Effect of colostrum on the acute-phase response in neonatal dairy calves

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ABSTRACT

The core part of the mammal innate immune system is the acute-phase response (APR), during which acute-phase proteins (APP) are synthesized. Colostrum contains immunomodulating factors such as proinflammatory cytokines and APP in large quantities. We looked at proinflammatory cytokines [IL-1β, IL-6, and tumor necrosis factor-α (TNF-α)] and APP [serum amyloid A (SAA) and haptoglobin (Hp)] in colostrum and in calves’ serum. The aim of this study was to evaluate the effects of colostrum on the calves’ systemic APR and the associations of the calves’ serum APR with short- and long-term weight gain (at the age of 1, 3, and 9 mo). A total of 143 female dairy calves were studied during their first 3 wk of life. The calves were separated from their mothers immediately after birth and bottle-fed 3 L of quality-controlled colostrum once within 2 h after birth. Serum samples were collected once a week during the first 3 wk of life (a total of 1–3 samples per calf). Mean sampling age (±standard deviation) was 4.3 (±2.0) d in the first week, 11.0 (±2.0) d in the second week, and 18.0 (±2.0) d in the third week. Linear regression models were used to study associations of colostrum APP and cytokine concentration with serum APR markers and for studying associations of colostrum and serum APR markers with calves’ average daily weight gain (ADWG). Mixed linear regression models were used to compare serum concentrations of APR markers by study weeks. The colostrum IL-6 concentrations were positively associated with serum IL-6 in the first 3 wk of life. Colostrum IL-1β was positively associated with calves’ serum IL-1β during the first week of life, and colostrum TNF-α was positively associated with calves’ serum TNF-α during the first 2 wk of life. Serum IL-1β concentrations differed over the 3 wk, being the highest during the first week and the lowest during the second week. For IL-6, the concentration during the first week was the highest, and for TNF-α, a steady decline in the concentration was observed. Serum SAA concentrations were elevated during the first 2 wk of life and subsequently declined during the third week. Albumin concentrations were lowest in the first week, whereas Hp concentrations were highest during the second week. Serum concentrations of SAA, Hp, IL-6, and TNF-α during the second week were negatively associated with ADWG at 9 mo of age. The SAA concentrations during the third week of age had a negative association with 9-mo ADWG. Serum Hp concentrations in the third week were negatively associated with 3-mo ADWG. The results of our study suggest that colostrum cytokines influence calf serum cytokine concentrations. Thus, they influence the newborn calves’ adaptation to the environment and the development of their immune system. Factors that activate an APR during the second and third week of life have a long-term influence on calves’ development.

Key words: colostrum, proinflammatory cytokines, neonatal calf, APR

INTRODUCTION

The neonatal period of calves is considered to be a crucial time for the animal to adapt to the environment, and the development of the immune system is part of that process. At the time of birth, dairy calves, like other ruminants, lack placental immunoglobulins and hence also lack adequate acquired immunity. Therefore, the intake of high-quality colostrum is critical for the survival of neonatal calves. In addition to providing immunoglobulin and essential nutrients for the calf’s energy needs, cow colostrum is rich in leukocytes, growth factors, hormones, enzymes, and other immunologically bioactive molecules, meaning that colostrum also has other immunomodulatory properties (Blum and Hammon, 2000; Barrington and Parish, 2001). Colostrum contains a variety of components associated with innate immunity, such as various peptides, small proteins, and enzymes with innate immune function (Vorbach et al., 2006). Colostrum ingestion supports the functional and morphological development of calves (Blum and Hammon, 2000). Therefore, deprivation of colostrum leads to poor weight gain, morbidity, and even increased
mortality after birth (Nocek et al., 1984). During the last decade, the importance of specific colostrum proteins has gained attention, yet the exact bioactive roles of different proteins in neonatal ruminants still need clarification (Hernández-Castellano et al., 2015). The understanding of innate immunity functions in neonatal calves and their role in resistance to infections and maintaining homeostasis cannot be underestimated.

Part of the innate immune system is the acute-phase response (APR), a first-line defense mechanism of the organism, initiated by infection, injury, tissue damage, stress, immunological disorders, or neoplasia (Baumann and Gauldie, 1994; Gruys et al., 2005). At the site of the inflammatory stimulus, monocytes and macrophages are the predominant cells that elicit the APR by releasing proinflammatory cytokines, of which tumor necrosis factor α (TNF-α) and IL-1β and IL-6 are the predominant ones (Baumann and Gauldie, 1994; Ceciliani et al., 2012). One of the main functions of these proinflammatory cytokines is the activation of a large group of serum proteins, known as acute-phase proteins (APP), which initiate an effective innate immune response. The APP are mainly produced in the liver and in lesser quantities in local tissues to restore the homeostasis of the body (Baumann and Gauldie, 1994; Koj, 1996). As the APR process is part of the first-line antibody-independent defense mechanism, it is crucial for neonate animals who are immunologically naïve to invading pathogens.

The APP has been commonly used in medicine as a quantitative sensitive diagnostic and prognostic biomarker (Schrödl et al., 2016). In cattle, the 2 major positive APP are serum amyloid A (SAA) and haptoglobin (Hp), and their concentrations increase notably during APR (Ceciliani et al., 2012). Albumin and transferrin are negative APP in cattle, whose concentrations decrease during APR (Petersen et al., 2004).

The SAA and Hp belong to the evolutionarily conserved set of APP (Uhlar and Whitehead, 1999; Wang et al., 2001). Serum amyloid A has many roles, mainly the binding of cholesterol; immunomodulatory functions via pro- and anti-inflammatory activities, such as chemotactic recruitment of inflammatory cells to sites of inflammation; and opsonization (Liang and Sipe, 1995; Uhlar and Whitehead, 1999; Shah et al., 2006; Ceciliani et al., 2012). Haptoglobin’s main function is to bind free hemoglobin from erythrocytes, providing antioxidant and antimicrobial activity by decreasing the available iron to microbes (Dobryszycza, 1997; Tóthová et al., 2014).

