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Blood and milk immune and inflammatory profiles in periparturient dairy cows showing a different liver activity index

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This paper reports the results of a study that aimed to assess whether liver functionality defined by liver activity index (LAI) is associated with inflammatory and immune parameters in blood and milk. LAI is an index including the average blood levels of albumin, lipoproteins and retinol-binding protein measured three times in the first month of lactation (at 5, 15 and 30 days in milk). The aim was to assess the relationship of this index with blood and udder immune and inflammatory status as a means of identifying as early as possible cows at risk of disease. The research was carried out using 10 multiparous Italian-Friesian dairy cows of average genetic merit. Cows were retrospectively ranked in three groups according the LAI level. Blood samplings were performed at different intervals before and after calving; quarter milk samples were taken only after calving with the same schedule as blood samples. Leucocytes, oxidative burst, blood lysozyme and N-acetyl-β-D-glucosaminidase (NAGase) curves showed large overlapping among the three LAI group curves during the follow-up period. Four blood (complement, sialic acid, haptoglobin and reactive oxygen metabolites) and three milk (somatic cell count, lysozyme and NAGase) parameters showed larger and more consistent differences among LAI groups. Complement showed higher values and sialic acid showed lower values in high LAI group when compared with the other two LAI groups. Two other markers of inflammatory status (haptoglobin and reactive oxygen metabolites) showed the lowest values in high LAI cows. A consistent and significant reduction of milk NAGase and milk lysozyme in high LAI group was observed. The results suggest that cows with the highest liver functionality index have also the highest levels of some immune markers and the lowest levels for inflammatory markers at blood (already before calving) and mammary levels. Finally, cows with low LAI index, being more susceptible to metabolic and infectious diseases, should be carefully monitored to identify as early as possible the development of a disease.

Keywords: Cow, periparturient period, liver activity, blood, milk, immunity, inflammation

The inflammatory process has received growing attention in the last decades for its implications with regard to some dangerous human health disorders such as cardiovascular disease, cancer, obesity and diabetes (Hotamisligil, 2006). Inflammation is also very harmful in farm animals and it can occur for several reasons (Bertoni et al. 2008): the immune response to bacteria, viruses, fungi or parasites; the response to tissue damage (e.g. trauma, injuries, burns, digestive bacteria endotoxins) and to oxidative stress (Gruys et al. 1999), which could reiterate the process (Rimbach et al. 2002).

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The interest in these aspects of inflammation continues to increase because not only can it be more dangerous than useful, but it can also reiterate itself, reducing the productive and reproductive efficiency of the cow as reviewed by Ingvartsen et al. (2003). Moreover, dairy cows are often susceptible, at calving time, to inflammations (Cappa et al. 1989; Sordillo et al. 1995; Bionaz et al. 2007). Pro-inflammatory cytokines—the main mediators of inflammation—trigger a sequence of events involving several organs and tissues, consequences of which are several endocrine and metabolic changes. Between them, they are responsible for a peculiar phenomenon at the liver level: e.g. a deviation of synthesis with an increase of many positive acute-phase proteins (APP: i.e. haptoglobin,

Table 1. Main characteristics of dairy cows utilized in the research, grouped according to liver activity index (LAI)

Cow	LAI group†	Parity, N°	Milk yield previous lactation, t/305 d	Length of dry period, d	Somatic cell count, linear score‡	Body Weight, kg§	BCS§
1	LO	4	10·50	70	5·63	732	2·10
2		3	12·11	48	3·98	785	2·50
3		2	10·66	71	4·29	764	2·80
	Mean ± SD	3·0 ± 0·8	11·1 ± 0·7	63·0 ± 10·6	4·63 ± 0·72	760·3 ± 21·8	2·47 ± 0·29
4	ME	5	13·24	47	0·88	790	2·50
5		2	11·55	52	2·26	909	2·80
6		2	13·60	50	††	772	2·55
7		2	10·80	63	NA	706	2·60
	Mean ± SD	2·8 ± 1·3	12·3 ± 1·2	53·0 ± 6·0	1·57 ± 0·69	794·3 ± 73·3	2·61 ± 0·11
8	HI	2	12·69	52	2·29	631	2·20
9		2	10·96	53	3·14	781	2·90
10		2	10·02	51	3·65	940	2·95
	Mean ± SD	2·0 ± 0·0	11·2 ± 1·1	52·0 ± 0·8	3·03 ± 0·56	784·0 ± 126·2	2·68 ± 0·34

