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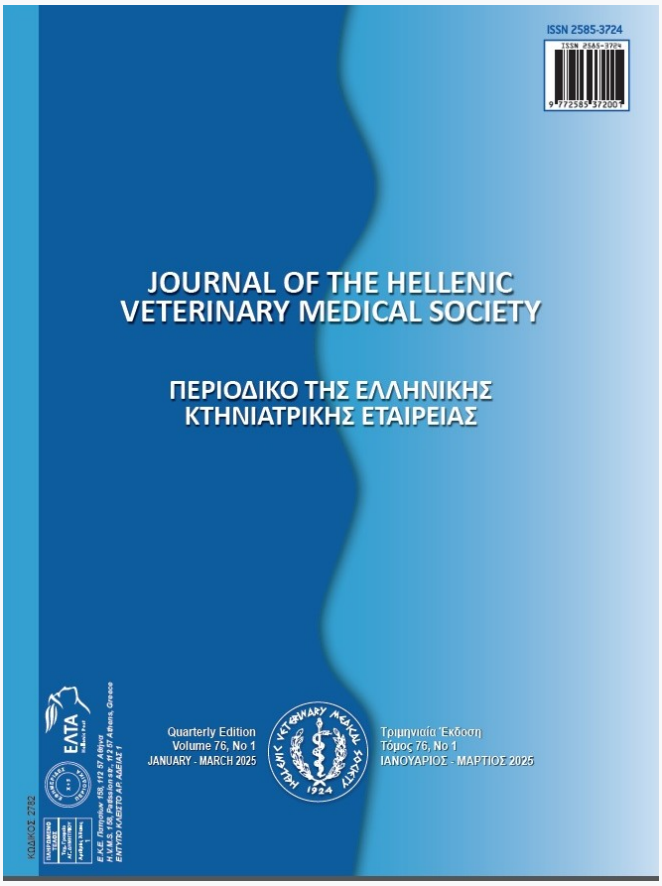

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Research article
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Redox balance in dairy cattle and their calves at birth and its relationship to colostral passive immunity

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ABSTRACT: Newborn calves are exposed to oxidative stress (OS) just after birth, and yet the role of colostrum in OS is not well detailed. The redox indicators, total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI)] along with immunoglobulin-G (IgG) concentrations were assessed in 21 healthy Swiss Brown mother-calf pairs. Furthermore, alterations in theseparameters and IgG concentrations were analysed in an additional 23 healthy calves during the first 45 days of life (day 0 = at birth, 1, 3, 7, 14, 28 and 45). TAS and TOS concentrations were determined calorimetrically and IgG level by commercial ELISA kit. A negative correlation was determined between colostral IgG and serum maternal TOS (r:-0.49, P<0.05) and colostral TAS (r:-0.54, P <0.05). Calves exhibited elevated levels of TOS (8.29±0.30), TAS (0.71 ± 0.02) and OSi (1.20 ± 0.08) at birth. However these levels decreased after the 24th hour (6.72 ±0.2, 0.65 ±0.03, and 1.05 ±0.05, respectively) and remained stable throught the study. In conclusion, our results suggest that newborn calves experience OS after birth and colostral antioxidants may highlights its potential role in mitigating OS in these calves. The results may be of help in improving antioxidant capacity, either by treating dams during pregnancy or by treating colostrum with substances with antioxidant properties.

Keywords: Calves; oxiditive stress; redox status; Passive transfer of colostral immunity; Newborn health

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INTRODUCTION

The neonatal period is crucial for calves, as most illnesses and deaths occur during this time, especially in the first days of life, which has negative economic and welfare implications for cattle farmers (Lora et al., 2018). Newborn calves are particularly vulnerable at birth because the syndesmochorial structure of the placenta prevents the transfer of many beneficial substances to the fetus such as immunoglobulins (Ig) and antioxidants, which they must absorb from the colostrum into the bloodstream via enterocytes, a process known as passive transfer of colostral immunity (PTCI) (Cuttance et al., 2019; Lombard et al., 2020). Failure of PTCI has been associated with increased risk of morbidity and mortality in neonatal or preweaning calves (Godden et al., 2019). Although studies on colostrum and colostrogenesis have largely focused on IgG, colostrum feeding may also promote bioactive factors that support calf growth, health and development (McGrath et al., 2016; Gökçe et al., 2019. Fischer-Tlustos et al., 2021).

