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Effect of colostrum redox balance on the oxidative status of calves during the first 3 months of life and the relationship with passive immune acquisition

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Abstract

New-born calves depend upon colostrum intake for the acquisition of immunoglobulins (Ig) and other beneficial substances. However, colostrum is also a source of reactive oxygen species (ROS). Intrinsic production of ROS also increases after birth, so the combination of colostral and intrinsic ROS could overwhelm the antioxidant capacity of the calf leading to oxidative stress (OS), a condition that has been shown to play a key role in the initiation and development of several pathological conditions.

The aim of this observational study was to assess the effects of the redox balance of colostrum on the oxidative status of calves and on passive immune transfer. Serum samples were taken from 20 calves on their day of birth, every week during their first month of life and at 2 and 3 months of age, and the concentrations of ROS and serum antioxidant capacity (SAC) assayed. The oxidative/anti-oxidative profile and IgG content of the colostrum were also assessed.

The redox balance of the colostrum had a significant effect on both calf oxidative status and on passive immune transfer (as measured by calf serum IgG concentration), which indicates that the oxidative/antioxidative profile of colostrum should be measured when colostrum quality is assessed. The highest risk of OS during the study period was found to be when the calves were fed artificial milk replacer; this suggests that calves should be supplemented with antioxidants during this period in order to minimize any harmful consequences of high ROS generation.

Keywords: Passive immune transfer; Oxidative stress; Antioxidants; Immunoglobulin G; Calf milk replacer.

Introduction

After birth, calves are exposed for the first time to an oxygen rich environment once they start to breathe and this results in an increase in the production of reactive oxygen species (ROS) (Saugstad, 2003; Wiedemann et al., 2003). If the production of ROS overwhelms the antioxidant capacity of the neonatal calf oxidative stress (OS) can develop. This is known to play a key role in the initiation and maintenance of conditions such as diarrhoea or pneumonia (Ranjan et al., 2006; Lykkesfeldt and Svendsen, 2007; Sordillo and Aitken, 2009)

New-born calves depend upon colostrum intake for the acquisition of immunoglobulins and other beneficial substances (McGuirk and Collins, 2004). Colostrum is a source of antioxidants (Przybylska et al., 2007), but it is also a source of ROS, as it is rich in macromolecules, such as lipids or proteins, that are easily oxidised and also has a significant population of immune cells, including macrophages, that use ROS generating systems to kill bacteria. Recent studies (Kankofer and Lipko-Przybylska, 2008; Albera and Kankofer, 2011) have shown that, compared to normal milk, colostrum has the same amount of oxidants but less antioxidants, with the concentrations of the latter increasing progressively from the first colostrum onwards. Calves may thus be at a higher risk of developing OS when fed colostrum rather than normal milk.

To the best of the authors' knowledge, there are no studies that have investigated the effect of the redox balance of colostrum on either the oxidative status of the calves or the acquisition of passive immunity. The present study aimed to evaluate, under field conditions, the effect of the redox profile of colostrum, as measured using lipoperoxide concentrations and the antioxidant activity of the colostrum, on serum ROS and serum antioxidant activity (SAC) in the first 3 months of life. Additionally, the effect of the redox profile of colostrum on passive immune transfer, as measured by determining serum immunoglobulin (Ig) G concentration, was assessed.

Materials and methods

All animal use was approved by the Bioethical Committee of the University of Santiago de Compostela.

Animals and housing

Twenty Holstein-Friesian calves (11 males, 9 females) born and kept on a commercial dairy farm in Arzúa – A Coruña (NW Spain) were used. Calves were housed in groups of three or four animals in a total of six pens, bedded on straw, allowing at least 3 m^2 per animal. All calves came from a full-term gestation and had been delivered by eutocic birth with little or no assistance. After delivery, calves were immediately separated from their dams, whose udder and teats were cleaned before being milked, and calves received 3 L of the first milked colostrum within the first 3 h of life. Thereafter they were fed milk replacer (Table 1) based on individual bodyweight every 12 h, giving them 2% of the calf's weight daily in milk replacer powder at a dilution rate of 15%, until the age of 6 weeks, when they were subsequently weaned. Grass hay, pelleted concentrate (Table 2) and water were offered ad libitum. Throughout the study period, calves were examined daily with assessment of appetite, rectal temperature, respiratory rate, umbilical and joint swelling and the presence/absence of diarrhoea.

