Revista Brasileira de Zootecnia

Passive immune transfer, health, pre-weaning performance, and metabolism of dairy calves fed a colostrum supplement associated with medium-quality maternal colostrum

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ABSTRACT - The objective of this study was to evaluate passive immunity transfer, health, performance, and metabolism of Holstein calves fed colostrum supplement associated with medium-quality colostrum. After birth, calves were blocked according to birth weight and date and distributed into the following treatments: high-quality colostrum (> 50 mg of Ig/mL; n = 15; 150 g kg^{-1} BW; positive control group); medium-quality colostrum (30-40 mg of Ig/mL; n = 14; 150 g kg^{-1} BW), and medium-quality colostrum (30-40 mg of Ig/mL; n = 15; 150 g kg^{-1} BW) + colostrum supplement (15 mL) given with the colostrum. Colostrum was given within the first 12 h of life in two meals by nipple bottle, and colostrum replacer was administered by a blister syringe. Blood samples were taken every 12 h up to 48 h of life for total serum protein (TSP) evaluation. After colostrum feeding, calves were fed 4 L of transition milking, split in two meals for a period of one to four days and received milk replacer thereafter. Calves were individually housed, with free access to water and concentrate and bucket-fed 6 L/d of milk replacer up to the sixth week of life, when they received 4 L/d until weaning, with eight weeks. Colostrum feeding protocol affected the TSP concentration in the first 48 h of life, while the concentrations of albumin, glucose, β-hydroxybutyrate, γ-glutamyl transferase, and alkaline phosphatase were not affected. During the milk-feeding period, the concentrate and total dry matter intake were not affected by the colostrum protocol, but increased as animals aged. Colostrum-feeding protocol did not affect performance or health of calves. Feeding colostrum supplement associated with medium-quality colostrum had no effect on passive immune transfer, performance, nor on the metabolism during the liquid-feeding phase.

Keywords: blood parameters, colostrum supply, immunity, immunoglobulin Y, intake

Introduction

High-quality colostrum intake is one of the factors that most affects the short and long-term performance of animals (Faber et al., 2005). Calves that consume higher colostrum volumes present higher serum immunoglobulins (Ig) concentrations and lower risk of morbidity and mortality (Davis and Drackley, 1998). Adequate immune passive transfer is associated with lower veterinary treatment costs, higher weight gain rates, increased milk production, and longevity of the herd (Williams et al., 2014). A calf is diagnosed with failure of passive immunity transfer (FPIT) when serum Ig concentrations are bellow 10 g IgG/mL (Weaver et al., 2000) or total serum protein (TSP) is lower than 5.5 g/dL (Godden, 2008). Several factors may contribute to the FPIT; however, colostrum quality is the most important one, since

2

Passive immune transfer, health, pre-weaning performance, and metabolism of dairy calves fed a colostrum...
De Paula et al.

calves need to consume about 104 g of IgG at the first meal to avoid this condition (Davis and Drackley, 1998). Colostrum is classified of high quality when Ig concentrations is higher than 50 mg/mL, while medium-quality colostrum presents Ig concentration between 20-50 mg/mL (Godden, 2008). However, recent research has suggested that the cut point for a high-quality colostrum should be of 80 mg/mL for colostrometer measurements and 23% Brix for the refractometer (Bartier et al., 2015).

Recent research about dairy calf management in the South and Southeast of Brazil has shown that more than 60% of the producers may allow calves to stay with the cows for more than 8 h and that 74% of producers do not freeze extra colostrum (Santos and Bittar, 2015). In addition, 89% of producers in Brazil do not evaluate colostrum quality, and 98% do not monitor serum total protein of the calves (Santos and Bittar, 2015), increasing the risk for FPIT. Besides, in Brazil, only about 22% of colostrum offered to newborn calves have nutritional and microbiological quality considered adequate (Santos et al., 2017).

Because of that, colostrum quality evaluation using a colostrometer (Fleenor and Stott, 1980) or a refractometer (Quigley et al., 2013) is vital for newborn calf management, helping to decide whether or not to give the colostrum. Even though several management errors may occur, tools to assist producers in case of colostrum shortage or inadequate quality have been developed. Colostrum supplement can be used when medium-quality colostrum is available, providing low doses of Ig (from 25-45 g Ig/dose when derived from lacteal secretions).