The mammary gland epithelium expresses SAA and Hp. In bovine colostrum, highly elevated levels of extrahepatically secreted mammary-associated SAA isoform 3 (SAA3) have been demonstrated, especially during the first few days after calving (McDonald et al., 2001; Thomas et al., 2016). Moderately elevated Hp concentrations are detectable in cow colostrum, which then decreases on the fourth day postcalving (Thomas et al., 2016). The role of elevated SAA levels in the mammary gland is associated with the cow’s health state, as SAA is involved in mammary gland defense against pathogens (Molenaar et al., 2009). Colostrum whey also contains high quantities of the cytokines IL-1β, IL-6, TNF-α, and IFN-γ; their concentrations are significantly higher in colostrum than in mature milk (Hagiwara et al., 2000).

Colostrum SAA has been assumed to have a protective role in offspring by modulating gastrointestinal immunity (Molenaar et al., 2009). The SAA induces mucin 2 gene expression in the gastrointestinal tract (Shigemura et al., 2014). This is a major component of the mucus layer in the intestines that separates bacteria from the epithelium and protects the gastrointestinal tract of newborns from pathogen colonization. It has been demonstrated that locally expressed SAA in intestinal epithelial cells has a role in intestinal immune homeostasis, as SAA reduces bacterial growth in vitro (Eckhardt et al., 2010). Therefore, colostrum SAA probably has a balancing effect during microbiota colonization at and after birth. The immunostimulatory effect of orally administered IL-1β in newborn calves has been found to affect the activation of neutrophils and proliferation of T cells (Hagiwara et al., 2001). The colostrum proinflammatory cytokines (IL-1β, TNF-α, and IFN-γ) improve the mitogenic response of peripheral blood mononuclear cells (Yamanaka et al., 2003). Thus, colostrum cytokines contribute to the maturation of neonatal immune functions (Yamanaka et al., 2003).

The concentrations of different APP change during the first few weeks of ruminant life and are termed age-related changes in APP. In lambs and goat kids, SAA concentrations increase during the first week of life, start to decrease during the second week, and then stabilize at the end of the third week (Eckersall et al., 2008; Ulutas et al., 2017; Niine et al., 2018a; Peetsalu et al., 2019; Dinler et al., 2020). It has been suggested that colostrum intake influences the APP concentration in calves, as their SAA concentrations were found to be low before colostrum consumption and then increased during the first 24 h after birth (Orro et al., 2008; Tóthová et al., 2015). Nevertheless, no direct transfer of SAA isoforms in calves was found in the study of Orro et al. (2008), who measured colostrum and calf serum SAA isoforms. A proteomic study in sheep demonstrated colostrum led to higher SAA concentrations in lambs (Hernández-Castellano et al., 2014). In our previous study, we found positive associations between colostrum SAA and lamb serum SAA at 1 to 5 d of age,
which indicates the important effect of ewe’s colostrum on lambs (Peetsalu et al., 2019).

In the same study, a negative association between SAA concentration during the second week of life and weight gain at 3 to 4 mo of age was evident. This suggests that the second week of life is important, as environmental factors and diseases at that time have a long-term effect on animal health, whereas the colostrum effect has already diminished. Similar negative associations between second-week SAA concentrations and future weight gain have also been found in reindeer, beef calves, and dairy calves reared for meat (Orro et al., 2006; Seppä-Lassila et al., 2017, 2018).

Based on our previous results, colostrum influence on APR of offspring was not proven. We hypothesized that proinflammatory cytokines and APP in colostrum are associated with the systemic APR of neonatal calves, and thus indirectly influence the offspring’s immune system. In addition, we evaluated the changes in calves’ APR marker concentrations during the neonatal period and their possible associations with weight gain, measured at the ages of 1, 3, and 9 mo.

**MATERIALS AND METHODS**

Sampling took place in 2015 on a dairy farm in Estonia, housing approximately 1,800 cows at the time, and the average milk production per cow was 10,000 kg (Eesti Põllumajandusloomnõud Jõudluskontrolli AS, 2015). The present study is part of a large-scale study conducted to describe the inflammatory response during an acute *Cryptosporidium parvum* outbreak in female dairy calves previously described by Niine et al. (2018b). For this study, we used fecal and serum samples from the first 3 wk, in addition to colostrum samples. Sample collection was conducted based on ethical permission issued by the Ethical Committee of Animal Experiments in the Estonian Ministry of Agriculture (no. 7.2-11/2).

**Animals**

All Holstein-Friesian female calves (n = 143) included in the present study were born between January 21 and March 16, 2015. The calves were separated from their mothers immediately after birth and bottle-fed 3 L of quality-controlled colostrum once within 2 h after birth (median ± SD; 61 ± 30 min). The colostrum given to the calves was collected from the dam and the quality examined visually and with a colostrum densimeter (Jørgen Kruuse A/S). In 2 cases colostrum quality was poor (specific gravity <1.035, or total protein <50 g/L), so deep-frozen colostrum from another cow was used. Obstetric aid at birth were recorded as spontaneous delivery (n = 85), aid by one person (n = 46), and aid by 2 persons (n = 12). Seventy-six mothers of the calves were primiparous (first time calving) and 67 multiparous (second time 28, third time 22, and 17 fourth time or more).

Detailed descriptions of the housing conditions, feeding, vaccinations, and prophylactic treatments of the animals are given in Niine et al. (2018b). In short, during the first 4 wk, the calves were kept in individual pens with a wooden floor and straw bedding. After that, they were moved to group pens (8–10 calves per pen) with concrete flooring, straw, and sawdust bedding. The calves were weaned at approximately 70 d of age. They were kept in a different barn after that until the age of 4 to 5 mo (on straw bedding). At the age of 6 to 7 mo, they were moved to a new barn (large stalls with concrete floors) where they remained until pregnancy.

