

Evaluation of blood and milk oxidative status during early *postpartum* of dairy cows

A. Rizzo¹, E. Ceci², M. Pantaleo¹, M. Mutinati¹, M. Spedicato¹, G. Minoia¹ and R. L. Sciorsci^{1†}

¹Department of Animal Production, Faculty of Veterinary Medicine, University of Bari, strada prov.le per Casamassima km3, 70010 Valenzano (BA), Italy;

²Department of Public Health and Zootechnic, Faculty of Veterinary Medicine, University of Bari, strada prov.le per Casamassima km3, 70010 Valenzano (BA), Italy

(Received 28 September 2011; Accepted 2 April 2012; First published online 8 June 2012)

In dairy cows, the intensity of metabolic activity, associated with the negative energy balance (NEBAL), is responsible for an increased production of reactive oxygen species (ROS) and, subsequently, for the development of the condition of oxidative stress, which may overwhelm the antioxidant potential of the bovine maternal organism, making it prone to the development of many puerperal dysfunctions, as well as to an alteration of colostrum and milk quality. Given these premises, the aims of this study are to evaluate serum and milk concentrations of ROS and lipoperoxides, vitamins A and E, on the 10th, 12th, 14th and 16th day postpartum of dairy cows, a particularly critical period during which the NEBAL reaches its nadir, and to compare the trends of these parameters in two different bovine breeds. The study was performed in pluriparous Italian Friesian and Brown dairy cows. On the 10th day postpartum, all cows underwent a clinical examination to exclude the presence of alterations; furthermore, on the same day, a milk sample was collected from each cow, in order to perform the somatic cell count (SCC; (CE) N. 853/2004) and to establish which of them had an SCC $\leq 400\,000/\text{ml}$ or $>400\,000/\text{ml}$. In this study, among the 110 cows that were initially selected, the evaluation of these parameters allowed the inclusion of 80 animals, which were divided into four groups of 20 subjects each: Group F and F1: Italian Friesian healthy cows, with SCC $\leq 400\,000/\text{ml}$ and $>400\,000/\text{ml}$, respectively; Group B and B1: Italian Brown healthy cows, with SCC $\leq 400\,000/\text{ml}$ and $>400\,000/\text{ml}$, respectively. On the 10th, 12th, 14th and 16th day postpartum, peripheral blood and milk samples were collected. The results obtained show that in group B1 there were higher concentrations of ROS and milk antioxidants compared with Friesian group cows. This datum let us suppose that even in the presence of higher ROS concentrations the antioxidant status found in group B1 seems to be able to counteract the oxidative damage, which is more likely to develop in these cows.

Keywords: dairy cow, *postpartum*, NEBAL, ROS, vitamins

Implications

This work investigates the physiological reactive oxygen species (ROS) and antioxidant concentrations in dairy cows between the 10th and the 16th day *postpartum* (peak of negative energy balance (NEBAL)) and highlights the importance of an adequate antioxidant system in dairy cows, particularly during NEBAL, in order to prevent and/or face the outcome of many *postpartum* pathologies and mastitis, which are easy to develop in such a critical period of the cow. The adequacy of an antioxidant support may thus reduce the economic losses and the prolongation of calving-first oestrus, calving-conception interval, related to the above-mentioned ROS-dependent alterations.

Introduction

The transition period is particularly critical to health, productivity and fertility of dairy cows (Leroy and Vanholder, 2008; Roche *et al.*, 2009). In the last months of gestation, the cow experiences a reduction in dry matter intake (DMI), which continues even after calving (Grummer *et al.*, 2004), whereas the late foetal growth, parturition and the onset of lactation imply a dramatic increase in energetic demands (Taylor *et al.*, 2003). As a consequence, the cow is unable to meet these energetic requirements (Taylor *et al.*, 2003) and this results in negative energy balance (NEBAL) that begins few days before calving and usually reaches its nadir about 2 weeks later (Butler and Smith, 1989; Bell, 1995). In such a forced metabolic condition, an increase in reactive oxygen species (ROS) generation may occur and, subsequently, oxidative stress may develop (Miller *et al.*, 1993; Mudron *et al.*, 1999; Bionaz *et al.*, 2007).

