Evaluation of potential biomarkers to determine adequate colostrum provision in male dairy-beef calves upon arrival at the rearing facility beyond 14 days of age

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ABSTRACT

Colostrum consumption is crucial for passive immunization and development of the newborn calf. However, the incidence on failed transfer of passive immunity in male calves destined to dairy-beef production remains high to date. In addition, the lack of an automated procedure to validate the immunization status upon arrival at rearing facilities in calves beyond 14 d of age impedes the identification of failed transfer of passive immunity, and therefore, of those calves at high risk of suffering diseases. For this study, 82 newborn male Holstein calves (43.3 ± 0.86 kg of body weight; mean ± standard error) from a commercial dairy farm were used to investigate potential serum biomarkers of colostrum provision. The potential biomarkers selected were IgG, IgG1, cholesterol, alkaline phosphatase, gamma-glutamyl transferase (GGT), and total protein (TP). Treatments were as follows: high-colostrum (HC; n = 49), in which calves received 4 L of colostrum within the first 2 h after birth and 2 L of colostrum in the next 3 feedings within the first 24 h after birth, for a total of 10 L of colostrum; and low-colostrum (LC; n = 33), in which calves received only 2 L of colostrum within the first 2 h after birth. After colostrum consumption, calves were allocated to individual hutches and fed 2 L of milk replacer twice daily at a concentration of 125 g/L as fed. Starter feed and water were offered ad libitum. At approximately 14 d of age (14.2 ± 0.81 d of age; mean ± standard error) calves were transported 2.5 h to a research unit at IRTA (Torre Marimon, Spain) simulating the arrival to a rearing facility. Blood samples were collected before feeding at birth, 48 h after birth, and at arrival to the rearing facility. Results on the serum concentrations of the potential biomarkers at arrival to the rearing facility showed that IgG, IgG1, GGT, and TP were greater for the HC calves compared with the LC calves. Serum concentrations of cholesterol and alkaline phosphatase did not show differences between treatment groups. Additionally, body weight losses from birth until arrival to the rearing facility were greater for the LC treatment compared with the HC. Because of their low cost, quickness, and ease of measurement, GGT and TP were good indicators of colostrum intake in calves arriving at rearing facilities beyond 14 d of age.

Key words: dairy-beef calf, colostrum provision, gamma-glutamyl transferase, total protein

INTRODUCTION

Male Holstein calves are considered by-products, or even waste products, from the dairy industry, and their postnatal care is not always a priority for producers (Devant and Marti, 2020). It has been estimated that, within a range of 12 to 43%, male calves entering the dairy-beef industry suffer failed transfer of passive immunity (**FTPI**; Pardon et al., 2015; Wilson et al., 2020). Even though the importance of colostrum provision in newborn calves is globally understood, FTPI continues to be of great concern in both the veal and the dairy-beef production systems. Accordingly, previous studies have demonstrated that female calves receive higher volumes of less contaminated (average bacterial counts) colostrum compared with male calves (Fecteau et al., 2002) which, in some cases, do not receive colostrum at all (Renaud et al., 2017). Colostrum consumption is crucial for the correct development of the immune system by the ingestion of immunoglobulins, particularly IgG (Godden, 2008) because newborn calves lack immunocompetence at birth (Hulbert and Moisá, 2016). Additionally, nutrient and bioactive compounds found in the colostrum have also been shown to modulate the development and function of the gastrointestinal tract of newborn calves (Blum, 2006). The

lack of information on the immunological background and health status of these male calves when they arrive at the rearing facilities reveals the large disconnection between the dairy and dairy-beef production industries. Also, the public is increasingly concerned about the health and welfare of male calves being marketed to the meat industry. Although the European Union has addressed some of these concerns by establishing minimal standards for the protection of calves reared for fattening, including housing, care, and nutrition (European Union, 2008), other standards, such as fitness of young calves for transport, are still poorly described (Regulation 1/2005).

Assessing FTPI in young calves at arrival to rearing facilities, when calves are normally older than 14 d of age, would provide important evidence on colostrum provision and, consequently, this information could be used to improve vaccination protocols, management, and nutrition considering calves` individual vulnerability, which in turn would affect antibiotics use. Several techniques are available for measuring FTPI; however, no one has developed a standardized method. Measurements of serum IgG concentration via radial immunodiffusion is considered the reference test (Beam et al., 2009). The IgG concentration, especially IgG1 (the primary immunoglobulin in bovine colostrum) is a good indicator of FTPI but controversy exists regarding which value ranges should be applied in calves that are a few days old as well as the age up to which those ranges should apply (Chigerwe et al., 2015; Pardon et al., 2015; Weaver et al., 2000). However, samples for radial immunodiffusion must be sent to a referral laboratory, which delays diagnosis and increases costs. Using a refractometer is considered to be more practical because of the immediate responses and the lower cost. This technique indirectly measures total protein (**TP**) concentration, which correlates well with the concentration of IgG (Tyler et al., 1996; Wilm et al., 2018). Concentration of maternal IgG has a half-life of approximately 10 d after colostrum provision (Hassig et al., 2007), and previous research has shown than TP concentration measured up to a week (Villarroel et al., 2013) or 9 d (Wilm et al., 2018) after colostrum consumption can be a good indicator of FPTI. However, the search for a biomarker able to detect colostrum intake in older calves remains necessary. Other serum biomarkers related to colostrum intake in preweaned calves are the plasma enzyme gamma-glutamyl transferase (**GGT**; Weaver et al., 2000), alkaline phosphatase (**ALP**; Thompson and Pauli, 1981), and cholesterol (**CHOL**; Renaud et al., 2018). Therefore, the objective of this study was to find a biomarker indicative of colostrum provision upon arrival at the rearing facility when calves are older than 14 d of age. This biomarker should potentially be economical and rapidly measurable to be practical at the farm level. We evaluated the use of IgG, IgG1, CHOL, ALP, GGT, and TP as potential biomarkers of colostrum provision.