Living organisms, including calves, possess an antioxidant mechanism that functions to combat free radicals, also known as metabolic residues (Mutinati et al., 2014). This process is integral to maintaining cellular health, and is known as redox balance, which refers to the balance between oxidants and antioxidants. In the absence of antioxidants, the balance shifts in favour of oxidants, resulting in oxidative stress, which can be dangerous because it leads to the disruption of cell membranes and damage to proteins, lipids, and DNA (Mittal et al., 2014; Celi et al., 2020; Hejel et al., 2021).

Colostrum, the first feed for newborns, reportedly contains substances that act as antioxidants [enzymatic (lactoperoxidase, superoxide dismutase-SOD, catalase, and glutathione peroxidases-GSH-Px) and non-enzymatic (α-tocopherol, β-carotene, vitamin C, ceruloplasmin, lactoferrin, selenium, copper, zinc, cysteine etc.)] which are essential against OS induced in newborns during and after parturition (Przybylska et al. 2007; Pandey et al., 2011). Since the substances like IgG in colostrum are absorbed by active pinocytosis in the intestine of calves, antioxidants may also be absorbed in the same way as IgG after colostrum ingestion leading to increased blood antioxidant concentrations.

Newborn calves may develop oxidative stress (OS) due to adaptation to adverse extrauterine conditions compared to intrauterine conditions, maternal OS; developed and possibly transmitted during birth, and even colostrum intake (Mutinati et al., 2014; Cigliano et al., 2014). The mechanisms of OS affecting growth, metabolism, development, and health in ruminants have been the subject of numerous studies (Celi and Gabai 2015; Jacometo et al. 2016; Cuervo et al., 2021, Nayak and Abuelo 2021). Although OS is a new area of research in ruminants, there is a clear evidence of ist role in the development, progression, and maintenance of pathological conditions such as sepsis, diarrhoea and pneumonia (Al-Qudah, 2009; Sordillo and Aitken, 2009; Colitti et al., 2019). The parameters of OS and its changes at birth in dam and their offspring, in colosturm, and in neonatal calves after colostrum feeding have not been studied in detail.

The relationship between redox status and IgG concentrations in the serum of dams and their offspring, as well as in colostrum, remains to be elucidated. Furthermore, the paucity of research investigating birth-related OS in calves has been compounded by the heterogeneity of the sampling and measurement methods employed, resulting in the generation of controversial results (Abuelo et al., 2019). The objectives of this study are i) to investigate the effects of birth on oxidative stress parameters and IgG concentrations in colostrum and calves and ii) to monitor physiological changes in oxidative stress parameters in healthy calves during the first 45 days of life in order to determine changes in oxidative stress parameters at birth and after colostrum intake.

MATERIALS AND METHODS

The study was approved by Kafkas University Institutional Ethical Committee for Animal Care and Use (Code: KAU-HADYEK/2008-20).

Animals

The animals included in the study were Swiss Brown dairy cows and their calves from the Research and Training Farm of Kafkas University in Turkey. There were a total of 42 cows on the farm and 41 calves born to them. Cows in their second lactation with an approximate live weight of 400 kg and an average milk yield of 20 litres/day, considered healthy based on clinical examination, with no twin births and no dystocia/intervention during parturition were included. All animals in the study were subjected to the same management and feeding regimes. During the dry period, each cow was fed a ration containing 7.5 kg grass and 3.5 kg concentrate (grains, corn, sunflower, cottonseed, soybean) whereas during the lactation period a ration containing 7 kg grass, 7 kg corn silage and 7.5 kg concentrate was provided. Calving took place in a 4 week period between January and Febru-

ary, 2012. There was no intervention during calving. Calves were bottle fed with colostrum at the amount of 6% of their birth weight within 4 hours of birth and then fed again with the same amount 8-10 hours later. Calves were fed by transition milk twice a day for the first 3 days of life, not exceeding 10% of body weight/day. Thereafter, calves were similarly fed with fresh pooled milk. They also had *ad libitum* access to grass hay, calf starter pellets consisting of grains, molasses, corn, cottonseed, soybean, and water.

Clinical Examination

Calves were subjected to daily clinical examinations during the study period (0 to 45 days of age) by the first author for the presence or absence of loss of appetite, umbilical and joint swelling, and diarrhea. In addition, the absence of findings related to systemic inflammatory response syndrome (abnormal rectal temperature, abnormal respiratory rate and heart rate per minute) was used as a criterion for inclusion. Those considered healthy based on clinical and haematological examinations were included in the study.