[†] E-mail: r.sciorsci@veterinaria.uniba.it

In physiologic conditions, living organisms have sophisticated antioxidant defence systems, both enzymatic and non-enzymatic, to counteract excessive ROS levels (Takata *et al.*, 2002; Locher *et al.*, 2011). Among non-enzymatic antioxidants, α -tocopherol (α -toc) and carotenoids need to be mentioned (Lindmark-Mansson and Akesson, 2000; Havemose *et al.*, 2004). α -Toc is the major out of eight components showing vitamin E activity and acts as a radical scavenger, protecting all phospholipid-containing membranes from peroxidation (Przybylska *et al.*, 2007). Bovine maternal blood concentrations of vitamin E decrease rapidly towards parturition mainly for the high accumulation of this vitamin in the colostrum (Goff *et al.*, 2002). β -Carotene is the main dietary precursor of vitamin A (retinol) in dairy cattle. This substance escapes rumen degradation and is metabolized in the intestinal mucosa to retinol and absorbed and transported to the liver with fat (Chew, 1987). This vitamin is also present in colostrum and milk at high levels (Calderón *et al.*, 2007) and is endowed with strong ROS-scavenging properties, which strengthen the resistance to infectious diseases, particularly mastitis (Przybylska *et al.*, 2007).

The increase in ROS generation likely to occur around calving may overwhelm the antioxidant potential of the bovine maternal organism, making it prone to the development of many ROS-related alterations (Rizzo *et al.*, 2007 and 2009) and infectious diseases such as mastitis (Malinowski and Gajewski, 2010). The leukocyte defences are depressed during the periparturient period. In particular, the neutrophil action could lead to inadequate clearance of the pathogens from the bovine udder (Contreras and Sordillo, 2011). Strategies designed to improve the immune cells of the diseased udder during immunosuppressive stages would greatly impact the ability of the animal to resist the pathogenic infection (Burvenich *et al.*, 2007; Spears and Weiss, 2008). Given these premises, the aims of this study are to evaluate ROS and antioxidant (vitamins A and E) concentrations in blood, lipoperoxide and antioxidant (vitamins A and E) levels in milk in dairy cows from the 10th to the 16th day *postpartum*, when it reached the nadir of NEBAL. We also compared the trends of these parameters in two breeds of dairy cows (Italian Friesian and Brown), in order to evaluate their eventual different resistance to oxidative stress.

Material and Methods

Animals

In this study, all procedures were conducted in accordance with the institutional guidelines for animal care and use.

A total of 110 pluriparous Italian Friesian and Brown dairy cows (4 to 7 years of age) during *postpartum* were initially selected for this study. The animals had a mean BW of 550 kg (range 450 to 650), body condition score: 2.5 to 3 and were maintained on farms (semi-intensive dairy cattle breeding) in the south of Italy (Bari, Puglia). All animals were fed with hay, concentrate and minerals. Water was available *ad libitum*. The animals were between the second and the

fourth lactation, with an average milk production ranging from 8300 to 8500 kg per lactation.

All animals considered in this study were free from common parasites (coccidian and strongyloides) and declared free from bovine diarrhoea, brucellosis, bovine leukosis virus, tuberculosis and diseases caused by haemoprotozoa.

On the 10th day *postpartum*, all cows underwent a clinical examination and trans-rectal palpation, trans-rectal ultrasonographic monitoring of the genital tract and the clinical examination of the mammary gland to exclude the presence of alterations; furthermore, on the same day, a milk sample was collected from each cow, in order to perform the somatic cell count (SCC; (CE) N. 853/2004) and to establish which of them had an SCC $\leq 400\,000$ /ml or $>400\,000$ /ml.

Among the 110 cows initially selected, the evaluation of these parameters allowed the inclusion of 80 animals, divided into four groups of 20 subjects each:

Group F and F1: Italian Friesian healthy cows, with SCC $\leq 400\,000$ /ml and $>400\,000$ /ml, respectively; Group B and B1: Italian Brown healthy cows, with SCC $\leq 400\,000$ /ml and $>400\,000$ /ml, respectively.

Blood and milk samples

Just before evening milking, peripheral blood samples were collected from the coccygeal vein in refrigerated serum vacutainer tubes from each subject, at the following time points:

- T10: 10th day *postpartum*
- T12: 12th day *postpartum*
- T14: 14th day *postpartum*
- T16: 16th day *postpartum*.

At the same time points, milk samples were collected in sterile refrigerated falcon tubes. Blood and milk samples were taken on ice to our laboratory (mean transportation time 20 ± 5 min); blood was centrifuged at $1620 \times g$ for 10 min at $+4^\circ\text{C}$ and the sera obtained were stored in Eppendorf tubes (1.5 ml). Both Eppendorf and milk-containing falcon tubes were stored at -20°C until analytical determination.

