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Oxidative indices as metabolic stress predictors in periparturient dairy cows

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ABSTRACT

The aim of the study was to determine changes in reactive oxygen species (ROS), serum antioxidant capacity (SAC) and oxidative stress index (OSi; ROS/SAC) from dry off to 30 days postpartum in dairy cows. Furthermore, the relationship between indices of oxidative status (ROS, SAC, OSi) and indices related to metabolic disorders (blood free fatty acids, FFA and β-hydroxybutyrate, BHB) and α-tocopherol (α-T) was determined. Blood samples were collected from 131 dairy cows belonging to four commercial farms, located in Italy and Greece. Blood samples were collected at dry-off, calving and 30 days postpartum. Results indicated that ROS and OSi were low at dry-off and 30 days postpartum and high at calving. The serum antioxidant capacity followed exactly the opposite trend. There was a negative correlation of ROS (rho: −0.282; $p \leq .01$) and OSi (rho: −0.267; $p \leq .05$) with α-T at all three sampling points, whereas no correlation was found between SAC and α-T. Reactive oxygen species were positively correlated with BHB at all three sampling points and with FFA levels only at dry-off. A negative correlation of SAC with FFA was found at dry-off and 30 days postpartum. The oxidative stress index was positively correlated with FFA and BHB at dry-off, with FFA at calving and with BHB at 30 days postpartum. Thus, associations between parameters related to oxidative status and those related to energy balance were found, mainly at dry-off and postpartum. Oxidative stress markers determined at dry off could be useful in preventing metabolic disorders.

HIGHLIGHTS

- Dairy cows around calving often incur metabolic disorders.
- Associations between oxidative and metabolic markers were found mainly at dry-off and postpartum.
- Oxidative markers could be used as a tool for metabolic disorders prevention.

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Oxidative stress; metabolic disorders; free fatty acids; β-hydroxybutyrate

Introduction

The occurrence of negative energy balance is the hallmark of metabolic disorders in dairy herds managed and fed for high milk yields. Severe negative energy balance impairs, among others, milk production, DM intake, energy efficiency and fertility in dairy cows. Fatty acids (FFA) and β-hydroxybutyrate (BHB) are the two markers used to assess the severity of the negative energy balance. In a previous study including four commercial farms located in Italy and Greece, our group showed that these two markers are not well correlated during the transition period (Pilotto et al. 2016). In fact, the concentration of free fatty acids in blood became maximal during calving and declined postpartum, while BHB continued to increase in the postpartum period. Furthermore, significant negative correlations between BHB and α-tocopherol (α-T) were found.

Except increased frequency of metabolic disorders, the majority of the evidence available suggests that dairy cows also experience oxidative stress around parturition (Bernabucci et al. 2005; Sordillo and Aitken 2009). Abuelo et al. (2013) reported that increased oxidation occurs in the periparturient period of dairy cows. Furthermore, they found that the oxidative stress index (OSi), which is the ratio between reactive oxygen species (ROS) and serum antioxidant capacity (SAC), predicts more accurately the oxidative status than ROS or SAC alone. Whether oxidative stress markers are associated with metabolic indices is not

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known with certainty. If a positive association does occur, one might envision that determination of oxidative stress markers can be used as tool in the prevention of metabolic disorders in dairy cows.

The fact that we have already determined FFA and BHB values in blood samples from four commercial farms (Pilotto et al. 2016) gave us the incentive to extend our previous investigation and to use the index proposed by Abuelo et al. (2013) on a larger scale, determining ROS, SAC and OSi. Our working hypothesis is that a positive association occurs between markers of oxidative stress and metabolic indices in dairy cows. The objectives of the present study were: (1) to determine changes in ROS, SAC and OSi levels at dry-off, calving and 30 days postpartum in four commercial farms located in Southern Europe, (2) to examine the relationship between the three markers (ROS, SAC, OSi) of oxidative status and the two metabolic indices (FFA, BHB) in dairy cows.

Materials and methods

A total of 131 Holstein cows belonging to four commercial farms were sampled in an observational field study. Two of the farms were located in Northern Italy and the other two in Northern Greece. A total of 59 cows belonged to the Italian farms (30 and 29 from each farm) and 72 belonged to the Greek farms (36 from each farm). Selected cows were pluriparous, in average in their third lactation and did not exhibit any health issues (e.g. metabolic, mastitis) on their previous lactation.