Blood Collection

Experiment 1

Blood samples were collected from the coccygeal arter or vein into clot-activated tubes (BD Vacutainer, BD, Franklin Lakes, NJ) and colostrum samples into 10-mL glass tubes (Venoject, Code: VT-100SU, Terumo) from 21 cows within the first 4 hours after birth. Blood samples were also collected from their calves from the *vena jugularis* (11 males, 10 females) at 24 hours of age.

Experiment 2

Blood samples were collected at birth (day 0) and on days [1, 3, 7, 14 (±1), 28 (±2) and 45 (±2)] after colostrum intake from 23 healthy calves (12 male, 11 female) from the jugular vein into clot-activated tubes.

Preparation of serum samples

Serum samples were harvested by centrifugation (Hettich Rotina 380R, Hettich, Germany) at 4000 rpm for 10 min and stored at -20°C until analyses.

Preparation of colostrum samples

Colostrum samples were centrifuged at 4000 rpm for 30 min to remove fat and sediment. Samples were stored in 1.5 mL microcentrifuge tubes at -20°C until analysis. Prior to analysis, the solid phase of the colostrum samples was diluted (100-fold) in physiological saline and was filtered twice through a nylon-66 syringe filter (0.45-µl Millipore, USA) to obtain transparent samples (Gokce et al., 2014; Atakisi et al., 2010). Transparent samples were used for the analyses detailed below.

Measurement of total antioxidant, and oxidant capacity

In colostrum (Transparent) and serum samples, total antioxidant (TAS, mmol Trolox equiv./L) and oxidant (TOS, µmol H2O2 equiv./L) capacities were determined colorimetrically (PowerWave XS, BioTek, Instruments, USA) using a commercially available kit (Rel Asssay Diagnostic, Gaziantep, Turkey). Briefly, total oxidation status (TOS) was determined according to the technique described by Erel, (2005) which is based on the oxidation of ferrous to ferric ion in the presence of different oxidative species in acidic medium and the measurement of the ferric ion by xylenol orange and total antioxidant capacity was assessed as described by Erel, (2004) which is based on the bleaching properties of a more stable 2,2- azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid - ABTS) radical cation induced by antioxidants. The inter and intra assay coefficient of variation for TAS and TOS kits were 2.8 and 3.3%, and 3.2%, and 3.9%, respectively. OSi was used as a reflection of the oxidant/antioxidant balance and was calculated using the following formula: OSI (arbitrary unit) = [(TOS)/(TAS) × 100] (Abuelo et al., 2013; Ranade et al., 2014; Cecchini et al., 2018) and expressed as arbitrary unit (au).

Serum and Colostrum IgG Assays

Serum and colostrum IgG concentrations were measured using a commercial ELISA kit (Bovine IgG ELISA kit, Bethyl Laboratories Inc., Texas, USA) as per manufacturer instruction. The inter and intra assay coefficient of variation for IgG were 6.1% and 2.6%, respectively.

Statistical analysis

Data were collected and entered into a database (Microsoft access). Statistical analysis of the data was performed using SPSS 26.0. Descriptive analyses of data were performed. Data were first tested for normality using the Kolmogorov Smirnov test. In the second experiment, serum IgG, TAS, TOS and OSi levels on days 0, 1, 3, 7, 14, 28 and 45 in healthy calves were compared using one-way repeated-measures ANOVA which was followed by post-hoc Tukey test. In the first experiment, the Tukey HSD ANOVA test was used

to compare IgG, TOS, TAS and OSi levels in colostrum and serum of cows and their calves. A model was built in which the dependent variable was 'Serum IgG' and the independent variables were 'TAS', 'TOS' and 'OSi'. Fixed effects included number of trials, number of days and colostrum intake. A boxplot was used to observe the distribution, spread, median and outliers of these dependent and independent variables on different days (0, 1, 3, 7, 14, 28 and 45). In addition, Duncan's test was used to compare the means between the groups on different days and the results are presented on the boxplot graph. The relationship between serum and colostrum IgG, TOS, TAS and OSi levels was examined by Pearson correlation. The results of statistical analyses are presented as least square means ± standard error (SE) of means. For all analyses, values of P<0.05 were considered significant.