MATERIALS AND METHODS

Animals, Treatments, and Feeding

All calves used in this study were managed following the principles and guidelines of the Animal Care Committee of Institut de Recerca i Tecnologia Agroalimentàries (Barcelona, Spain; RD 53/2013; project no. 11211). A total of 82 male Holstein calves (43.3 ± 0.86 kg of BW; mean $\pm$ SE) born at a commercial dairy farm (Granja Selergan, S.A., Lleida, Spain) in October 2020 [average ambient temperature of $9.3°C$ (range = $3.1–16.5°C$) and 87% humidity (range = $81–100\%$)] were used in this study. Treatments were as follows: the high-colostrum (**HC**; n = 49; 42.4 ± 0.94 kg of BW) group received 4 L of colostrum within the first 2 h after birth, and 2 L of colostrum in the next 3 feedings within the first 24 h after birth; in total those calves received 10 L of colostrum ($22.9 \pm 0.30\%$ of birth BW). The low-colostrum (**LC**; n = 33; 44.1 ± 0.77 kg of BW) group received only 2 L of colostrum within the first 2 h after birth ($4.8 \pm 0.36\%$ of birth BW). All feedings were administered via esophageal tube. Only high-quality colostrum was used for all calves (average of 24.5% Brix). Colostrum samples of each pool given to the animals (a total of 43 pools) were collected. For the analysis of the composition, an equal quantity from different pools used during the study was mixed to create a single sample that was later analyzed for CP (56.28%), fat (29.48%), and lactose (10.07%). This colostrum pool was also analyzed for GGT ($36,910$ IU/L), lactoferrin ($1,828.84$ ng/mL), IgG (145.44 mg/mL), and IgG1 (33.11 mg/mL) concentrations. After colostrum consumption, calves were allocated to individual hutches and fed 2 L of milk replacer twice daily at a concentration of 125 g/L as fed (21.86% CP, 16.59% fat, 45.50% lactose; Schils). Calves had ad libitum access to starter feed (15.2% CP, 15.0% NDF, 5.0% ADF, 29.2% starch, 4.9% ether extract on a DM basis, with the main ingredients being 13.9% soybean meal, 38.9% corn, 9% wheat, 17% barley, 3% sunflower expeller, 1.9% palm oil, and 1.3% calcium carbonate), and water. At approximately 14 d of age (14.2 ± 0.81 d of age; mean $\pm$ SE), calves were transported 2.5 h from their origin dairy farm to an experimental research unit at IRTA (Torre Marimon, Spain) simulating the arrival at a rearing facility [average ambient temperature of $11°C$ (range = $4.4–17°C$) and 87.5% humidity (range = $82–90\%$)]. Calves with respiratory or digestive prob-

lems or that were repeatedly treated with antibiotics at the dairy farm were not selected to be transported to the experimental research unit.

Measurements and Sample Collection

Body weight was recorded at birth and at arrival to the experimental research unit (simulating the arrival at a rearing facility) at approximately 14 d after birth. Blood samples were collected at birth before feeding and 48 h after birth to confirm if the colostrum administration in each treatment was correctly done and on arrival at the rearing facility before feeding to identify potential biomarkers of colostrum consumption. Samples were obtained from the jugular vein, using evacuated serum tubes (BD Vacutainer Plus Plastic Serum Tubes), and then centrifuged at $1,500 \times g$ at 4°C for 15 min. A serum sample was used to measure TP by using a clinical refractometer (KERN refractometer, ORA-AL). The remaining serum was aliquoted and stored at −20°C until further analysis. As previously mentioned, colostrum samples from different pools used during the study were collected and mixed to obtain a single sample.

Chemical Analysis

Blood samples were processed to obtain serum for analysis of potential biomarkers of colostrum provision (IgG, IgG1, CHOL, ALP, GGT, and TP). Serum IgG and IgG1 concentrations were determined by an ELISA (species-specific Bovine IgG and Bovine IgG1, Bethyl Laboratories Inc.). Serum concentration of GGT, CHOL, ALP, and TP were analyzed by using a Beckman Coulter AU480 analyzer.

The intra- and interassay coefficients of variations of IgG, IgG1, GGT, CHOL, ALP, and TP were 3.2 and 3.3%, 3.37 and 9.26%, 0.8 and 1%, 0.4 and 1.1%, 0.9 and 1.5%, and 0.38 and 0.74%, respectively. The starter feed was analyzed for DM (24 h at 103°C), ash (4 h at 550°C), CP by the Kjeldahl method (method 981.10; AOAC International, 1995), and ADF and NDF (method 973.18; AOAC International, 1996). Samples from the milk replacer used in this study were analyzed for DM (24 h at 103°C), ash (4 h at 550°C), CP by the Kjeldahl method (method 981.10; AOAC International, 1995), and sugars (HPLC–Refractive Index; method 984.22; AOAC International, 2002). Colostrum samples were analyzed for fat by the gravimetric method (ISO 1211/IDF 1: 2010; ISO, 2010), CP by the block-digestion method (ISO 8968–3/IDF 20–3:2004; ISO, 2004), and ashes (BOE-A-1997-1661; BOE, 1997). Lactose was calculated removing the fat, CP, and ashes from the TS content.