Samplings and measurements

Blood was obtained by jugular venepuncture, into tubes without anticoagulants, 2 h after the ingestion of colostrum on the day of birth (day 0), and 6 (range: ±1), 13 (±1), 21 (±1), 29 (±1), 60 (±2) and 90 (±2) days later. Collected tubes were rapidly cooled on crushed ice and then centrifuged at 2000 *g* for 20 min, and serum collected. This was stored at -20 ºC until analysis up to 3 months after collection. A 20 mL sample of the colostrum fed to each animal was also obtained and kept frozen at -20 ºC until analysis.

Serum oxidative status determinations

The determinable reactive oxygen metabolites were quantified as an indicator of ROS (Alberti et al., 2000; Trotti et al., 2001), for which the d-ROMs Test (Diacron International) was used. This test determines hydroperoxides, breakdown products of lipids as well as other organic substrates, generated by the oxidative attack of ROS, through their reaction with the chromogen N, N-diethylparaphenylenediamine. Results are expressed in arbitrary 'Carratelli Units' (CarrU), where 1 CarrU is equivalent to the oxidizing power of 0.08 mg H_2O_2/dL.

Serum antioxidant capacity (SAC) was estimated as described by Trotti et al. (2001) using the OXY-Adsorbent Test (Diacron International). This test exploits the capacity of a solution of hypochlorous acid (HClO) to oxidise the complete pool of antioxidants in serum, and thus SAC is a measure of 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. The oxidative stress index (OSi) (Abuelo et al., 2013) was calculated as ROS/SAC.

Colostrum redox balance assessment

The levels of lipoperoxides (LPO), as markers of oxidative damage on lipids, were assayed in colostrum using the Lipocell test (Diacron International). This test is based on the ability of LPO to facilitate the oxidation of Fe^{2+} to Fe^{3+}; the Fe^{3+} produced binds to thiocyanate producing a coloured complex. The increase in absorbance is directly proportional to the concentration of lipoperoxides present in the sample. Results are expressed as nanoequivalents of hydroperoxides/mL. For this measurement, 10 mL of colostrum was defatted (centrifugation at 2500 *g*, 15 min, 4 ºC) and the supernatant assayed. In order to make the sample more watery so it could blend better with the reagents, it was incubated for 1 min at 37 ºC, prior to assay.

Antioxidant activity of the colostrum (‘barrier to oxidation’; CBO), was determined in the subnatant fraction using the OXY-Adsorbent Test (Diacron International). The balance between pro- and antioxidants was estimated using LPO/CBO.

IgG determinations in serum and colostrum

The concentration of IgG in both serum and colostrum samples were determined with a bovine specific ELISA commercial kit (FastELISA, RD-Biotech). The intra- and inter-analysis CVs were established as 5.3% and 8.7%, respectively.

Statistical analysis

All statistical analyses were performed using SPSS v.20 for Windows (IBM). A generalized linear mixed model (GLMM) with repeated measurements was used. Calf was the experimental unit, serum IgG the outcome variable, LPO, CBO, colostrum IgG concentration, ROS, SAC, OSi and parity of the dam were fixed effects and sex of the calf a random effect. Models like this one were built also for the outcome variables ROS, SAC and OSi, with the difference that serum IgG was a fixed effect in these models. The variance-covariance structure between 'time' was assumed to have a first-order autoregressive correlation (AR-1). Bonferroni corrections were included for post-hoc analysis. Correlations between measures in colostrum (LPO, CBO and IgG) and those analysed in serum (ROS, SAC, OSi and IgG) on day 0 and day 6 were investigated using Pearson's correlation.