The calves were fed 2 to 3 kg of warmed unpasteurized raw milk twice per day with free access to hay and starter feed (Prestarter, Agrovarustus OÜ) up to 15 to 17 d of age and afterward a milk powder (Josera Gold-enSpezial, Josera GmbH and Co. KG) solution and free access to starter feed and hay. Around weaning time (70–80 d of age), the calves received 2 × 2 L/d of the milk powder solution. After weaning, the calves had ad libitum access to starter feed (Starter, Agrovarustus OÜ), hay, and silage.

The calves were weighed with a digital scale immediately after birth, at approximately 1 mo of age (mean ± SD; 29.6 ± 4.5 d) and at 9 mo of age (264 ± 6.5 d). At 3 mo of age (101.4 ± 10.6 d), the calves’ weight was estimated using a measuring tape (ANImeter, Albert Kerbl GmbH) because the digital scale was not available. Average daily weight gain (ADWG, g/d) was calculated for a period from birth to 1 mo of age (n = 122), from birth to 3 mo of age (n = 120), and from birth to 9 mo of age (n = 121).

The calves were vaccinated against parainfluenza virus type 3 and bovine respiratory syncytial virus (Rispens, Zoetis Belgium SA) on their second day of life and against bovine herpesvirus-1 (Hirapovis, Laboratorios HIPRA, S.A.) at 3 mo of age. Toltrazuril (Cevazuril, Ceva Santé Animale) was used once at the age of 25 to 65 d as prophylactic treatment against *Eimeria* spp. infection. During the acute outbreak of cryptosporidiosis, halofuginone lactate (HL; Halocur, Intervet International B.V.) was used. The prophylactic HL mass treatment for controlling the outbreak of diarrhea caused by *Cryptosporidium* spp. was started on February 17 and ended on March 22, 2015 (the study period was from January 21 to March 16, 2015). All calves younger than 14 d were treated. In all, 110 calves were treated an average of 6 times (range 1–9 d).
Based on the HL treatment regimen, the calves were retrospectively divided into 3 groups: (1) no treatment, (2) incorrect treatment (daily treatment started >48 h after birth and lasted <7 d), and (3) correct treatment (according to the manufacturer’s instructions; daily treatment started <48 h after birth and lasted ≥7 d). Detailed descriptions of HL treatments and Cryptosporidium spp. infection by individual calves are published in the paper by Niine et al. (2018b). No other clinical diseases were diagnosed or treated during the study period.

**Sample Collection**

Serum and fecal samples collected from 143 female calves once a week during the first 3 wk of life (1–3 samples per calf) were used in the present study. Calves that did not have fecal matter in the rectum at the time of sampling were not included in the models of that study week (their serum sample of that day was also excluded). To avoid further stress, the calves were not caught and restrained a second time on that day. Thus, not every calf included in the study has samples from all 3 wk available. In total, 103 samples were available from the first week, 112 from the second, and 114 from the third, making for an overall sample size of 329. Mean sampling age (±SD) was 4.3 (±2.0) d in the first week, 11.0 (±2.0) d in the second week, and 18.0 (±2.0) d in the third week.

Blood samples were collected into sterile evacuated test tubes with an 18-G sterile needle from the jugular vein. Samples were centrifuged (1,800 × g, 10 min) and the serum was separated and stored in aliquots at −20°C until further analysis. A sample of the colostrum was collected before it was provided to the newborn. These were collected into sterile 10-mL vials and stored in aliquots at −20°C until further analysis. Before the laboratory analyses, the colostrum samples were skimmed by centrifugation (7,840 × g, 10 min, 4°C) followed by removal of the fat layer.

**Laboratory Analysis**

Fecal samples were prepared and analyzed for the detection of *Cryptosporidium* and *Giardia* approximate oocysts or cyst counts (oocysts per gram of feces, OPG; and cysts per gram of feces, CPG) as modified by Niine et al. (2018b) using an immunofluorescence method. For staining, fluorescein isothiocyanate (FITC)-conjugated anti-*Cryptosporidium* and anti-*Giardia* monoclonal antibodies (Crypto/Giardia Cel, Cellabs Pty Ltd.) were used. For statistical analysis, the calves were divided into 3 groups based on the *Cryptosporidium* oocyst count each week (negative, no oocysts; low oocyst level, oocyst count below the median value; and high oocyst level, oocyst count above the median value; Table 1). Because only 16 fecal samples were positive for *Giardia* and *Giardia* infection did not have an association with the APR (Niine et al., 2018b), *Giardia* data were not used in the present study (data not shown).

The concentrations of SAA in the serum and colostrum samples were measured using a commercial ELISA kit (Phase BE kit, Tridelta Development Ltd.), and Hp was measured using a method defined by Makimura and Suzuki (1982), with minor modifications, namely using tetramethylbenzine (60.0 mg/L) as the substrate and using microtitration plates (Alsemgeest et al., 1994). The detection limits for SAA and Hp were 0.3 and 60.0 mg/L, respectively. Cytokine concentrations in the serum and colostrum samples were determined using bovine IL-1β, IL-6, and TNF-α ELISA kits (Cusabio Biotech) according to the manufacturer’s instructions. The detection limits for IL-1β, IL-6, and TNF-α were 15.6, 2.5, and 50.0 ng/L, respectively. The IgG concentrations were measured using a commercial ELISA kit (BIO K 165/2 kit, Bio-X Diagnostics S.A.). The albumin concentration in the serum was determined by using a commercial photometric colorimetric method based on bromocresol green dye binding (Accent-200 Albumin II Gen, PZ Cormay S.A.).