Statistical Analysis

Based on the outcome of Cuttance et al. (2017) investigating diagnostic tests for FTPI in dairy calves, estimating prevalence of FTPI with a 95% confidence level at the herd level using different diagnostic tests required sample sizes varying from 42 to 61. Therefore, considering all the diagnostic tests with their sensitivity and specificity used in the current study it was estimated that between 30 and 54 calves/treatment would be required to detect differences. Calf was the experimental unit. The study design was a randomized unbalanced complete block design with a covariate adjustment. Animals were distributed using a stratified randomization where 1 of 3 animals balanced by parity and calving time was assigned to the LC treatment and 2 were assigned to the HC treatment. The model included the random effect of pen and the fixed effect of treatment (amount of colostrum), and initial BW and age as a covariate. The blocks were parity (primiparous and multiparous) and calving time (nighttime born or daytime born). Serum concentration of IgG, IgG1, CHOL, ALP, GGT, TP, and BW loss were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc.). A normality test was conducted, and the nonnormal data were log-transformed to achieve normal distribution. Pearson correlation analyses were performed between serum IgG, IgG1, CHOL, ALP, GGT, and TP at 48 h after birth and at arrival. Additionally, the Pearson correlation analyses were also performed between serum IgG and GGT, between TP measured by refractometry and TP measured at the laboratory, and between TP measured by refractometry and serum IgG, all of them measured at arrival. Furthermore, IgG at 48 h was also correlated with TP measured at the laboratory, TP measured by refractometry, and GGT at arrival. All Pearson correlations were analyzed using JMP (version 16.0.0 SAS Institute Inc.). Lin's concordance correlation coefficient (**CCC**) was used to assess agreement between TP measured by refractometry and TP measured at the laboratory. The Lin's CCC was calculated using a SPSS package (IBM SPSS Statistics version 28.0) and an adapted syntax file from Garcia-Granero (2009). Differences were declared significant at $P \leq 0.05$, and trends were discussed at $0.05 \leq P \leq 0.10$ for all models.

RESULTS AND DISCUSSION

Failed Transfer of Passive Immunity

The total amount of IgG consumed by the HC calves was 1,450 g in 10 L of colostrum administered in 4 feedings, and the total amount of IgG consumed by the LC

Table 1. Serum IgG, IgG1, cholesterol (CHOL), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and total protein (TP) concentrations at birth and 48 h after birth in male Holstein calves[1]

| Item | Treatment | | SEM | P-value[2] |
	HC	LC		Trt
Potential biomarker (at birth)				
IgG, mg/mL	0.23	0.80	0.241	0.15
IgG1, mg/mL	0.09	0.29	0.083	0.21
CHOL, mg/dL	18.7	19.8	1.46	0.58
ALP, ng/mL	5.13	5.18	0.092	0.71
GGT, ng/mL	11.00	12.1	0.79	0.49
TP, g/dL	4.22	4.2	0.064	0.89
Potential biomarker (48 h after birth)				
IgG, mg/mL	72.6[a]	29.9[b]	3.98	<0.01
IgG1, mg/mL	27.0[a]	13.2[b]	1.71	<0.01
CHOL, mg/dL	29.4	27.5	1.25	0.30
ALP, ng/mL	5.53	5.44	0.082	0.43
GGT, ng/mL	1,946[a]	562[b]	148.5	<0.01
TP, g/dL	7.14[a]	5.63[b]	0.143	<0.01

[a,b]Values with different superscript within a row differ at $P \leq 0.05$.

[1]Calves in the high-colostrum group (HC) were fed 4 L of colostrum within the first 2 h after birth and 2 L of colostrum in the next 3 feedings within the first 24 h after birth. Calves in the low-colostrum group (LC) were fed only 2 L of colostrum within the first 2 h after birth.

[2]Trt = effect of the amount of colostrum provision on serum concentration of potential biomarkers.

calves was 290 g in 2 L of colostrum administered in 1 feeding. All potential biomarkers except for CHOL and ALP showed greater ($P < 0.01$; Table 1) concentrations for the HC calves compared with the LC by 48 h after birth, confirming that the amounts of colostrum given were different between treatments. However, the LC treatment did not achieve what is considered to be an FTPI condition. Failed transfer of passive immunity has been described as <1.0 g/dL serum IgG concentration in calves that are between 1 and 7 d old (Tyler et al., 1996). In a more recent study, 4 categories have been proposed to assess FTPI as follows: excellent, good, fair, and poor with serum IgG levels of $\geq$25.0, 18.0–24.9, 10.0–17.9, and <10 g/L, respectively (Lombard et al., 2020). In the present study, results showed that IgG serum concentrations 48 h after birth were 72.6 ± 3.61 mg/mL and 29.9 ± 4.35 mg/mL (mean ± SE) for the HC and LC groups, respectively. Based on Tyler et al. (1996) our calves did not suffer FTPI and based on Lombard et al. (2020) both treatments fell into the "excellent" category. Even though we reached a high immunoglobulin level in both treatments, 2 L of colostrum may not be sufficient to provide the additional benefits of nutrients and bioactive substances contained in the colostrum that modulate for, for example, the development of the gastrointestinal tract (Hammon et al., 2020). To achieve FTPI in the LC calves it might be necessary to increase the time between birth and colostrum administration or to provide a low-quality colostrum. The LC treatment was selected based on commercial practices with the limitation of not achieving an FTPI condition. However, the amount of colos-

trum fed to the HC calves compared with commercial practices for research purposes allowed us to see differences between treatments.

The generally accepted cut-off points for measuring FTPI using TP is between 5 and 5.5 g/dL in calves from 1 to 8 d old (Tyler et al., 1996). In this study, TP concentrations by 48 h after birth were 7.1 ± 0.09 g/dL and 5.6 ± 0.11 g/dL (mean ± SE) for the HC and LC group, respectively (Table 1), suggesting that TP is probably the most sensitive of all biomarkers to define FTPI because the LC calves had a TP concentration close to the limit described by Tyler et al. (1996).

As mentioned, although the treatments applied were different enough to see differences on the concentration of IgG, IgG1, GGT and TP, it seems that providing 2 L of good-quality colostrum within 2 h after birth did not cause FTPI. However, adequate colostrum provision (amount, time, quality) is a concept that goes beyond FTPI. Colostrum contains nutrients (carbohydrates, proteins, lipids), minerals, vitamins, and growth promoters among other bioactive compounds that exert important morphological and functional changes in calves (Blum, 2006). This means that colostrum provision can exert positive effects on calves' lifetimes beyond the immune protection at an early age resulting from immunoglobulins. These positive effects are relevant for the development of the gastrointestinal tract in the newborn calf (Blum and Hammon, 2000); it has been demonstrated that high amounts of consumed colostrum increased the intestinal epithelial cells' growth, promoting intestinal absorption (Blättler et al., 2001). Ingestion of colostrum has also been shown

to increase crypt cell proliferation, decrease apoptosis, and stimulate villus growth in repeatedly colostrum-fed calves (Blum, 2006). Additionally, the interactions of the many different bioactive substances (vs. their individual actions) contained in colostrum have been described to promote intestinal growth and cell proliferation (Hammon et al., 2020). In the present study, even if biomarkers were not indicative of FTPI, they might be indicative of calves' colostrum consumption at their farm of origin. In this scenario, being able to detect colostrum provision even when the quality remains unknown would still provide valuable information that might allow producers to make decisions over management, vaccination, and nutritional protocols for the newly arrived calves.