Supplements may be made from animal plasma, lacteal secretion, or eggs produced by hyperimmunized chickens, which contain IgY (Quigley et al., 2005). However, because of Brazilian restrictions, only supplements derived from eggs or colostrum are allowed. Nevertheless, no studies were found in the literature evaluating these products in subtropical productions systems such as those in Brazil. Supplements produced from egg yolks are commonly presented in a paste form and stored in a blister. Unfortunately, literature data shows that IgY has a low absorption efficiency, which may turn these products recommended for feeding after closure (Erhard et al., 1997), improving local immunity rather than participating in the passive immunity transfer process.

The review of Xu et al. (2011) described very few studies in which supplementation of IgY during the milk feeding period was efficient to reduce diarrhea ocurrence in calves. However, literature is scarce in regard to the effects of feeding colostrum supplement produced from eggs (IgY) at birth to improve passive immune transfer when only medium-quality colostrum is available.

The objective of this study was to evaluate the passive immune transfer, performance, metabolism, and health of dairy calves fed colostrum supplement associated with bovine medium-quality colostrum.

Material and Methods

The local Animal Ethics Committee approved all animal procedures (case number 2014-18). The experiment was carried out in Piracicaba, SP, Brazil (22°43' S, 47°38' W, 547 m).

Forty-four male Holstein newborn calves from a commercial farm were used. Parturition was monitored, and calves were separated from their mothers as soon as they were born to avoid colostrum suckling. Navel treatment was performed using a 7% iodine solution during three consecutive days.

The cows were milked, and colostrum quality was measured using a colostrometer (Suprivet, Divinópolis, MG, Brazil) and a digital Brix refractometer (Hanna Instruments – Model HI 96811). Calves were nipple bottle-fed fresh colostrum in a volume corresponding to 150 g kg^{-1} of BW split in two meals within 12 h. The first meal was given within 4 h after birth. Animals were blocked by weight and date of birth and ramdomly distributed in one of the following treatments, according to colostrum quality and supplement feeding: high-quality colostrum (> 50 mg of Ig/mL; n = 15, BW = 39.2±3.09 kg; positive control group); medium-quality colostrum (30-40 mg of Ig/mL; n =14, BW = 38.0±3.11 kg); and medium-quality colostrum (30-40 mg of Ig/mL; n = 15, BW = 39.1±3.10 kg)

3

Passive immune transfer, health, pre-weaning performance, and metabolism of dairy calves fed a colostrum...
De Paula et al.

+ colostrum supplement (Feedtech™, DeLaval Ltda., Jaguariúna, SP, Brazil), orally given in two doses of 15 mL each, with the colostrum.

Colostrum was diluted with commercialized milk (128.3 g kg^{-1} solids, 41.5 g kg^{-1} fat, 32.0 g kg^{-1} protein) to obtain required IgG values, estimated by the colostrometer, for the different treatments. Colostrum supplement was composed of 600 g kg^{-1} soybean oil, 200 g kg^{-1} powdered eggs (Salmonella-free), dextrose, vitamins, and minerals, presenting 110 g kg^{-1} crude protein (CP), 810 g kg^{-1} ether extract (EE), 00.2 g kg^{-1} crude fiber (CF), and 58 g kg^{-1} ashes. Considering literature data reviewed by Xu et al. (2011), since each egg yolk (15 mL) has around 100-200 mg IgY, each blister (200 g kg^{-1} powdered eggs) should provide approximately 60 mg IgY. However, only 20 to 100 mg g^{-1} are specific Ig. Concentrations of IgY in the commercial product were not analyzed in the present study.

After colostrum feeding, at the commercial farm, calves were fed 4 L of second and third milkings, split in two meals (07.00 and 15.00 h). Calves were group-housed, with free access to water and concentrate, during a period that varied from one to five days, when they were transported to the experimental calf facility (about 120 km away), in a cattle truck bedded with straw. The study was conducted from February to April 2015, with average temperature of 24.7±1.8 °C and precipitation of 226±97.1 mm.