### Table 1. *Cryptosporidium* oocyst count (oocysts per gram of feces; OPG) of fecal samples in 143 dairy calves sampled during the first 3 wk of life (1–3 samples per calf)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Statistics</th>
<th>1–7 (n = 103)</th>
<th>8–14 (n = 112)</th>
<th>15–22 (n = 114)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cryptosporidium</em> (OPG)</td>
<td>Positive samples, n</td>
<td>17</td>
<td>59</td>
<td>78</td>
</tr>
<tr>
<td>Median</td>
<td>1,871</td>
<td>475,293</td>
<td>418,237</td>
<td></td>
</tr>
<tr>
<td>(Minimum–maximum)</td>
<td>(69–866,119)</td>
<td>(208–5,764,653)</td>
<td>(69–10,602,130)</td>
<td></td>
</tr>
<tr>
<td><em>Cryptosporidium</em> group¹</td>
<td>Negative, n</td>
<td>86</td>
<td>53</td>
<td>36</td>
</tr>
<tr>
<td>Low level, n</td>
<td>8</td>
<td>29</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>High level, n</td>
<td>9</td>
<td>30</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>

¹Positive samples were divided into low and high oocyst count categories using median values in the same week.
**Statistical Analysis**

Pearson correlation with Sidak-corrected P-values for pairwise comparisons was used to study the correlations between the measured variables in the colostrum and serum samples.

To compare the serum inflammatory markers (APP and cytokines) and IgG concentration differences by study week, linear mixed models (for SAA, Hp, albumin, IgG, IL-6, IL-1β, and TNF-α) and a mixed Tobit model (for IL-1β) were used. The Tobit regression model was chosen because >60% of the sample IL-1β concentrations were under the detection limit of the assay (15.6 ng/L), violating the regression model assumption of a normal distribution of the response variable. In the Tobit regression, all cases falling above (or below) a specified threshold value are censored, although these cases remain in the analysis (Long, 1997). Histograms of response variables were used to evaluate normal distribution. Except for albumin, all response variables had to be logarithmically transformed to achieve normal distribution.

The calf was included as a random intercept and an isotropic spatial exponential covariance structure was used to model the correlation between repeated samples from the same calf because it had the best fit to the data (lowest Akaike information criterion of the models). Sample week was included as a categorical variable (1, 2, or 3) and maternal parity as a binary variable (primiparous or multiparous). Because Cryptosporidium infection influences inflammatory markers, Cryptosporidium oocyst level group and HL treatment group were included in these models along with obstetric aid (spontaneous delivery, help by 1 person, or help by 2 persons) because dystocia can trigger APR in calves. A total of 103 samples were available from the first week, 112 from the second, and 114 from the third, making for an overall sample size of 329.

To investigate associations between the colostrum and serum variables by study week, multiple linear regression models were used. In mixed linear models that included all samples, interaction terms for all predictor variables by study week needed to be included as well, resulting in very complicated models. Thus, separate models for each study week were used for every response variable. The APR markers (SAA, Hp, albumin, IL-6, and TNF-α) and IgG serum concentrations by study week were used as response variables in those models. Tobit regression models were used for IL-1β. Colostrum APP, cytokine, and IgG concentrations were included as covariates. All models initially included age at sampling (d) and the time from birth to colostrum ingestion (min) as covariates, and maternal parity as the categorical explanatory variable. The final models were produced by backward elimination of the variables from the initial models. The linear relationship between response variable and covariates was checked and confounders were controlled (change of the coefficient by more than 10% after variable elimination). Possible cofounders (maternal parity) were identified by use of a causal diagram (Figure 1). Colostrum variables with a high correlation (IL-6 and TNF-α) were included separately to avoid collinearity.

To investigate associations between the calves’ ADWG and the concentrations of IgG, SAA, Hp, IL-6, TNF-α, and IL-1β in the colostrum, 3 separate linear regression models were used. The ADWG at 1 (n = 121), 3 (n = 119), and 9 mo (n = 120) of age was used as a response variable in each of the models. Colostrum concentrations of SAA, Hp, albumin, IgG, IL-6, TNF-α, and IL-1β, age at weighing, and birth weight were used as covariates. Because HL treatment

![Figure 1. Causal diagram describing the studied variables relationship with calves' neonatal acute-phase response (APR) and future weight gain. HL = halofuginone lactate; obstetric aid = spontaneous, aid by 1 or 2 persons.](image-url)
group was associated with weight gain, it was included in these models as an independent variable. Maternal parity was included as the binary categorical variable. Colostrum variables with a high correlation (IL-6 and TNF-α) were included separately to avoid collinearity.

Similar models were used to investigate associations between ADWG at 1, 3, and 9 mo of age and the calves’ serum concentrations of SAA, Hp, albumin, IgG, IL-6, TNF-α, and IL-1β by study week. Sample sizes by week for ADWG models at 1 mo were n = 86, n = 102, and n = 108, respectively. Sample sizes by week for ADWG models at 3 mo were n = 83, n = 98, and n = 104, respectively. Sample sizes by week for ADWG models at 9 mo were n = 86, n = 100, and n = 105, respectively. Serum concentrations of the markers, age at sampling, age at weighing, and birth weight were used as covariates. The HL treatment group, Cryptosporidium oocyst level group at the time of sampling, and maternal parity were included as categorical explanatory variables. A stepwise backward elimination procedure was used for the final models. The linear relationships between response variable and covariates were checked, and interactions and possible confounders according to the causal diagram (Figure 1) were controlled (change of the coefficient by more than 10% after variable elimination). Highly correlated variables in the serum (SAA and Hp; IL-6 and TNF-α) were included separately in these models to avoid collinearity.

The fit of all models was controlled using normality and scatter plots of the model residuals, and results were considered as statistically significant when $P \leq 0.05$. Analyses were performed using Stata/IC 14.0 statistical software (StataCorp LP). Least squares means (LSM) by age at sampling were derived using the margins command in Stata and the delta method was used to calculate LSM standard errors. DAGitty 3.0 (http://www.dagitty.net) was used to create the causal diagram describing the relationships between studied variables.