ROS

ROS serum concentrations were obtained by a photometric analytical system (FREE[®], Diacron, Parma, Italy). FREE[®] measures reactive oxygen metabolites (ROMs), a variety of free radicals that are characterized by an odd number of electrons around the external orbital of oxygen. ROMs react with a chromogen that, if correctly buffered, forms a coloured compound that can be measured photometrically (maximum absorbance peak at 505 nm). Once the absorbance value is determined, the instrument automatically converts the data into the appropriate arbitrary Carr Unit (1 U.Carr = 0.08 mg H₂O₂/100ml).

Vitamins

Fluid samples were shaken before the collection of aliquots (0.2 ml), which were heated to 85°C while mixing with

magnetic stirring. Once this temperature was reached, 0.1 ml samples (in quadruplicate) were collected and, after addition of 0.1 ml of ethanolic b-cryptoxanthin (as internal standard) and 0.9 ml of ethanol, were vortexed for 1 min. Each sample was extracted using hexane stabilized with butylated hydroxytoluene (0.01%) and methylene chloride (5:1), and the mixture was placed in an ultrasonic bath for 5 min. The sample was centrifuged (5000 r.p.m., 5 min) and the extraction was repeated. Organic phases were pooled, evaporated under nitrogen atmosphere, reconstituted and filtered to be injected onto the HPLC system. An HPLC (Model 1100, Agilent, Santa Clara, CA, USA) equipped with two pumps and an autosampler was used. The column was a Gemini C18 column, 25 × 0.46 cm, particle diameter 5 μm (Phenomenex, Torrance, CA, USA) with a matching guard cartridge. Water–acetonitrile–methanol (4:1:95, v/v/v) was used as the mobile phase, working in isocratic mode. Analytes were simultaneously detected with a photodiode array detector (Agilent) set at 323 nm for vitamin A (μg/dl) and 292 nm for vitamin E (g/dl).

Milk parameters

The determination of SCC in milk samples was carried out by a fluoro-opto-electronic counting method using FOSSOMATIC 90[®] (Foss Electric, Hillerod, Denmark).

The fat percentages in milk samples were calculated using LactoScope (AIA, Rome, Italy). An aliquot of about 20 ml was heated in water bath at 40°C. The sample was then subjected to reading through the LactoScope, which makes a nephelometric measurement.

Lipoperoxides

The Association of Official Analytical Chemists (Official Method 965.33 peroxide value of oils and fats) was employed for the determination of milk lipoperoxide values. Peroxide values were expressed as milliequivalent peroxide/kg sample (meq O₂/kg).

Weigh 5.00 ± 0.05 g sample into 250 ml glass-stoppered Erlenmeyer flask. Add 30 ml CH₃COOH-CHCl₃, (a), and swirl to dissolve. Add 0.5 ml saturated KI solution, (b), from Mohr pipet, let it stand with occasional shaking for 1 min and add 30 ml H₂O. Slowly titrate with 0.1N Na₂S₂O₃ with vigorous shaking until yellow is almost gone. Add Ca 0.5 ml 1% starch solution, and continue titration, shaking vigorously to release all I₂ from CHCl₃ layer, until blue just disappears. If <0.5 ml 0.1N Na₂S₂O₃ is used, repeat determination with 0.01N Na₂S₂O₃.

Conduct blank determination daily (must be 0.1 ml 0.1N Na₂S₂O₃). Subtract from sample titration. Peroxide value (meq O₂/kg sample) = $S' N' / 1000/g$ sample, where S = ml Na₂S₂O₃ (blank corrected) and N = normality Na₂S₂O₃ solution.

Statistical analysis

All values were expressed as mean ± s.d. and underwent statistical analysis by means of GLM (with *post hoc* least significant difference test), for comparisons within each group and one-way ANOVA for comparisons between the groups. Correlations between ROS and serum vitamins A

and E, lipoperoxides and milk vitamins A and E, were investigated. A value of $P < 0.05$ was set as significant level.

Results

The results are shown in Figures 1 to 6.

The fat percentages in milk did not evidence any statistically significant difference either within or between the groups, even though these percentages were quite higher in Brown breed than in the Friesian one (Tabel 1).

