Serum and saliva immunoglobulin (immunoglobulin G, immunoglobulin A, and immunoglobulin M) dynamics in newborn calves and their association with health status during the first week of life: An exploratory study

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ABSTRACT

The transfer of passive immunity is essential to ensure the health and welfare of newborn calves. Although the dynamics of serum Ig concentrations in these animals are well-described, data about saliva Ig are limited. This study aimed to evaluate serum and saliva Ig (i.e., IgG, IgA, and IgM) concentrations during the first week of life in healthy and sick calves. Blood and saliva samples were collected from 20 dairy calves from a dairy farm located in Portugal, at birth (30 min before colostrum intake) and on d 1, 2, and 7 of life. Calves were fed with 3.8 ± 0.64 L (mean $\pm$ SD) of maternal colostrum (44.7 ± 16.56 g/L IgG) within 96 ± 73 min of birth. Calves were examined 2 times daily by the farm staff, and health scores were recorded before sample collection (i.e., d 0, 1, 2, and 7), using a modified version of the Wisconsin calf health scoring system. Based on the health scores, calves were categorized as healthy (HC; n = 11; absence of physical signs of disease, active, and with a positive demeanor during the experimental period) or diarrheic (DC; n = 9; loose or watery feces observed during the experimental period). The Ig and total protein (TP) concentrations in serum and saliva were evaluated with linear mixed models including health status (HC vs. DC), time (d 0, 1, 2, and 7), and the interaction between both as fixed effects. The significance was set as $P < 0.05$. Except for diarrhea, no other health condition was observed. At birth, all Ig isotypes were present in saliva, with IgA being the most relevant. In serum, IgA and IgG were detected during the entire experimental period, but IgM concentrations were only detectable after d 1. Both serum and saliva Ig concentrations increased after colostrum intake (d 1), although saliva IgG concentration declined on d 2, unlike serum IgG concentration, which only decreased on d 7. All saliva Ig declined from d 1 to 2 but only in the HC group. Saliva Ig concentrations in the DC group did not change from d 1 to 7. A tendency for lower saliva IgA and M concentrations was observed in DC calves compared with HC on d 1. Similarly, SC [AU1: Define SC.]calves showed a reduced proportion of IgA in saliva relative to serum compared with HC on d 1 and a tendency for higher saliva TP on d 7. In conclusion, calf health status was associated with saliva Ig concentrations during the first week of life, with the calves from the DC group showing lower saliva Ig concentrations before clinical signs were observed.

Key words: colostrum, blood, dairy, neonatal calf diarrhea

INTRODUCTION

Newborn calves are hypogammaglobulinemic at birth (Weaver et al., 2000), as there is no transcytosis of Ig through the bovine synepitheliochorial placenta (Chucri et al., 2010; Baumrucker and Bruckmaier, 2014). Consequently, colostrum intake is essential to achieve a sufficient transfer of passive immunity (**TPI**; Baumrucker and Bruckmaier, 2014; Hernández-Castellano et al., 2014) to protect the calf against pathogenic microorganisms while endogenous production of Ig gradually increases after birth (Weaver et al., 2000). The evolution of serum

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

Ig concentrations in newborn calves is well-described. However, the immunological components present in saliva of newborn ruminants need further research. Saliva is a watery fluid that contains proteins, ions, and other organic compounds synthesized mainly by the salivary glands, with some compounds derived from blood (Lamy and Mau, 2012). The main advantage of saliva is that its collection is easy and of low invasiveness, allowing to have systemic and gland driven information (Cerón, 2019). Immunoglobulin A is the most abundant Ig in cow saliva (Mach and Pahud, 1971; Fouhse et al., 2017). Consequently, most studies on calf saliva have focused on determining saliva IgA concentration (Quezada-Mendoza et al., 2011; Goodier et al., 2012; Heinrichs et al., 2013). After colostrum intake, a large mass of IgG is transferred from the intestinal lumen into the bloodstream (Baumrucker and Bruckmaier, 2014). This process is very efficient during the first hours after birth, decreasing gradually during the first days of life (Wilm et al., 2018). Once in the blood, IgG can reach extravascular sites, such as the salivary glands, increasing the concentration of IgG in saliva (Johnsen et al., 2019). However, intestinal absorption of Ig depends not only on colostrum quality but also on the physiological status of the calf at birth (Sangild, 2003). Indeed, even under standardized colostrum feeding managements (i.e., similar volume, IgG concentration, and age), the IgG apparent efficiency of absorption (**AEA**) may not be constant among animals (Halleran et al., 2017). Serum IgG concentrations are associated with health outcomes (Renaud et al., 2018). However, in opposition to blood, little is known about the changes in saliva Ig concentrations in the event of disease during the neonatal period. Thus, determining saliva Ig concentration in sick calves is relevant due to the critical role of the adaptive mucosal immunity in the defense against enteric pathogens (Fischer et al., 2016). Therefore, this study hypothesizes that IgG, IgA, IgM, and total protein concentrations in serum and saliva are associated with calf health status during the first week of life. Based on this, the present study aimed to evaluate whether the calf health status relates with (1) serum and saliva Ig (i.e., IgG, IgA, and IgM) and total protein concentrations, (2) the IgG, IgA, and IgM AEA, and (3) the proportion of Ig (i.e., IgG, IgA, and IgM) in saliva relative to serum.

