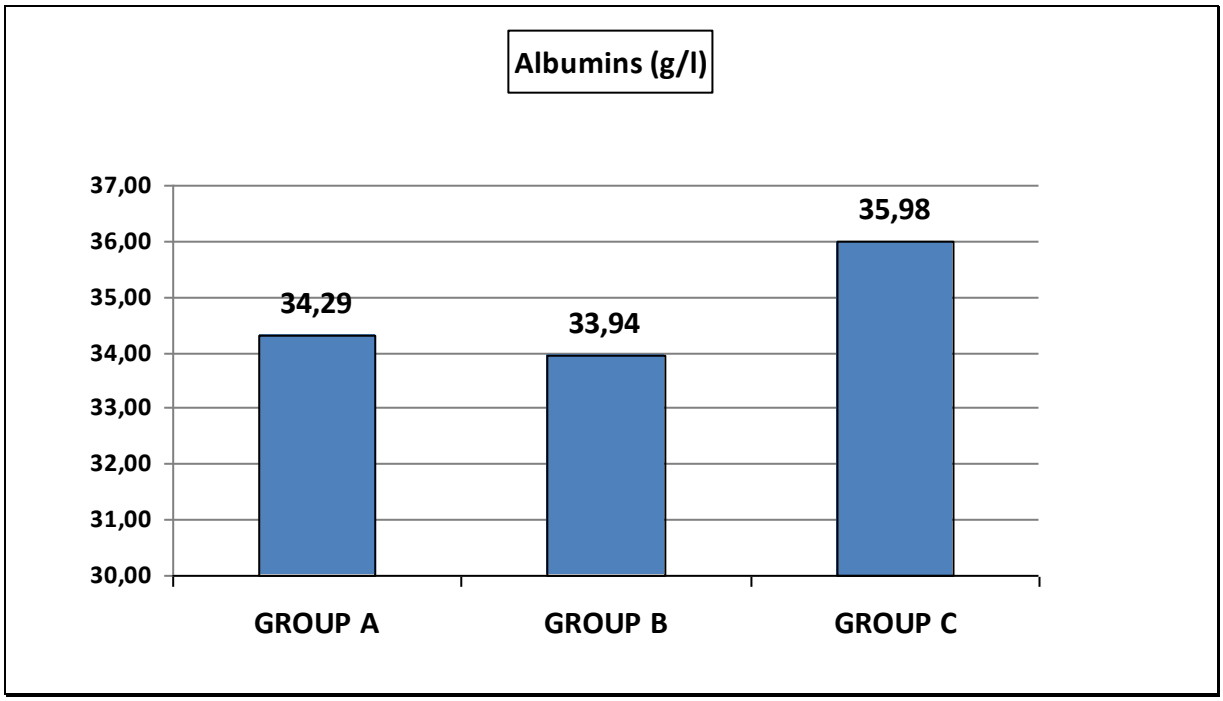
Total proteins and globulins in herd class are characterized by an increase in the SARA group even if there is not statistically significant difference (graphic 16). Total proteins were analyzed also by two way ANOVA to verify DIM class effect but any significant difference was found, even if in class DIM2 the trend became more linear (graphic 17). Total proteins and total globulins trend are very similar.

Graphic 17: mean total proteins in group A, B and C (herd and DIM classification; A=normal herds, B=herds at risk for SARA, C=herds with SARA)



Albumins (graphic 18), that is an important parameter to evaluate the acute phase response, lightly decreased in the risk group (A= 34.29 ± 2.83; B=33.94 ± 4.56) but strongly increased in group with SARA (C=35.98 ± 3.08). Albumins were analyzed also by two way ANOVA to verify DIM class effect: the effect of different levels of herd class does not depend on what level of DIM class is present and there is not a statistically significant interaction between herd class and DIM class. It is interesting to note that albumins is a sinificative parameters if introduced in a logistic regression model and it affect the probability to pass from herd class A to the herd class B and C.

Graphic 18: albumins in herd class (A=normal herds, B=herds at risk for SARA, C=herds with SARA)



About minerals, there is not an important variation except for the sodium that is strongly higher in the group (B).

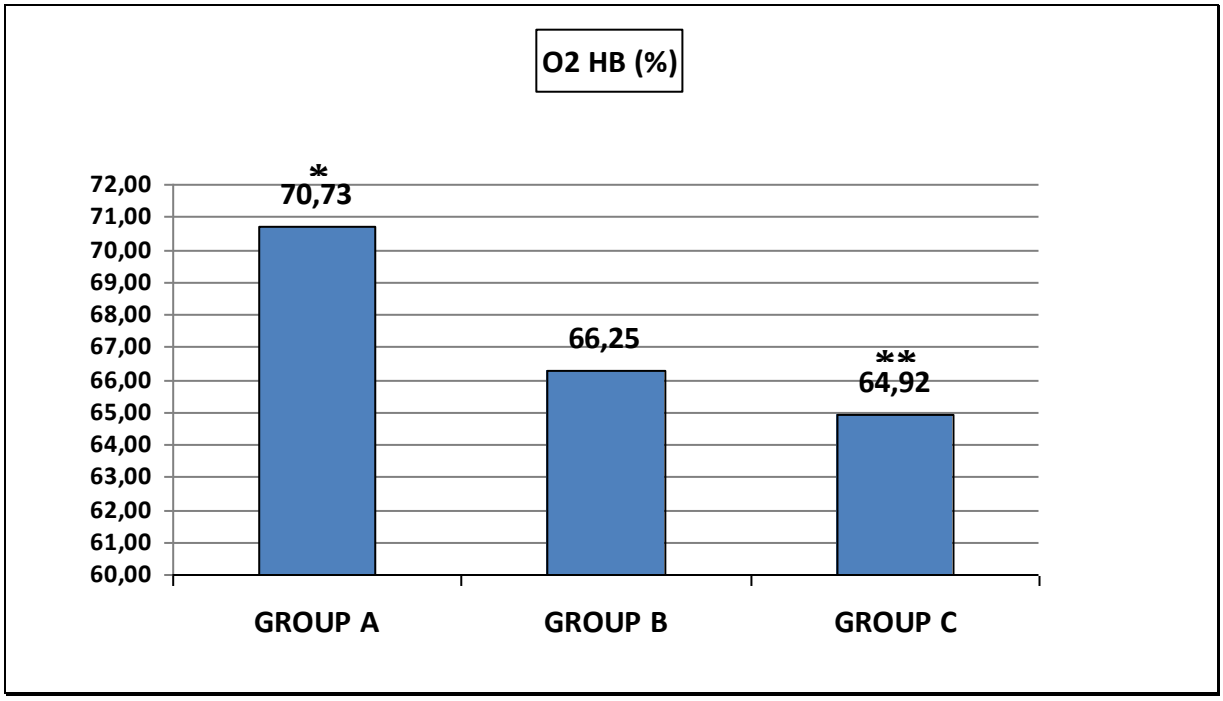
Blood gas analysis results are shown in the following table.