Diets on all farms have been reported in detail in a previous study from our group (Pilotto et al. 2016).

Blood samples were collected from the tail vein after the morning milking from all cows at dry-off (58.94 ± 9.34 days from calving), at calving, and at 30 days postpartum. Serum was obtained following centrifugation of the blood samples, and it was frozen at −80 °C until analysis. Reactive oxygen species were determined using the method of Trotti et al. (2001) by the spectrophotometric d-ROM test (Diacron International, Via Zircone 8, 58100 Grosseto, Italy). Results are expressed in arbitrary 'Carratelli Units' (CarrU), where 1 CarrU is equivalent to the oxidising power of 0.08 mg H_2O_2/dL. Serum antioxidant capacity was determined with the OXY-Adsorbent Test (Diacron International, Via Zircone 8, 58100 Grosseto, Italy) (Trotti et al. 2001). Results are expressed as mmol HClO/mL. Oxidative stress index was calculated as the ratio of ROS/SAC.

The amounts of FFA, BHB and α-T were determined, and the results were described in detail in a previous paper published by our group. Furthermore, information concerning milk production and milk composition can be found in the same paper (Pilotto et al. 2016).

Statistical analysis

The statistical analysis was performed using a linear mixed model with two fixed factors (farm and sampling point) and three repeated measures for each cow. Cow was considered as a random factor nested within farm. We used heterogeneous first order autoregressive covariance structure, resulting in the smallest Akaike information criterion. The model used was as follows:

$$Y_{ijk} = \mu + F_i + T_j + F_i \times T_j + C_{k(i)} + e_{ijk} \qquad (1)$$

where Y_{ijk} is the individual value for each dependent variable (ROS, SAC, OSi); μ is the overall mean; F_i is the fixed effect of farm (1 and 2 = Italian farms and 3 and 4 = Greek farms); T_j is the fixed effect of 3 repeated measures factor 'sampling point' for each cow (1 = dry-off, 2 = calving, 3 = 30 days postpartum); $C_{k(i)}$ is the random cow effect, nested within farm; and e_{ijk} is the random error assumed to be normally and independently distributed with zero expectation and common variance σ^2. Values in the tables are presented as least squares means (±SEM). The Bonferroni test for *p*-values was used when performing multiple comparisons and assigned significance at an α level of 0.05 unless otherwise noted. All analyses were performed by PROC MIXED in SAS, version 9.0 (SAS Institute 2004). Spearman's rho bivariate correlations of variables at sampling points were estimated by PROC CORR statement in SAS.

Results and discussions

Table 1 shows that ROS and OSi were highest at calving and lower values were found at dry-off and 30 days postpartum ($p < .05$); conversely, SAC was lowest at calving while higher values were found at dry-off and 30 days postpartum ($p < .05$). Our data, taken together, suggest that significant differences occur in all three markers of oxidative status across the periparturient period, which implies they may be useful biomarkers for oxidative stress.

This is in contrast with Abuelo et al. (2013) who reported that OSi was more accurate in the detection of oxidative stress when compared to ROS and SAC. This discrepancy cannot be properly explained but it should be pointed out that Abuelo and colleagues' study involved less animals ($n = 22$) than the current study. All the markers of oxidative status considered, namely ROS, SAC and OSi, are able to detect oxidative stress conditions in dairy cows around calving. However,

Table 1. Changes in levels of reactive oxidative substances (ROS, CarrUnits), serum antioxidant capacity (SAC, µmol HClO/mL) and oxidative stress index (OSi, ROS/SAC) during the periparturient period in dairy cows from four farms[1].