MATERIALS AND METHODS

Animals and Management

The study was approved by the Ethics Committee for Animal Welfare (ORBEA) at Universidade Trás-os-Montes e Alto Douro (UTAD, Portugal) under the reference 2664-e-DZ-2023.

This study included 20 dairy calves (14 Friesian and 6 cross-Friesian) from a commercial dairy farm located in Évora (Portugal). Only calves delivered by cows that required no assistance or easy assistance (i.e., simple hand traction from the caretaker without any obstetric device) were included in this study. Calves were separated immediately after birth from the dam and were not allowed to suck colostrum. Calf sex was recorded, and birth BW was measured using an electronic scale (Tru-Test 702, Tru-Test Datamars, Lugano, Switzerland). Calves were then allocated to individual straw-bedded pens located [AU2: Confirm changes to sentence retain meaning.]indoors and provided area of 1.2 m^2. Before colostrum feeding, animals were ear-tagged, and their umbilical cords were disinfected with an iodine solution. Calves were fed with 3.8 ± 0.64 L (mean $\pm$ SD) of maternal colostrum within 96 ± 73 min (mean $\pm$ SD), using a nipple bottle (n = 15) and an esophageal tube feeder if the calf did not voluntarily consume at least 3 L of colostrum (n = 5). Colostrum was provided from the farm colostrum bank (i.e., colostrum stored from individual cows), and the volume consumed was recorded. After that, animals were fed with transition milk from d 1 to 3 after birth, and then fed with milk replacer (Bovimilk, Vetlima, Vila Nova da Rainha, Portugal) until d 7. Both transition milk and milk replacer were provided 2 times daily (i.e., 3 L at 0700 and 3 L at 1600 h). Calves had free access to fresh water. In case of calves showing loose to liquid feces, a solution consisting of electrolyte salts (i.e., Na^+, $K+$, and Cl^-), pectin substances, dextrose, and lactic acid bacteria (ReVital, R2 Agro A/S, Hedensted, Denmark) was provided following manufacturer instructions. The ethophysiological profile (**EPP**) of each calf was assessed by a single observer immediately after birth using a methodology adapted from Schulz et al. (1997), Mee (2008), and Murray (2014). Calves were scored from 0 to 3, where 0 represents the best and 3 the worst condition. General physical and behavioral signs were observed, such as the presence and extension of meconium staining and head or tongue edema (or both), degree of response to a physical stimulus, suckling reflex, time taken to achieve independent locomotion, and rectal temperature. The health status of the calf during the first week of life was evaluated using a modified version of the Wisconsin calf health scoring system (McGuirk and Peek, 2014; Renaud et al., 2018; McCarthy et al., 2021). Calves were examined 2 times daily by the farm staff, and health scores were recorded before sample collection by a single trained researcher. A calf with a respiratory score (nose, eye, ear, cough, and rectal temperature) equal or greater than 5 was considered to have a respiratory disease. A calf with a fecal score equal or greater than 2 was diagnosed with diarrhea (McGuirk, 2008). Cleanliness and dehydration status (combined score of enophthalmos

and skin elasticity) were also recorded. Omphalitis was detected using a scoring system based on the system proposed by Steerforth and Van Winden (2018), where 0 corresponds to a normal umbilical stomp, 1 to an umbilical stomp lightly swollen without presence of pain or local heat, 2 to an umbilical stomp lightly swollen, wet, or with local heat or with the presence of pain, 3 to an umbilical stomp swollen, with local heat, pain, and with possible presence of discharge. A calf was considered with omphalitis if scored equal or greater than 2. General responsiveness and demeanor were assessed by the calf attitude toward the observer, the activity level, and suckling reflex. Additional information about these evaluations can be found in Supplemental File S1**[AU3: Please call out Supplemental Tables S1 and S2 instead of the overall file.]** (see Notes). Based on the health status (**HS**) evaluation, calves were classified either as healthy (**HC** group; n = 11) or diarrheic (**DC** group; n = 9).

Sample Collection

Two colostrum samples (50 mL) were collected directly from the colostrum bottle used to feed each calf. The bottle was stirred to ensure homogeneity of the sample. At birth, saliva samples were collected 30 min before colostrum intake (d 0). Following collections were made 22 to 26 h (d 1), 2 d (d 2), and 7 d (d 7) after birth. Saliva samples were collected at least 2 h after feeding to avoid milk residues in the samples, using Salivette cotton swabs (Sarstedt GmbH, Nümbrecht, Germany). After saliva sampling, blood samples were obtained from the jugular vein and transferred into a plasma collection tube containing EDTA (1.4 mL, Primavette, Germany) and into a serum collection tube (4.9 mL, Primavette, Germany). All samples (i.e., colostrum, blood, and saliva) were refrigerated (4°C) and transported to the laboratory at the Universidade de Évora. Plasma tubes were centrifuged (Megafuge 1.0 R, Heraeus, Hanau, Germany) at $1,660 \times g$ for 10 min at 24°C. Serum tubes were stored at room temperature for 10 min and then centrifuged at $1,660 \times g$ for 10 min at 24°C. Plasma, serum, and colostrum samples were aliquoted into 1.5 mL propylene tubes and stored at −80°C until further analysis. Saliva samples were centrifuged (Z 323 K, Hermle LaborTechnik GmbH, Wehingen, Germany) at $9,000 \times g$ for 5 min at 4°C and then stored at −80°C until analysis (Lamy et al., 2017).