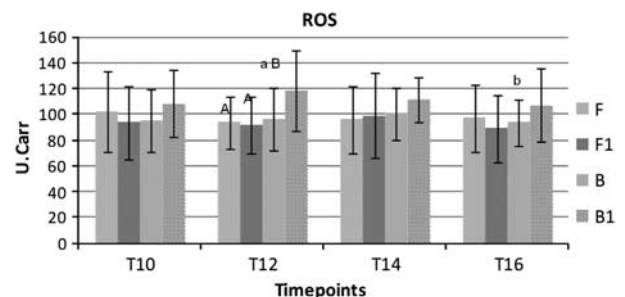


Figure 1 ROS concentrations (mean ± s.d.; U.Carr) in the experimental groups F, F1, B, B1 at T10, T12, T14 and T16 (10th, 12th, 14th and 16th day *postpartum*). Within-group: a, b: $P < 0.05$; between-groups: A, B: $P < 0.05$. ROS = reactive oxygen species.

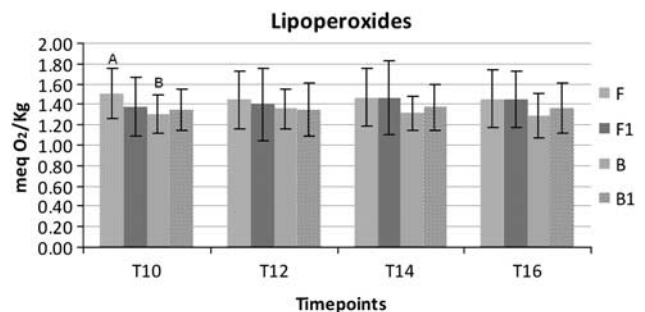


Figure 2 Lipoperoxide concentrations (mean ± s.d.; meq O₂/kg) in the experimental groups F, F1, B, B1 at T10, T12, T14 and T16 (10th, 12th, 14th and 16th day *postpartum*). Between-groups: A, B: $P < 0.05$.

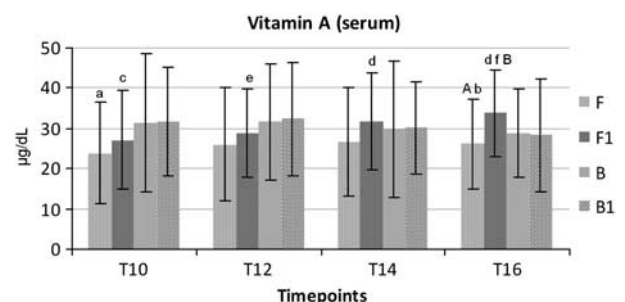


Figure 3 Vitamin A serum concentration (mean ± s.d.; μg/dl) in the experimental groups F, F1, B, B1 at T10, T12, T14 and T16 (10th, 12th, 14th and 16th day *postpartum*). Within-group: a, b, c, d, e, f: $P < 0.01$; between-groups: A, B: $P < 0.05$.

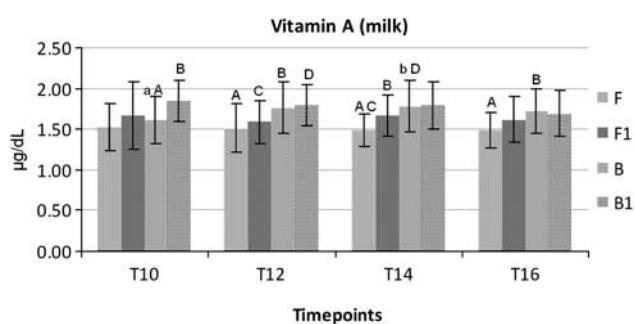


Figure 4 Vitamin A milk concentration (mean \pm s.d.; $\mu\text{g/dl}$) in the experimental groups F, F1, B, B1 at T10, T12, T14 and T16 (10th, 12th, 14th and 16th day *postpartum*). Within-group: a, b: $P < 0.05$; between-groups: A, B, C, D: $P < 0.05$.

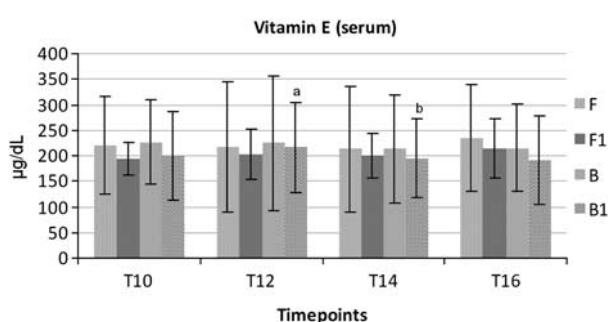


Figure 5 Vitamin E serum concentration (mean \pm s.d.; $\mu\text{g/dl}$) in the experimental groups F, F1, B, B1 at T10, T12, T14 and T16 (10th, 12th, 14th and 16th day *postpartum*). Within-group: a, b: $P < 0.05$.