† LO = low LAI index; ME = medium LAI index; HI = high LAI index
‡ Average of last 2 weeks before dry off
§ Body condition score at 30 d before calving
†† NA = Not available

ceruloplasmin, serum amyloid A etc.) and in turn a reduction of the negative APP, including apolipoproteins (Fleck, 1988). These changes do not have important effects on liver cell integrity, as demonstrated by the mild variations of liver enzymes (Bertoni et al. 2008). Changes of APP parameters have been utilized to monitor the inflammatory consequences on the liver functionality. An aggregated index (liver activity index or LAI) of three parameters (albumins, lipoproteins as total cholesterol and retinol-binding protein as vitamin A) in the first month of lactation has been set up by Trevisi et al. (2001). The cows with low values of LAI (e.g. high inflammatory status) have shown a lower dry matter intake (Trevisi et al. 2002), a lower energy efficiency (Trevisi et al. 2007) and a lower milk yield and fertility (Bertoni et al. 2008).

The importance of increasing our knowledge of cow homeostatic capacity and of the relationships between immune system parameters, oxidative stress, and mammary gland defence capacity, has been emphasized (Ingvartsen et al. 2003). Within this framework, this paper aimed to explore some of these relations and to assess whether liver functionality defined by the LAI index (Trevisi et al. 2001; Bertoni et al. 2008) could be correlated with blood and udder immune and inflammatory status.

Materials and Methods

Animals, management and diet

The research was carried out using 10 multiparous (2·6 ± 1·0 parity) Italian-Friesian dairy cows, of average genetic merit, at the Università Cattolica del S. Cuore. Cows were housed in artificially lighted and ventilated tie-stalls. This barn was maintained under constant climatic conditions: ~20 °C, 60–70% relative humidity, 14 h of light and 10 h of dark (light: 6·00–20·00). Main cow characteristics are described in Table 1.

The feed-diet characteristics were almost constant during the trial. Cows were individually fed ad libitum with forages and concentrate. On average, dry cows received daily 9–12 kg of grass hay, 8–10 kg of maize silage and 1–2 kg of concentrate. After calving, the daily diet included 2 kg of grass hay and 3 kg of alfalfa, while concentrate and maize silage were gradually increased (the latter to 22 kg). Forages were offered in two equal meals at 7·30 and 19·30, while concentrate was distributed by an autofeeder in equal meals: two in the dry period (10·30 and 22 : 30) and eight in the lactating period (one every 3 h).

All cows were treated at drying-off with a dry-cow intramammary preparation (penethamate hydroiodide, bentamin penicillin, framycetin sulphate) and intramuscular treatments with tylosin. Milking procedures included cleaning of the teat with a clorexidine-moistened paper towel, forestripping and milking cluster attachment.

Health status

Occurrence of all health problems (e.g. retained placenta, metritis, ketosis, mastitis), drug treatments, and outcomes of two gynaecological visits were accurately recorded for each cow throughout the entire experimental period. In addition, rectal temperature was measured every morning at feeding time. Herd manager, milkers and practitioner were responsible for clinical disease monitoring.

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Sampling procedure and sample preparation

Blood sampling was carried out before feed distribution in the morning. Samplings were performed at 30, 15, 10, 5, 2 d (±1 d) prior to calving, at calving and then at 2, 5, 10, 15, 25, 30 d after calving (±1 d); quarter milk samples (QMS) were taken only after calving with the same schedule as blood samples and immediately delivered to the laboratory in a refrigerated box.