Laboratory Analysis

Colostrum DM was determined using the air oven loss-on-drying method, according to AOAC Official Method **[AU4: Add AOAC Official Method to reference list, and cite in text.]**925.23. Fat content in colostrum was determined using the Gerber method, according to the AOAC Official Method **[AU5: Add AOAC Official Method to reference list, and cite in text.]**2000.18-2004. Colostrum protein content was determined using the LECO Protein Analyzer (FP528, LECO Corporation, St. Joseph, MI). A nitrogen conversion factor (i.e., 6.38) was used to calculate the protein content in each sample. In addition, colostrum SCC was determined using a DeLaval cell counter (DeLaval, Tumba, Sweden). Samples with a SCC $\geq 3,000 \times 10^3$ cells/mL were diluted 1:5 (vol/vol) with saline solution as described by Kawai et al. (2013).

Hematocrit was determined using 2 capillary tubes collected from the plasma tube before centrifugation. The 2 capillary tubes were then centrifuged (Haematokrit 20, Hettich Zentrifugen, Tuttlingen, Germany) at 9,503 $\times g$ for 5 min at 24°C. Serum total protein (**TP**) concentration was measured using a clinical Zuzi refractometer (model 50303020, Auxilab, Navarra, Spain). Saliva TP concentration was determined using the Bradford method (Bradford, 1976). Briefly, saliva was diluted using ultrapure water at 1:4, 1:8, and 1:12 (vol/vol) and then 10 μL were transferred into a 96-well plate using BSA as standard. The absorbance was read at 600 nm using a multiplate reader (Glomax, Promega, WI). Immunoglobulin concentrations (i.e., IgG, IgA, and IgM) were measured in colostrum, serum, and saliva using commercial ELISA kits (Bethyl Laboratories, Montgomery, TX). The intra-assay coefficients of variation in colostrum, serum, and saliva matrices were 4.3%, 6.0%, and 4.5% for IgG, 3.9%, 2.8%, and 3.2%, for IgA, and 4.4%, 3.9%, and 3.2% for saliva, respectively. The interassay CV in colostrum, serum, and saliva matrices were 4.7%, 3.9%, and 5.7% for IgG, 10.4%, 3.2%, and 1.4% for IgA, and 1.0%, 1.2%, and 5.2% for IgM, respectively.

Statistical Analysis

Statistical analyses were performed with R (R Core Team, 2024). The sample size (n = 9 per group) was calculated using the R "pwr" package, considering differences between 2 independent groups (t-tests of means) with an $\alpha = 0.05$, an 80% power, and a hypothesized effect size of 1, setting the estimated means as 9.48 and 19.08 g/L and the SD as 9.6 g/L of serum IgG concentration in newborn calves (Lopez et al., 2020) as the main variables to perform the calculation. A linear mixed model (**LMM**) with HS (HC vs. DC), time (**T**; d 0, 1, 2, and 7), and the interaction between both HS $\times$ T as fixed effects (independent variables), and the calf included as a random factor was used to analyze IgG, IgA, IgM, and TP concentrations in serum and saliva (dependent variables). The Wald test was used to assess the significance of the main effects,

Table 1. Least squares means and SEM of colostrum composition according to health status (HS): healthy calves (HC; n = 11) and diarrheic calves (DC; n = 9)

| Variable | HC | | DC | | Fixed effect, *P*-value |
	LSM	SEM	LSM	SEM	HS
DM, %	23.31	1.20	24.99	1.41	0.378
Protein, %	13.13	0.53	14.30	0.62	0.168
Fat, %	4.93	0.92	4.86	1.07	0.958
IgG, mg/mL	39.28	4.54	53.63	5.32	0.042
IgA, mg/mL	3.42	0.43	2.04	0.51	0.089
IgM, mg/mL	3.03	0.54	2.33	0.64	0.419
SCC, cells/mL	2.76	0.99	3.57	1.05	0.566

and pairwise comparisons with Tukey adjustment were performed to examine the differences within T and HS.

The AEA on d 1 was calculated for each serum Ig class (i.e., IgG, IgA, and IgM) using the following equation (Halleran et al., 2017):

$$\mathrm{AEA}\left(\%\right) = \frac{\mathrm{serum\ Ig}\left(\frac{\mathrm{g}}{\mathrm{L}}\right) \times \mathrm{BW}\left(\mathrm{kg}\right) \times 0.07}{\mathrm{colostrum\ Ig}\left(\frac{\mathrm{g}}{\mathrm{L}}\right) \times \mathrm{volume\ of\ colostrum\ administered}\left(\mathrm{L}\right)} \times 100.$$

The proportion of Ig (i.e., IgG, IgA, and IgM) in saliva relative to serum **[AU6: Confirm change to sentence retains meaning.]**(PSS) at 24 h of age (d 1) that was transferred through colostrum was calculated as follows:

$$\mathrm{PSS}\ \left(\%\right) = \frac{\mathrm{saliva\ Ig}\left(\frac{\mathrm{g}}{\mathrm{L}}\right)\mathrm{at\ d\,1} - \mathrm{saliva\ Ig}\left(\frac{\mathrm{g}}{\mathrm{L}}\right)\mathrm{at\ d\,0}}{\mathrm{serum\ Ig}\left(\frac{\mathrm{g}}{\mathrm{L}}\right)\mathrm{at\ d\,1} - \mathrm{serum\ Ig}\left(\frac{\mathrm{g}}{\mathrm{L}}\right)\mathrm{at\ d\,0}} \times 100.$$

An LMM with HS, Ig (IgG, IgA, and IgM), and the interaction between both HS × Ig as fixed effects and the calf included as a random factor was used to analyze the AEA and PSS.

