Oxidative stress index (OSi) as a new tool to assess redox status in dairy cattle during the transition period

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Oxidative stress (OS) plays a key role in the initiation or progression of numerous diseases, and dairy cows undergo OS at the transition period. However, discrepancies between methodologies make it difficult to make comparisons between studies, and therefore research on this topic may not be implemented in farms. This study aims to test under field conditions the use of an oxidative stress index (OSi) as a combined measurement through a ratio between pro-oxidants and antioxidants throughout the transition period in dairy farms. Serum samples of high-yielding dairy cows were taken, and markers of oxidative damage and antioxidant capacity were measured in four different production stages: (i) late lactation (LL; 2 to −1 months); (ii) prepartum (PrP; −1 month until parturition); (iii) postpartum (PsP; delivery to +1 month); and (iv) peak of lactation (PkL; +1 to +2.5 months). Values were compared between production stages and against a metabolic baseline status (CTR, 4th to 5th month of gestation). To the best of our knowledge, this is the first report in the literature that discusses the values of these oxidative stress biomarkers (and the OS index) for cows with low metabolic demands, as to date most research in this area has focused on the transition period. With the joint evaluation through the OSi, differences were found that were not present with the separate evaluation of pro-oxidants or antioxidants, thus supporting our hypothesis that the OSi indicates more accurately the oxidative status of the animals. It was also confirmed that dairy cows undergo OS after parturition, and that antioxidant supplementation from 1 month before parturition until the peak of lactation may be needed to reduce the risk of OS.

Keywords: oxidative status, peripartum period, vitamin supplementation, dairy cow, antioxidants

Implications

There is strong evidence that oxidative stress (OS) plays a central role in the initiation, progression and maintenance of several pathologies. However, at present, there is disagreement as to whether or not dairy cows suffer an oxidative challenge around parturition, partly because of the differences in the methodologies used. This study demonstrates that measurement of both pro-oxidants and antioxidants allows determination of an oxidative stress index (OSi) that was utilized to quantify and demonstrate that the transition period is a time of increased OS in dairy cows. Moreover, the better timeframe for antioxidant supplementation, in terms of OS, was identified to minimize the incidence of postpartum diseases.

Introduction

Oxidative stress (OS) plays a key role in several pathological conditions connected with animal production, reproduction and welfare (Lykkesfeldt and Svendsen, 2007), and is attributable to an imbalance between oxidant and antioxidant substances in the body. OS can be particularly dangerous as no clinical symptoms are shown.

One of the most critical moments in dairy health, with consequences for production variables, is the transition period (Goff and Horst, 1997) when the capacity of antioxidant defenses is exceeded by the production of reactive oxygen substances (ROS). The influence of OS in ruminant health in this period is a relatively recent field of research; however, unfortunately, differences between models and methodologies make it difficult to make meaningful comparisons (Celi, 2011) with practical conclusions.

Therefore, it is necessary to provide useful OS markers that would help to analyze it with accuracy to define protective nutritional strategies on the basis of antioxidant supplementation. We consider that it is important to evaluate not only concentrations of oxidants and antioxidants separately, but also analyze their relationship through a proportion or ratio, because it is the imbalance between oxidants and the antioxidants that defines the concept of OS (Castillo et al., 2005).

The first approach using this ratio was made in human medicine by Sharma et al. (1999). For dairy cattle, Celi (2011)
in a review article proposed the use of the ratio pro-oxidants/antioxidants as an index indicative of an animal’s risk to develop disease. Thus, an increase in the ratio indicates risk of OS because of the increase in ROS production or defensive antioxidant consumption.

In line with these considerations, the approach we have used aims to test, under field conditions in a commercial dairy herd, the use of an Oxidative Stress index (OSI), based on the ratio between ROS and serum antioxidant capacity (SAC), that is, ROS/SAC, comparing the information given by this parameter with those given by ROS and SAC separately. Moreover, the usefulness of this index is evaluated in the most complex stage: around parturition (2 months before parturition to 2.5 months after it) that includes the transition period.

Material and methods

This study involved the collection of serum samples at different stages of the transition from gestation to lactation in dairy cows in order to compare the differences between these stages in terms of oxidative status. Permission for the procedures of the experiment was granted by the Bioethical Committee of the University of Santiago de Compostela (Spain).