Li-heparin and K3-EDTA tubes were immediately cooled in an ice-water bath, then Li-heparin tubes were centrifuged at 3520 ***g*** for 16 min; plasma samples were divided into five aliquots stored at −20 °C until further analyses. Serum for lysozyme and N-acetyl-β-glucosaminidase (NAGase) analyses was obtained from centrifugation of blood at 3520 ***g*** at room temperature for 16 min, then aliquoted into two 500-µl tubes, and immediately frozen at −80 °C for the enzyme analyses. Whey was obtained from skimmed milk by centrifugation at 60 000 ***g*** at 4 °C for 30 min then stored and processed as described for serum.

Polymorphonuclear lymphocytes (PMN) were isolated from blood by blood hypotonic lysis (Piccinini et al. 2004, 2005).

Leucocyte counts

Blood samples were analysed by the means of a haemocytometer with specific settings for bovine blood (MS4, Melet Schloesing Laboratories, France).

Biochemical assays

The parameters on plasma were analysed at 37 °C using a clinical auto-analyzer (ILAB 600, Instrumentation Laboratory, Lexington MA, USA) according to methods previously described by Bionaz et al. (2007) and Bertoni et al. (2008). Assays included liver tests: functional (total bilirubin) and biochemical [aspartate aminotransferase (GOT/AST), γ-glutamyl-transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP)]; inflammatory response tests: haptoglobin, reactive oxygen metabolites (ROM), thiol groups, total antioxidant activity; immunological tests: globulins, haemolytic complement, sialic acid and other tests: albumin, cholesterol as lipoprotein index, paraoxonase, vitamins A and E as an index of the corresponding carrier proteins. Sialic acid was determined by a colorimetric assay kit purchased from Roche (Roche Diagnostics GmbH, Penzberg, Germany), adapting it to the ILAB 600 conditions, while the haemolytic complement was determined using the analytical method described by Barta & Barta (1993).

In addition to the previous markers, lysozyme was assessed in duplicate by the procedure described by Metcalf et al. (1986) with slight modifications (Piccinini et al. 2004, 2005) and NAGase was assessed in duplicate by the procedure described by Kitchen et al. (1978) with slight modifications (Piccinini et al. 2004, 2005).

Respiratory burst (RB) was assessed by luminol-enhanced chemiluminescence as previously described (Piccinini et al. 2004, 2005).

Milk bacteriological analysis

At the laboratory, an aliquot (0·01 ml) of each QMS was spread on blood agar plate. Colonies were isolated and identified by official methods according to National Mastitis Council (NMC, 1999). Somatic cell counts (SCC) were performed on a Bentley Somacount 150 (Bentley Instruments, Chaska MN, USA).

Data handling

Data were retrospectively grouped into three classes on the basis of LAI as described elsewhere (Trevisi et al. 2001; Bertoni et al. 2008): low (LO, cows with the lowest values of LAI, $n=3$), medium (ME, cows with intermediate values of LAI, $n=4$) and high (HI, cows with the highest values of LAI, $n=3$). This index includes the average blood levels (5, 15 and 30 days in milk) of albumin, lipoproteins (indirectly measured as total cholesterol) and retinol-binding protein (RBP, indirectly measured as vitamin A) (Bertoni et al. 2008).

Statistical analysis

To assess the pattern of the different blood and milk components during the periparturient period by different LAI groups, data were analysed using the MIXED procedure of SAS 9.1 (SAS Institute, Cary NC, USA). To verify the study hypothesis a relatively simple model was applied which included the interaction between time from calving (days in milk, DIM) and LAI groups (LAIg) and the random effects accounting for variance within cows. Effects of repeated samples were accounted for by choosing the appropriate covariance structure by Akaike's information criteria.