RESULTS

Experiment 1

The serum and colostrum TAS concentrations of the dam at birth were 0.42 ± 0.02 and 0.50 ± 0.03 mmol Trolox equiv./L, respectively (Figure 1A). These values were significantly lower than those observed in their calves' serum TAS (0.78 ±0.06; mmol Trolox Equiv./L; p <0.001). Colostrum and serum TOS concentrations of dams at birth and calves at 24h of age were 8.87 ± 0.99, 6.14 ± 0.28 and 8.71 ± 0.82 µmol H2O2 equiv./L, respectively (Figure 1B). The TOS level in maternal serum was significantly lower than that in colostrum and calf serum (P <0.008) (Figure 1C). Serum OSi levels in mothers (1.27 ± 0.06 au) and their calves (1.18 ± 0.11 au) were similar, but significantly lower (P <0.001) when compared with colostrum (2.34 ± 0.33 au). The serum IgG concentrations of the dams (868.4 ± 87.3 mg/dL) and their calves (1277.9 ± 106.7 mg/dL) were similar (Figure 1D), but significantly lower (P <0.001) compared with colostrum (3340.9 ± 343.9 mg/dL).

Colostral TAS had a negative significant correlation with colostral IgG (r:-0.54, P <0.05). Similarly, a statistically significant negative correlation was noted between colostral IgG and maternal serum TOS (r:-0.49, P<0.05). A statistically significant positive correlation was found between maternal serum OSi and calf serum TAS (r: 0.51, P<0.05) (Figure 2).

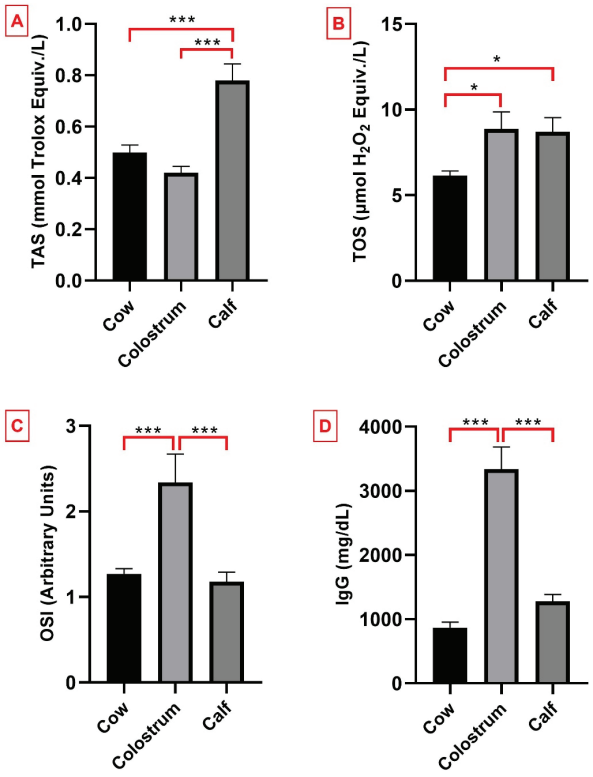

Figure 1. Changes in oxidative stress parameters and IgG concentration measured at birth in colostrum and serum of cows and serum of calves at 24 h of life. A: Total Antioxidant Status (TAS), B:Total Oxidative Status (TOS), C: Oxidative Stress Index (OSi), D: Immunoglobulin G (IgG). *P<0.05, ***P<0.001.