**RESULTS**

**Colostrum and Serum Concentrations**

Concentrations of APP (SAA, Hp, albumin), cytokines (IL-6, TNF-α, IL-1β), and IgG in the colostrum and serum (by weeks of age) are presented in Table 2. Model-based LSM of the APP and cytokines are presented in Figures 2 and 3 by day of age. The colostrum concentrations of IL-6 and TNF-α were correlated ($r = 0.69, P < 0.001; n = 143$). Other inflammatory markers and IgG in the colostrum were not correlated with each other ($r < 0.2$). Calves’ serum concentrations of IL-6 and TNF-α up to 3 wk of age were positively correlated ($r = 0.79, r = 0.92$, and $r = 0.64$, all $P < 0.001$). Serum SAA and Hp concentrations were positively correlated during the second and third weeks of life ($r = 0.45$ and $r = 0.36$, both $P < 0.001$). All other pairwise correlations between serum protein concentrations during the first 3 wk of age were nonsignificant ($r < 0.3$).

There were different changes in the calves’ serum APP concentrations during the first 3 wk of life (Table 2 and Figure 2). Cryptosporidium infection and HL treatment were included in all models to control the possible influence on the proteins changing patterns. Sample size in these mixed linear regression models was 329 (102, 112, and 114 by study weeks). The SAA concentrations were elevated during the first 2 wk and then declined in the third week. The Hp concentrations were highest in the second week compared with the first and third weeks. Albumin concentrations were lowest in the first week and then increased and stabilized during the second and third weeks. Serum IgG was highest in the first week and declined constantly over the 3-wk period investigated. Age-dependent changes in the serum concentrations of the cytokines are presented in Table 2 and Figure 3. Serum concentrations of IL-6 in the first week were higher than in the second and third weeks. The TNF-α concentrations were highest during the first week of life and then declined. The IL-1β concentrations were lower during the second week than during the first and third weeks.

**Effect of Colostrum and Cryptosporidium Infection**

Colostrum marker concentrations had some associations with all measured protein concentrations in the calves’ serum (Figures 2 and 3). Colostrum SAA had a negative association with calves’ SAA serum concentration in the first week of life (coefficient for log mg/L ± SEM: $-0.158 \pm 0.066 \; \text{log mg/L}; P = 0.018$; Figure 4). Colostrum IL-6 concentration was positively associated with serum IL-6 concentrations during all 3 wk (coefficients for ng/L ± SEM: $0.008 \pm 0.002 \log \text{ng/L}, 0.006 \pm 0.001 \log \text{ng/L}, \text{and } 0.006 \pm 0.002 \log \text{ng/L}; \text{all } P < 0.001$). Colostrum TNF-α concentration was positively associated with the same cytokine serum concentrations during the first 2 wk (coefficients for ng/L ± SEM: $0.134 \pm 0.025 \log \text{ng/L}$ and $0.118 \pm 0.021 \log \text{ng/L}; \text{both } P < 0.001$), and colostrum IL-1β concentration was associated with serum IL-1β during the first week (coefficient for ng/L ± SEM: $0.001 \pm 0.0002 \log \text{ng/L}; P < 0.001$). Colostrum IgG concentration was positively associated with calves’ IL-6 (coefficient for g/L ± SEM: $0.014 \pm 0.006 \log \text{ng/L}; P = 0.031$) and TNF-α (coefficient for g/L ± SEM: $0.013 \pm 0.005 \log \text{ng/L}; P = 0.008$) concentrations in the second week, and negatively with serum albumin concentration during the first week (coefficient for g/L ± SEM: $-0.100$}
Table 2. Colostrum and serum sample concentrations of proinflammatory cytokine and acute-phase protein concentrations and IgG in 143 dairy calves sampled once a week during the first 3 wk of life (1–3 samples per calf)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Statistics</th>
<th>Colostrum (n = 143)</th>
<th>Serum sample (d of age)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1–7 (n = 103)</td>
<td>8–14 (n = 112)</td>
</tr>
<tr>
<td>SAA (mg/L)</td>
<td>Mean (± SD)</td>
<td>65.7 (47.1)</td>
<td>146.6 (66.5)*</td>
</tr>
<tr>
<td></td>
<td>Median (minimum–maximum)</td>
<td>52.1 (7.5–277.0)</td>
<td>128.9 (22.4–347.7)</td>
</tr>
<tr>
<td>Hp (mg/L)</td>
<td>Mean (± SD)</td>
<td>190 (62)</td>
<td>376 (430)*</td>
</tr>
<tr>
<td></td>
<td>Median (minimum–maximum)</td>
<td>175 (104–480)</td>
<td>701 (658)b</td>
</tr>
<tr>
<td>Alb (g/L)</td>
<td>Mean (± SD)</td>
<td>NA</td>
<td>29.1 (5.8)*</td>
</tr>
<tr>
<td>IgG (g/L)</td>
<td>Mean (± SD)</td>
<td>55.1 (12.0)</td>
<td>16.8 (8.7)*</td>
</tr>
<tr>
<td>IL-6 (ng/L)</td>
<td>Mean (± SD)</td>
<td>55.3 (33.5)</td>
<td>16.3 (19.5)</td>
</tr>
<tr>
<td>TNF-α (ng/L)</td>
<td>Mean (± SD)</td>
<td>43.2 (10.2–501.4)</td>
<td>9.8 (2.5–130.7)</td>
</tr>
<tr>
<td></td>
<td>Median (minimum–maximum)</td>
<td>3.126 (3.681)</td>
<td>450 (70–2,820)</td>
</tr>
<tr>
<td>IL-1β (ng/L)</td>
<td>Mean (± SD)</td>
<td>551.0 (1,142.1)</td>
<td>102.8 (227.6)*</td>
</tr>
</tbody>
</table>

*Different letters indicate significantly (P < 0.001) different concentrations by week evaluated with mixed linear regression models.