Animals, nutrition and husbandry

The study was conducted on a commercial dairy herd located in Arzúa (Galicia, NW, Spain), with an average 305 days normalized milk production of 10.235 kg/cow, and where economics play the main role in farm decisions. At this herd, we sampled fortnightly 25 cows from 2 months before the expected date of parturition until 2.5 months after it. The data obtained from three animals were discarded because one animal needed a caesarean section at calving, and another two of them developed clinical mastitis and left displacement of the abomasum, respectively; therefore, only another two of them developed clinical mastitis and left displacement of the abomasum, respectively; therefore, only another two of them developed clinical mastitis and left displacement of the abomasum, respectively; therefore, only another two of them developed clinical mastitis and left displacement of the abomasum, respectively; therefore, only another two of them developed clinical mastitis and left displacement of the abomasum, respectively; therefore, only another two of them developed clinical mastitis and left displacement of the abomasum, respectively; therefore, only another two of them developed clinical mastitis and left displacement of the abomasum, respectively; therefore, only another two of them developed clinical mastitis and left displacement of the abomasum, respectively; 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Grosseto, Italy), which determines hydroperoxides (breakdown products of lipids as well as of other organic substrate, generated by the oxidative attack of ROS) through their reaction with the chromogen N,N-diethylparaphenylenediamine. Results are expressed in arbitrary ‘Carrañelli Units’ (CarrU), where 1 CarrU is equivalent to the oxidizing power of 0.08 mg H$_2$O$_2$/dl. Intra- and inter-assay CV were 3.22% and 8.19%, respectively.

SAC was estimated with the OXY-Adsorbent Test (Diacron International) (Trotti et al., 2001). This test exploits the capacity of a massive solution of hypochlorous acid (HClO) to oxidize the complete pool of antioxidants in serum (albumin, bilirubin, uric acid, thiol groups, vitamins, glutathione, glutathione peroxidase, superoxide dismutase, catalase, etc.). Thus, SAC considers the cumulative action of all the antioxidants present in serum, rather than simply the sum of measurable antioxidants. Results are expressed as μmol HClO/ml. Intra- and inter-assay CV were 2.85% and 5.10%, respectively.

Both variables were used previously in bovine studies and managed according to the manufacturer’s instructions. The OSi was calculated as ROS/SAC, expressed as CarrU/(mol HClO/ml). Both ROS and SAC were measured on an UV/VIS absorption spectrophotometer (Clima MC-15; RAL Técnica para el Laboratorio, Barcelona, Spain).

### Statistical procedure

Data for each parameter were checked for normal distribution with the Kolgomorov–Smirnov test. A repeated measurement ANOVA was used to compare means among the different stages of the transition period, with the individual cows as experimental unit. Following the ANOVA, significant inter-group differences were detected by Bonferroni test. To compare the means of each transitional stage with the mean of the CTR group, the Student t-test was used. The criterion for statistical significance was established at $P < 0.05$. All statistical procedures were carried out with the IBM SPSS v19.0 for Windows software package.

### Results

Table 2 shows the results of the oxidative status markers in all the studied stages. Values in CTR cows can be considered as the baseline values for dairy cows under field conditions, taking into account that they were obtained in animals with the theoretically lowest metabolic burdens than can be achieved in lactating cows in a commercial dairy farm. Thus, the values obtained from cows at the transitional stages will be compared and discussed with those values obtained from CTR cows.

Mean ROS values did not differ significantly between control and any stage of the transition period. ROS progressively increased from LL to PkL with a slight decrease in the next stage. Although values in PkL were higher than those before parturition, they did not achieve statistical significance.

Similarly, mean SAC values did not statistically differ between CTR and transitional cows at any stage; nonetheless, it can be noted that there was an increase in the antioxidant barrier in PrP, with a subsequent decrease after delivery, reaching the lowest activities at PkL.

Despite the lack of statistical significance in the differences among transitional stages, and with the CTR group in either ROS or SAC separately, the evaluation of the oxidative status of the animals with the OSi found a significant difference between the means of OSi in lactating and dried animals. However, the values of CTR cows only differed significantly with the animals at PkL. It is noted that the values in dried animals were lower than in CTR ones; which were considered to be the baseline levels.

### Discussion

This experiment studied the differences between the separate and the joint evaluation of pro-oxidants and antioxidants at blood level throughout the transition period of dairy cows. Serum samples were taken at different stages of the transition from gestation to lactation and compared among them and a control group.

### Pro-oxidants

Changes in ROS during the study were in accordance with previous reports that showed an increase in oxidant species after parturition, attributable to the metabolic challenges associated with this stage (Bernabucci et al., 2005; Dobbelaar et al., 2010). Furthermore, during lactation, energy partitioning associated with milk production contributes to maintain a metabolic stress, favoring high ROS production (Castillo et al., 2006).

### Table 2 Mean values of oxidative status markers throughout the studied stages

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>LL</th>
<th>PrP</th>
<th>PsP</th>
<th>PkL</th>
<th>CTR (n = 40)</th>
<th>RMSE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS</td>
<td>CarrU</td>
<td>121.4</td>
<td>129.8</td>
<td>153.0</td>
<td>145.1</td>
<td>135.6</td>
<td>35.3</td>
<td>0.074</td>
</tr>
<tr>
<td>SAC</td>
<td>μmol HClO/ml</td>
<td>481.1</td>
<td>516.8</td>
<td>489.8</td>
<td>425.8</td>
<td>480.0</td>
<td>117.8</td>
<td>0.169</td>
</tr>
<tr>
<td>OSi</td>
<td>CarrU/(μmol HClO/ml)</td>
<td>0.26a</td>
<td>0.26a</td>
<td>0.34bc</td>
<td>0.37c</td>
<td>0.30ab</td>
<td>0.10</td>
<td>0.003</td>
</tr>
</tbody>
</table>

ROS = reactive oxygen substances; SAC = serum antioxidant capacity; OSi = Oxidative Stress index; CTR = control cows (between the 4th and 5th month of gestation); LL = late lactation (between 1 and 2 months before parturition); PrP = prepartum (from 1 month before parturition until delivery); PsP = postpartum (from delivery until 1 month after calving); PkL = peak of lactation (from 1 month after parturition until peak lactation); RMSE = root mean squared error.