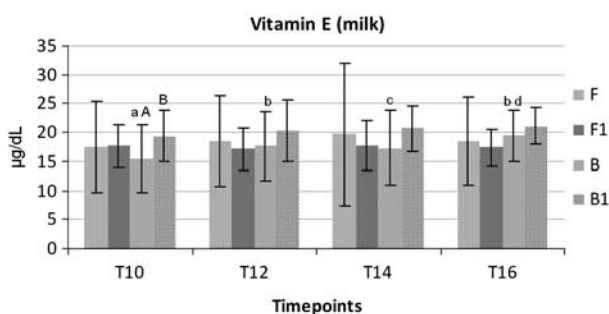


Figure 6 Vitamin E milk concentration (mean \pm s.d.; $\mu\text{g/dl}$) in the experimental groups F, F1, B, B1 at T10, T12, T14 and T16 (10th, 12th, 14th and 16th day *postpartum*). Within-group: a, b, c, d: $P < 0.05$; between-groups: A, B: $P < 0.05$.

ROS displayed quite constant trends, except for group B1, in which a statistically significant difference occurred between T12 and T16 ($P < 0.05$; Figure 1). With regard to comparison among groups, two statistically significant differences were recorded at T12, at F1 *v.* B1 and at B *v.* B1 ($P < 0.05$).

Liperoxides gave rise to a homogeneous trend in the four groups, as well, showing a statistically significant difference at T10 between groups F and B ($P < 0.05$; Figure 2).

With regard to serum levels of vitamin A, statistically significant differences arose both within and between the groups F and F1 (Figure 3). For what concerns milk, vitamin A levels showed similar trends at all the time points, producing

Table 1 Fat milk concentrations (%) in the experimental groups F, F1, B, B1 at T10, T12, T14 and T16 (10th, 12th, 14th and 16th day *postpartum*)

Groups	Times			
	T10 (%)	T12 (%)	T14 (%)	T16 (%)
F	4.18 \pm 1.48	4.16 \pm 0.61	4.17 \pm 0.91	4.20 \pm 0.92
F1	4.78 \pm 1.38	4.75 \pm 2.39	4.12 \pm 0.89	4.34 \pm 1.24
B	4.44 \pm 0.94	4.72 \pm 2.07	4.57 \pm 1.29	4.48 \pm 1.13
B1	4.64 \pm 1.27	4.91 \pm 2.50	4.22 \pm 0.99	4.36 \pm 1.50

T10 = 10th day *postpartum*; T12 = 12th day *postpartum*; T14 = 14th day *postpartum*; T16 = 16th day *postpartum*.

statistically significant differences mainly among groups (Figure 4).

For what concerns serum concentrations of vitamin E, a significant difference was noted only within the group B1, between T12 and T14 ($P < 0.05$; Figure 5). Milk levels of vitamin E did not significantly change in the groups F, F1 and B1, whereas statistical increases arose in group B ($P < 0.05$; Figure 6). With regard to the comparison among groups, a statistical increase was detected between B and B1 at T10 ($P < 0.05$).

Between serum ROS and vitamin E, a significant negative correlation was detected at T12 ($r = 0.5$) in group F ($P < 0.05$), whereas two positive ones were found at T14 ($r = 0.635$) and T16 ($r = 0.664$) in group B1 ($P < 0.01$).

In milk, positive correlations were observed between liperoxides and vitamin A at T12 ($r = 0.623$; $P < 0.01$) and T16 ($r = 0.578$; $P < 0.05$) in group F1, whereas two positive correlations were found between liperoxides and vitamin E, at T12 in group B ($r = 0.509$) and B1 ($r = 0.589$; $P < 0.05$).

Discussion

NEBAL is easy to develop in the early *postpartum* of dairy cows, because of an alteration between energy intake and output, caused by a decrease in DMI and the increased energetic demands occurring at calving and at the beginning of lactation (Drackley *et al.*, 2005). In such a stressful period, a rapid metabolic adaptation develops, during which the increased mitochondrial activity may lead to the overproduction of ROS (Albera and Kankofer, 2011).