1SAA = serum amyloid A; Hp = haptoglobin; Alb = albumin; TNF-α = tumor necrosis factor-α; NA = not analyzed.
Colostrum IgG concentration was positively associated with serum IgG concentrations during all weeks \((P < 0.001; \text{Table 2})\). Serum SAA was higher in the high Cryptosporidium oocyst level group than in the Cryptosporidium negative group during the second week \((P = 0.002)\), whereas Hp was higher in the first 2 wk of life \((P = 0.007\) and \(P < 0.001\), respectively). The same association existed between high Cryptosporidium oocyst levels and serum IL-6 concentrations in the second and third weeks \((P = 0.038\) and \(P = 0.004\)) and serum TNF-\(\alpha\) in the third week \((P = 0.005)\).

The differences of the marker concentrations between the 3 study weeks could be influenced by the calves’ systemic immune response to the Cryptosporidium infection; thus, Cryptosporidium oocyst level group and HL treatment group had to be controlled in all the statistical models.

**Effect of Colostrum and Serum Concentrations on Weight Gain**

The average birth weight of the calves was 41.2 ± 5.8 kg (ranging from 27 to 52 kg). At the age of 1 mo, ADWG (g/d) was 419.5 ± 149.2 \((n = 121)\), at the age of 3 mo 783.8 ± 130.8 \((n = 119)\), and at 9 mo 688.8 ± 166.9 \((n = 120)\).

Colostrum protein concentrations did not have statistically significant associations with any age period ADWG (g/d), and the serum protein concentrations did not have associations with the calves’ ADWG at 1 mo of age. Serum Hp concentration in the second week was borderline significantly negatively associated with 3-mo ADWG \((\text{coefficient for mg/L ± SEM: } -0.03 ± 0.02 \text{ g/d}; \ P = 0.067; \ n = 98)\) and in the third week significantly negatively associated \((\text{coefficient for mg/L ± SEM: } -0.06 ± 0.03 \text{ g/d}; \ P = 0.025; \ n = 104, \text{ Table 3})\). Age at the blood sampling time, HL treatment group, Cryptosporidium oocyst level group, and parity of the cow were included in these multiple linear regression models as possible confounders \((\text{Table 3})\).

Serum concentrations of SAA and IL-6 during the second week of age were negatively associated with ADWG at 9 mo of age \((\text{Table 4})\). A significant positive correlation was found between serum SAA and Hp \((r = 0.36)\) and IL-6 and TNF-\(\alpha\) \((r = 0.92)\); thus, the associations between Hp and TNF-\(\alpha\) of the second week of age and 9-mo ADWG (g/d) were evaluated separately from SAA and IL-6 in different models. In these separate models, serum Hp concentration \((\text{coefficient for mg/L ± SEM: } -0.035 ± 0.015 \text{ g/d}; \ P = 0.023)\) and serum TNF-\(\alpha\) concentration \((\text{coefficient for ng/L ± SEM: } -0.04 ± 0.02 \text{ g/d}; \ P = 0.041)\) were negatively associated with 9-mo ADWG. Serum SAA concentra-
tion in the third week of age was negatively associated with 9-mo ADWG (coefficient for mg/L ± SEM: −0.45 ± 0.17 g/d; \( P = 0.010; n = 105 \)). Age at the time of sampling, age at weighing, maternal parity, HL treatment group, and Cryptosporidium oocyst level group were included in these multiple linear regression models as confounders (Table 4).

**DISCUSSION**

This study found that colostrum cytokines have a direct association on the calves’ immune response mainly during the first week of life and even after that (e.g., IL-6), whereas colostrum APP do not directly affect the calves’ systemic innate immune response, from which we suggest that they may instead have a local protective effect in the gastrointestinal tract of young calves after ingestion.

High levels of proinflammatory cytokines (IL-6, IL-1β, and TNF-α) and APP (SAA and Hp) were measured in colostrum in this study. Higher concentrations of cytokines in colostrum compared with mature milk have been found in bovines (Hagiwara et al., 2000). The levels of the cytokines IL-6 and TNF-α were correlated in the colostrum, suggesting that they are both influenced by the same factors. These cytokines have also been found to potentiate immunological functions (Yamanaka et al., 2003). Higher concentrations of SAA and Hp in colostrum compared with mature milk have also been demonstrated before (McDonald et al., 2001; Thomas et al., 2016).

A proteomics study in sheep demonstrated the effect of colostrum on SAA concentrations in lambs’ serum (Hernández-Castellano et al., 2014). In calves, it has been shown that colostrum SAA isoforms do not directly cross calf intestines (Orro et al., 2008). Our previous studies demonstrated that ewe colostrum SAA and lamb serum SAA were positively associated during the first 5 d of life, and we hypothesized that colostrum has a direct influence on the systemic innate immune response of lambs (Niine et al., 2018a; Peetsalu et al., 2019). The present study did not confirm this hypothesis, but because lambs, in contrast to calves, have ad libitum access to colostrum, the colostrum effect may be different in these 2 species.

