The Veterinary Journal 199 (2014) 295–299

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

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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ARTICLE INFO

Article history:
Accepted 23 October 2013

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

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/anti-oxidative 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.

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.

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http://dx.doi.org/10.1016/j.tvjl.2013.10.032

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 (IU/kg)	50,000
Vitamin D_3 (IU/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

Table 2
Composition of the pelleted concentrate offered ad libitum to the calves 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	2000
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

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 °C 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 °C until analysis.

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	182.9–853.3
CBO (μmol HClO/mL)	293.8 ± 23.67	350.0	98.62–388.28
LPO/CBO (Arbitrary units)	1.9 ± 0.44	1.12	0.69–7.88
IgG (g/L)	69.3 ± 3.91	62.5	50.5–109.6

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 oxidising 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 were defatted (centrifugation at 2500 *g*, 15 min, 4 °C) 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 °C, 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