This increase in ROS generation, if not properly counterbalanced by an efficient antioxidant defence, may be a cofactor in the determinism of many puerperal dysfunctions, as well as responsible for an alteration of colostrum and milk quality. As a consequence, the maintenance of an equilibrium between ROS and antioxidants is crucial, above all in such a critical period of the dairy cow, as its abruption may also contribute to the outcome of many *postpartum* pathologies, which imply a prolongation of calving-first oestrus and calving-conception intervals and, consequently, economic losses (Kankofer, 2001; Rizzo *et al.*, 2007 and 2009).

Some studies, reported in literature, have been conducted during the transition period in the dairy cow, evaluating

the oxidative status as plasma levels of malondialdehyde (MDA), a degradation product of lipid peroxidation and the total antioxidant status (TAS; Kumagai and Chaipan, 2004; Castillo *et al.*, 2005 and 2006).

Kumagai and Chaipan (2004) reported that plasma and colostrum α -toc concentrations in the multiparous cows were significantly higher than those of the primiparous cows from 60 days before expected calving to 90 days of lactation ($P < 0.05$), with multiparous cows receiving a ration higher in α -toc concentrations. This implied that also the plasmatic α -toc levels in the calves of multiparous cows after birth were significantly higher, probably because of a higher α -toc transfer via placenta or α -toc secretion in the colostrum.

Castillo *et al.* (2005) reported that TAS obtained in late lactation and pregnant cows was lower than the one observed in a previous report on dairy cows at their lactation peak (Castillo *et al.*, 2003), and these results were in agreement with Wachter *et al.* (1999), which observed a progressive decline in antioxidant activity as lactation progresses, probably because of the depletion of fat-soluble antioxidants by milk.

On the other hand, Castillo *et al.* (2006) reported that the metabolic adaptation to the onset of lactation leads to an overproduction of free radicals, which cause lipid peroxidation and high MDA values. However, in this period, the antioxidant system can cope with this condition effectively, whereas the achievement of peak of lactation is accompanied by a stabilization of the metabolic status that is reflected by a stabilization of the antioxidant status as well.

Our study is the first, to the best of our knowledge, to investigate serum and milk concentrations of ROS and lipoperoxides, vitamins A and E, 10 to 16 days *postpartum* of Friesian and Brown dairy cows, that is, in a particularly critical period for the cow, during which NEBAL is likely to develop and reach its nadir (Drackley *et al.*, 2005).

We thought of allocating the cows of each breed into two groups, based on SCC (\leq or $>400\,000$ /ml (CE) N. 853/2004), since the higher SCC, the higher the possibility of an inflammatory process (clinical or subclinical), which in turn may affect the oxidative status of the organism (van den Borne *et al.*, 2011).

ROS concentrations detected in all the experimental groups were higher than those observed in a previous study from our group in which physiological cows in dioestrus were enrolled (55.13 ± 1.96 (U.Carr; mean \pm s.e.m.) at Day 12; 56.53 ± 1.96 (U.Carr; mean \pm s.e.m.) at Day 16; Rizzo *et al.*, 2007). Thus, ROS production, which is known to accompany steroidogenesis (Sugino, 2006), is lower than the one occurring during the intense metabolic changes characterizing NEBAL (Albera and Kankofer, 2011).

The results of this study show a general negative (even if not significant) for vitamin A correlation among ROS and serum vitamins A and E, in the Friesian cows with SCC $\leq 400\,000$ /ml. This datum confirms the role of vitamins in scavenging ROS, a process during which these non-enzymatic antioxidants undergo reduction (Takata *et al.*, 2002; Locher *et al.*, 2011). In fact, in the Friesian cows with

SCC $>400\,000$ /ml, in which a subclinical inflammatory process is likely to have been present, a mechanism able to grant a higher serum vitamin concentration is supposed to be activated, in order to counteract the eventual increase in the ROS expected (Takata *et al.*, 2002; Locher *et al.*, 2011).

Among the four groups considered, B1 was the one in which the highest serum ROS were recorded; moreover, in both B and B1 groups, positive correlations were found among ROS and vitamins A and E. This datum suggests that the Brown breed may be endowed with more efficient non-enzymatic antioxidant defences than the Friesian one; this is in accordance with the suggestions of some authors who state that the Brown breed is highly rustic and resistant (Zicarelli *et al.*, 1999).

The results of our study seem to confirm that the rusticity typical of this breed could also be correlated to the effectiveness of the antioxidant system.