Table 11: Average values of blood gas parameters, expressed in their conventional units together with the related standard deviations in the dairy cows of Group A (Normal herds), B (Risk herds) and C (SARA herds), and statistical significance observed.

Parameters	Group A	Group B	Group C
BE ECF (mmol/l)	6,37 ± 2,48	5,66 ± 3,37	6,59 ± 2,79
HCO3- (mmol/l)	30,72 ± 2,35	30,13 ± 3,15	30,99 ± 2,47
MET HB	0,61 ± 0,31	0,76 ± 0,39	0,66 ± 0,36
O2 HB (%)	70.73* ± 8.52	66.25±8.95	64.92** ± 8.63
PCO2 (mmHg)	44,78 ± 4,11	44,47 ± 3,65	45,28 ± 3,35
PH	7,44 ± 0,03	7,43 ± 0,02	7,44 ± 0,03
PO2 (mmHg)	41.18* ± 5.03	33.43** ± 4.95	34.67** ± 5.01
SO2 C (%)	76.70* ± 7.97	64.31** ± 7.88	66.57** ± 7.84
SO2 M (%)	70.80 ± 8.64	66.19 ± 8.05	64.96 ± 8.95
TCO2 (mmHg)	32,10 ± 2,45	31,49 ± 3,24	32,37 ± 2,54

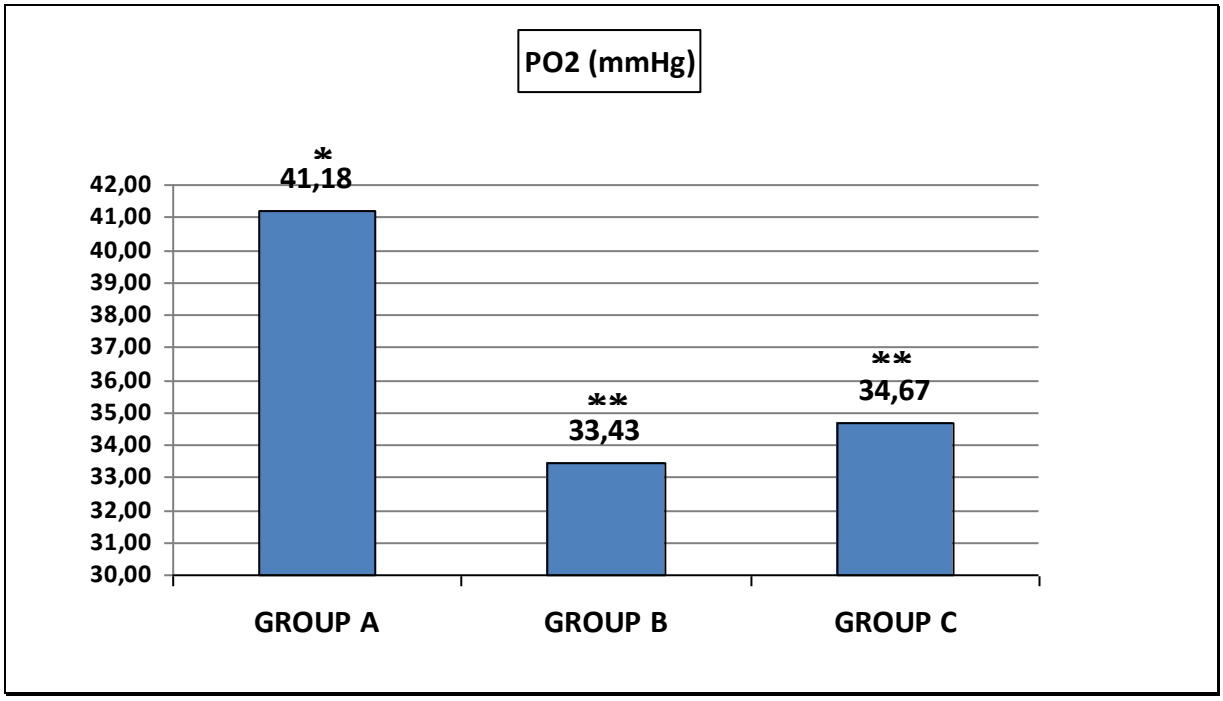
Blood bicarbonates, pH, partial pressure of CO2 and measured oxygen saturation didn't show significant differences but oxyhemoglobin (%O2 Hb), partial pressure of O2 (pO2) and calculated oxygen saturation (sO2c and sO2m) were significantly different in herd class. In particular %O2 Hb was 70.73±8.52, 66.25±8.95, 64.92±8.63 - pO2 was 41.18±5.03, 33.43±4.95, 34.67±5.01 mmHg - %sO2c was 76.70±7.97, 64.31±7.88, 66.57±7.84 in group A, B and C respectively. Table 11 shows the average values of blood gas parameters, expressed in their conventional units together with the related standard deviations in the dairy cows of Group A (Normal), B (Critical) and C (Acidosis) and statistical significance observed. Graphics 19-21 show the mean values of principal parameters among the three classes of herds.

Graphic 19: mean O2HB in herd class (A=normal herds, B=herds at risk for SARA, C=herds with SARA)