At the experimental calf facility, calves were individually housed in wood shelters, being attained by a collar and a chain. The animals were fed a milk replacer diluted to 125 g kg^{-1} solids (200.5 g kg^{-1} CP, 154.6 g kg^{-1} EE, 6.5 g kg^{-1} neutral detergent fiber [NDF]; Feedtech, De Laval Ltda., Jaguariúna, SP, Brazil), at 07.00 and 17.00 h. Milk replacer was bucket-fed in a volume of 6 L/d until the sixth week of age and 4 L/d during the seventh and eighth weeks, when calves were abruptly weaned. In addition, calves had free access to water and starter concentrate (250 g kg^{-1} CP, 33 g kg^{-1} EE, 130 g kg^{-1} NDF; Agroceres Multimix, Rio Claro, SP, Brazil). The pelleted concentrate was offered *ad libitum* in the morning (06.00 h), with orts being weighted to calculate daily intake. The initial quantity offered was 200 g/d and increased or reduced according to intake during the previous day.

For the evaluation of passive immune transfer, blood samples were harvested by venipuncture using vacuolated tubes without anticoagulants or with sodium fluoride as antiglucolytic and potassium EDTA as anticoagulant (VACUETTE do Brasil, Campinas, SP, Brazil). Sampling was carried out at birth and each 12 h until calves were two days old and every week, always after morning feeding for metabolism evaluation. An aliquot was used for packed cell volume (hematocrit) determination, through centrifugation (SPIN 1000 – MICROSPIN) at 12,000 × g for 10 min. The remaining sample was centrifuged at 2,000 × g for 20 min at 4 °C for plasma or serum harvesting. After that, samples were freezer-stored in plastic tubes for subsequent analysis using an Automatic Biochemistry System (SBA – 200, CELM, Barueri, SP, Brazil) and commercial kits (LABTEST Diagnóstica S.A., Lagoa Santa, MG, Brazil) of TSP (Ref.: 99), albumin (Ref.: 19), γ-glutamil transferase (γ-GT, Ref.: 105), and alkaline phosphatase (Ref.: 40), all to evaluate the passive immune transfer success (Tyler et al., 1999; Slosarkova et al., 2014). Samples taken weekly were also centrifuged and freezer-stored for later analysis of TSP and albumin in the serum, and glucose (Ref.: 85) and β-hidroxybutyrate (BHBA, RANBUT – Ref.: RB1007, RANDOX Laboratories – Life Sciences Ltd., Crumlin, UK) in the plasma. Glucose and BHBA were evaluated to understand shift metabolism because of rumen development (Nussio et al., 2003).

For performance evaluation, body weight, withers height, hip width, and heart girth were measured weekly, always before morning feeding, until the eighth week, when the study ended. Body weight was meaured using a mechanical scale (ICS-300, Coimma Ltda., Dracena, SP, Brazil); withers height and hip width were measured using a ruler, and heart girth with a flexible tape, both with scale in centimeters. Average daily gain (ADG) was calculated according to the formula: ADG = [(Current animal weight) − (Previous weight)] / (Days between the two weighings). Feed efficiency was calculated by dividing ADG by the average daily feed intake.

For the evaluation of animal health, fecal scores were daily recorded, according to fluidity as 1 - normal and firm; 2 - soft; 3 - aqueous; and 4 - fluid (Larson et al., 1977). Diarrhea was considered when animals presented fecal score higher than 3 for more than one day. When diarrhea was diagnosed, a blister of a commercial oral rehydration solution (3.2 g sodium chloride; 3.2 g potassium chloride; and

4

Passive immune transfer, health, pre-weaning performance, and metabolism of dairy calves fed a colostrum...
De Paula et al.

18.33 g sodium acetate in 50 mL - Glutellac, Bayer Saúde Animal, Brazil) was given together with the milk replacer for a maximum period of seven days, according to manufacturer's recommendations. The number of diarrhea episodes was considered when the calf presented a fecal score higher than 3 for more than one day, and the duration was the number of days that the calf presented a fecal score higher than 3. Days of rehydration was recorded as the number of days that calves received the oral solution. In addition, all the health events, duration, and veterinary treatments were recorded. Temperature of calves was measured daily from day 1 to week 8 of life, using a digital thermometer placed into the rectum, and the days with fever was considered when calves presented more than 39.4 °C.