A repeated measurement ANOVA with cow as experimental unit was used to compare the means within the transitional stages, whereas the means of these stages were compared with the means of the CTR group through the Student t-test. Means with different superscript alphabets within rows are significantly different ($P < 0.05$).
Antioxidants
Unlike other studies (Sharma et al., 1999) that used the biological antioxidant potential (BAP) as an estimation of antioxidant capacity, we decided to estimate the SAC by the plasma barrier to oxidation (OXY-Adsorbent test). This was because, in addition to the ‘scavengers’ antioxidants (those determined by BAP), this test can also measure the so-called ‘shock adsorbers’, that is, all the antioxidants that are not active from the chemical point of view but are able to ‘plug’ the oxidant action of ROS. This provides information on the structural component of the antioxidant barrier of which the period of recovery is relatively slow, in comparison with the antioxidants of lower weight, or at least a more rapid turnover, included in the BAP determination. Therefore, we are measuring the cumulative capacity of the antioxidant defense against a particular oxidant aggression (how the animal has been accumulating and disposing its antioxidants reserves to specific, predictable and expected situations such as delivery and early lactation), rather than measure the short-term antioxidants at the time of sampling.

The prepartum period is characterized by a depleted antioxidant status and, consequently, OS (Bernabucci et al., 2002 and 2005), and therefore these cows were supplemented with a vitamin complex before parturition, which is a recommended practice to minimize the risk of postpartum diseases (Politis, 2012). This fact prevents us from getting the clear picture of the natural cycle of OS; however, on the other hand, it shows the oxidative status that might be observed in dairy cows in commercial farms. Under these conditions, the slight increase in SAC values at PrP can be considered as a result of the preventive vitamin complex administration (Dobbelaar et al., 2010). The small drop at PsP is not only the consequence of the utilization of antioxidants in colostrum production (Goff and Horst, 1997), but also the consequence of antioxidant consumption, in an attempt to cope with the metabolic production of oxidants. As lactation progresses, antioxidants continue to decline because of the depletion of fat-soluble antioxidants by milk, in combination with their consumption by endogenous ROS production (Castillo et al., 2005 and 2006).

OSi
Currently, there is no agreement on whether or not dairy cows undergo OS during the transition period. Previous studies suggested that dairy cows experience OS during the periparturient period (Bernabucci et al., 2005; Castillo et al., 2005). However, in contrast with these studies, some authors do not report an oxidative challenge during the periparturient period (Wullepit et al., 2009; Dobbelaar et al., 2010). While with a separate evaluation of our results of ROS and SAC, apparently these cows did not undergo OS, as no significant difference was found between the studied stages neither for ROS nor for SAC. When the oxidative status is studied by a combined evaluation of pro-oxidants and antioxidants with the OSi, statistical differences between the foregoing and subsequent parturition stages were found, suggesting in fact that these cows experienced an oxidative challenge after parturition. This finding suggests that it may be a better practice to evaluate jointly both oxidants and antioxidants rather than separately, as OS could either be a consequence of an excessive production of ROS production and/or a decrease in the body antioxidant defense, and therefore these parameters are strictly inter-dependent.

This agrees with a study in human medicine, in which the relationship between the level of OS and pathology was higher when oxidants and antioxidant defense measurements were combined as a ratio (Sharma et al., 1999). However, care must be taken when interpreting these results, taking into account the higher variance associated with the base measurements. Marked individual variations were already reported for other oxidative status biomarkers in periparturient dairy cows, with many factors influencing it (Castillo et al., 2005 and 2006).

Of particular interest is the observation that in peak lactation, when theoretically the cow is metabolically adapted to milk production (Castillo et al., 2006), there is the maximum risk for OS, with values of the OSI significantly higher than CTR cows. This reason can be attributed not only to the slight decrease in ROS, as the results of a lesser metabolic burden, but also to the larger decrease in SAC. For these reasons, and although no clinical symptoms of disease were observed in the studied animals, these findings suggest us the convenience of extending antioxidant supplementation from the dry period until peak of lactation.

Conclusions
Under the conditions of this study, the OSI provides an objective assessment of the relationship between oxidants and antioxidants, not seen by the determination of both components separately. In addition, baseline levels of oxidative status biomarkers under field conditions for commercial high-yielding dairy cows are reported, which will bring a step forward to their applicability in farms. It was also found that dairy cattle show an increase in the levels of OS after parturition, and hence to develop preventive actions that would minimize the effects of production diseases after parturition, further studies should study the effects of antioxidant supplementation from 1 month before parturition until the peak of lactation.

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