Results

The mean ± SEM, median and range values of the measures analysed in colostrum are summarised in Table 3. Table 4 shows the effects of the different variables considered in the GLMM, while Figs. 1, 2, 3 and 4 illustrate, respectively, the change with time of the serum variables ROS, SAC, OSi and IgG. The degree of correlation between the serum and colostrum variables is shown in Table 5.

Mean ROS was significantly lower on day 0 than on all other days; it then remained stable from 6 to 90 days of age (Fig. 1). Serum ROS values on day 0 were positively correlated with colostrum LPO and colostrum redox profile (LPO/CBO), and on day 6 with the latter measure alone (Table 5). In the mixed model ROS was significantly influenced by SAC and OSi.

SAC did not vary significantly within the first month, but mean SAC on day 0 was lower than that recorded at months 2 and 3 (Fig. 2). In the mixed model SAC was significantly influenced by ROS, OSi and CBO, but SAC on both days 0 and 6 was not significantly correlated with any colostral measure.

On day 6 mean OSi was 87% higher than on day 0 (the higher value, the higher risk of OS), thereafter it remained stable until day 29 and decreased to levels similar to day 0 by 2 months (Fig. 3). A positive correlation was found on day 0 between OSi and the colostrum's redox profile, but not on day 6. The mixed model showed that OSi was influenced by ROS, SAC, CBO and LPO/CBO.

Age, LPO, LPO/CBO, colostrum IgG and the parity of the dam all had a significant effect on serum IgG in the mixed model. Serum IgG was higher 2 h after the ingestion of colostrum (day 0) than at any other time point (Fig. 4); after this time point mean IgG concentration remained constant until 1 month of age and then declined. On day 0, but not on day 6, serum IgG concentration was positively correlated with CBO. In contrast, serum IgG concentration was positively correlated with colostrum IgG concentration on day 6 but not on day 0 (see Table 5). This statistically significant correlation persisted from day 6 to day 21 (data not shown).

Discussion

The positive correlations of LPO/CBO with ROS at days 0 and 6, together with the positive correlation at day 0 between LPO and ROS and the negative one between CBO and ROS at day 6, suggest that calves may absorb pro-oxidant substances from colostrum, and that these substances can contribute to the reported marked increase of pro-oxidants just after delivery (Gaál et al., 2006; Albera and Kankofer, 2011). However, neither LPO nor LPO/CBO affected ROS, which may indicate that this relationship between colostrum and serum pro-oxidants has only a transient effect that only lasts for the first week of life.

In contrast with other studies, our results do not show a higher concentration of ROS in serum on day 0 than in older calves, although previous reports have suggested that this increase in pro-oxidants is transitory, with pro-oxidants levels declining by day 3 (Gaál et al., 2006) or within 24 h (Albera and Kankofer, 2011) of calving. Although we did not study pre-colostral ROS levels, taking together our results with these two studies, it seems that the increase in pro-oxidants associated with birth (Saugstad, 2003; Wiedemann et al., 2003) begins to decreases within 2 h after colostrum ingestion. However, at 6 days of age, ROS levels were higher than on day 0, as a consequence of the oxidative metabolism resulting from the increase in feed consumption and the independence of calves; as showed previously by Albera and Kankofer (2011).

As previously reported (Gaál et al., 2006; Albera and Kankofer, 2011), SAC remained stable during the first weeks of life, reaching higher values in the second and third months than recorded 2 h after colostrum intake. Our results indicate that the development of the antioxidant capacity took place over time. The lack of an increase in SAC between day 0 and weaning, suggests that supplementation of antioxidants to calves being fed artificial milk replacer may be of benefit. Such antioxidant activity is not usually taken into account when calf milk replacers are formulated (Soberon et al., 2012). Further research on the benefits of such anti-oxidant supplementation is needed.