Potential Biomarkers of Colostrum Provision at Arrival

Differences in IgG and IgG1 concentrations were found between HC and LC calves. Serum concentrations of IgG and IgG1 were greater ($P < 0.01$; Figure 1A and B, respectively) on arrival at the rearing facility for the HC treatment compared with the LC treatment. Three main classes of immunoglobulins are found in serum, milk, and colostrum in bovines: IgG, IgM, and IgA. Additionally, IgG is subdivided in 2 subclasses: IgG1 and IgG2. Of these 2, IgG1 represents more than 75% of the immunoglobulins in colostrum (Korhonen et al., 2000). Both immunoglobulins can be used as indicators of colostrum consumption; however, IgG1 could be a better estimate based on its predominance in colostrum. Several methods have been developed to determine IgG concentration in the blood of newborn calves as an approach to detect FTPI or colostrum consumption. However, even though results are promising in differentiating calves with different colostrum provision using IgG or IgG1, direct measurement of immunoglobulins by radial immunodiffusion, turbidimetric immunoassay, or ELISA is expensive because these tests require laboratory interpretation and trained personnel. Therefore, the lack of less costly and automated techniques makes it difficult to control colostrum provision in herds based on these measurements.

The use of CHOL as a biomarker of colostrum consumption has been previously explored (Marcato et al., 2018). Cholesterol can be found in higher concentrations in the colostrum compared with mature milk (Shope and Gowen, 1928). Consequently, neonatal calves' serum CHOL concentration after birth might be indicative of the amount of colostrum consumed (Renaud et al., 2018). Results from this study showed no differences ($P = 0.39$; Figure 2 A) for serum CHOL concentration between HC or LC calves. Cholesterol
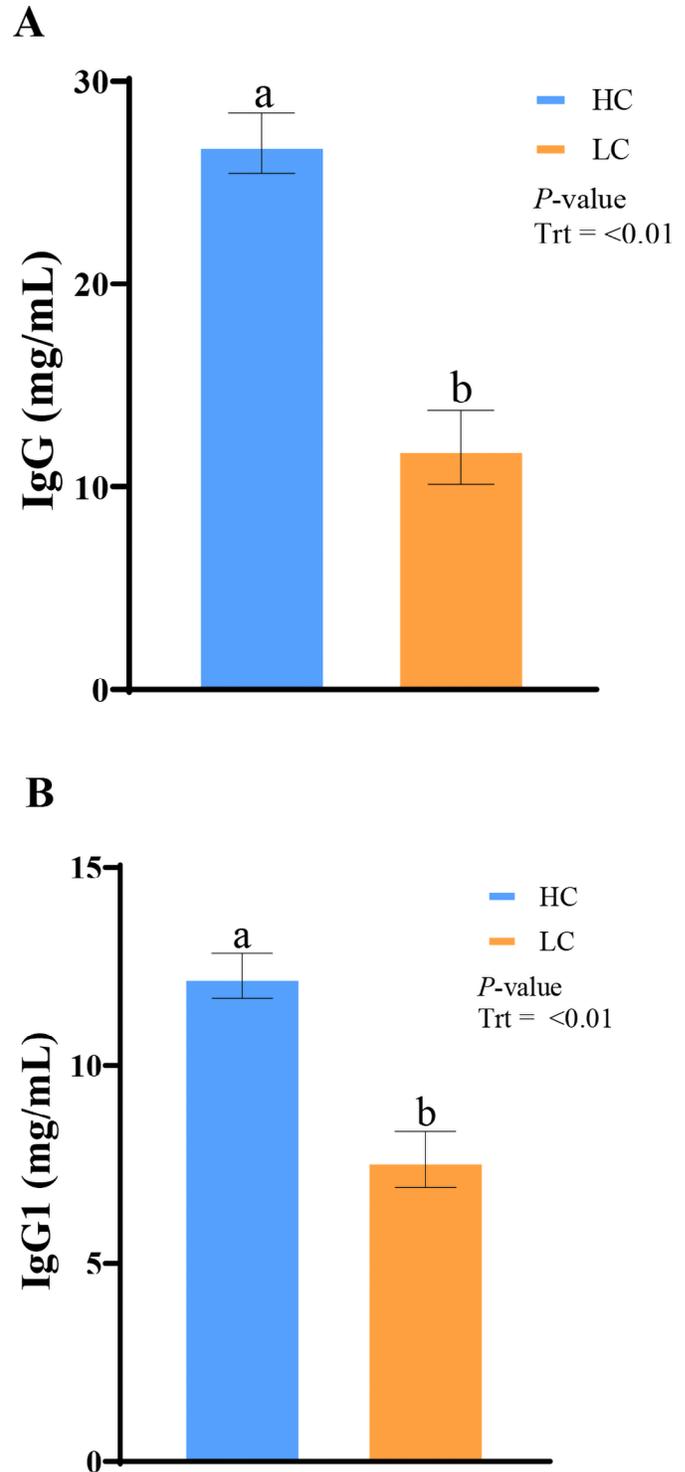
Figure 1. Serum concentrations (mean ± SE) of IgG (A), and IgG1 (B) in male Holstein in 2 treatment groups. Calves in the high-colostrum group (HC) were fed 4 L of colostrum within the first 2 h after birth and 2 L of colostrum in the next 3 feedings within the first 24 h after birth. Calves in the low-colostrum group (LC) were fed only 2 L of colostrum within the first 2 h after birth. Different letters within a time point denote differences among treatments ($P < 0.05$); order of the letters denotes the treatment with the highest value. Trt = treatment.
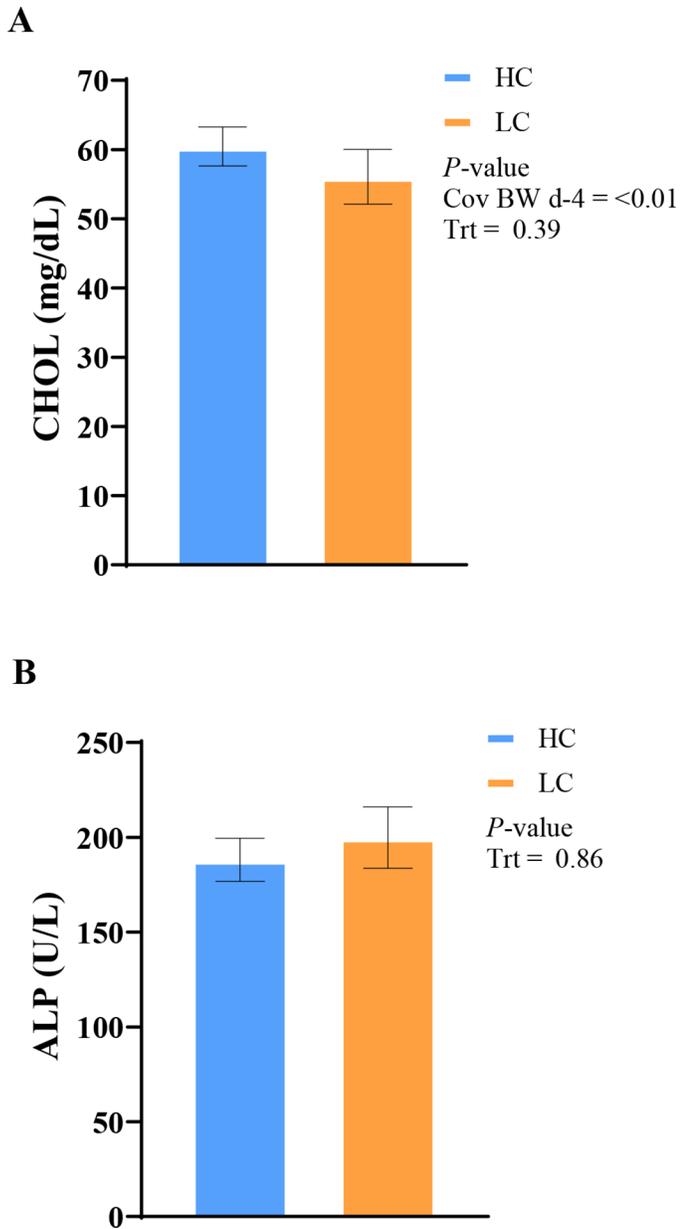
A