Data for concentrate intake, BW, ADG, body measurements, as well as blood parameters were analyzed as repeated measurements using the PROC MIXED from SAS software (Statistical Analysis System, version 9.4) according to model (1). The best covariance structure was identified from different covariance structures by comparing the AIC_C statistic (Corrected Akaike Information Criteria). Calf date of birth and weight were used as blocking criteria, and there were 15 blocks, one of them incomplete. Block was used as a random factor in the statistical model. Treatments were compared according to the adjusted Tukey test, and differences were considered significant at P<0.05 unless otherwise stated.

$$Y_{ijk} = \mu + T_i + B_j + W_k + T_iW_k + E_{ijk}, \tag{1}$$

in which Y_{ijk} is the response variable, μ is the overall mean, T_i is the treatment effect, B_j is the block effect, W_k is the age effect, T_iW_k is the interaction of treatment and age effects, and E_{ijk} is the residual effect.

Results

The colostrum supplied to the animals was considered of high and medium quality, as previously planned (Table 1). Colostrum quality was measured with different tools, a colostrometer and a Brix refractometer, whose results were similar. Calves fed high-quality colostrum received colostrum with 70.6 mg Ig/mL and > 24.4% Brix, while the other groups received inferior colostrum. The high-quality colostrum values are in agreement with suggestions made by Godden (2008) for newborn calves. However, even with the attempt to feed calves with medium-quality colostrum (30-40 mg Ig/mL), diluting colostrum with milk decreased Ig concentration to an average of 41.8 mg/mL.

Regarding metabolic parameters during the first two days of life, TSP was affected by colostrum protocol, with higher values observed for calves fed the high-quality colostrum, as compared with those fed the medium-quality colostrum (P<0.05; Table 1 and Figure 1), with no difference with calves fed medium-quality colostrum associated with the supplement. In addition, there was a time effect for TSP concentration, with increasing values from 0 to 48 h (P<0.0001).

Considering the cut point of 5.5 g/L of TSP, only three calves from the group fed medium-quality colostrum presented FTIP. All calves from the other two groups presented higher values at 48 h. Serum albumin concentrations were not affected by colostrum protocol (P>0.05); however, there was an age effect, with decreasing values up to 24 h followed by an increase thereof. The concentrations

Table 1 - Quality of colostrum provided to newborn calves and passive immune transfer

	Colostrum feeding[1]			SEM	P-value
	High quality	Medium quality	Medium quality + Supplement		
Colostrum quality estimation					
Colostrometer (mg Ig/mL)	70.6a	42.7b	41.6b	1.66	0.0001
Refractometer (% Brix)	24.4a	18.8b	18.6b	0.44	0.0001
Total serum protein[2] (g/dL)	6.7a	6.1b	6.4ab	0.13	0.0014

SEM - standard error of the mean.
[1] High-quality colostrum (>50 mg Ig/mL); medium-quality colostrum (30-40 mg Ig/mL); medium-quality colostrum (30-40 mg Ig/mL) + supplement.
[2] Time effect with P-value <0.0001 and treatment and time interaction effect with P-value <0.05.
a,b - Means in the same row with different superscript differ at P<0.05.

5

Passive immune transfer, health, pre-weaning performance, and metabolism of dairy calves fed a colostrum...
De Paula et al.

of γ-GT and alkaline phosphatase were also not affected by colostrum feeding protocol (P>0.05). However, there was an age effect (P<0.0001; Figure 1), with increasing concentrations up to 12 h for both enzymes.

During the milk feeding period, concentrate and total dry matter intake were not affected by colostrum feeding protocol (P>0.05; Table 2) and increased throughout the experiment (P<0.0001). After the sixth week of age, when volume of liquid diet offered was reduced from 6 to 4 L/d, calves increased daily intake in higher rates than in previous weeks (average of 320 g increase from one week to the other); however, there was a statistical difference from one week to the other troughtout the whole period. Body weight, ADG, and corporal measurements were not affected by colostrum feeding protocol (P>0.05; Table 2), even though there was an age effect for these variables (P<0.0001; Figure 3). Feed efficiency was not affected by colostrum feeding protocols (P>0.05; Table 2), but there was also an age effect for all treatments, with increasing values during the experimental period (P<0.0001).