We also found that CBO influenced the SAC of calves, indicating that the early development of the antioxidant defence of the calf depends on its dam; and as CBO and

SAC were not significantly correlated, it might be possible that the mechanism by which the CBO stimulates the development of the calf's SAC involves more complex processes than simply absorption from colostrum.

The balance between pro- and anti-oxidants was assessed by the calculation of the oxidative stress index (OSi), where an increase in the ratio indicates higher risk of OS due to increase in ROS production or defensive antioxidant consumption. The same approach was used for colostrum, and its redox profile is expressed as the LPO/CBO ratio. Results on OSi cannot be easily compared with the literature, since, to the best of our knowledge, this is the first report that uses this approach in new-born calves. Nevertheless, taking as an increase in ROS production (and thus an increase in OSi) is associated with birth (Saugstad, 2003; Wiedemann et al., 2003; Gaál et al., 2006; Albera and Kankofer, 2011), our finding that calves showed the lowest OSi value 2 h after colostrum intake indicates that colostrum intake helps the calf to counteract the after calving increase in ROS production. In line with this, the association between OSi and both CBO and LPO/CBO in this study, support our hypothesis that colostrum redox balance has an influence on the calf oxidative status and consequently more attention should be paid to the redox balance of colostrum, since it can directly increase the susceptibility of the calf to develop OS and its harmful consequences.

Gaál et al. (2006) reported that calves had greater serum pro-oxidants concentration than their dams at parturition. In the present study, the oxidative status of the dam was not evaluated; however, comparing the results on the oxidative status of the calves with those of adult cattle around parturition from another study of our research group (Abuelo et al., 2013), in which we used the same serum parameters, calves showed on day 0 a mean ROS concentration 64% lower than the lowest mean ROS concentration found in cattle in the studied time frame (121.4 ± 4.64; mean ± SEM). The lowest SAC value found in adult cattle throughout the transition period (425.8 ± 25.29; mean ± SEM) was 122% higher than mean calf SAC at day 0; and during the first 3 months of life, SAC values of calves were never more than the 68% of the lowest SAC of cattle. This suggests that calves have lower serum levels of ROS than cows and a lower antioxidant capacity. This is reflected in our findings that from day 6 to day 29 calves showed higher values of OSi than the highest OSi value showed by adult cattle in the study by Abuelo et al (2013). This further supports our suggestion that antioxidant supplementation may be of benefit in calves fed artificial milk replacer.

All of the colostrum samples showed an IgG concentration > 50 g/L, which is often considered as the cut-off point for high-quality colostrum (Kehoe et al., 2011). However, the methodology employed in previous studies that assessed redox balance in colostrum samples (Kankofer and Lipko-Przybylska, 2008; Albera and Kankofer, 2011), yielded results that are not comparable with our results, as they either used in-house protocols that differ in the basis of the commercial test we employed or measured only single oxidants/antioxidants. The age of the calf, the number of parturition of the dam and the concentration of IgG in colostrum all affected serum IgG of calves. The highest concentration of IgG in serum was found 2 h after the ingestion of colostrum; and although the maximal concentration of immunoglobulins are usually not achieved until 12-24 h after birth (Tizard, 2008), all calves after 2 h showed an IgG concentration above the 10 g/L failure of passive transfer of immunity threshold (Weaver et al., 2000). After absorption ceases, these passively acquired antibodies decline through normal catabolic pathways, and the calf must start to produce its own immunoglobulins, hence the decrease in IgG concentration compared to day 0. The effects of the dam's parity and the colostrum's IgG concentration are attributable to the fact that older Holstein cows produce colostrum with higher concentrations of IgG (Kehoe et al., 2011), and when a high amount of IgG is fed, serum IgG concentration in calves increases (Besser et al., 1985).