With regard to milk, higher lipoperoxide concentrations would have been expected in Brown cows than in Friesian cows, given the higher fat percentages found in Brown subjects. However, the higher the fat percentage, the wider is supposed to be the distribution of liposoluble vitamins inside it (Ramos-Lledó *et al.*, 2001).

Our results, considered in the light of the available literature by Wachter *et al.* (1999) and Castillo *et al.* (2003), show a progressive decrease of serum antioxidants in Brown cows with the progress of lactation (from the 12th to the 16th day *postpartum*), probably because of the depletion of fat-soluble antioxidants by milk.

Furthermore, lipoperoxides maintain a homogeneous trend throughout the experiment, whereas vitamins in milk undergo a progressive increase. This is also strengthened by the positive correlations observed among lipoperoxides and vitamins A and E. The constant amount of lipoperoxides in milk during the experimentation may be interpreted as a result of the counteracting effect exerted by vitamins, in order to grant the same quality and quantity of milk composition along with the early *postpartum*.

Conclusions

This study investigates ROS, lipoperoxide and vitamin concentrations in serum and milk of Friesian and Brown dairy cows, during NEBAL. The results show that even in presence of higher ROS concentrations the amount of antioxidant vitamins found in Brown cows with an elevated concentration of somatic cells ($>400\,000$ /ml) may have been able to counteract the oxidative damage, which is more likely to develop in these cows, compared with Friesian cows.

However, given their scavenging properties, the administration of vitamins A and E, should be recommended in all cows, in order to reinforce their endogenous antioxidant defences during NEBAL and to prevent or attenuate reproductive and productive failure *postpartum*.

Acknowledgement

This work was supported by Regione Puglia CIP PS_019.