	Farms (F)				Sampling time (T)			Effects		
	1 ($n=30$)	2 ($n=29$)	3 ($n=36$)	4 ($n=36$)	Dry-off	Calving	30 days postpartum	*F*	*T*	FxT
ROS	69.578[a] ± 1.829	80.179[b] ± 1.860	58.349[c] ± 1.674	56.973[c] ± 1.673	60.924[A] ± 1.141	73.871[B] ± 1.223	64.015[A] ± 1.275	***	***	ns
SAC	453.556[a] ± 10.889	458.460[a] ± 11.075	408.717[b] ± 9.958	415.375[bc] ± 9.956	64.015[A] ± 1.275	380.412[B] ± 6.847	461.863[A] ± 6.555	**	***	**
Osi	0.163[a] ± 0.006	0.183[a] ± 0.006	0.148[b] ± 0.005	0.143[b] ± 0.005	0.134[A] ± 0.003	0.200[B] ± 0.004	0.143[A] ± 0.004	***	***	***

[a,b,c,A,B] Means within the same row followed by different superscript for each parameter ([a,b,c]) between farms and ([A,B]) between sampling times, differ significantly $p \le .01$; ns: not significant; $^{**}p \le .01$; $^{***}p \le .001$. [1]Dairy cows from 4 farms: 1 and 2 from Italy; 3 and 4 from Greece. All values are LSM ± SEM.

Italian values are, on average, higher during the whole experimental period compared with Greek farms (ROS= 74.60 ± 1.39 CarrUnits vs. 57.67 ± 1.24 CarrUnits; SAC= 457.58 ± 6.86 µmol HClO/mL vs. 412.11 ± 6.99 µmol HClO/mL; OSi= 0.172 ± 0.01 vs. 0.145 ± 0.01). This could be due to a major effect of the production level on the general health status of the animals; indeed, as reported by Pilotto et al. (2016), milk yield was ∼20% higher in the Italian farms than in the Greek ones (Pedernera et al. 2010).

α-tocopherol (α-T) is the main antioxidant vitamin in dairy cows (Baldi et al. 2000; Politis 2012). As expected, our results show that ROS and OSi were negatively correlated with α-T at all three sampling points ($p < .01$ at calving; $p < .001$ at dry off and postpartum) (Table 2). Our findings show similarities with those reported by Politis et al. (2012), who found that α-T was inversely related with two measures of oxidative stress, namely ROS and thiol groups, during the periparturient period.

Surprisingly, SAC was not correlated with blood α-T at any of the three sampling points. Serum antioxidant capacity was lower by 18% at calving compared with the corresponding value at dry off ($p < .01$) and increased by 21% postpartum ($p < .01$). Pilotto et al. (2016) on the same cows showed α-T levels during the dry period decreasing by 50% at calving and then increasing by 100% postpartum. Thus, changes in SAC are less pronounced than those in α-T.

Moreover, the lack of expected positive correlation between SAC and α-T can be attributed to the inability of OXY adsorbent test to correlate with the single antioxidant measure (Costantini 2011); the test indeed accounts simultaneously for the contribution of different antioxidants families that could affect the total antioxidant capacity.

Table 3 presents the Spearman's rho correlation coefficients between the three markers of oxidative status (ROS, SAC, OSi) and the two markers of negative energy balance. Reactive oxygen species were positively correlated with BHB at all three sampling points (Spearman's rho correlation coefficients were calculated equal to 0.274 ($p \le .002$; $n = 131$), 0.184 ($p \le .036$; $n = 130$) and 0.272 ($p \le .002$; $n = 130$) at dry-off, calving and 30 days postpartum sampling time, respectively) but only at dry-off with FFA (rho: 0.233; $p \le .008$; $n = 131$).

Oxidative stress index was positively correlated with BHB at dry-off (rho: 0.261; $p \le .003$; $n = 131$) and 30 days postpartum (rho: 0.254; $p \le .004$; $n = 130$), and with FFA at dry-off (rho: 0.327; $p \le .000$; $n = 131$) and calving (rho: 0.215; $p \le .014$; $n = 130$). Serum antioxidant capacity was negatively correlated with FFA at dry-off (rho: –0.197; $p \le .024$; $n = 131$) and 30 days

Table 2. Spearman's rho correlations between reactive oxygen substances, serum antioxidant capacity, oxidative stress index and blood α-tocopherol in dairy cows ($n = 131$)[a].