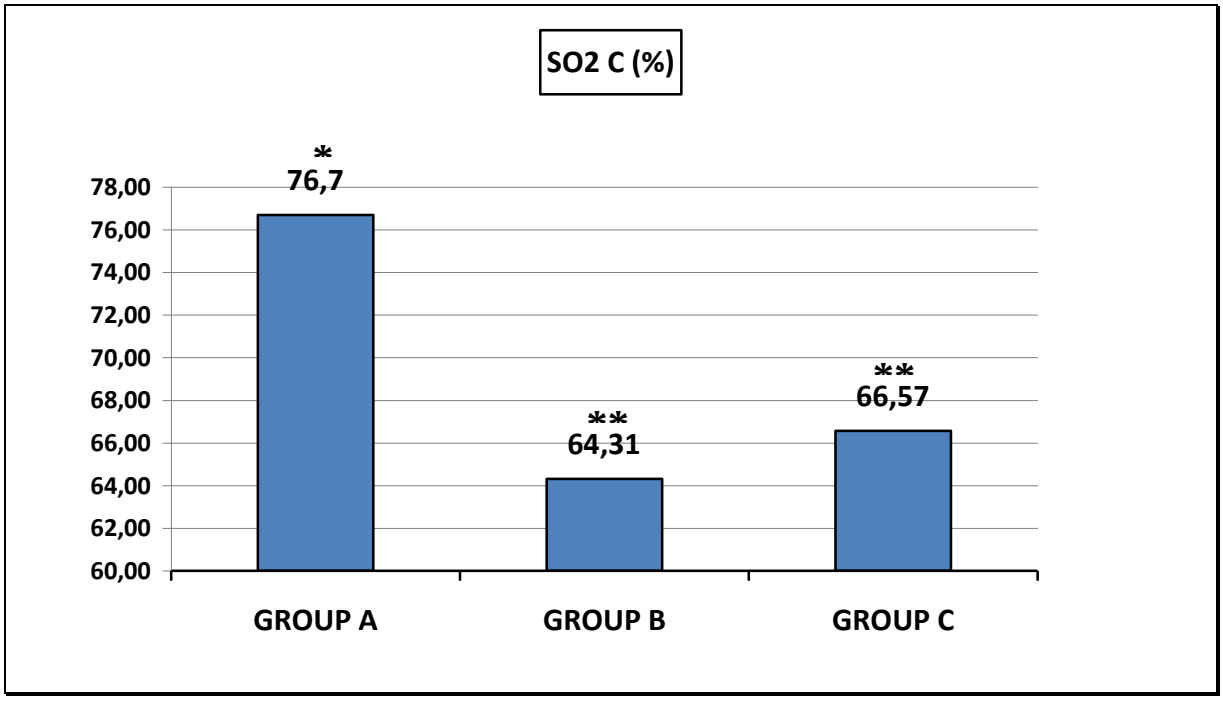


In bovine class there was not blood gas parameters statistically significant.

Graphic 20: mean PO2 in herd class (A=normal herds, B=herds at risk for SARA, C=herds with SARA)

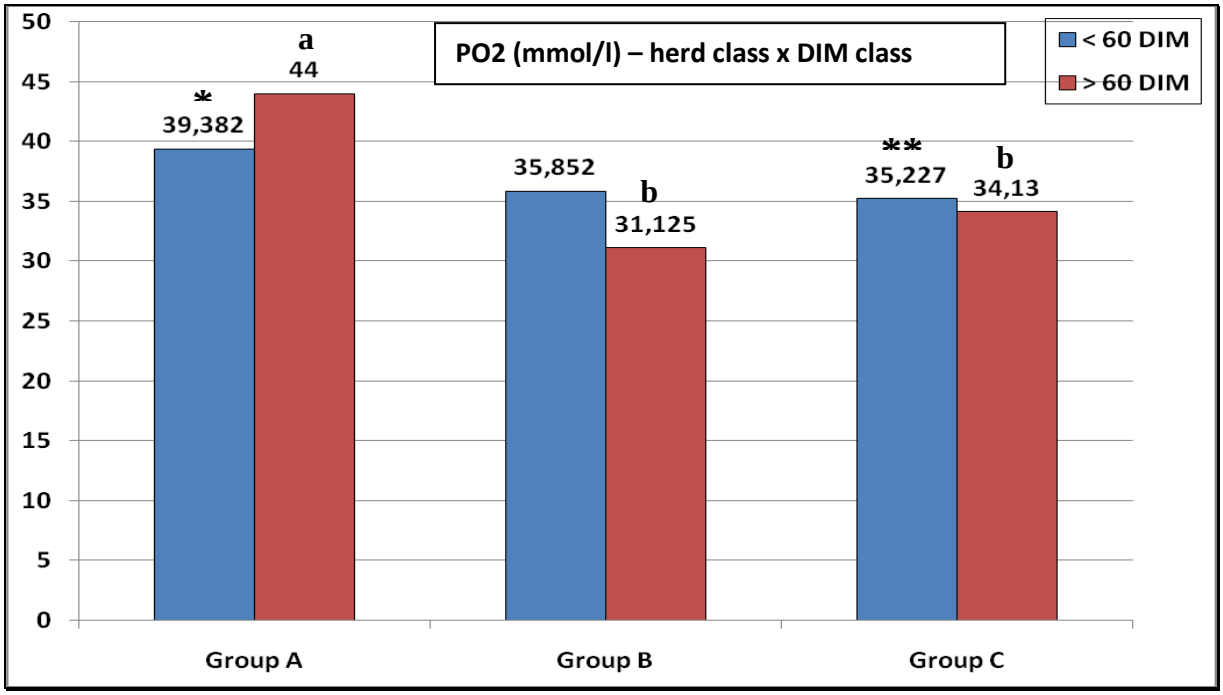


Graphic 21: mean SO2 C in herd class (A=normal herds, B=herds at risk for SARA, C=herds with SARA)



Blood gases were then analyzed using a two way ANOVA to understand if there was an influence of the DIM class.

Graphic 22: mean PO2 in group A, B and C (herd and DIM classification; A=normal herds, B=herds at risk for SARA, C=herds with SARA)