References

- Albera E and Kankofer M 2011. The Comparison of antioxidative/oxidative profile in blood, colostrums and milk of early post-partum cows and their newborns. *Reproduction in Domestic Animals* 46, 763–769.
- Bell AW 1995. Regulation of organic nutrient metabolism during transition from pregnancy to early lactation. *Journal of Animal Science* 73, 2804–2819.
- Bionaz M, Trevisi E, Calamari L, Librandi F, Ferrai A and Bertoni G 2007. Plasma paraoxonase, health, inflammatory conditions and liver function in transition dairy cows. *Journal of Dairy Science* 90, 1740–1750.
- Burvenich C, Bannerman DD, Lippolis JD, Peelman L, Nonnecke BJ, Kehrl ME Jr and Paape MJ 2007. Cumulative physiological events influence the inflammatory response of the bovine udder to *Escherichia coli* infections during the transition period. *Journal of Dairy Science* 90, E39–E54.
- Butler WR and Smith RD 1989. Interrelationship between energy balance and postpartum reproductive function in dairy cattle. *Journal of Dairy Science* 72, 767–783.
- Calderón F, Chauveau-Duriot B, Martin B, Graulet B, Doreau M and Nozière P 2007. Variations in carotenoids, vitamins A and E, and color in cow's plasma and milk during late pregnancy and the first three months of lactation. *Journal of Dairy Science* 90, 2335–2346.
- Castillo C, Hernandez J, Lopez-Alonso M, Miranda M and Benedito JL 2003. Values of plasma lipid hydroperoxides and total antioxidant status in healthy dairy cows: preliminary observations. *Archives of Animal Breeding* 46, 227–233.
- Castillo C, Hernandez J, Bravo A, Lopez-Alonso M, Pereira V and Benedito JL 2005. Oxidative status during late pregnancy and early lactation in dairy cows. *The Veterinary Journal* 169, 286–292.
- Castillo C, Hernández J, Valverde I, Pereira V, Sotillo J, Alonso ML and Benedito JL 2006. Plasma malondialdehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. *Research in Veterinary Science* 80, 133–139.
- Chew BP 1987. Vitamin A and β -carotene on host defense. *Journal of Dairy Science* 70, 2732–2743.
- Contreras GA and Sordillo LM 2011. Lipid mobilization and inflammatory responses during the transition period of dairy cows. *Comparative Immunology, Microbiology and Infectious Diseases* 34, 281–289.
- Drackley JK, Dann HM, Douglas GN, Guretzky NAI, Litherland NB, Underwood JP and Looor JJ 2005. Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. *Italian Journal of Animal Science* 4, 323–344.
- Goff JP, Kimura K and Horst RL 2002. Effect of mastectomy on milk fever, energy and vitamins A, E and β -carotene status at parturition. *Journal of Dairy Science* 85, 1427–1436.
- Grummer RR, Mashek DG and Hayirli A 2004. Dry matter intake and energy balance in the transition period. *Veterinary Clinics of North America: Food Animal Practice* 20, 447–470.
- Havemose MS, Weisbjerg MR, Bredie WLP and Nielsen JH 2004. Influence of feeding different types of roughage on the oxidative stability of milk. *International Dairy Journal* 14, 563–570.
- Kankofer M 2001. Antioxidative defence mechanisms against Reactive oxygen species in bovine retained and not-retained placenta: activity of glutathione peroxidase, glutathione transferase, catalase and superoxide dismutase. *Placenta* 22, 466–472.
- Kumagai H and Chaipan Y 2004. Changes of vitamin E status of periparturient dairy cows and newborn calves. *Animal Science Journal* 75, 541–547.
- Leroy J and Vanholder T 2008. Nutrient prioritization in dairy cows early postpartum: mismatch between metabolism and fertility. *Reproduction in Domestic Animals* 43, 96–100.
- Lindmark-Mansson H and Akesson B 2000. Antioxidative factors in milk. *British Journal of Nutrition* 84, 103–110.
- Locher L, Sattler T and Wittek T 2011. Relevance, measurement and assessment of the antioxidative status in farm animals. *Berlin Munchen Tierarztl Wochenschr* 124, 419–431.
- Malinowski E and Gajewski Z 2010. Mastitis and fertility disorders in cows. *Polish Journal of Veterinary Sciences* 13, 555–560.
- Miller JK, Brzezinska-Slebodzinska E and Madsen FC 1993. Oxidative stress, antioxidants, and animal function. *Journal of Dairy Science* 76, 2812–2823.
- Mudron P, Rehage J, Qualmann K, Sallman HP and Scholz H 1999. A study of lipid peroxidation and vitamin E in dairy cows with hepatic insufficiency. *Journal of the American Veterinary Medical Association* 46, 219–224.
- Przybylska J, Albera E and Kankofer M 2007. Antioxidants in bovine colostrum. *Reproduction in Domestic Animals* 42, 402–409.
- Ramos-Lledó P, Vera S and San Andrés MP 2001. Determination of vitamins A and E in milk samples by fluorescence in micellar media. *Fresenius' Journal of Analytical Chemistry* 369, 91–95.
- Rizzo A, Minoia G, Trisolini C, Manca R and Sciorsci RL 2007. Concentrations of free radicals and beta-endorphins in repeat breeder cows. *Animal Reproduction Science* 100, 257–263.
- Rizzo A, Minoia G, Trisolini C, Mutinati M, Spedicato M, Jirillo F and Sciorsci RL 2009. Reactive Oxygen Species (ROS): involvement in bovine follicular cyst etiopathogenesis. *Immunopharmacology and Immunotoxicology* 31, 631–635.
- Roche JR, Friggens NC, Kay JK, Fisher MW, Stafford KJ and Berry DP 2009. Invited review: body condition score and its association with dairy cow productivity, health, and welfare. *Journal of Dairy Science* 92, 5769–5801.
- Spears JW and Weiss WP 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. *The Veterinary Journal* 176, 70–76.
- Sugino N 2006. Roles of reactive oxygen species in the corpus luteum. *Animal Science Journal* 77, 556–565.
- Takata J, Matsunaga K and Karube Y 2002. Delivery systems for antioxidant nutrients. *Toxicology* 180, 183–193.
- Taylor VJ, Beever DE and Wathes DC 2003. Physiological adaptations to milk production that affect fertility in high yielding dairy cows. Occasional Publication No. 29. In *Dairying, using science to meet consumer needs* British Society of Animal Science., pp. 37–71. Nottingham University Press, Nottingham, UK.
- van den Borne BH, Vernooij JC, Lupindu AM, van Schaik G, Frankena K, Lam TJ and Nielen M 2011. Relationship between somatic cell count status and subsequent clinical mastitis in Dutch dairy cows. *Preventive Veterinary Medicine* 102, 265–273.
- Wachter CM, McDaniel BT, Whitlow LW and Pettyjohn S 1999. Genetics of antioxidant activity in Holsteins and Jerseys: associations with various traits. *Journal of Dairy Science* 82 (Suppl. 1), 31.
- Zicarelli L, Tafuri V and Di Palo R 1999. La produttività delle principali razze da latte allevate nell'Italia meridionale e in Sardegna. La razza Bruna Italiana 2, 31–37.