B

Figure 2. Serum concentrations (mean ± SE) of cholesterol (CHOL, A), and alkaline phosphatase (ALP, B) in male Holstein calves in 2 treatment groups. Calves in the high-colostrum group (HC) were fed 4 L of colostrum within the first 2 h after birth and 2 L of colostrum in the next 3 feedings within the first 24 h after birth. Calves in the low-colostrum group (LC) were fed only 2 L of colostrum within the first 2 h after birth. Cov BW d-4 = BW on d 4 as covariate; Trt = treatment.

was first described as a marker of calves' mortality risk during the first 21 d after arrival at rearing facilities by Renaud et al., (2018). Cholesterol plays an important role in intestinal signaling, mediating intestinal lipid absorption, and regulating plasma lipid concentration (Thurnhofer and Hauser, 1990), which in neonatal calves represent the major energy source to

satisfy the demands of tissue growth (Ontsouka et al., 2016). Additionally, CHOL regulates lactase activity, an enzyme facilitating lactose absorption in the intestine and whose low concentration has been associated with the risk of morbidity and diarrhea in the newborn calf (Kien et al., 1996). In the present study we were expecting to see differences in CHOL concentration between treatments based on the amounts of colostrum consumed. However, it seems that even when feeding different amounts of colostrum, CHOL concentration did not vary significantly. Because we were unable to generate an actual FTPI condition, it remains unclear how this could have affected CHOL concentration.

Increased serum ALP concentration has been previously correlated with increases in serum GGT in calves (Thompson and Pauli, 1981; Zanker et al., 2001). Additionally, Britti et al. (2005) found an increase in ALP concentration after colostrum consumption and a positive correlation between ALP and IgG in newborn lambs. In disagreement, the present study showed no differences between treatments for serum ALP concentration ($P = 0.86$; Figure 2B). Thompson and Pauli (1981) described a large initial drop followed by a gradual decline in ALP concentration after colostrum intake in calves. This pattern seems to act similarly in lambs. However, because the activity of ALP in ewe's colostrum is lower than in lambs' serum, ALP appears to be derived from the brush border of the intestine and its concentration might be associated with feeding but not necessarily ingestion of colostrum (Thompson and Pauli, 1981). Pauli (1983) proposed that a similar phenomenon exists in calves. However, based on the results from the present study, serum ALP cannot be considered a good biomarker of colostrum consumption in calves older than 14 d of age. Inconsistencies in the literature to date make it difficult to validate ALP serum concentration as an indicator of colostrum consumption.