![Figure 4. Negative association of calves’ (n = 103) serum amyloid A (SAA) and colostrum SAA concentrations during the first week of life. The solid line represents the regression line (with 95% CI; dotted lines) evaluated with a multivariable regression model, in which calves’ age at sampling and maternal parity (primiparous or multiparous) were included. Coef. = coefficient.](image)

**Table 3.** Results of multivariable linear regression model for detecting association of calves’ (n = 104) serum proinflammatory cytokine and acute-phase protein concentrations during the third week of life (15–21 d of age) with average daily weight gain (g/d), measured at approximately 3 mo of age (mean ± SD; 101.4 ± 10.6 d)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Coefficient</th>
<th>SEM</th>
<th>( P )-value</th>
<th>Wald test ( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at sampling (d)</td>
<td>42</td>
<td>−0.39</td>
<td>5.77</td>
<td>0.946(^2)</td>
<td>0.032</td>
</tr>
<tr>
<td>Hp serum concentration (mg/L)</td>
<td>41</td>
<td>−0.06</td>
<td>0.03</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>HL treatment group(^3)</td>
<td>47</td>
<td>−95.16</td>
<td>37.03</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>16</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incorrect treatment</td>
<td>41</td>
<td>−43.17</td>
<td>35.76</td>
<td>0.230</td>
<td></td>
</tr>
<tr>
<td>Correct treatment</td>
<td>47</td>
<td>−95.16</td>
<td>37.03</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>Cryptosporidium group(^4)</td>
<td>47</td>
<td>−95.16</td>
<td>37.03</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>No oocysts found</td>
<td>35</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low oocyst level (below the median)</td>
<td>36</td>
<td>−18.16</td>
<td>29.31</td>
<td>0.537</td>
<td></td>
</tr>
<tr>
<td>High oocyst level (above the median)</td>
<td>33</td>
<td>−35.45</td>
<td>34.14</td>
<td>0.302</td>
<td></td>
</tr>
<tr>
<td>Parity of the cow</td>
<td>50</td>
<td>−44.47</td>
<td>23.59</td>
<td>0.062</td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>50</td>
<td>−44.47</td>
<td>23.59</td>
<td>0.062</td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>54</td>
<td>914.06</td>
<td>111.81</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

\(^{1}\)Hp = haptoglobin; \( \text{HL} = \) halofuginone lactate.

\(^{2}\)Nonsignificant variable was retained from the model because of confounder effect.

\(^{3}\)Incorrect treatment: daily treatment started >48 h after birth and lasted <7 d. Correct treatment: daily treatment started ≤48 h after birth and lasted ≥7 d.

\(^{4}\)Positive samples were divided into low and high oocyst count categories using median values of the same week.
is also possible that in sheep, colostrum SAA directly transfers to the lambs.

In neonatal pigs, it has been shown that colostrum Hp is transferred to piglets and the endogenous Hp synthesis is also stimulated by colostrum (Hiss-Pesch et al., 2011). Studies on the relationship between colostrum consumption of calves and their serum Hp concentrations are inconclusive. Dairy calves that received milk-based formula had higher Hp serum concentrations than colostrum-fed calves (Sadri et al., 2020), a tendency that was also observed in colostrum-deprived lambs (Hernández-Castellano et al., 2015). Possible explanations include a lack of immunoglobulin and a stress reaction resulting in a lack of energy intake or higher inflammatory stimulus because of weaker passive immunity transfer. In contrast, in another study, higher plasma concentrations of Hp were found in the group that received colostrum than in the group that received milk-based formula (Liermann et al., 2020). However, the present study showed no direct positive associations of colostrum SAA or Hp with the calves’ serum levels of the same parameters. We hypothesize that colostrum APP have local effects on the gastrointestinal tract or systemic effects through their proinflammatory properties. Because SAA has direct opsonizing properties, it also acts locally by stabilizing the intestinal environment (Reigstad et al., 2009). Serum amyloid A is assumed to respond to microbial colonization with the suppression of systemic neutrophil activation and bactericidal activity (Murdoch et al., 2019). A study of piglet diarrhea indicated that colostrum SAA has a beneficial effect on newborns, as litters from sows with higher colostrum SAA concentrations showed less diarrhea in the first week of life (Hasan et al., 2019). In humans, colostrum contains SAA1, which is suggested to have a role in the development of newborn intestinal defense maturation and immune responses (Sack et al., 2018). We found that colostrum SAA and calf serum SAA had a negative association during the first week of age, suggesting that the calves’ own inflammatory response was sooner activated when less protection from colostrum SAA was available. This further emphasizes the protective role of colostrum SAA shortly after birth (approximate 1-wk period).

In this study, positive associations between the colostrum cytokines IL-1β, TNF-α, and IL-6 and the same cytokines in calves’ serum (IL-1β (first week), TNF-α (first and second week), and IL-6 (during all 3 wk)) were found. In a study by Yamanaka et al. (2003), among the examined cytokines (IL-1β, IL-6, TNF-α, and IFN-γ), only IL-1β was detected before colostrum consumption. We suggest that colostrum proinflammatory cytokines (IL-6, IL-1β, and TNF-α) may have a direct influence on the calves’ immune system during the first week of life.

We conclude that colostrum may have a systemic effect on neonatal calves’ APR through the impact on the calves’ cytokine milieu.

Concentrations of serum SAA and Hp fluctuate in neonate ruminants before they stabilize at adult levels. In our study, the SAA concentrations of dairy calves increased during the first 2 wk before stabilization, whereas Hp concentrations peaked in the second week of life but decreased thereafter. Studies of clini-

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Coefficient</th>
<th>SEM</th>
<th>P-value</th>
<th>Wald test P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (kg)</td>
<td>311</td>
<td>−5.06</td>
<td>2.02</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>Age at sampling (d)</td>
<td>311</td>
<td>−9.19</td>
<td>4.84</td>
<td>0.061</td>
<td></td>
</tr>
<tr>
<td>Age at weighing (d)</td>
<td>317</td>
<td>4.48</td>
<td>1.49</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>SAA serum concentration (mg/L)</td>
<td>311</td>
<td>−0.36</td>
<td>0.12</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>IL-6 serum concentration (ng/L)</td>
<td>311</td>
<td>−1.48</td>
<td>0.63</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>HL treatment group²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No treatment</td>
<td>16</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incorrect treatment</td>
<td>37</td>
<td>−96.61</td>
<td>27.47</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Correct treatment</td>
<td>47</td>
<td>−152.41</td>
<td>30.47</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Cryptosporidium group³</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.158⁴</td>
</tr>
<tr>
<td>Negative</td>
<td>50</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low oocyst level (below the median)</td>
<td>21</td>
<td>−19.12</td>
<td>25.04</td>
<td>0.447</td>
<td></td>
</tr>
<tr>
<td>High oocyst level (above the median)</td>
<td>29</td>
<td>33.69</td>
<td>26.19</td>
<td>0.201</td>
<td></td>
</tr>
<tr>
<td>Intersect</td>
<td></td>
<td>−9.57</td>
<td>387.22</td>
<td>0.980</td>
<td></td>
</tr>
</tbody>
</table>