Homoscedasticity was assessed by visual analysis of the scatterplots of the residuals against the predicted values in every model, and normality of the residuals was analyzed by inspection of normal probability plots and analysis of skewness and kurtosis for each dependent variable. To evaluate the presence of possible outliers, the studentized deleted residuals were generated by regressing the observation by the predicted values. Variables with non-normal distribution were $\log_{10}$-transformed. Data are expressed as LSM ± SEM. Log-transformed and back-transformed results are presented as geometric mean (CI at 95%); Lee, 2020). Significant values were considered as *P* < 0.05 and tendencies as *P* < 0.1.

RESULTS

The minimum number of animals obtained from the power analysis was n = 18, although 2 additional calves were included in the experiment to compensate for possible losses unrelated to the experiment. Calf birth BW was 42.70 ± 4.90 kg (mean ± SD), and the EPP was 1.55 ± 1.61 (mean ± SD). Colostrum composition by group (i.e., HC and DC) is shown in Table 1. No differences in colostrum composition used for feeding either the HC or the DC group were detected (*P* > 0.168), except for IgG concentration which was higher (*P* = 0.042) in the colostrum consumed by the DC calves (53.63 ± 5.32 mg/mL) than the one consumed by the HC calves (39.28 ± 4.54 mg/mL). In addition, a tendency (*P* = 0.089) for higher IgA concentration in the colostrum consumed by the HC calves (3.42 ± 0.43 mg/mL) compared with the one consumed by the DC calves (2.04 ± 0.51 mg/mL) was observed. Colostrum intake was similar between HC (3.97 ± 0.18 L) and DC (3.52 ± 0.20 L; *P* = 0.122). The total mass of IgG, IgA, and IgM consumed by the calves did not differ (*P* ≥ 0.099) between the HC (158.42 ± 24.46, 14.18 ± 2.33, and 12.27 ± 2.10 g, respectively) and the SC **[AU7: Define SC.]**(193.25 ± 28.68, 7.18 ± 2.73, and 8.04 ± 2.47 g, respectively) groups. There were no cases of respiratory disease or omphalitis during the experimental period. On d 7, 45% (9/20 cases) of calves had diarrhea, which started from d 4 to 7, with a mean of 2.2 d (min = 1 and max = 4 d) with fecal score ≥2 (Supplemental Figure S1, see Notes). The rectal temperature on d 0 (38.45 ± 0.10°C) was similar to d 1 (38.50 ± 0.10°C) and then increased on d 2 (38.90 ± 0.09°C; *P* ≤ 0.002), decreasing again on d 7 (38.69 ± 0.09°C; *P* ≥ 0.08). The hematocrit decreased from d 0 (39.37% ± 1.26%) to d 1 (35.28% ± 1.26%; *P* < 0.001) and then maintained on d 2 (34.18% ± 1.26%) and d 7 (34.25% ± 1.26%).

Serum IgG, IgM, and TP concentrations were affected by time (*P* < 0.001) but not by HS (*P* ≥ 0.161; Figure
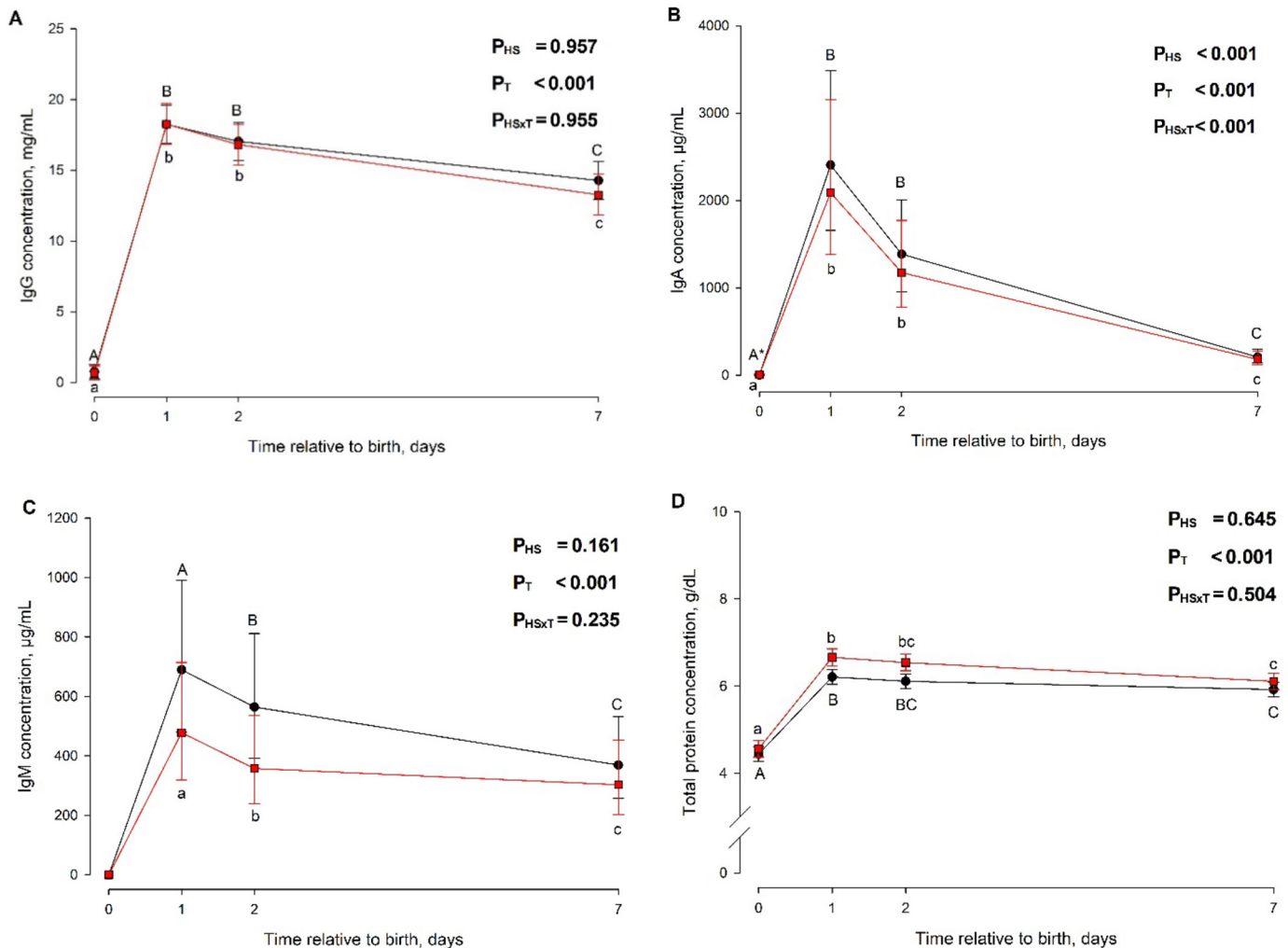
Figure 1. Serum IgG (A), IgA (B), IgM (C), and total protein (D) concentrations in healthy calves (HC; black circle, ●) and diarrheic calves (DC; red square, ■) during the experimental period (i.e., d 0, 1, 2, and 7). Different letters (A–C) indicate significant differences between days (d 0, 1, 2, and 7) in HC calves. Different letters (a–c) indicate significant differences between days (d 0, 1, 2, and 7) in DC calves. Significant differences between both groups are represented with (*). Results are expressed as LSM ± SEM (IgG and total protein) or geometric mean ± CI at 95% (IgA and IgM). HS = health status; T = time.