Measurements of serum GGT concentration have been previously studied as a potential biomarker for FTPI (Perino et al., 1993; Parish et al., 1997; Buczinski et al., 2020). In the present study, GGT serum concentration was greater ($P < 0.01$; Figure 3A) in the HC group compared with the LC group upon arrival at the rearing facility. Gamma-glutamyl transferase is an enzyme involved in amino acid transport and produced in the mammary gland ducts during colostrogenesis (Baumrucker, 1979; Blum and Hammon, 2000). For this reason, its concentration in bovine colostrum is higher than in milk (Cuttance et al., 2019). After colostrum consumption, GGT is absorbed in the small intestine of the calf via the same nonselective passage that is used by IgG (Parish et al., 1997). After absorption, GGT can be found in calves' serum at concentrations
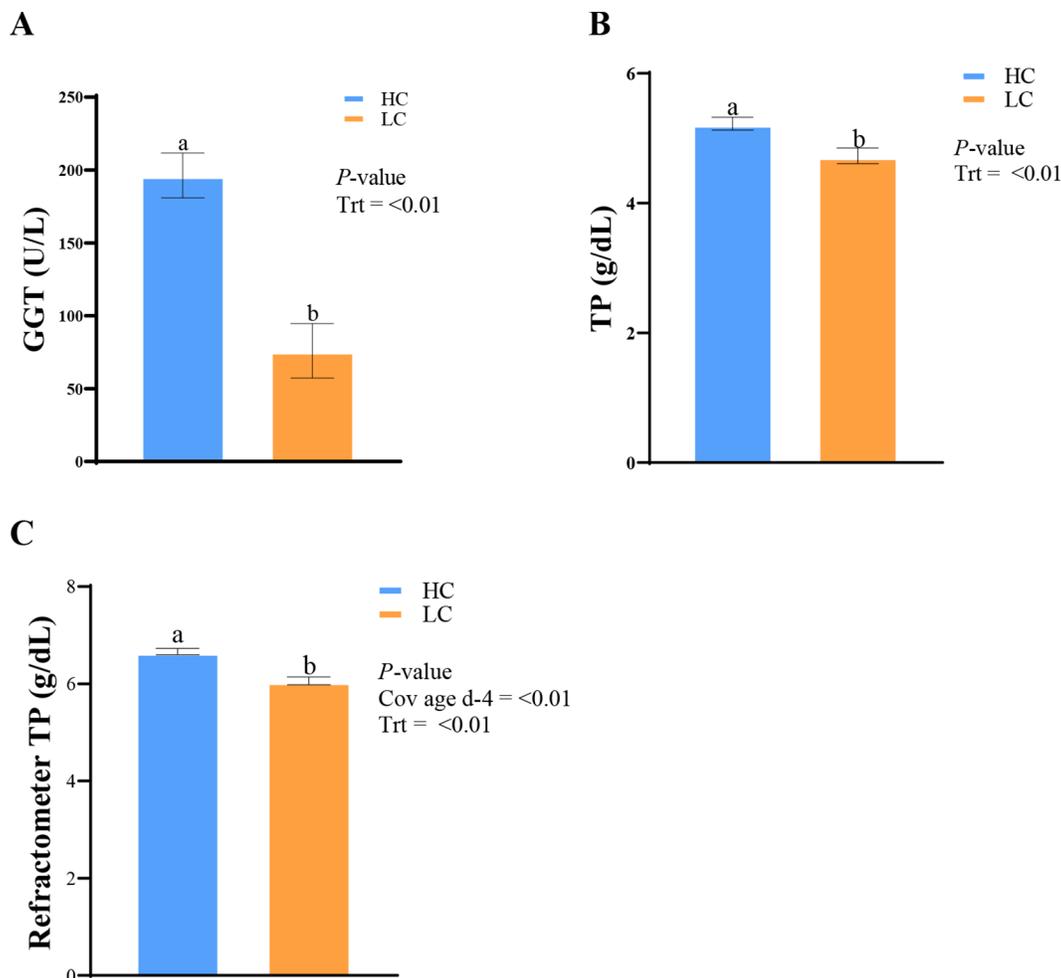
A

B

C

Figure 3. Serum concentrations (mean ± SE) of gamma-glutamyl transferase (GGT, A), total protein (TP, B), and TP calculated with a refractometer (C) in male Holstein calves in 2 treatment groups. Calves in the high-colostrum group (HC) were fed 4 L of colostrum within the first 2 h after birth and 2 L of colostrum in the next 3 feedings within the first 24 h after birth. Calves in the low-colostrum group (LC) were fed only 2 L of colostrum within the first 2 h after birth. Different letters within a time point denote differences among treatments ($P < 0.05$); order of the letters denotes the treatment with the highest value. Cov BW d-4 = BW on d 4 as covariate; Trt = treatment.

60–160 times greater than in adult cattle, declining to adult activities by 5 wk of age (Thompson and Pauli, 1981; Yu et al., 2019). Some studies have shown positive correlations between serum GGT and serum immunoglobulin concentrations (Thompson and Pauli, 1981; Perino et al., 1993; Parish et al., 1997; Weaver et al., 2000). Results from the present study showed a positive correlation ($P < 0.01$) between serum IgG and GGT concentrations at arrival, although with a low coefficient of determination ($R^2 = 0.53$; CI 95% = 0.60–0.82; $P < 0.001$). However, a high concentration of GGT in a calf is not necessarily indicative of having been fed a good-quality colostrum because there is no biological relation between GGT and colostrum IgG concentration (Weaver et al., 2000; de Souza et al., 2021). In this scenario, measurements of GGT could

be used only as an indicator of colostrum intake (Blum and Hammon, 2000). Further studies evaluating GGT upon arrival at rearing facilities and correlating the amount, timing, and quality of colostrum offered may provide more information on the potential of GGT as a biomarker of colostrum provision in calves older than 14 d of age because, as mentioned previously, measurements of IgG and IgG1 are expensive and tedious, whereas GGT determination is cheaper, quicker, and can be conveniently automated (Hogan et al., 2015).