Results

Data description

Overall 275 blood samples and 208 QMS were considered. None of the cows showed signs of any clinical diseases, mastitis included. Table 2 reports the distribution of bacteriological results obtained by culturing QMS in the three groups of cows considered. These analyses showed that overall udder health was good. Indeed, major pathogens (environmental Streptococci) were isolated in few quarters (1·5–2·4%), while nearly 50% of the quarters were infected with coagulase-negative Staphylococci. They are considered opportunistic pathogens, and they

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Table 2. Distribution of bacteriological results from 208 quarter milk samples by the groups as defined by liver activity index (LAI)

Result	Low	Medium	High
Negative	48·5	50·1	51·7
Coagulase-negative Staphylococci	50·0	47·5	46·7
Environmental Streptococci	1·5	2·4	1·7

are not generally associated with a significant increase of clinical and subclinical mastitis frequency. A large set of markers has been assessed as reported in the Material and Methods section, but only some of them showed to be significantly influenced ($\alpha=0{\cdot}05$) by time from calving and LAI classification (blood leucocyte counts and oxidative burst, blood lysozyme, NAGase, complement, sialic acid, haptoglobin and ROM) (Table 3). Similarly, when milk samples were considered (Table 4), the interaction DIM and LAIg was always statistically significant for all the markers included in the table.

Blood parameters

All the curves obtained by plotting time from calving and LAI groups were analysed. However, blood leucocyte counts and oxidative burst, blood lysozyme and NAGase curves showed large overlapping among the three LAI group curves. Therefore, they were not further considered. Instead, we focused on four blood parameters (complement, sialic acid, haptoglobin and ROM) and on three milk parameters (SCC, lysozyme and NAGase) that showed larger and more consistent differences among LAI groups during the follow-up period.

Cows classified in the high-LAI group (Fig. 1) showed consistently higher levels of complement activity, whilst in comparison the other two groups (low and medium) showed overall lower levels. Only around calving, the three groups showed values that largely overlapped. Complement values showed an increasing trend after calving in low and medium groups while in the high group the peak observed at 10 d after calving was followed by a decrease.

When sialic acid was considered (Fig. 1), we observed lower levels in the high-LAI group, while the other two groups showed similar higher mean values. In all the three groups mean values were lower before calving than after calving, with larger increases for low and medium LAI groups.

Levels of haptoglobin (Fig. 2) were very similar before calving in the three groups of cows; however, as calving came closer, some differences could be observed. Indeed, immediately after calving, there was a large increase in haptoglobin values both in low-and medium-LAI groups and only a slight increase in high-LAI group. At 10 DIM, a general decrease of haptoglobin values was observed, but only high LAI group achieved the pre-calving value by 20 DIM, while in the other two groups, values remained higher than pre-calving ones.

The pattern of ROM (Fig. 2) was very similar in the three groups of cows. However, the mean levels were clearly different during the whole follow-up period. Indeed, the high-LAI group showed the lowest levels, while the medium group showed the highest. In all three groups an increase could be observed moving towards calving, then values started to decrease in the high-LAI group, but not in the other experimental groups.

Milk parameters

Figure 3 shows the mean SCC values after calving in the three groups of cows. In all the groups the observed values were below 100 000 cells/ml (Log 5), in the first sample after calving. The pattern was similar in the three groups even though cows in the high-LAI group showed lower values overall.

A completely different pattern was observed when milk NAGase was considered (Fig. 3). Indeed, cows in the high-LAI group showed significantly lower levels of the enzyme in all samples. On the contrary, cows in the medium group showed significantly higher values of NAGase between 5 and 20 DIM.

Milk lysozyme (Fig. 3) was significantly lower in the high-LAI group, while values in the medium group were always the highest among the three groups of cows. The trend was increasing in medium-LAI group, while it was decreasing in the high-LAI group and erratic in the low-LAI group.