1). Serum IgG concentration increased from d 0 (0.72 ± 0.99 mg/mL) to d 1 (18.24 ± 0.99 mg/mL) and then decreased from d 2 (16.92 ± 0.99 mg/mL) to d 7 (13.77 ± 0.99 mg/mL). While none of the calves showed failure of TPI (**FTPI**), defined as serum IgG <10 g/L, on d 1 and 2, by d 7, 20% (4/20) of the calves were considered to have FTPI. Serum IgM concentration was below the detection limit on d 0 but was detectable on d 1 (574.13 [437.92, 752.61] μg/mL), decreasing on d 2 (449.34 [342.68, 589.09] μg/mL) and d 7 (334.52 [255.06, 438.61] μg/mL). Serum TP concentration increased from d 0 (4.50 ± 0.13 g/dL) to d 1 (6.43 ± 0.13 g/dL), and then constantly decreased until d 7 (6.01 ± 0.13 g/dL). An interaction HS × T was observed for serum IgA concentration (P < 0.001; Figure 1). In the HC group, serum IgA concen-

tration increased on d 1 (2,404.18 [1,657.10, 3,487.88] μg/mL), and decreased on d 7 (207.12 [142.47, 300.89] μg/mL). In the DC group, serum IgA concentrations increased on d 1 (2,088.77 [1,384.20, 3,151.72] μg/mL), and decreased on d 7 (179.61 [118.72, 271.48] μg/mL). However, serum IgA concentrations on d 0 were lower in the HC (1.49 [0.72, 2.61] μg/mL) than in the DC group (6.53 [3.99, 10.36] μg/mL), although no differences were detected between groups for the rest of the experimental period.

An interaction HS × T was observed for saliva IgG, IgA, and IgM (P ≤ 0.003; Figure 2). Saliva IgG concentration in the HC group increased from d 0 (2.58 [1.35, 4.44] μg/mL) to d 1 (124.88 [83.24, 187.11] μg/mL), decreased on d 2 (47.58 [31.51, 71.60] μg/mL), and remained

similar to d 7 (25.37 [16.65, 38.41] µg/mL). Saliva IgG concentration in the DC group increased from d 0 (3.26 [1.59, 5.99] µg/mL) to d 1 (85.39 [54.41, 133.69] µg/mL), showing a tendency to decline on d 2 (36.52 [23.06, 57.49] µg/mL; $P = 0.052$). Saliva IgA concentration in the HC group increased from d 0 (31.12 [23.09, 41.93] µg/mL) to d 1 (92.10 [69.26, 122.48] µg/mL), decreased on d 2 (28.30 [20.65, 38.78] µg/mL), and showed similar concentration on d 7 (44.32 [32.34, 60.73] µg/mL). Saliva IgA concentrations in the DC group were similar during the entire experimental period (40.77 [33.08, 50.25] µg/mL). In addition, saliva IgA concentration tended to be higher in the HC than in the DC group on d 1 (92.10 [69.26, 122.48] and 49.35 [36.01, 67.64] µg/mL, respectively; $P = 0.083$). Saliva IgM concentration increased in the HC group from d 0 (0.50 [0.05, 1.14] µg/mL) to d 1 (5.12 [3.37, 7.58] µg/mL), decreased on d 2 (1.89 [1.06, 3.04] µg/mL), and remained similar on d 7 (1.25 [0.61, 2.15] µg/mL). Saliva IgM concentration in the DC group increased from d 0 (0.31 [0.001, 0.90] µg/mL) to d 1 (1.86 [0.97, 3.16] µg/mL), being similar until the end of the experimental period. Additionally, the HC tended to show higher saliva IgM concentration than the DC group on d 1 (5.12 [3.37, 7.58] and 1.86 [0.97, 3.16] µg/mL, respectively; $P = 0.068$). Saliva TP concentration was affected by time ($P = 0.010$; Figure 2), increasing from d 0 (0.08 ± 0.01 g/dL) to d 1 (0.12 ± 0.01) and decreasing again on d 2 (0.08 ± 0.01 g/dL), for both groups. In addition, a tendency for the interaction between HS and T was observed ($P = 0.082$), showing higher saliva TP concentrations in DC (0.14 ± 0.02 g/dL) than in HC (0.08 ± 0.01 g/dL) on d 7.