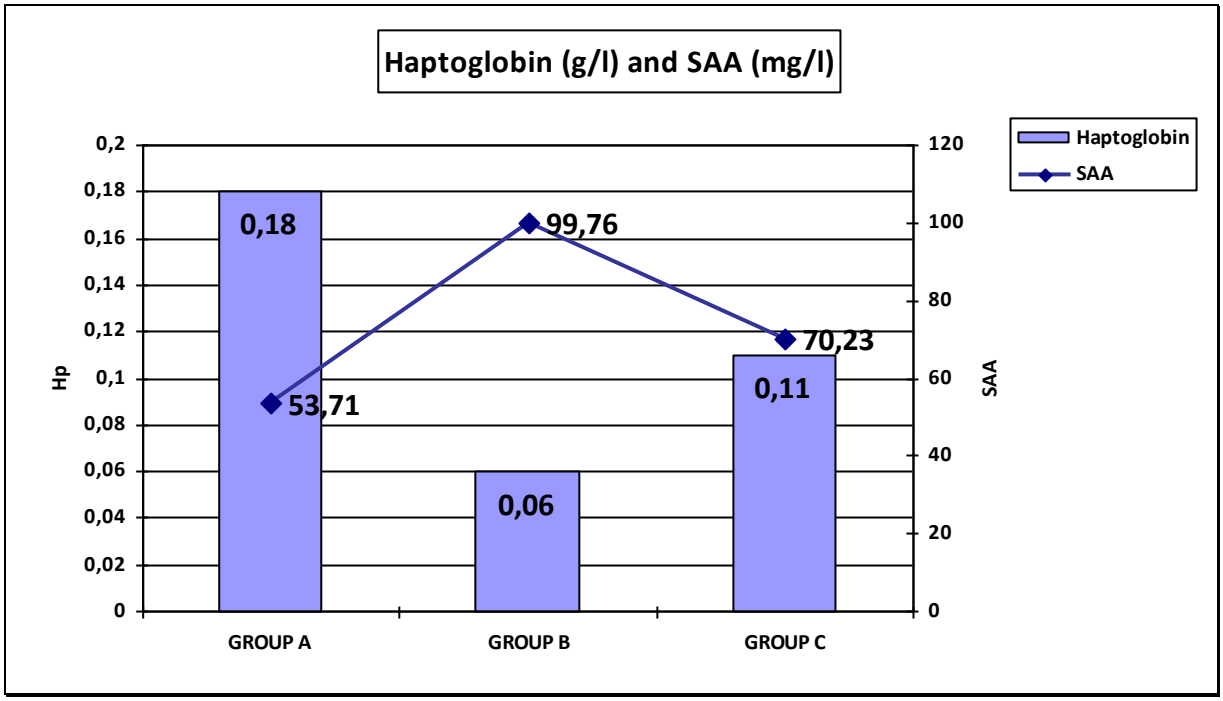


More blood parameters analyzed were homocysteine, vitamine B12, and acute phase proteins (haptoglobin and Serum Amyloid A).

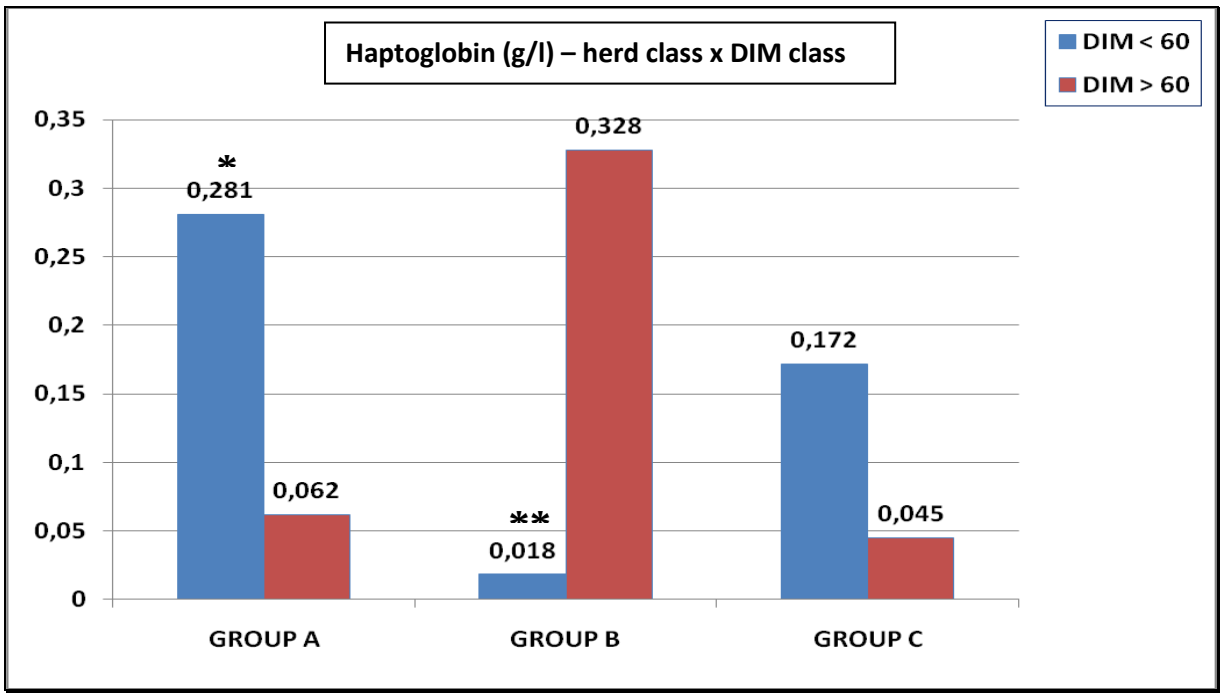
Table 12: Average values and standard deviations of homocysteine, vitamin B12 and acute phase proteins (haptoglobin and SAA) expressed in their conventional units in the dairy cows of Group A (Normal herds), B (Risk herds) and C (SARA herds), and statistical significance observed.

Parameters	Group A	Group B	Group C
Homocysteine (µmol/l)	4.83 ± 1.41*	3.09 ± 1.43**	4.38 ± 1.60*
Vitamin B12	222,22 ± 75,21	228,50 ± 136,51	228,64 ± 88,18
Haptoglobin (g/l)	0.18 ± 0.42	0.06 ± 0.14	0.11 ± 0.06
SAA	53,712 ± 126,05	99,764 ± 297,39	70,228 ± 150,53

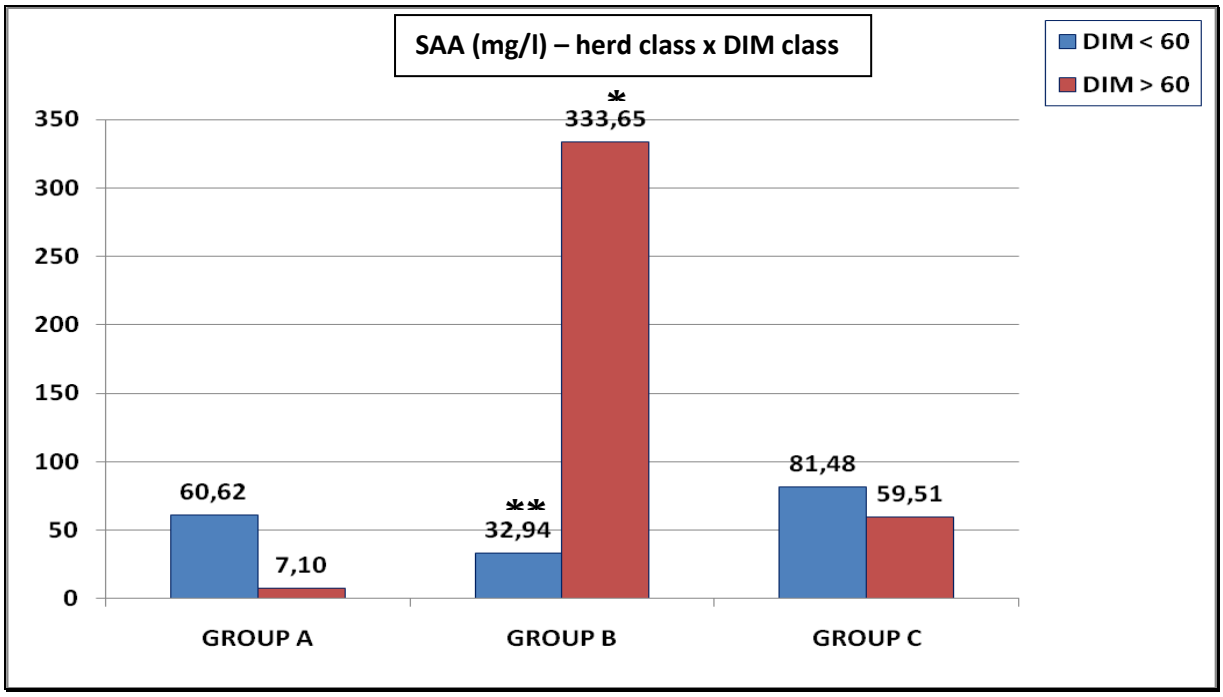
Graphic 23: haptoglobin and SAA in herd class (A=normal herds, B=herds at risk for SARA, C=herds with SARA)