Discussion

The periparturient period is one of the most critical periods in the productive life of the dairy cow. It has been demonstrated that in this period some impairment of immune defences can be observed (Mallard et al. 1998; Piccinini et al. 2004). This impairment could increase the frequency of production and reproductive diseases (Ingvartsen et al. 2003). However, field studies showed that both blood and milk immune and inflammatory response during periparturient period are significantly influenced by herd factors (Piccinini et al. 2005). Namely, the role of nutrition and metabolism on immune system and inflammatory response is also widely accepted and, for some aspects, also demonstrated (Ingvartsen et al. 2003; Lacetera et al. 2005). If identifying accurate markers to define the immune and inflammatory status of the cow is still a matter of discussion, then the need for accurate markers to define the metabolic status of the cow is even more controversial. However, the availability of these markers will increase our capability to identify cows at risk and thus prevent further diseases (Ingvartsen et al. 2003; Whist et al. 2007) and/or metabolic deviations (Bertoni et al. 2008). This paper aimed to explore some of these relationships and to

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Table 3. Mean values (±SD) for temperature and blood immunological and inflammatory markers (see text for key to abbreviations) observed during follow-up period (±30 days from calving) in cows classified by liver activity index (LAI) (all the interactions between time from calving x LAI group were statistically significant at $\alpha=0{\cdot}05$)

	Low			Medium			High		
Parameter	Pre calving	Calving	Post Calving	Pre calving	Calving	Post Calving	Pre calving	Calving	Post Calving
Temperature, °C	38·70±0·30	38·75±0·28	38·99±0·40	38·9±0·2	38·9±0·5	38·8±0·3	38·9±0·2	38·8±0·3	38·9±0·2
LAI parameters									
Albumin, g/l	37·19±1·48	35·42±0·99	36·43±1·82	36·62±1·63	35·26±0·94	35·99±1·70	37·95±1·29	37·45±1·42	37·87±1·19
Vitamin A, µg/100 ml	30·55±6·57	18·48±4·88	26·93±9·72	34·09±7·54	19·16±4·94	25·67±8·66	36·93±7·49	22·77±6·15	33·26±8·88
Cholesterol, mmol/l	1·68±0·32	1·39±0·33	2·32±0·93	2·17±0·54	1·63±0·21	2·93±1·06	2·17±0·44	1·48±0·20	2·90±1·15
Blood cells									
Leucocytes, cells/µl	8·29±2·50	9·08±4·25	7·27±2·02	8·74±1·63	11·95±7·97	7·25±1·65	7·97±1·30	11·08±1·98	7·72±1·97
Polymorphonuclear lymphocytes, %	53·65±15·02	65·34±15·67	62·83±10·13	53·64±16·74	55·96±18·93	61·57±10·70	63·72±6·00	69·71±8·28	61·87±10·90
Monocytes, %	3·49±1·05	2·82±1·19	3·08±0·67	3·56±1·05	3·13±2·30	3·37±0·60	3·74±0·80	3·69±2·00	3·87±0·87
Lymphocytes, %	40·55±17·99	31·83±15·39	34·29±10·03	42·79±16·91	40·91±18·62	35·07±11·03	32·56±5·77	26·60±6·64	34·22±10·68
Platelets, cells/µl	224±100	261±163	367±142	196±84	249±102	412±190	256±105	253±84	474±244
Immune and inflammatory parameters									
Complement, haemolytic unit 50%/150 µl	17·90±3·66	19·47±4·05	25·02±3·08	19·89±3·68	19·02±5·62	21·41±5·71	25·08±6·68	23·08±4·19	29·49±7·69
SHp, µmol/l	314±20	355±62	374±44	300±42	312±83	334±72	297±37	330±46	362±90
Sialic acid, g/l	0·44±0·06	0·49±0·14	0·64±0·10	0·45±0·08	0·45±0·1	0·63±0·09	0·40±0·08	0·40±0·06	0·54±0·06
Haptoglobin, g/l	0·14±0·10	0·66±0·44	0·75±0·6	0·18±0·11	0·67±0·55	0·58±0·35	0·11±0·10	0·29±0·24	0·24±0·23
ROM, mg H_2O_2/100 ml	13·18±2·01	15·16±3·83	16·01±2·29	13·65±1·91	14·93±1·96	16·51±1·71	10·24±1·91	11·51±2·39	12·89±1·87
NAGase, Units	192±83	339±148	172±67	201±92	306±97	265±115	207±85	265±.164	198±73
Lysozyme, µg/ml	3·89±6·54	0·00±0·00	8·89±11·65	13·64±16·85	16·29±23·95	22·72±30·72	0·00±0·00	0·00±0·00	4·44±4·58
Oxidative burst, mV	465±471	248±210	209±181	746±941	127±83	278±169	295±150	110±104	330±254