An interaction HS × Ig was observed for the AEA and PSS ($P = 0.040$ and $P = 0.005$, respectively, Table 2). In both HC and DC groups, IgA AEA (70.16% and 82.90%, respectively, SEM = 6.85%) was higher than IgG (44.54% and 28.16%, respectively, SEM = 6.85%) and IgM (26.28% and 21.09%, respectively, SEM = 6.85%) AEA. In the HC group, the IgA PSS (3.07% ± 0.50%) was higher than the IgG (0.77% ± 0.48%) and IgM PSS (1.00% ± 0.48%). In addition, the IgA PSS in the HC (3.07% ± 0.50%) was higher than in the DC group (0.52% ± 0.50%).

DISCUSSION

Monitoring the TPI is crucial in newborn calves and is usually assessed by measuring serum IgG or TP concentrations (Godden et al., 2019). However, the assessment of other Ig transferred through colostrum (i.e., IgA and IgM) is also relevant, as they play a crucial role in the mammalian immune response (Boes, 2000; Estes, 2010). Furthermore, given the important role of mucosal immunity as the first line of adaptive defense, under-

standing the dynamics of saliva Ig during early life is relevant (Fábián et al., 2012; Fischer et al., 2016). Therefore, this study focused on observing changes in saliva and serum IgG, IgA, IgM, and TP concentrations in HC and DC newborn calves during the first 7 d of life. It should be emphasized that the ELISA kits used for the quantification of IgG, IgA, and IgM in this study have not undergone full validation for bovine saliva matrices. While intra- and interassay precision were acceptable, further validation including recovery percentage or matrix effects would be necessary to confirm the suitability of these assays for this matrix. Future work will address this limitation. The results of this study showed that both serum and saliva Ig (i.e., IgG, IgA, and IgM) and TP concentrations are increased after colostrum intake. Unlike serum Ig concentrations, saliva Ig concentrations showed different patterns in HC and DC during the first week of life.

As described by Murphy et al. (2014), circulating IgA and IgM have shorter half-life compared with IgG, which could explain the decline in IgA and IgM concentrations on d 2, while serum IgG concentrations remained similar. Although IgA and IgM concentrations in colostrum were similar, IgM AEA was lower than IgA AEA. This suggests that these Ig are not equally absorbed at the intestinal epithelium. As described by Husband et al. (1972), differences in the chemical structure of IgG, IgA, and IgM may influence the absorption rate of each Ig. Thus, IgM absorption seems to be negatively affected by its total mass in the intestinal tract, in contrast to IgG and IgA, whose absorption rates are not affected by their total mass in the intestine (Stott and Menefee, 1978; Bush and Staley, 1980). After intestinal uptake, IgG, IgA, and IgM can be transported into different extravascular regions, depending on their molecular structure (Kruse, 1970; Smith et al., 1976). Therefore, it might be hypothesized that Ig concentrations in extravascular regions, such as saliva, likely depend on their intestinal absorption and decaying rates.

Saliva proteins can be either synthesized and secreted by the salivary glands, pass from blood to the salivary glands, and then to saliva or pass directly from blood to saliva through the crevicular route (Lehner, 1969; Brandtzaeg, 2007; Pfaffe et al., 2011). Neither the mechanisms underlying the transfer of Ig from blood to saliva nor the production of saliva Ig have been deeply investigated in newborn calves yet. As described in humans, both serum-derived and local synthesized IgG are actively transported to the mucosal surfaces through the neonatal Fc receptor (Yoshida et al., 2004; Horton and Vidarsson, 2013). However, most IgG present in saliva is derived from blood by passive leakage through the gingival crevices (Brandtzaeg, 2013). In contrast, IgA is mainly synthesized by plasma cells within the salivary

Table 2. Apparent efficiency of absorption (AEA) and proportion in saliva relative to serum at 24 h of age (PSS) of IgG, IgA, and IgM in healthy calves (HC; n = 11) and diarrheic calves (DC; n = 9)[1]

| Variable | HS[2] | | SEM[3] | Fixed effect, P-value | | |
	HC	DC		HS	Ig	HS × Ig
AEA			7.32	0.173	<0.001	0.040
IgG, %	44.54	28.16				
IgA, %	70.16	82.90				
IgM, %	26.28	21.09				
PSS			0.50	<0.001	<0.001	0.005
IgG, %	0.77	0.51				
IgA, %	3.07	0.52				
IgM, %	1.00	0.44				