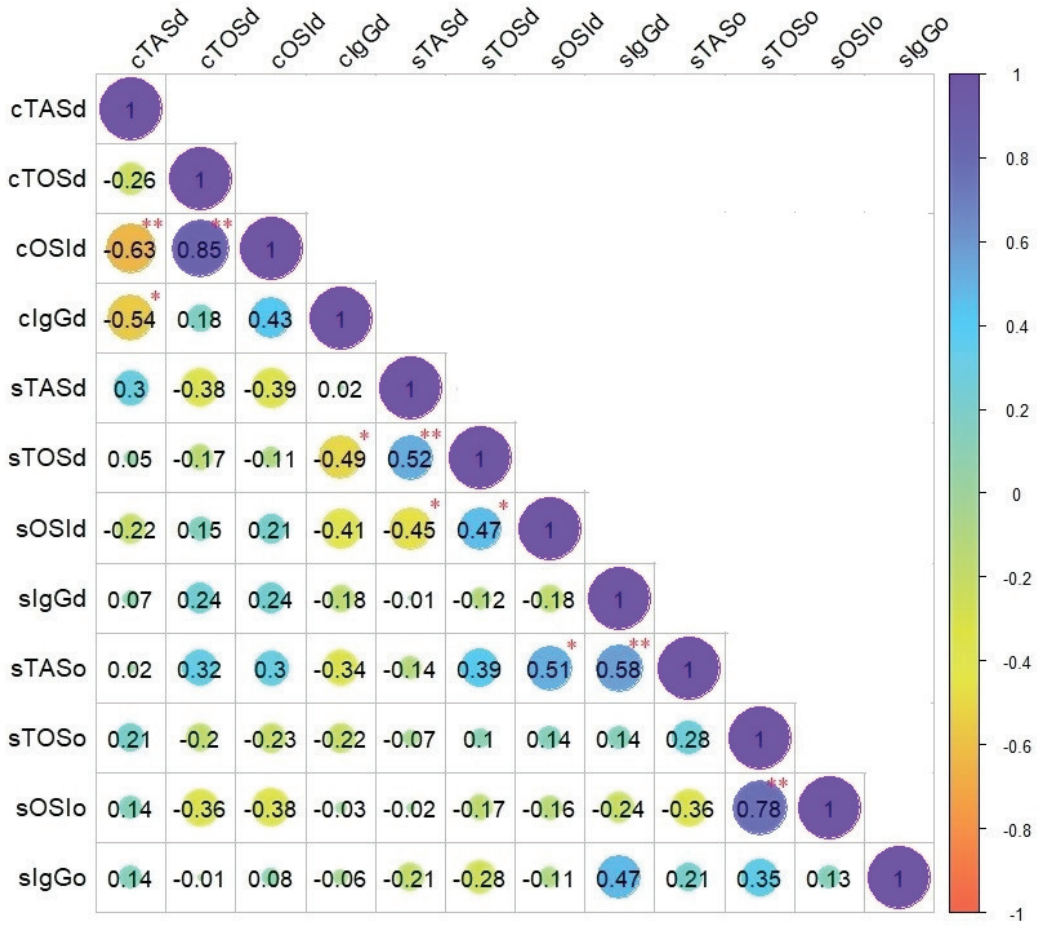

Figure 2. Pearson's correlation coefficients between TAS, TOS, OSi and IgG levels of dam and their calves. cTASd: Colostral Total Antioxidative Status of dam, sTASd: Serum Total Antioxidative Stats of dam, sTASo: Serum Total Antioxidative Status of offspring, cTOSd: Colostral Total Oxidative Status of dam, sTOSd: Serum Total Oxidative Status of dam, sTOSo: Serum Total Oxidative Status of offspring, cOSid: Colostral Oxidative Stress Index of dam, sOSid: Serum Oxidative Stress Index of dam, sOSio: Serum Total Oxidative Stress Index of offspring, cIgGd: Colostral Immunoglobuline G of dam, sIgGd: Serum Immunoglobuline G of dam, sIgGo: Serum Immunoglobuline G of offspring, * P<0.05, ** P<0.01

Experiment 2

Serum TAS was higher before colostrum feeding and decreased slightly after colostrum feeding. It remained stable throughout the study except on day 3 when a statistically significant decrease was observed (P=0.037). TAS concentrations at birth (before colostrum intake), days 1, 3, 7, 14, 28 and 45 were 0.71 ± 0.02, 0.65±0.03, 0.61±0.02, 0.68±0.01, 0.68±0.02, 0.65±0.03 and 0.63±0.03 mmol/Trolox equiv/L, respectively (Figure 3A). Serum TOS concentrations decreased significantly (P<0.001) after colostrum intake and remained low during the neonatal period (first 28 days of life), but increased slightly and insignificantly on day 45. Serum TOS concentrations on days 0, 1, 3, 7, 14, 28 and 45 were 8.29±0.30, 6.72±0.2, 5.46±0.27, 5.77±0.18, 5.42±0.32, 28=5.44±0.17 and 6.21±0.21 μmol H2O2 equiv/L, respectively (Fig. 3B). Calculated OSi values decreased (P<0.001) during the neonatal period (day 1=1.05 ± 0.05, day 3=0.89 ± 0.04, day 7=0.80 ± 0.03, day 14=0.82 ± 0.05, day 28=0.89 ± 0.06 and day 45=1.1 ± 0.62 au) when compared with the value determined before colostrum intake (day 0=1.20 ± 0.08 au) (Figure 3C). The serum IgG (mg/dL) concentration of the calves increased significantly after colostrum feeding (P<0.001) and remained high throughout the study. The values were 14.66 ± 1.40, 1332 ± 168, 1638 ± 273, 1332 ± 214, 990 ± 76, 977 ± 59 and 974 ± 94 for days 0 (pre-colostrum intake), 1, 3, 7, 14, 28 and 45, respectively (Figure 3D).