1Hp = haptoglobin; SAA = serum amyloid A; HL = halofuginone lactate.
2Incorrect treatment: daily treatment started >48 h after birth and lasted <7 d. Correct treatment: daily treatment started <48 h after birth and lasted ≥7 d.
3Positive samples were divided into low and high oocyst count categories using median values of the same week.
4Nonsignificant variable was retained from the model because of confounder effect.
cally healthy calves (dairy and cross-breed) showed the same tendency (Orro et al., 2008; Tóthová et al., 2015). This leads to the conclusion that some of the age-related changes in SAA concentration occur independently from clinical disease and are most probably caused by some physiological process or subclinical infections.

At birth, albumin is the most prominent protein fraction of total serum proteins (Tóthová et al., 2016). Its relative concentrations have been shown to decrease significantly 1 d after colostrum intake but then increase gradually over the first month (Tóthová et al., 2015). Other studies have confirmed a progressive increase in albumin after birth in calves (Bertoni et al., 2009; Piccione et al., 2009). It has been previously shown that IL-6 inhibits the production of albumin (Tanaka et al., 2014). In our study, the albumin concentration increased during the first 3 wk of life, and a negative association between colostrum IgG and albumin during the first week of life was evident. This emphasizes the inhibiting effect of colostrum globulins on neonatal albumin production.

Several explanations for the changes in APP concentrations after birth have been considered. One possibility could be the birth process itself, which is traumatic and could elicit APR (Marchini et al., 2000). Another hypothesis considers colostrum to be the trigger for APP changes (Tóthová et al., 2015), but we did not find any associations of colostrum APP and cytokines after the first week of life.

As changes in APP concentrations after birth seem to be physiological, we can assume that they have beneficial effects. Nevertheless, prolonged elevated levels of neonatal APP may negatively reflect the animal’s performance (e.g., weight gain; Orro et al., 2006; Seppä-Lassila et al., 2017, 2018; Peetsalu et al., 2019).

Postnatal growth is also controlled by growth hormone and IGF-1 (Strle et al., 2004), which in turn are inhibited by the proinflammatory cytokines IL-1β and TNF-α (O’Connor et al., 2008). In this study, there were no associations between high serum cytokine levels and short-term weight gain during the first week of life, but a negative association was evident between the second week cytokine levels and long-term weight gain, similar to the associations of SAA and Hp. After the first week of life, the protection of colostrum starts to fade, and environmental factors, possibly leading to subclinical infections, may cause long-lasting immunomodulatory effects and through that negatively affect long-term weight gain. There were no associations between colostrum components and weight gain, meaning that especially the second and third week, when the colostrum effect has ceased, represents an important period for neonatal adaptation.

High shedding of Cryptosporidium had a considerable effect on the APR of neonatal calves in this study, so it has to be considered in field studies during the time in which shedding of Cryptosporidium spp. occurs. Cryptosporidium spp. is very common on cattle farms worldwide (O’Handley and Olson, 2006). In Estonia, Cryptosporidium spp. shedding in neonatal calves was found in 66% of investigated farms (Santoro et al., 2019). Halofuginone lactate is used as standard care for prophylaxis of Cryptosporidium spp. by reducing the excretion of oocysts (Silverlås et al., 2009), and it needs to be considered as a possible factor influencing the outcomes of our study (Figure 1). Because cryptosporidiosis is very prevalent in young calves and prophylactic treatment is widely used, our results represent a common situation in dairy farms.

One of the shortcomings of this study is the possibly high level of noise due to other factors activating the calves’ APR. The calves in this study did not present symptoms of any other clinical disease (e.g., respiratory, umbilical, or joint disease) during the first 3 wk of life. The Cryptosporidium spp. outbreak or HL treatment (or both) may have masked the clinical signs of other infections. Before the Cryptosporidium spp. outbreak, veterinarians on the study farm had diagnosed several infections causing diarrhea (coronavirus, rotavirus, and Escherichia coli). In addition, the herd had tested positive for bovine viral diarrhea virus at the time of the study (Niine et al., 2018b). This suggests that other infections may have been involved. In addition, the results of the present study may not be representative of large populations because the study took place on only one farm, and there was an acute outbreak of cryptosporidiosis. However, the negative association between APR markers in the second week of life with weight gain has been reported in different ruminant species under different management conditions. This suggests that the results of this study are not only specific to this one farm but are also applicable more generally.

This study was an observational field cohort study describing the real-life situation in many dairy farms. However, these kinds of studies can raise more questions because they are influenced by different uncontrollable factors. These studies can eventually lead to results that can be directly transferable to farm management practices.

**CONCLUSIONS**

The associations between colostrum cytokines and calves’ systemic immunity, predominantly during the first week of life, show that there may be an effect of colostrum beyond maternal antibodies. However, colostrum cytokines may not influence calves’ serum
APP. Concentrations of inflammatory markers, such as APP and proinflammatory cytokines, in calves’ serum go through time-related changes during the neonatal period, and after the first week, have a negative association with long-term weight gain. Future studies should examine environmental factors (e.g., pathogen exposure) and microbial colonization during the neonatal period more closely because they may have a long-term impact on animal health and production.

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