Table 4. Mean values for milk immunological and inflammatory markers observed during follow-up period (±30 days from calving) in cows classified by liver activity index (LAI) (all the interactions between time from calving × LAI group were statistically significant at $\alpha = 0{\cdot}05$)

	Low		Medium		High	
Parameters	Mean	SD	Mean	SD	Mean	SD
Somatic cell count, $\log_{10}$/ml	4·37	1·16	4·24	1·04	4·16	0·97
N-acetyl-β-D-glucosaminidase, Units	31·68	23·12	42·82	37·98	8·14	5·95
Lysozyme, µg/ml	36·62	16·60	51·30	27·14	23·79	8·66

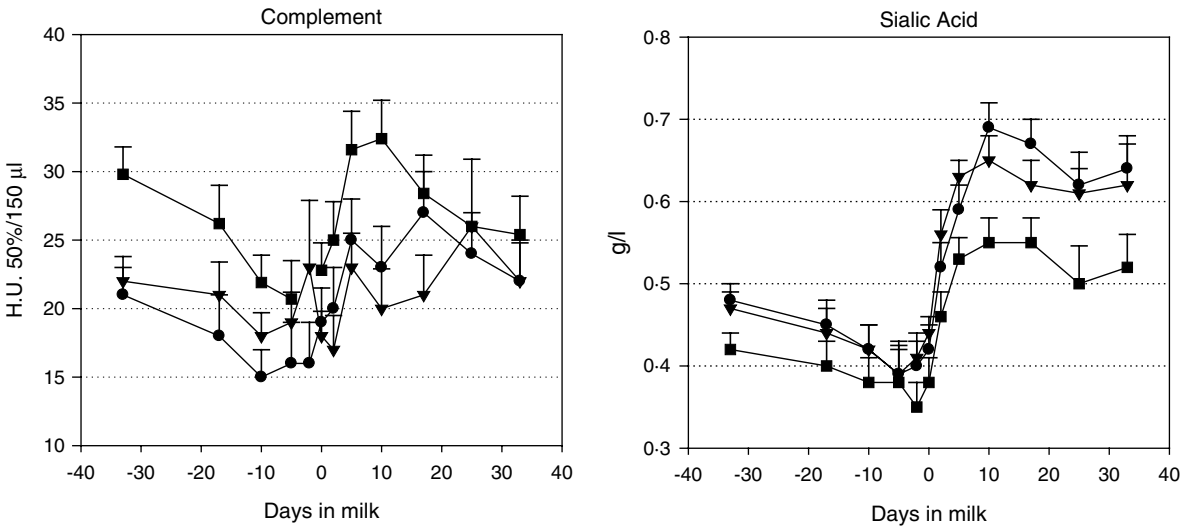

Fig. 1. Blood complement and sialic acid mean values (+ SE) observed during follow-up period (±30 d from calving) by liver activity index (LAI) classification (● low, ▼ medium, ■ high).