The correlation between serum TAS and TOS was negative on day 0 and 28 and positive on other days, but it was only statistically significant on day 28 (P<0.01). The correlations between serum IgG and TOS, TAS or OSi were not significant (Table 1).

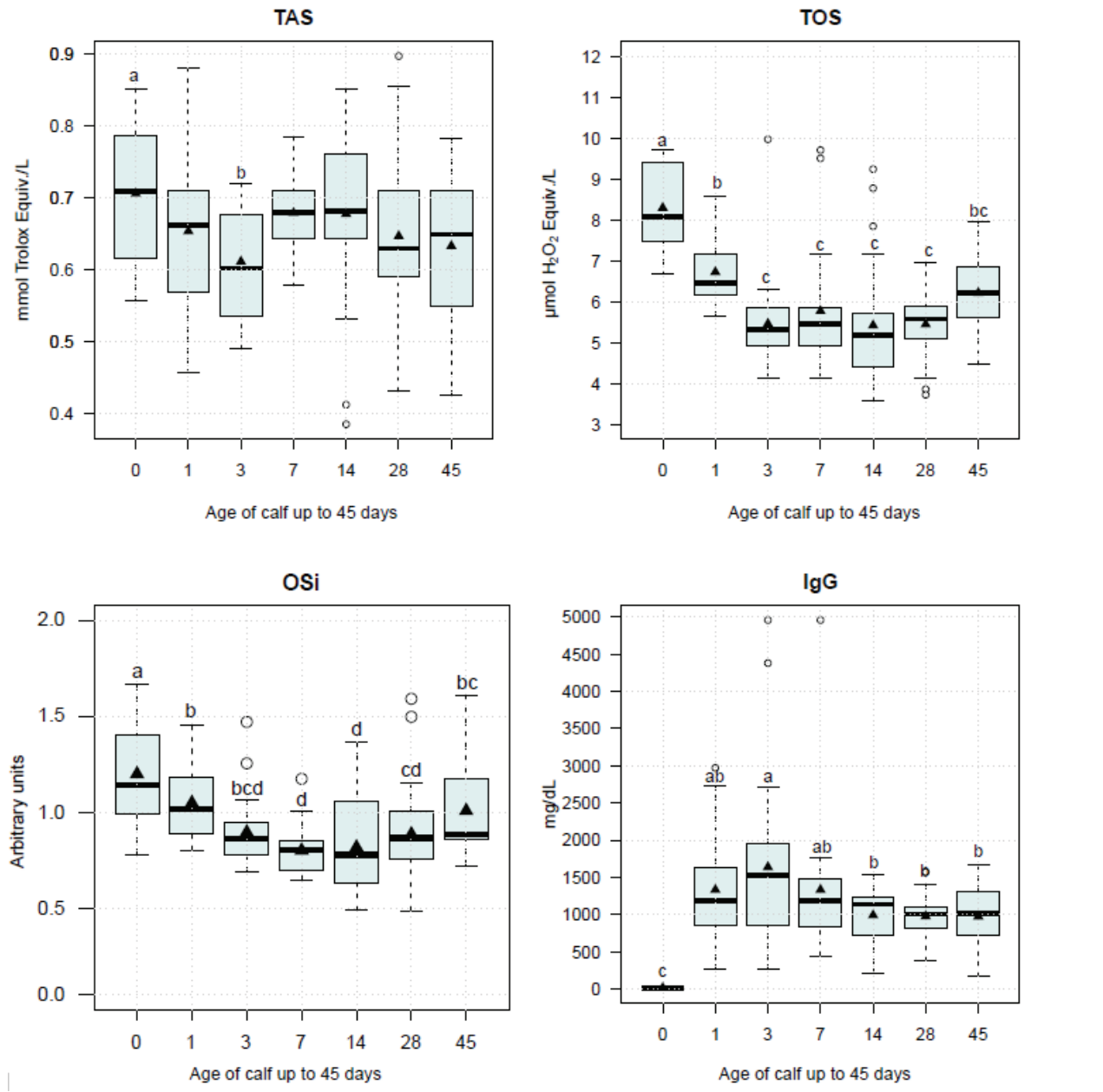

Figure 3. Changes in redox balance and IgG concentration measured before (Day 0) and after colostrum intake (Days 1, 3, 7, 14, 28 and 45) in calves (n = 23). A: Total Antioxidant Status (TAS), B:Total Oxidative Status (TOS), C: Oxidative Stress Index (OSi), D: Immunoglobulin G (IgG). Different letters refer to significant differences between the values (P<0.05).