		α-T[b]		
Item		Dry-off	Calving	30 days postpartum
ROS[c]	Rho	−0.500	−0.282	−0.671
	p-Value	***	**	***
SAC[d]	Rho	0.123	0.100	0.178
	p-Value	NS	NS	NS
OSi[e]	Rho	−0.552	−0.267	−0.634
	p-Value	***	**	***

[a]Dairy cows from four herds, two of them in Italy and two in Greece. [b]α-tocopherol. [c]Reactive oxidative substances. [d]Serum antioxidant capacity. [e]Oxidative stress index. **Correlation is significant at $p < .01$; $^{***}p < .001$ (2-tailed); NS: nonsignificant differences.

Table 3. Spearman's rho correlations between the levels of blood free fatty acids, β-hydroxybutyrate, reactive oxidative substances, serum antioxidant capacity and oxidative stress index in dairy cows ($n = 131$)[a].

Time of sampling	Item		ROS[b]	SAC[c]	OSi[d]
Dry-off	FFA[e]	Rho	0.233	−0.197	0.327
		p-Value	**	*	***
	BHB[f]	Rho	0.274	−0.004	0.261
		p-Value	**	NS	**
Calving	FFA	Rho	0.121	−0.138	0.215
		p-Value	NS	NS	*
	BHB	Rho	0.184	0.169	0.040
		p-Value	*	NS	NS
30 days postpartum	FFA	Rho	0.037	−0.400	0.162
		p-Value	NS	***	NS
	BHB	Rho	0.272	−0.023	0.254
		p-Value	**	NS	**

[a]Dairy cows from four herds, two of them in Italy and two in Greece. [b]Reactive oxidative substances. [c]Serum antioxidant capacity. [d]Oxidative stress index. [e]Free fatty acids. [f]β-hydroxybutyrate. *Correlation is significant at $p < .05$; $^{**}p < .01$; $^{***}p < .001$ (2-tailed); NS: nonsignificant differences.

postpartum (rho: −0.400; $p \leq .000$; $n = 131$), but was not correlated with BHB at any of the three sampling points (rho: −0.004; $p = .968$, rho: 0.169; $p = .055$ and rho: −0.023, $p = .794$, at dry-off, calving and 30 days postpartum, respectively).

These findings, in agreement with observations by Bernabucci et al. (2005), suggest that a positive correlation may exists between oxidative and metabolic markers, especially for fatty acids. Indeed, according to Bionaz et al. (2007), fatty acids in the liver undergo to an intense process of oxidation that increase ROS production. As consequence, oxidative stress may occur. The main part of FFA is metabolised by hepatocytes via β-oxidation to acetyl-coenzyme A (Acetyl-CoA) that is then metabolised in ketone bodies, such as BHB (Abdelli et al. 2017). However, even if BHB derives from FFA, they are not correlated, or at least weakly, to each other (McCarthy et al. 2015; Pilotto et al. 2016); indeed, FFA and BHB follow different trends during the peri-parturient period.

Thanks to the correlation with FFA and BHB, ROS could be the most cost-effective oxidative stress marker to be used on large datasets, also for the prevention of metabolic disorders. However, on smaller datasets, Osi has been demonstrated to be more accurate in the detection of oxidative stress, compared to ROS and SAC alone (Abuelo et al. 2013) and as consequence could be a good option as preventive tool. However, attention should be paid to the effect of dry-off on metabolic, oxidative and inflammatory potential biomarkers responses (Putman et al. 2018).

Conclusions

In conclusion, calving was associated with the highest values of two measures of oxidative stress (ROS and OSi) and the lowest value of serum antioxidant capacity. Our findings suggest that a positive relationship does exist mainly at dry-off and 30 days postpartum between markers of oxidative stress (OSi and ROS) and those of negative energy balance (FFA, BHB). Determination of oxidative stress markers as early as the dry off can provide an early warning and they could be potentially used as a management tool to reduce the frequency of metabolic disorders. Further investigations will be needed to determine if ROS, SAC and Osi are truly associated with disease outcomes.

Ethical approval

All animal rearing and handling procedures were carried out in accordance with the European Commission recommendation 2007/526/EC and Directive 2010/63/UE on revised guidelines for the accommodation and care of animals used for experimentation and other scientific purposes.

Disclosure statement

No potential conflict of interest was reported by the authors.

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