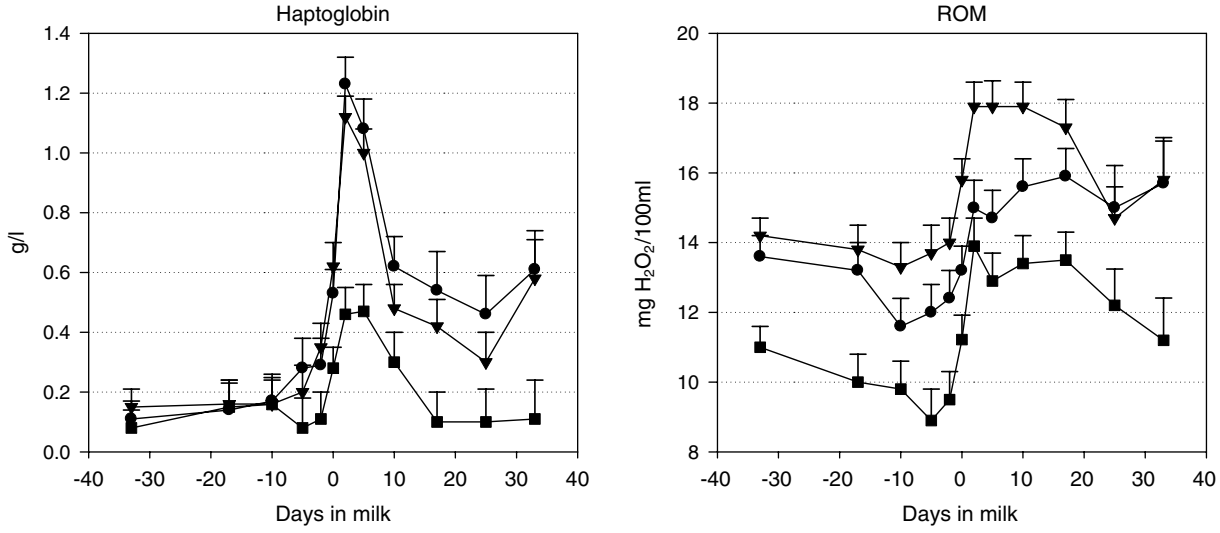

Fig. 2. Blood haptoglobin and reactive oxygen metabolites (ROM) mean values (+SE) observed during follow up period (±30 d from calving) by liver activity index (LAI) classification (● low, ▼ medium, ■ high).

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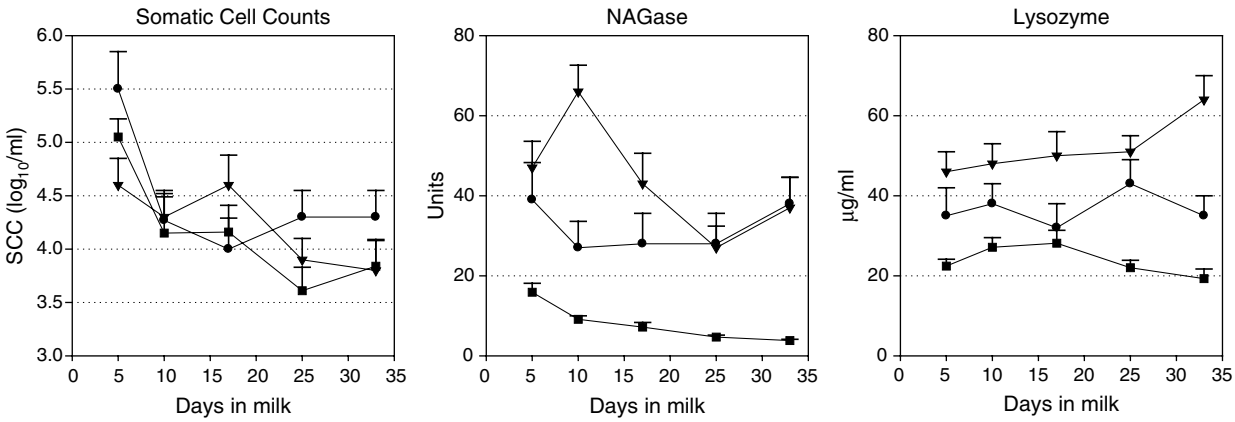

Fig. 3. Milk somatic cell counts, N-acetyl-β-D-glucosaminidase (NAGase)and lysozyme mean values (+SE) observed after calving by liver activity index (LAI) classification (● low, ▼ medium, ■ high).

assess whether liver functionality defined by the LAI index (as a metabolic index) could be correlated with blood and udder immune and inflammatory status.