Table 1. Correlation between total antioxidant status (TAS), total oxidative stress (TOS), oxidative stress index (OSi) and immuglobilin G (IgG) concentrations in healthy dairy calves (n=23).

	Days						
Parameters	**0*** **	**1**	**3**	**7**	**14**	**28**	**45**
IgG and TAS	0.26	-0.218	0.434	0.11	-0.115	-0.2	0.372
IgG and TOS	-0.145	-0.043	0.478	0.244	0.171	-0.037	0.104
TAS and TOS	-0.403	0.311	0.356	0.204	0.239	-0.588**	0.126
OSi and TOS	0.835**	0.343*	0.853**	0.866**	0.694**	0.829**	0.572*
OSi and TAS	-0.832**	-0.776**	-0.172*	-0.251*	-0.515*	-0.898**	-0.722**
OSi and IgG	-0.242	0.206	0.243	0.116	0.116	-0.222	-0.218

= *P<0.01, *=P<0.05, day 0**=before colostrum intake

DISCUSSION

The neonatal period, especially the first weeks of life, is the most critical time for calves, as they are exposed to an unfavourable environment and many pathogens while their immune system is not yet sufficiently developed. Therefore it is important to understand the mechanisms involved in the adaptation of neonatal calves to extrauterine life (Cecchini and Fazio, 2021; Surai and Earle-Payne 2022). Oxidative stress experienced during parturition and the role of colostrum in coping with OS was evaluated in this study.

Methods that determine antioxidants/oxidants as a whole more accurately reflect the redox status and are also simpler, more sensitive, more practical (Erel, 2004; Erel, 2005) and more reliable when compared to those methods that measure parameters individually (malondialdehyde, nitric oxide, SOD, GSH-Px etc.) (Kankofer and Lipko-Przybylska, 2008). In addition, it may be useful for determining globally accepted reference intervals, assessing treatment efficacy and animal welfare, designing antioxidant treatments, and monitoring nutritional status (Celi, 2011; Soriano et al., 2015). Measurement of total oxidant status (TOS) as reactive oxygen metabolites (ROMs) and total antioxidant status (TAS) as biological antioxidant potential (BAP) by commercial kits has become an attractive options to assess oxidative stress (Celi, 2010). Researchers have recently used oxidative stress index (OSi) to better reflect OS status as OSi (ratio of oxidants to antioxidants) more sensitively reflects oxidative stress (Abuelo et al., 2014; Ranade et al., 2014). In this study commercial kits were used to determine oxidant/antioxidant status in calves.

Our results indicated changes in redox balance at birth and after colostrum intake and a relationship between redox status and IgG in the neonatal period. Total oxidant status, the indicator for all ROS, was high at birth and gradually decreased and generally remained stable throughout the study period, which correlated well with colostrum intake. This stability of TOS has been attributed to robust maintenance of redox haemostasis (Ranade et al., 2014), as reports suggest that oxidative stress is present at birth, but newborns calves are able to deal with it (Gaal et al., 2006). It has been previously reported that the increase in OS is transient and decreases within the first week of life (Abuelo et al., 2014; Ranade et al., 2014). This transient increase was explained by the immature defence system against superoxide radicals in neonates (Inanami et al., 1999). However, adequate, good quality and timely intake of colostrum by calves is important to mitigate the effects of oxidative stress, as previous studies reported a decrease in OS at 24 hours (Albera and Kankofer 2011), and 3 days of age (Gaal et al., 2006). Similarly, OS parameters were high before colostrum intake and then decreased in our study. This finding may reflect that antioxidants, possibly present in colostrum, enter the circulation by the same mechanism as IgG in ruminants and actively combat OS. A previous study also reported that OS concentrations remained relatively stable during the first 18 weeks of life as a result of possible antioxidant activity in calves (Ranade et al., 2014). However, it remains to be investigated in detail whether the gradual decline in OS after birth is due to the transfer of antioxidants via colostrum, the activation of antioxidant pathways in the calf, as TAS was higher at birth in our study, or a combination of both (Abuelo et al., 2019).