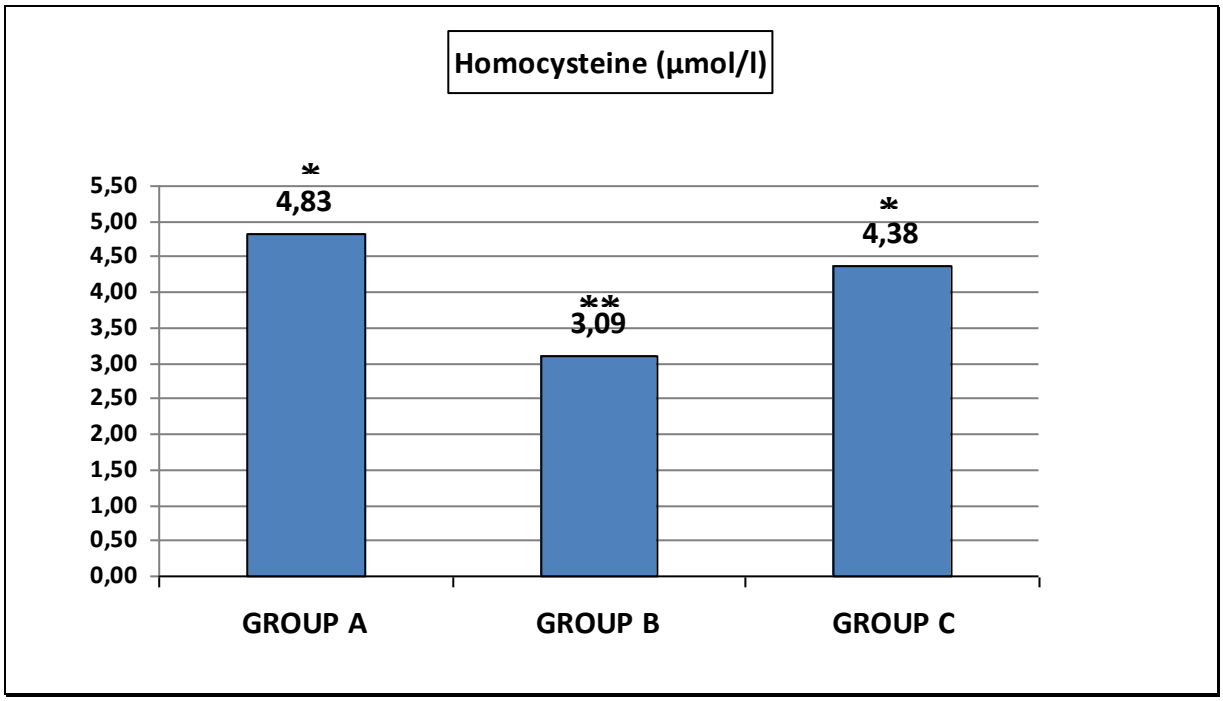
Graphic 24: mean haptoglobin in group A, B and C (herd and DIM classification; A=normal herds, B=herds at risk for SARA, C=herds with SARA)



Graphic 25: mean SAA in group A, B and C (herd and DIM classification; A=normal herds, B=herds at risk for SARA, C=herds with SARA)



Graphic 26: mean homocysteine levels in herd class (A=normal herds, B=herds at risk for SARA, C=herds with SARA)



Discussion

The objective of this study, as stated above, was to analyze some blood and urinary and faecal parameters that are supposed to be involved during subacute ruminal acidosis and to understand their variations. The animals were homogeneous for BCS, daily milk production, parity and general health condition. Data have been analyzed especially within the herd class because this trial confirmed that SARA can be considered at a farm level (see table 2): animals within a herd are usually homogeneous in their ruminal condition. Herds were representative of north Italy dairy herds for management and structures characteristics. It has been demonstrated that low rumen pH can be seen in mid/late lactation cows (Oetzel et, al., 2007) and that there is not a correlation between rumen pH and DIM; data of this trial confirm that mid/late lactation SARA is quite common in Italian herds and the fact that a two way ANOVA (herd class x DIM class) applied for many parameters did not change significant differences or determined a linear trend in the group DIM2 (more then 60 DIM) confirms the existence of SARA over the 60 days in milk cows.

I considered at first field data because they are easy to collect and they could became an easy diagnostic tool. After that I considered the acute phase response because a lot of works have been done on this subject in experimental conditions but less is known about field conditions.

Then I considered blood gas parameters since they are supposed to change during altered rumen conditions because of the altered situation of VFA in the rumen and increased absorption of them in the systemic circulation.

Urine pH and faeces pH

Urine pH and faeces pH show similar trend in herd class; the differences between the three groups are statistically significant especially for group B (risk group) that is lower then group A (normal) and C (SARA). The two way ANOVA is again very similar for urine and faeces pH and emphasize the significativity of normal group (group A) compared to the other two, especially if we consider class DIM2 (cows with more than 60 days in milk) where the difference of group B and C from group A is statistically significant. Even if there is not statistically significant interaction between herd class and DIM class (P=0.083), more studied could probably be done within the group of cows with more than 60 DIM because in this case the statistic difference is good (P<0,001) and could be interesting to understand if this condition is directly related to rumen pH class belonging. However it is important to note that this parameters do not have a regular trend and the differences are so limited that is very difficult to use them as prognostic for rumen situation; moreover if urine pH is used in the

logistic regression model it results not relevant to determine the probability for a herd to be classified in A, B or C group. Literature confirms that fecal pH is normally not related to ruminal pH (Enemark et al., 2004) unless large amount of starch by-pass the rumen and result in hindgut fermentation. In SARA cases, the faeces are bright, yellowish, have a sweet-sour smell (Kleen et al., 2003), appear foamy with gas bubbles and contain more than normal amounts of indigested fiber or grain; intermittent diarrhea and the presence of indigested particle indicates inadequate digestion and fast passage of feed (Enemark, 2008) but this is not enough to diagnose SARA.