At arrival to the rearing facility, the concentration of TP was greater ($P < 0.01$; Figure 3 B) for the HC group compared with the LC group when TP concentration was determined with a biochemical analyzer. As expected, serum concentration of TP exhibited a similar pattern compared with IgG1 (Tyler et al., 1996;

Table 2. Correlation between serum IgG, IgG1, cholesterol (CHOL), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and total protein (TP) concentrations 48 h after birth and at arrival to the rearing facility in male Holstein calves[1]

Item	R^2	Correlation	CI 95%	*P*-value
IgG, mg/mL	0.29	0.53	0.36–0.68	<0.001
IgG1, mg/mL	0.09	0.30	0.09–0.49	<0.01
CHOL, mg/dL	<0.001	0.01	−0.21–0.23	0.91
ALP, ng/mL	0.12	0.34	0.13–0.52	<0.01
GGT, ng/mL	0.49	0.70	0.57–0.80	<0.001
TP, g/dL	0.22	0.46	0.28–0.62	<0.001

[1]Calves were fed 4 L of colostrum within the first 2 h after birth, and 2 L of colostrum in the next 3 feedings within the first 24 h after birth (HC) and fed only 2 L of colostrum within the first 2 h after birth (LC)

Dawes et al., 2002; Godden, 2008). Determining FTPI by measuring TP is based on the fact that the ingestion of immunoglobulins via colostrum intake (major contributor of serum proteins in newborn calves) increases TP concentration in serum (Hogan et al., 2015). Concentration of TP was also measured by using a clinical refractometer. This technique is inexpensive, easy for farm personnel to perform and interpret, and offers immediate results. Likewise, results showed greater ($P < 0.01$; Figure 3 C) serum concentration of TP measured by refractometry for the HC calves compared with the LC calves at arrival to the rearing facility. However, when assessing colostrum provision by measuring TP some aspects need to be considered. The first consideration is the age of the animals. Previous studies have demonstrated that age can influence IgG serum concentration mainly based on a protein catabolism process (Cuttance et al., 2019; de Souza et al., 2021). Cuttance et al. (2019) suggested that calves should not be older than 1 wk of age, de Souza et al. (2021) proposes 24 to 48 h, if possible, and Wilm et al. (2018) described that serum TP concentration up to 9 d of age can provide reliable estimates of FPTI in calves. Second, this test should be performed in healthy nondehydrated calves because a process of dehydration or protein-losing enteropathy will alter protein concentration in serum (Hogan et al., 2015) leading to misinterpretations of TP concentration. This is an important consideration because transported calves arrive at the rearing facilities dehydrated (Renaud et al., 2018; Wilson et al., 2020). Therefore, the time when TP should be measured is crucial for the reliability of the results. In this study, calves were transported only 2.5 h from their origin dairy farm to the rearing facility and did not exhibit signs of dehydration (e.g., sunken eyes or skin turgor). Thus, the differences in TP concentration found between HC and LC calves can be considered reliable. Measuring TP 24 h after arrival when calves have been rehydrated and fed after a long-distance transportation could be an optimal time to perform the test. However, further studies should investigate the

interactions between transport duration, dehydration, and TP concentration in unweaned calves to better understand the veracity of TP concentration as a tool to estimate FTPI.

Results of the Pearson correlation analysis between the different biomarkers at 48 h after birth and at arrival when calves are approximately 14 d of age showed that IgG, IgG1, ALP, and TP had a positive significant correlation but with a low coefficient of correlation or determination (Table 2). A higher coefficient of determination was observed for GGT (Table 2); however, an R^2 of 49% is still low as a prediction of the concentration of GGT over time. The Pearson correlation analysis performed between serum TP concentration obtained by refractometry and TP measured at the laboratory at arrival showed a significant positive correlation ($P < 0.01$) between techniques although the coefficient of determination was lower than expected ($R^2 = 0.35$; CI 95% = 0.43–0.59; $P < 0.001$). Similar results were obtained with Lin's CCC (CCC = 0.185; CI 95% = 0.113–0.255), demonstrating that the agreement of the TP analyzed by refractometry and by laboratory techniques was low.

The reason why the correlation between those measurements was low is unknown and further comparisons should be done by sending the samples to different laboratories or using other lab techniques. When the correlation between the IgG at 48 h and TP measured at the laboratory at arrival ($R^2 = 0.08$; CI 95% = 0.06–0.47; $P = 0.01$) was compared with the correlation between IgG at 48 h and TP concentration obtained by refractometry at arrival ($R^2 = 0.26$; CI 95% = 0.32–0.66; $P < 0.001$), the association was improved. On the other hand, a Pearson correlation with an $R^2 = 0.61$ (CI 95% = 0.67–0.85; $P < 0.001$) was observed between serum TP calculated by refractometry and serum IgG concentration at arrival. The high correlation between serum IgG and serum TP concentration measured by refractometry has been studied in newborn calves (McBeath et al., 1971; $R^2 = 0.72$). However, in our study TP values measured either

at the laboratory or with the refractometer at arrival were not good enough to predict the level of IgG 48 h after birth and may be reliable only when compared with IgG at the same time point. However, most of the literature has only shown levels of IgG or TP until 7 d after birth (Rauprich et al., 2000; Renaud and Pardon, 2022), and comparisons when calves are older could not be made. Rauprich et al. (2000) showed how IgG concentration decreased rapidly 3 d after birth, while the decrease in TP was slower. Additionally, a recent study showed inconsistencies in the use of TP measured by refractometry as an estimate of IgG concentration in calves due to the existence of residual nonprotein solutes contained in colostrum, which accounted for approximately 36% of the variation in results (Schalich et al., 2021). Recently, measuring TP by using a refractometer has been questioned and other methods have been proposed (Lombard et al., 2022; Schalich and Selvaraj, 2022). In the present study, Pearson correlations between TP by refractometer at arrival and IgG at 48 h ($R^2 = 0.26$; CI 95% = 0.32–0.66; $P < 0.001$) had a higher coefficient of determination than the correlation between GGT at arrival and IgG at 48 h ($R^2 = 0.17$; CI 95% = 0.21–0.58; $P < 0.001$). The sensitivity and specificity (**Sp**) analysis using IgG (Lombard et al., 2020) and TP (Tyler et al., 1996) at 48 h after birth showed a sensitivity of 0.9%, Sp of 0.31%, and an accuracy of 0.81%. The sensitivity and Sp analysis using IgG (Lombard et al., 2020) and GGT (Parish et al., 1997) at 48 h after birth showed a sensitivity of 0.91%, Sp of 1%, and an accuracy of 0.91%. The greater Pearson correlation between GGT at 48 h and GGT at arrival (Table 2) and the high accuracy and Sp between IgG at 48 h and GGT at 48 h could make the GGT concentration as good as TP as a biomarker of colostrum consumption and with the advantage that it might not be affected by the dehydration status of the animals.