[1]Results are expressed as LSM ± SEM.
[2]HS = health status.
[3]Largest SEM.
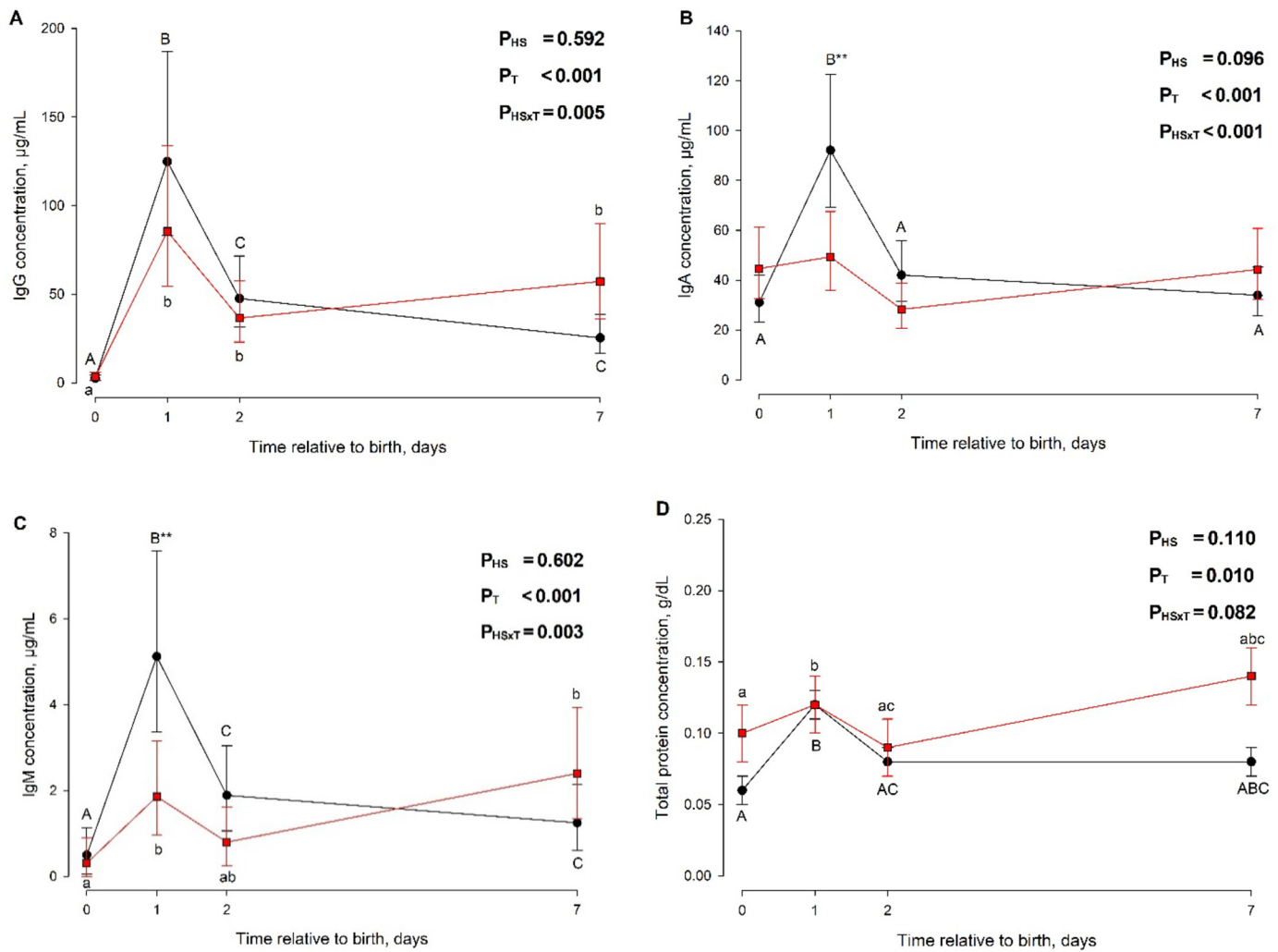
Figure 2. Saliva IgG (A), IgA (B), IgM (C), and total protein (D) concentrations in healthy calves (HC; black circle, ●) and diarrheic calves (DC; red square, ■) during the experimental period (i.e., d 0, 1, 2, and 7). Different letters (A–C) indicate significant differences between days (d 0, 1, 2, and 7) in HC calves. Different letters (a–c) indicate significant differences between days (d 0, 1, 2, and 7) in DC calves. Significant differences between both groups are represented with [AU14: Single asterisk does not appear in figure; remove here?](*) and tendencies with (**). Results are expressed as geometric mean ± CI at 95% (IgG, IgA, and IgM) or LSM ± SEM (total protein). HS = health status; T = time.