Acute Phase Response

It was very interesting to analyze the acute phase response of cows classified in the three different herd groups. Many different parameters were considered to define the presence of an acute phase response. Data resulted within the normal ranges with the exception of haptoglobin, total proteins and globulins (which were generally above the physiological level). We have seen that WBC in herd classification increased only in group B; in the two way ANOVA this parameters increased with a linear trend if we consider only the class DIM2 (days in milk >60) and there is a statistically significant difference between normal and SARA group. Probably the WBC count in the class DIM1 (days in milk <60) is influenced by a calving effect, since we know that WBC count is significantly high at parturition (Meglia et al., 2001) but in DIM2 the WBC count could be related to SARA status (even if it is not statistically significant).

At the same time lymphocytes increased with linear trend in herd class but they are almost constant in class DIM1 (with two way ANOVA) while they linearly increase in class DIM2. We can refer again to Meglia et al. (2001) work were it was demonstrated that lymphocytes did not differ significantly at calving but they were almost constant or lightly decreased. Cows with more than 60 days in milk show an increasing trend from class A to C but levels are lower than the normal range or just at the lower limit; we can say that all cows have a mild lymphopenia that is typically associated to a stress leukogram. In the logistic regression model that use herd class as variable, lymphocytes results an important parameter to determine the probability for a herd to pass from class A to class B or C.

Neutrophiles are also important parameters to verify acute phase status but they shown little variation and the trend is not regular in herd class; there in not statistically significant interaction between herd class and DIM class (P=0,144) but a linear trend is seen from group A to group C if we consider only DIM2 class: this light neutrophilia could be attributed to an

inflammatory leukogram but also to a stress leukogram since we can observe at the same time an alteration in neutrophils/lymphocytes ratio.

Haptoglobin (Hp), that is one major acute phase protein in cows, appear increased in class A compared to the other two classes. It is important to underline that in SARA group (group C) there is the great majority of cows with elevated DIM, that means that we could probably assist to a physiological-induced increase since haptoglobin has been cited as calving-inducted protein. The two way ANOVA (herd class x DIM class) shown an increase of Hp in group B if we consider DIM2 class, but if we consider DIM1 class we can see an increase in normal group (group A) but also in SARA group (even if more moderate). This situation is very different from what is described in literature probably because in experimental condition, grain-induced SARA determine a defined inflammatory status more comparable to an acute/inflammatory situation while in field condition it is very different. In normal group we can suppose again a calving effect on cows in early lactation (DIM<60).

Serum Amyloid A increased in group B (risk) but there is a light increase also in SARA group (group C) in herd classification. This situation could be explained with results obtained by Emmanuel et al. (2008): in his trial LPS in the rumen reached peak on day 10 and then declined, moreover we know that SAA is marker of acute response. The role of SAA in the organism is not still well known; many roles have been described so far: endotoxins detossification, inhibition of lymphocytes and endothelial cell proliferation, inhibition of platelet aggregation, inhibition of T-lymphocytes adhesion to extracellular proteins; it is implicated in chemotactic functions for inflammatory cells in local reaction; it has a role of up-regulation in the inflammatory process and it is secreted also by cells of intestinal epithelium. SAA and Hp increase in peripheral blood has been demonstrated in several works. Abrupt SARA induction in heifers and SARA induced by gradual adaptation from a forage based diet to a concentrate diet was associated to increase of these APP in many trials but other authors indicated that while grain-induced SARA has reportedly increased APP concentration in blood, SARA induced by reducing fibre particle size did not (Mulligan and Doherty, 2008) and this second situation is probably more similar to field situation in North Italy. Using a two way ANOVA analysis we can see that SAA is constantly low if DIM<60, except for a light increase in group C, but markedly increase in group B if we consider DIM>60. It is well known that glucocorticoids generally enhance the stimulatory effects of cytokines on the production of acute-phase proteins, whereas insulin decreases their effect on the production of some acute phase proteins: this must be considered since we found a linear

trend for glucose which increased from class A to B and then to C class, and also because we suspect a stress condition in cows affected by SARA.

Pearson correlations were calculated between SAA, haptoglobin, albumins and neutrophils/lymphocites ratio within the different groups. At first correlations between the three groups A (normal herds), B (herds at risk) and C (herds with SARA) were calculated and any statistically significant correlation were found. Then Pearson correlations were calculated within each group ulteriorly divided into DIM classes, less than 60 days in milk and more than 60 days in milk.

In normal groups we did not found any correlation between SAA and Hp for DIM1 but in this group there is a mild negative correlation between Hp and albumin ($P<0,001$; $R^2 = -0,58$). Since in this group the haptoglobin is very high we could suppose a calving effect because, as I previously mentioned, this acute phase protein has been associated to peripartum period. In group DIM2 the correlation is high but both levels of SAA and Hp are within or near normal levels.

In risk groups we found a high correlation between SAA and Hp which are both high in DIM>60 group; in this group we found also a high positive correlation between Hp and neutrophils/lymphocites ratio ($P<0,001$; $R^2 = 0,928$). We can state that there is an inflammatory status in these cows but it is hard to link it to risk to develop SARA since in risk group with DIM<60 there is not any correlation and levels are low and the increased neutrophil/lymphocites ratio, which is present also in DIM1 group, lead to suppose a stress situation more then an inflammatory one. This could be confirmed by high APP levels since we know that acute phase proteins could be released also for stress: it is widely accepted that, in humans and experimental animals, physical and psychological stress elevates plasma IL-6 and APP levels (Deak et al., 1997; Nukina et al., 2001). There is also evidence in cattle that physical stress can induce APP (Murata and Miyamoto, 1993; Alsemgeest et al., 1995; Lomborg et al., 2008). Activation of the hypothalamic–pituitary–adrenal (HPA) axis by stress signals may be a trigger of systemic or local (intra-pituitary) cytokine production, thereby augmenting hepatic APP synthesis and release into the bloodstream (Murata, 2004).

In SARA groups we can see a high positive correlation between SAA and Hp ($P<0,0001$; $R^2 = 0,918$) and also a mild correlation between SAA and albumin ($P=0,01$; $R^2 = -0,558$) for DIM1 (less then 60 DIM) group: this situation is clearly related to an inflammatory status and could be related to SARA condition but in group DIM2 the correlation between SAA and Hp is strongly lower ($P<0,01$; $R^2 = 0,562$) and there is not correlation between SAA and albumin and Hp and albumin.