Colostrogenesis begins several weeks before parturition, with the mammary gland accumulating IgG (Brandon et al., 1971). However, colostrum itself is a source of redox reactions (Kankofer and Lipko-Przybylska, 2008) and immunoglobulins are molecules with a high susceptibility to peroxidation (Margiloff et al., 1998). In colostrum with high IgG concentrations, antioxidants may be consumed in an attempt to protect the antibodies, which might explain the negative correlation found in colostrum between antioxidants and IgG. Furthermore, our data showed that the redox balance of the colostrum plays a significant role in IgG absorption. This may explain the findings of Kamada et al. (2007) who demonstrated that selenium supplementation of colostrum increases IgG absorption; our results would suggest that selenium supplementation enhanced the antioxidant properties of the colostrum. Further research is required to confirm this hypothesis.

Conclusions

The redox balance of the colostrum plays a key role in counteracting the oxidative challenge associated with birth in calves and also in the passive transfer of immunity from the colostrum to the calf. Assessing the redox status of colostrum may be an additional measure of colostral quality which should be used alongside other measures such as IgG concentration. Additionally, the highest risk of oxidative stress was found in calves prior to weaning while they were being fed artificial milk replacer and the benefits of supplementing milk replacer with anti-oxidants should be further investigated.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Table 1: Composition of the milk replacer administered to the calves during their first 45 days of life.

Chemical composition (%)	
Crude protein	22,50
Fat	18,50
Ashes	7,30
Calcium	0,85
Phosphorous	0,60
Sodium	0,45
Crude fibre	0,20
Vitamins	
Vitamin A (I.U./kg)	50.000
Vitamin D_3 (I.U./kg)	10.000
Vitamin C (mg/kg)	500
Vitamin E (all-rac α-tocopherol) (mg/kg)	160
Oligoelements (mg/kg)	
Zinc	60
Iron	50
Manganese	35
Cupper	7
Cobalt	0,60
Selenium	0,45
Iodine	0,15
Antioxidants (mg/kg)	
Propyl gallate	26,5
Butylated hydroxytoluene	9

377 **Table 2: Composition of the pelleted concentrate offered ad libitum to the calves**
378 **throughout the study period.**

Chemical composition (%)	
Crude protein	17,00
Ashes	6,50
Fibre	5,50
Fat	4,20
Sodium	0,43
Vitamins (IU/kg)	
Vitamin A	10.000
Vitamin D_3	2.000
Vitamin E (all-rac α-tocopherol)	40
Dietary minerals (ppm)	
Zinc	80
Manganese	40
Iron	16
Cupper	5
Iodine	0,40
Cobalt	0,30
Selenium	0,30
Antioxidants (ppm)	
Butylated hydroxytoluene	0,75

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Table 3: Mean values (± standard errors of the mean) of lipoperoxides (LPO), colostrum barrier to oxidation (CBO), colostrum redox profile (LPO/CBO) and immunoglobulin G (IgG) in colostrum samples.

	Mean ± SEM	**Median**	**Range**
LPO (nanoEq of hydroperoxides/mL)	422.1 ± 41.72	370.8	182.9 to 853.3
CBO (µmol HClO/mL)	293.8 ± 23.67	350.0	98.62 to 388.28
LPO/CBO (Arbitrary units)	1.9 ± 0.44	1.12	0.69 to 7.88
IgG (g/L)	69.3 ± 3.91	62.5	50.5 to 109.6

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Table 4: Effects of calf age, serum antioxidant activity (SAC), reactive oxygen species (ROS), oxidative stress index (OSi), serum immunoglobulin G (IgG), colostrum's lipoperoxides (LPO), barrier to oxidation (CBO), redox profile (LPO/CBO) and IgG content and parity of the dam on serum measures of passive immunity and redox status.