glands as a dimer linked to the polymeric Ig receptor (**pIgR**; Staley et al., 2018), with only a small proportion transferred from blood (Strober et al., 1970). However, the combination of a low number of circulating B cells, elevated cortisol levels, and the presence of maternal antibodies and hormones in newborn calves results in a prolonged lack of endogenous antibody response (Chase et al., 2008). Thus, endogenous synthesis of Ig does not reach significant concentrations until 8 to 32 d after birth (Husband et al., 1972; Husband and Lascelles, 1975; Chase et al., 2008). Similar to IgA, IgM is synthesized by local plasma cells and actively transported to saliva by the same receptor-mediated epithelial excretion mechanism (Brandtzaeg, 2013). However, nasal and conjunctival mucous membranes may be only permeable to maternal IgG, and not to IgA and IgM, as suggested by Smith et al. (1976) in newborn lambs. In this study, all Ig concentrations (i.e., IgG, IgA, and IgM) increased in both serum and saliva after colostrum intake, suggesting that a large proportion of the Ig detected in saliva on d 1 also depends on maternal Ig transferred through colostrum. The decline in IgA and IgM concentrations in both saliva and serum after d 1 may be related to their short half-life, as mentioned previously. However, the faster decline of IgG in saliva than in serum can be caused by a reduction in the passive diffusion of IgG from blood to saliva, reflecting a decrease in the permeability of mucosal membranes after the first day of life (Selner et al., 1968). In addition, the development of the salivary glands after birth may increase the volume of saliva, diluting the concentration of IgG. The extent of saliva Ig decrease was lower in the HC compared with the DC group. Because this difference was not observed in serum, this seems likely to be caused by higher transfer of Ig from serum to saliva after colostrum intake in the HC compared with the DC group. Furthermore, a lower IgG concentration in colostrum did not seem to interfere with saliva IgG concentration, as colostrum fed to HC had a lower IgG concentration than the colostrum fed to DC. As reported in previous studies, calves fed with different colostrum Ig concentrations **[AU8: Confirm change to sentence retains meaning.]**of serum IgG concentration may not be affected by colostrum IgG concentration.

In addition, saliva Ig are also transported throughout the upper gastrointestinal tract, thereby contributing to the protection of the upper gastrointestinal mucosal surfaces (Fábián et al., 2012). Indeed, IgA has a relevant role in the mucosal defense against virus, bacteria (Vlasova and Saif, 2021), and gastrointestinal parasites (Castilla Gómez de Agüero et al., 2023). Although calves from the HC group received colostrum with a lower IgG concentration than DC calves, the total mass of IgG consumed by the calves did not differ between groups, which may explain the similar serum IgG concentrations observed

between HC and DC groups. The association between diarrhea and the reduced concentrations of Ig in saliva observed in the DC group is intriguing and deserves further investigation to clarify its biological relevance. The lower saliva IgA and IgM concentrations in DC compared with HC on d 1 of life may indicate a reduced defense capacity. This was further supported by the fact that the IgA PSS was lower in the DC than in the HC group.

Increased TP concentrations in both serum and saliva can be indicative of dehydration (Walsh et al., 2004; Marcato et al., 2018), which may explain the tendency for higher saliva TP concentrations on d 7 in the DC compared with the HC group. During gastrointestinal inflammation, proinflammatory proteins may increase in saliva (Botía et al., 2023; Ortín-Bustillo et al., 2023; Rodrigues et al., 2023), which could have also contributed to the increased saliva TP concentrations observed in the DC compared with the HC group. Proinflammatory proteins such as IL, lead to the activation of immune cells (Kany et al., 2019). Some IL, such as IL-17, can upregulate pIgR, a key mediator of mucosal IgA and IgM secretion (Brandtzaeg, 2013). In fact, the saliva expression of Ig is regulated by mucosal and gut-associated lymphoid tissue in humans as part of the immune response (Mestecky et al., 1978; Czerkinsky et al., 1991; Cesta, 2006). Therefore, although no differences in saliva Ig concentrations were observed between HC and DC on d 7, the present results do not exclude a humoral immune response in the DC group on d 7, although additional sampling days would be required to confirm this hypothesis. Because the mucosal immune system can act independently of the systemic immune system (Cesta, 2006), this may explain the different pattern observed in serum.

In the present study, saliva samples were collected before feeding to avoid the presence of residual colostrum or milk components in the oral cavity. Carlander et al. (2002) described that oral-administered IgY may be still detectable (i.e., very low concentrations) in the human oral cavity 8 h after the administration. Similar studies about colostrum-derived Ig in the calf oral cavity are not available, however, it is likely that these Ig may still be detectable a few hours after colostrum intake. As saliva samples were collected 21 to 26 h after colostrum intake and 2 to 7 h after transition milk intake, the observed results are not likely dependent on residual Ig. However, given the absence of information in the literature regarding residual Ig in the calf oral cavity after colostrum intake, further studies will be needed to better understand the dynamics of saliva Ig in newborn calves.

CONCLUSIONS

This study showed that IgG, IgA, IgM, and TP concentrations in saliva, in the first days of life, were associated

with calf health, with lower concentrations detected on calves that showed signs of enteric disease during the first week of life. In addition, it seems that the oral mucosa membranes in dairy calves are permeable to Ig during the first hours of life, increasing their diffusion from blood to saliva. This fact highlights the importance of saliva Ig in establishing mucosal protection and preventing enteric disease in newborn calves. Further studies are needed to better understand the role of Ig acquired from colostrum in the mucosal defenses of newborn calves.

NOTES

Nonstandard abbreviations [AU11: Please confirm that all nonstandard abbreviations are included in the list and that the definitions are correct.]used: AEA = apparent efficiency of absorption; DC = diarrheic calf; EPP = ethophysiological profile; FTPI = failure of TPI; HC = healthy calf; HS = health status; LMM = linear mixed model; pIgR = polymeric Ig receptor; PSS = proportion of saliva relative to serum; T = time; TP = total protein; TPI = transfer of passive immunity.

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