Acute phase response discussion:

The situation in field is very different from what Gozho and other described inducing SARA with barley grain and from studies in which LPS were infused directly regardless of sequelae before they are supposed to translocate in portal vein. It is not still clear by which mechanism LPS, when it passed ruminal barrier, can pass portal circulation and hepatic barrier and stimulate an APR; LPS can be detossified by the liver before reaching general circulation (Andersen, 2000) but many receptor for cytochines are present in kupfer cells (Bode e Heinrich, 2001) and then the first proinflammatory citochines could be released before detossification start.

MCV and RBC increased in risk and SARA group; also HGB, HCT increased in these classes: this is against an anemic status hypothesised by many authors during SARA (considered as a chronic inflammation) but they could be relative increases caused by hemoconcentration/dehydration that is also confirmed by increase in total protein and increase of globulins and albumine at the same time.

Total proteins functions are to maintain oncotic pressure and buffer; blood proteins decrease in lactation (we can see lower levels in DIM>60) and this lead to consider less important alteration in DIM1 class (days in milk <60) where the trend is not regular.

Since also albumin increased (and it's a negative APP) we can start to exclude an inflammatory status. The trend seen in herd class is confirmed also by the two way ANOVA (herd class x DIM class) and there is not statistically significant differences (normality test is positive with P=0,076). It is therefore interesting to note that albumin is negatively correlated to the probability for a herd to pass from normal class to risk or SARA class applying a logistic regression model. This means that herd with low albumin levels have a high probability to experience SARA but we do not know if this albumin condition is the consequence of an inflammatory status determined by SARA or if a subclinical infection or a stressor or other external disturbs determined albumin decrease and SARA developed as consequence of an altered behavior or an altered gastro-intestinal motility or disturbed VFA absorption.

If there is hemoconcentration, this could be also the cause of the leukocytosis (and not only an inflammatory status). Neutrophyles and lymphocytes increase in SARA group and this could contribute to increase WBC as well but there isn't a linear trend and it isn't statistically significant; moreover normal ratio Neu/Lym (1/2) is varied towards neutrophyles increase in every class, could we say that it is stress leukogram caused by cortisol? Hypotalam-hypophisis axis can modulate APP synthesis: glicocoticoids decrease pro-inflammatory

citokines functions and positively influence anti-inflammatory citokines (H.Murata et al., 2004). Clinical signs attributed to SARA become manifest after a certain delay to the initial insult: we don't know if herds that we diagnosed with SARA are having problems afterwards and if normal herds have had problems before, then it is difficult to relate APP variations to other clinical signs or more difficult to subclinical signs. We can then confirm that SARA must be considered as a group-disease (see the difference in the distribution of animals in herd class and bovine class) since individual variations can disturb the diagnosis and it is necessary to exclude individual effect using a statistically significant number of animals.

Blood gas analysis, vitamin B12 and homocysteine

Blood acidosis can results from either excessive production or insufficient removal of acid. With metabolic abnormalities, excess acid production or absorption decrease pH and bicarbonate in body fluids due to accumulation of acids or loss of fixed bases from the body. A base-excess normally is present in blood, but an acid load can decrease this base-excess and can overcome the buffering capacity of bicarbonate. Acids of concern include those absorbed from the digestive tract plus L-lactate produced by muscular activity. Acid absorption or production is of concern only at the site of absorption or when acids accumulate (i.e., when rate of entry exceeds the rate of metabolism). Among the VFA, only acetate normally reaches the peripheral blood stream; much of the butyrate is converted to beta-hydroxybutyrate during absorption through the rumen wall and all of the propionate is converted to glucose by the liver. Presumably, VFA should not accumulate in blood plasma at sufficient concentrations to depress blood pH, but exactly how blood VFA concentrations change under acidotic conditions has not been determined. However, metabolism of the ruminal wall and the liver may be compromised during acidosis. Further complicating the situation, the liver is faced with L-lactate from tissue metabolism plus the D- and L-lactate absorbed from the digestive tract. Indeed, when ruminal glucose concentrations are high, as seen with acidosis, glucose being absorbed is partially converted to L-lactate by the digestive tract (Seal and Parker, 1994); if excessive, capacity of the liver to catabolize lactate may be overloaded (Naylor et al., 1984). Individual animals with a larger (wheat pasture cattle) or more adapted liver have greater capacity for metabolizing lactate and thereby may be less likely to experience blood acidosis (Owens, 1998).

It is important to underline that in normal conditions VFA do not accumulate at sufficient concentration in the rumen to reduce pH drastically; however when the rate of acid production exceeds the rate of acid absorption, due either to rapid production, inhibited absorption or reduced dilution, VFA accumulate to higher concentrations. Usually lactate is supposed to be

the cause of ruminal pH drop (because of altered balance between “lactate producer” and “lactate users” microbes) but in some studies ruminal pH falls below 5.0 even without lactate being present. In our study VFA concentration in the rumen was characterized by total rumen VFA concentration increased in herd that were experiencing SARA but not related to lactic acid while they were related to propionate and acetate concentration (that were statistically significant) and valerate concentration (these data are not shown in this work).

Our data suggest that cows attended by SARA probably don't have any problem in acid-base equilibrium because the compensative mechanism explained is sufficient to maintain BE thanks to kidneys compensation (that could lead to have acidic urine); this explain why BEecf, pH and bicarbonate are similar in the three classes and there is not statistically significant differences. But SARA condition could be related with some problems in blood oxygenation status: we can see statistically significant differences on oxygenation parameters and this probably occur when lung can not compensate perfectly and a respiratory acidosis (compensatory and transitory) can be instaured.

However, applying a logistic regression model, blood pH appear among significant data to determine the probability for a herd to pass from healthy status (group A) to the other two classes (B and C).

Vitamin B12 and homocysteine data look different from expected levels, since we supposed to see a decrease of vitamin B12 levels from normal (A) to risk (B) and SARA (C) groups related to an increase of homocysteine levels. Vitamin B12 levels are higher in risk and SARA groups (B and C) and homocysteine levels are higher in normal group and we found a statistically significant difference between this group and group B (risk). It is interesting that if we consider vitamin B12 in a logistic regression model using individual bovine classification (bovine class), this parameter becomes significant to determine the probability for a cow to pass from normal class to risk or SARA class. This parameter is then probably more related to individual variations and should be considered for individual and not for herd diagnosis.