As mentioned previously, colostrum has potential beneficial effects on gut development beyond providing immunity through immunoglobulins. These additional effects that colostrum has on calves' gastrointestinal tracts and physiology might be responsible for the differences observed for BW losses in the present study. Body weight loss was calculated as the difference between BW at birth and BW at arrival to the rearing facility. Results showed greater ($P < 0.01$; Figure 4) BW losses for the LC calves compared with the HC calves. At dairy farms, when male calves are fed a low plane of nutrition, their dietary requirements are not fully covered, which causes BW losses (Winder et al., 2016). Based on the results on BW losses, it could be assumed that administrating lower amounts of colostrum at birth directly affects calves' performance at arrival. Low BW
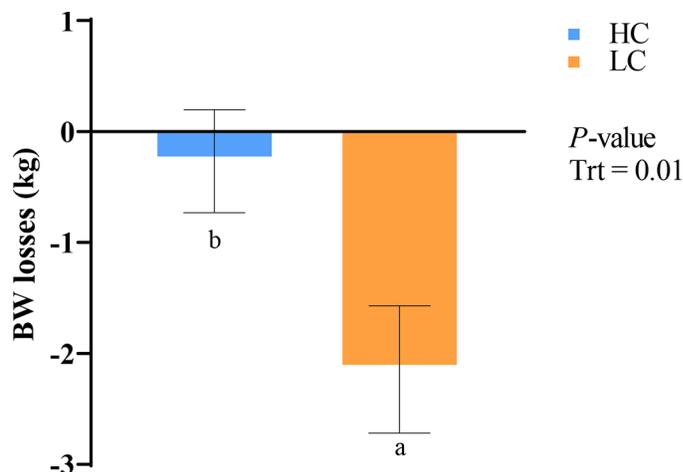

Figure 4. Body weight losses in male Holstein calves in 2 treatment groups. Calves in the high-colostrum group (HC) were fed 4 L of colostrum within the first 2 h after birth and 2 L of colostrum in the next 3 feedings within the first 24 h after birth. Calves in the low-colostrum group (LC) were fed only 2 L of colostrum within the first 2 h after birth. Differences were calculated between BW at birth and BW at arrival to the rearing facility. Different letters within a time point denote differences among treatments ($P < 0.05$); order of the letters denotes the treatment with the highest value. Trt = treatment.

at arrival to veal farms has been previously associated with an increase of mortality risk (Fallon et al., 1987; Moore et al., 2002; Winder et al., 2016). Additionally, it has been demonstrated that malnourished calves have increased cortisol levels, which can affect their immune response (Drackley, 2005) and their capacity to deal with pathogens, especially those provoking diarrhea (Fallon et al., 1987; Paré et al., 1993), a disease of great incidence in young calves. All together, these conditions aggravate calves' health and performance at a time in their lives when they are overexposed to stressful situations like transportation, commingling, feed restriction, and undeveloped immunity, among others (Roland et al., 2016). For this reason, dairy farmers should be encouraged to ensure an adequate plane of nutrition for male calves from birth until arrival at the rearing facilities and to collect BW records to avoid transportation of unfit calves at risk of suffering from disease in order to decrease morbidity and mortality rates upon arrival at the rearing facilities.

In summary, the use of biomarkers that could detect colostrum consumption will offer important information that can be used by veal and dairy-beef producers to classify, isolate, and treat calves depending on their individual risk. Previous studies have evaluated the concentration of these biomarkers in newborn calves to assess colostrum consumption or FTPI (Thompson and Pauli, 1981; Blum and Hammon, 2000; Marcato et al., 2018). However, their application in older calves arriving at rearing facilities remains unclear. Among

the potential biomarkers of colostrum consumption considered in this study, GGT and TP measured by refractometer might be good indicators of colostrum intake in calves arriving at rearing facilities because they are faster, less expensive, easier to automate, and more consistent techniques compared with measuring immunoglobulins. The age at arrival to a rearing facility for dairy-beef calves varies widely but is normally beyond 14 d of age. One of the benefits of using serum GGT concentration as an alternative to TP as a marker of colostrum consumption is that, based on the literature, GGT concentration decreases to adult activities by 5 wk of age (Thompson and Pauli, 1981). This could allow the detection of differences in concentration in calves arriving at rearing facilities until approximately 1 mo of age. Additionally, it is still unknown until what age TP could be detected in serum to have a good correlation with IgG. Another advantage is that GGT concentration in serum is not affected by the hydration status of the calves upon arrival at the rearing facility. And, as previously mentioned, dehydration after long-distance transportation periods is quite common in dairy-beef calves. On the other hand, TP provides more information on the quality of the colostrum and can be easily measured by using a refractometer, a test that can be performed at the farm. Additionally, even though BW is not routinely recorded at the dairy farms, measurements of BW loss could also be a good estimator of risk during the first weeks after arrival. Even though these biomarkers might seem promising in assessing colostrum consumption in dairy-beef calves, further investigation should be conducted on stablishing valid ranges in serum considering if calves were HC or LC at the farm of origin. Additionally, the most appropriate time to measure the concentration of these biomarkers in addition to the study of better correlations needs to be further defined in the search of a validated tool to assess colostrum consumption upon arrival at rearing facilities.

CONCLUSIONS

Serum concentrations of GGT and TP appear to be the most reliable indicators of colostrum consumption in calves beyond 14 d of age. In the present study, even though calves were fed high-quality colostrum and did not suffer FTPI, HC calves showed greater concentrations of most of the biomarkers evaluated. These results obtained under experimental conditions might show even greater differences when evaluating colostrum consumption in the practice where transported male calves are vulnerable to stressors like commingling, mixing, contagious diseases, environmental conditions, and feed restriction, among many others that increase their risk.

Further research should be undertaken to investigate if these additional transport stressors confirm the results observed in the present study.

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