	Effect																			
Item	Age		SAC		ROS		OSi		Serum IgG		LPO		CBO		LPO/CBO		Colostrum IgG		Number of previous parturitions	
	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Serum IgG	**100.62**	**<0.01**	3.82	0.053	0.15	0.70	3.69	0.057	--	--	**12.4**	**<0.01**	2.21	0.14	**8.62**	**<0.01**	**11.2**	**<0.01**	**3.84**	**0.012**
OSi	0.34	0.562	**215.42**	**<0.01**	**555.90**	**<0.01**	--	--	1.91	0.17	0.82	0.37	**10.87**	**<0.01**	**5.82**	**0.017**	0.21	0.65	0.49	0.69
ROS	0.10	0.75	**117.2**	**<0.01**	--	--	**371.5**	**<0.01**	0.004	0.95	0.16	0.69	2.06	0.15	2.92	0.09	0.47	0.50	0.64	0.59
SAC	0.97	0.33	--	--	**116.0**	**<0.01**	**154.6**	**<0.01**	1.85	0.18	0.554	0.46	**4.06**	**0.046**	1.75	0.19	0.11	0.74	1.20	0.31

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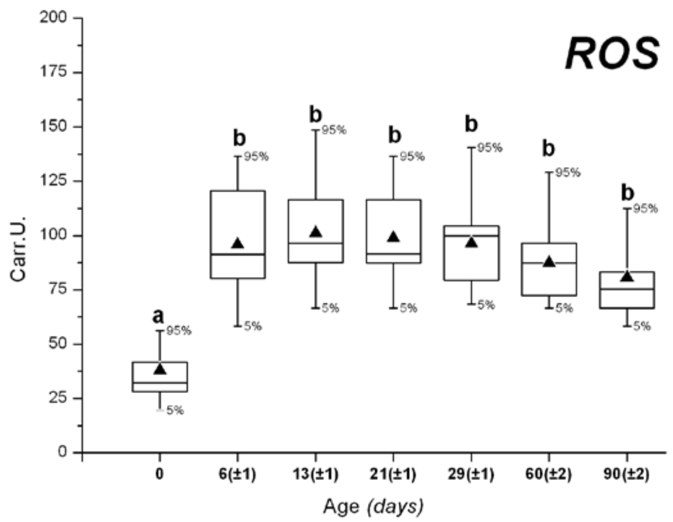
Table 5: Correlations between the redox balance and the immunoglobulin G concentration of colostrum with serum measures of passive immunity and redox status in calves.

	Colostrum	Calves							
		Age = 0 days [a]				Age = 6(±1) days			
	IgG	ROS	SAC	OSi	Serum IgG	ROS	SAC	OSi	Serum IgG
LPO	n.s.	0.49*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CBO	-0.56**	-0.28**	n.s.	n.s.	0.50*	-0.38*	n.s.	n.s.	n.s.
LPO/CBO	0.41**	0.61**	n.s.	0.43*	n.s.	0.45*	n.s.	n.s.	n.s.
Colostrum IgG	--	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.59**

[a], The first sampling took place on the day of birth, 2 h after the ingestion of colostrum; LPO, lipoperoxides in colostrum; CBO, colostrum barrier to oxidation; LPO/CBO, colostrum redox profile; IgG, immunoglobulin G; ROS, Reactive Oxygen Species; SAC, Serum Antioxidant Capacity; OSi, Oxidative Stress Index; *, P <0.05; ** P <0.01; n.s., P >0.05

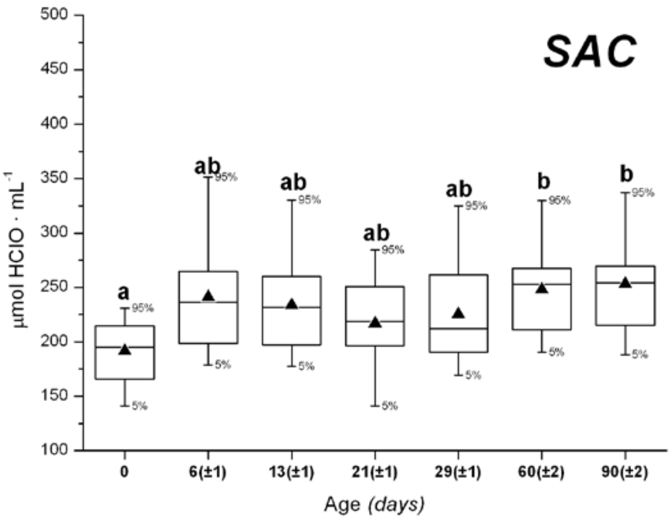
Figure legends

Fig. 1. Changes in time of the reactive oxygen species (ROS), in calves' serum throughout the study.