Conclusions

Our group has a service for practicioner that consist on advisory in case of suspected SARA in a farm. This means that we go in a farm when the vet or the nutritionist call us and we collect ruminal fluid (with rumenocentesis) and blood (from jugular vein) from 12 cows between 5 and 100 DIM average, anamnestic and epidemiologic data, production data, ration composition and data about management to define a diagnosis relative to SARA and say if the herd has SARA, if it is at risk to develop SARA or if there isn't SARA (according to Nordlund and Garret classification). This diagnosis is possible just with rumen pH (that is measured immediately with a portable pHmeter) but all the other data are necessary to understand the global situation and discuss with the farmer, the vet and the nutritionist about the herd problem (since it is well known that very often SARA is linked to management and ration preparation even if the chemical composition is correct).

With these data (during these years we have collected samples from almost 50 farms and approximately 500 cows) we have an idea about epidemiological situation in the North of Italy (Morgante et al., 2008), but we have never found any important and significative relationship between SARA and blood parameters (metabolic profile or gas-analysis) and SARA and other symptoms or alterations.

In this specific project we collected also urine and feces and we measured also APP: the absence of strong correlation between SARA and APR made us think that SARA as described in the literature is not really SARA that we find in field. All our data are relevant to field situation, we never induced SARA experimentally. Probably SARA exists in field more than we think but we still don't know what really is SARA and I dare to say that I am quite sure that SARA is different from what we have described so far in experimental situation.

According to our data we can not say that there is a direct relationship between SARA and metabolism; I think that there could be a secondary effect, but we don’t know how. Some intermediate products like homocysteine or other toxic products like valerate could affect general metabolism but this subject must be investigated more. Probably we could hypothesize that at the base of SARA onset in our region there is a gastro-intestinal motility problem but this is very difficult to evaluate because of practice limitations in stalls and bovine anatomy. It probably should be interesting to evaluate motility during the hole day and not at a precise moment, because the problem should be an alteration in circadian rhythm of gastro-intestinal motility.

About valerate: our data show that this VFA is present in bigger quantities (compared with physiological situation) in the rumen fluid when SARA is diagnosed but since we do not know metabolic pathway of this VFA we can't say if this could affect metabolism. Valerate could also be involved in gastro-intestinal motility. Some authors investigated the influence of the composition of cecal contents on cecal motility. Svendsen and Kristensen surgically created a cecal fistula in 3 Jersey cows and evaluated the effect of various feeding regimens (high concentrate vs high roughage) on cecal motility: addition of concentrate to the diet resulted in a significant decrease in the number of cecal contractions and pH of cecal contents compared with feeding only hay. In another study researchers infused acetic, propionic and butyric acid into the cecum of fistulated sheep and recorded cecal motility: force of cecal contraction was significantly decreased when 10 mMol butyric acid was administered compared with 10 mMol acetic acid. Some authors reported a significantly higher concentration of butyric and valerianic acids in digesta samples from the cecum and proximal loop of the ascending colon of cows with cecal dilatation-dislocation compared with concentration in healthy control cows. Other authors tried then to demonstrate that this VFA would reduce contractility of specimens from the cecum and colon after challenge with CH but results were not positive because they indicated that these VFA did not affect contractility of ex-vivo specimens via muscarinic antagonism. However inhibition of signal conduction by VFA via mediation by other transmitters of the enteric nervous system cannot be excluded on the basis of that study. In fact other reports (Huizinga et al., 1997; Briejer et al., 1999) provide evidence of an intermediary role of the interstitial cells of Cajal in neuro-transmission; these cells or a mechanism of signal conduction that is not mediated by muscarinic receptors at the smooth muscle membrane might be involved in motility disorders of the gastrointestinal tract. More investigation might be done to more precisely characterize the role of butyric and valerianic acids as possible etiopathogenetic factors of altered gastro-intestinal motility in cows (Alleman et al., 1999). Some studies with sheep and steers showed metabolic interactions between butyrate and valerate, indicating that these acids compete for the same metabolic pathways in the ruminal epithelium (Kristensen et al., 2000; Kristensen and Harmon, 2004). If butyrate and valerate are truly competing in the ruminal epithelium, increasing absorption rates of valerate will affect butyrate metabolism. It was hypothesized that the metabolism of valerate, caproate, and heptanoate interacts with butyrate and thereby affects splanchnic metabolism in ruminants both indirectly and directly: results of study from Kristensen and Harmon (2005) indicated that the relative importance of the volatile fatty acids with a long chain length compared with their ruminal concentration is caused by their higher

content of carbon per mole of acid and by their high fractional ruminal absorption rates, which implies that valerate, caproate, and heptanoate might be important for interpreting ruminal volatile fatty acid data and modeling ruminant metabolism (Kristensen and Harmon, 2005). Even if VFA absorption form the rumen epithelium is regulated by passive mechanism, could we suppose that valerate accumulation depend on an alteration of absorption functions? Is this accumulation a cause or a consequence of SARA status? Could valerate accumulation cause an alteration in gastrointestinal motility and splancnic metabolism? Could we suppose that SARA is enhanced by a decrease in VFA absorption and not by an increase of VFA production? Trials made in vivo about this subject could be very interesting.

I think that in field condition there is not an inflammatory response clearly related to SARA and what is described in the literature (see Gozho, Emmanuel and other) is not SARA but an experimental situation more "acute".

Since very often we found more nervous cows in herds with SARA and considering that we found alteration in blood parameters (leukocytosis with stress-like leukogram that included altered neutrophils/lymphocytes ratio, albumin alteration, some alteration in APP that could be related to stress as well), I think that this fermentative/metabolic problem could be related to a general unhealthy status and welfare decrease. It would be interesting to investigate more on causes and effects of SARA to understand if this fermentative disturbs is the consequence of stressful situations: neuroendocrine conditions, management and environment could be related to SARA since several neuropeptides control voluntary feed intake and their effect may result in sorting out feeds from the diet. Moreover, if the main cause to develop SARA is truly linked to welfare, we can easily connect SARA to structures and management that could determine lameness or other problems and explain many related pathologies that probably are not caused by SARA but they are the causes. In effect in our studies we often saw that SARA is a management-related problems: free stall overcrowding, inadequately bedded or otherwise uncomfortable stalls and excessive parlor holding times may also alter feed intake patterns and animal feeding behavior while the ration is very similar in a “chemical point of view”.

The fact that parameters found with logistic regression model for herd class are completely cancelled if we introduce the DIM class (a days in milk classification), confirm that SARA as it is in our region is not only related to early lactation period and confirm the important role of management in SARA onset. This statement leads to confirm that SARA must be considered as a herd problem and even if the transition period is the most delicate in the cow’s carrier SARA must be considered in every lactation stage and related not only to feeding

management (fiber content –peNDF content-, steaming up, feed manipulation and ration preparation) but to the equilibrium between the animals and the environment (general herd management, structures and welfare of cows).

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