This study was performed with a relatively small number of cows; however, very frequent samplings were performed to carefully identify the changes in the parameters considered. Cow characteristics are described in Table 1 and, as expected, some differences among cows can be seen. Indeed, high-LAI and medium-LAI groups included mainly younger cows. Since LAI index is related to cow liver activity, age-related differences were expected, and these data suggest that young cows could have a higher liver activity.

The present results confirm that blood changes observed around calving (±30 d from calving) significantly affect the pattern of several cellular and humoural immune (complement, lysozyme) and inflammatory markers (sialic acid, haptoglobin, ROM, SCC, NAGase). Moreover, these changes could be associated with the hepatic response to inflammatory stimuli, i.e. reduction of usual protein synthesis (and related indices), the so-called negative APP (Bertoni et al. 2008).

When two markers related to innate immunity (complement) and inflammation (sialic acid) were considered, we observed consistently higher values for haemolytic complement and lower values for sialic acid in the high-LAI group. Complement is considered as a primer for humoural immune response; being a natural adjuvant, it increases B-cell activation and promotes B-functions (Mastellos & Lambris, 2002). Therefore, theoretically, high-LAI cows were shown to be better protected against infections. Sialic acid is a part of the larger chemical family of glycans and it is generally considered as a marker for APP release. Its measurement is used to assess disease risk in humans, and its increase is associated with several diseases (Varki, 2008). The pattern observed in the high-LAI group for these two parameters suggests that cows in this group have higher immune capacity still before calving and, thus, lower inflammatory stimulus and consequently status. This is the desirable status, and the reduction of the immune capacity indicates that cows are more susceptible to infectious and metabolic diseases.

These observations are supported by the pattern of two other markers of inflammatory status (haptoglobin and ROM) which showed lower values in high-LAI cows when compared with the other two LAI groups.

Haptoglobin is an APP which increases several times during infection and inflammation around calving, also without clinical symptoms (Bionaz et al. 2007). It has been proposed as one of the most useful markers for acute inflammation in dairy cows (Humblet & Godeau, 2005; Aring et al. 2006; Bertoni et al. 2008). ROM are markers for reactive oxygen substances produced by cells and they are currently used in human medicine to assess the risk for many diseases, and their assessment is considered a useful tool to monitor oxidative stress (Conner & Grisham, 1996). The consistent ROM low levels during the follow-up period and the lower and short-lasting haptoglobin peak around calving suggest again that cows in the high-LAI group can cope more efficiently with pathogens or with tissue damage during the periparturient period, avoiding the consequences of an increase in oxidative stress on tissues.

It appears furthermore, worthwhile to underscore that previous better conditions of the high-LAI cows allows a very modest loss of usual liver function as suggested by albumin, lipoprotein and RBP levels (Bertoni et al. 2008).

The pattern observed for the blood parameters is reflected in milk parameters. Indeed, SCC was below 100 000 cells/ml in all groups, out of first sampling in the low-LAI group. More interesting is the observation of a consistent and significant reduction of NAGase and lysozyme in the high-LAI group when compared to the other two groups. The pattern of these enzymes in milk is different from the one observed in blood, confirming that the major source in milk is mammary gland epithelial cells (Piccinini et al. 2005, 2007). Moreover, a previous study

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showed that, after calving, such levels are highly correlated to a reduced frequency of mastitis (Piccinini et al. 2007).

Conclusions

The need for accurate and suitable markers to assess metabolic, immune and inflammatory status in dairy cows is recognized worldwide, but still a general consensus on the markers to be used has not been reached. The present study confirms that a liver functionality index could be related to the immune and inflammatory status both at blood and milk level. Indeed, cows with the highest liver functionality index have also the highest levels of some immune markers and the lowest levels for inflammatory markers already before calving. In this study we did not find any association between udder health and the LAI index, but this could be influenced by the low number of cows in the study. The next step will be to investigate the association between the LAI index and the immune and inflammatory markers with a large number of periparturient cows characterized as being healthy or having subclinical or clinical production diseases.

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