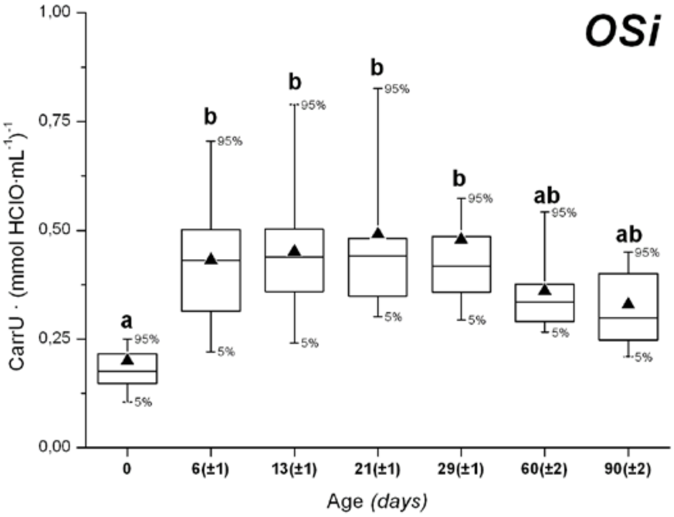
Boxes are delimited by the 25th and 75th percentiles. The horizontal line within the box represents the median, whereas the mean is symbolized by a triangle. Whiskers represent the 5% and 95% confidence limits. Values without a common superscript are statistically different (*P*<0.05).

Fig. 2. Changes in time of the serum antioxidant capacity (SAC) in calves' serum throughout the study.



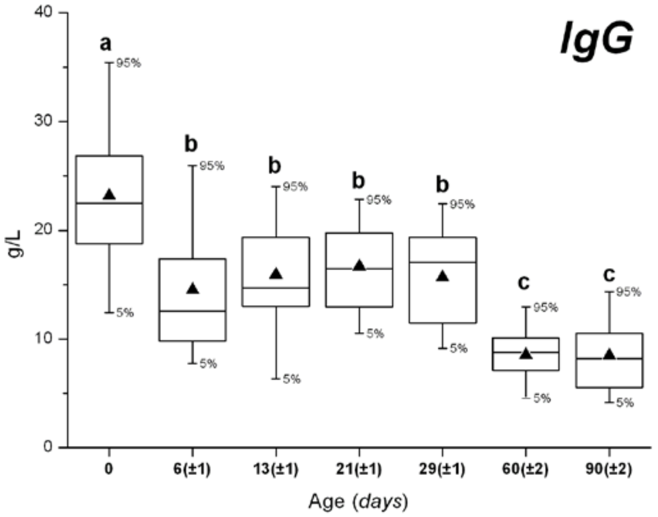
Boxes are delimited by the 25th and 75th percentiles. The horizontal line within the box represents the median, whereas the mean is symbolized by a triangle. Whiskers represent the 5 and 95% confidence limits. Values without a common superscript are statistically different (P<0.05).

Fig. 3. Changes in time of the oxidative stress index (OSi) in calves' serum throughout the study.



Boxes are delimited by the 25th and 75th percentiles. The horizontal line within the box represents the median, whereas the mean is symbolized by a triangle. Whiskers represent the 5 and 95% confidence limits. Values without a common superscript are statistically different (P<0.05).

Fig. 4. Changes in time of the immunoglobulin G (IgG) concentration in calves' serum throughout the study.



Boxes are delimited by the 25th and 75th percentiles. The horizontal line within the box represents the median, whereas the mean is symbolized by a triangle. Whiskers represent the 5 and 95% confidence limits. Values without a common superscript are statistically different (P<0.05).