OSi is an indicator of redox balance. Its increase indicates raised ROS production or depletion of antioxidants (Abuelo et al., 2019). In parallel with OS, OSi was also higher at birth and then gradually decreased after colostrum ingestion in our study. This finding is consistent with previous studies conducted in dairy cattle (Abuelo et al., 2013), in dairy calves in the first 90 days of life and colostrum (Abuelo et al., 2014), in dairy heifer calves in the first 18 weeks of life (Ranade et al., 2014), sheep (Cecchini et al., 2018), and lambs (Chauhan et al., 2016; Gökçe et al., 2022). In the present study, the detection of a decrease in TOS and OSi levels from the first day of colostrum intake shows that the calves are successfully combatting oxidative stress at birth and thereafter. A gradual increase in total antioxidant capacity in milk after birth (first 12 days) could explain this decrease in OSi (Albera and Kankofer 2011). Similarly, decreased OSi values within the first months of life in calves when compared with the value obtained at 2 h after birth was reported (Abuelo et all., 2014).

The colostrum quality is of paramount importance not only for good neonatal management but also for reducing the risk of neonatal diseases as colostrum contains many substances such as lactoferrin, lactoperoxidase, Ig, vitamins which may also interact (Gokce et all., 2021a; Gokce et all., 2021b; Soufleri et al., 2021). Oxidants are reported to be associated with decreased IgG concentrations in colostrum of cattle and serum of their ofspring, while antioxidants were associated with higher Ig concentrations in colostrum

and serum of calves (Singer et al., 2009; Çolakoğlu et al., 2021). In our study, a negative correlation was found between maternal serum TOS and colostral IgG concentration. This finding indicate that the concentration of colostral IgG may decrease as maternal OS markers such as TOS increase. Similarly, an adverse effect of oxidative stress on immune function and serum IgG concentrations has been reported in different conditions (Ercal et al., 2000, Abuelo et al., 2014; Bertoni et al., 2004), presuemably due to the detrimental effect of free radicals on the functions of proteins including IgG (Çolakoğlu et al., 2021). Studies indicate that colostral redox balance may appears to play an important role in immunoglobulin absorption in calves.

It is not surprising that significant and negative correlations were found between colostral IgG and TAS in our study. This finding confirms previous data (**Abuelo et al., 2013;** Abuelo et al., 2014). The previous results led researchers to add selenium, an antioxidant, to colostrum, in order to increase the IgG absorbtion (Kamada et al., 2007). Colostrum contains easily oxidized macromolecules such as lipid or proteins (immunglobulins) and also has a significant immune cell population including macrophages that use ROS generating systems to lyse bacteria. This makes colostrum sensitive to oxidant reactions which may cause the depletion of antioxidants. The increase in ROS production in the transition period and the loss of vitamins and minerals during the production of colostrum contribute to the decrease in antioxidant capacity of colostrum (Abuelo et al., 2014). Therefore, a negative correlation can be observed between IgG and antioxidant in colostrum, as was also the case in our study. Similar to our findings, the antioxidant activation in colostrum has been determined to be low when compared with that in plasma of cows and their calves (Albera and Kankofer 2011). The supplementation of colostrum and mother during transition period with feed or drugs with antioxidant properties before birth can protect calves against OS and reduce mortality rates by increasing IgG concentrations (Heinrichs et al., 2020; Robbers et al., 2021). However, since the mechanisms of these hypotheses can not be elucidated, further studies are needed.

In contrast to the finding that TAS concentrations of sheep and their lambs did not differ on the first day of life (Soriano et al., 2015), we determined that calves had higher serum antioxidant concentrations than their mothers, which decreased after colostrum ingestion and then remained stable during the study period as previously reported (Gaal et al., 2006; Albera and Kankofer 2011; Ranade et al., 2014).

A negative and significant correlation between OSi and biological antioxidant potential after birth in calves has been reported (Ranade e al., 2014). This is an expected situation, as antioxidants act to counteract oxidant substances in order to maintain the redox balance. This was also the case in our study, there were negative correlations between TAS and TOS in healthy calves, but it only became significant at day 28.

The present study was carried out on relatively small number of cattle and their offspring. To better understand the relationship between redox balance and passive immunity and changes in redox status after colostrum feeding further studies with large number animals are required.

In conclusion, our findings indicate that the calves developed OS at birth, and that feeding calves with fresh milk did not cause a significant adverse change in antioxidant capacity. It also indicates the disruption of redox hemostasis in the mother consequently caused decreases in colostral IgG concentrations. Results may also be taken as bases for the idea that supplementation of mothers or/and colostrum with antioxidant would be beneficial in terms of health status.

CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest

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