Regarding the metabolic parameters during the liquid feeding period, colostrum feeding protocol had no effect on hematocrit (P>0.05), with average values of 20%. The average TSP during the milk feeding period was affected by the colostrum feeding protocol (P<0.05), with calves fed high-quality colostrum presenting higher volumes than those fed medium-quality colostrum, with no differences when compared with calves fed medium-quality colostrum with supplementation. On the other hand, treatments did not differ in albumin (P>0.05), glucose (P>0.05), or BHBA (P>0.05) concentrations. However, there was an age effect for all metabolites (P<0.05; Figure 3). Albumin decreased until the third week of age, increasing thereafter; glucose decreased during the first two weeks and increased latter; and BHBA increased during the whole evaluation period (Figure 4).
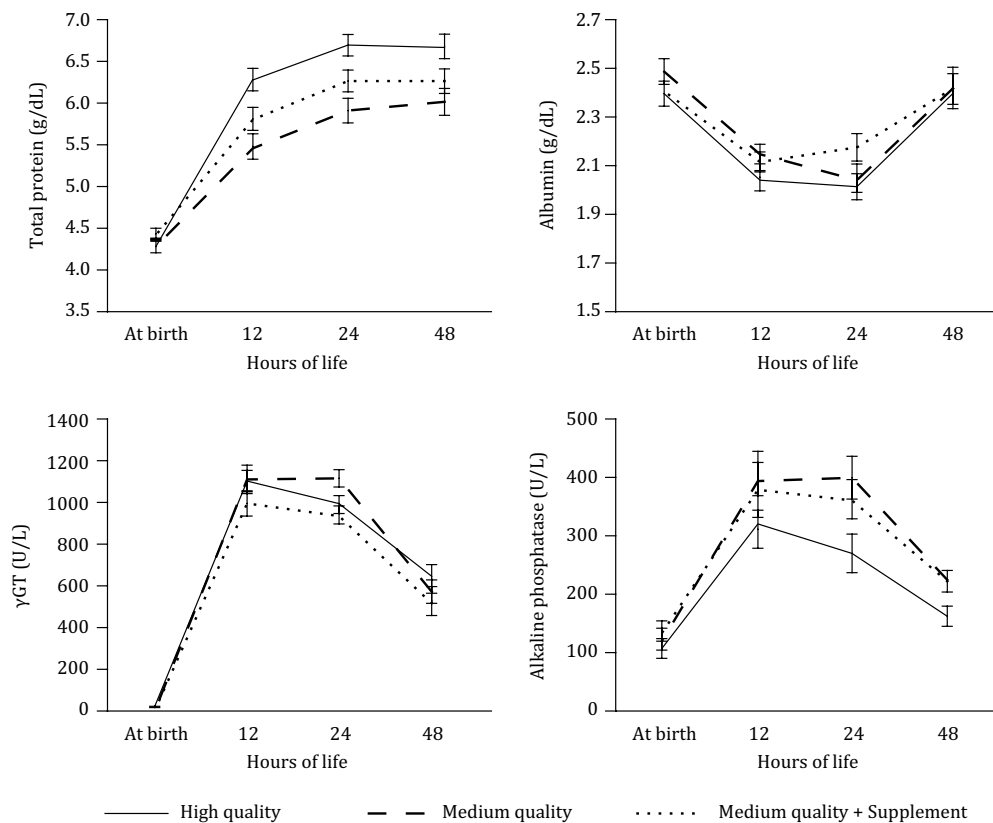
P-values refer to the age effect.

Figure 1 - Total serum protein (g/dL; treatment effect with P = 0.0014), albumin (g/dL; P = 0.2896), γGT (U/L; P = 0.0174), alkaline phosphatase (U/L; P = 0.0939) concentrations during the first 48 h after birth of calves fed high-quality colostrum, medium-quality colostrum, or medium-quality colostrum associated with a supplement.

6

Passive immune transfer, health, pre-weaning performance, and metabolism of dairy calves fed a colostrum...
De Paula et al.

Colostrum feeding protocol had no effect on fecal score and number and duration of diarrhea episodes (Table 3). For fecal score evaluation, diarrhea occurrence was considered when score was higher than 3. Thus, the higher frequency of diarrhea occurred between the second and third week of age (Figure 5). However, calves fed medium-quality colostrum presented higher frequency of score 4 (severe diarrhea), with animals presenting this score up to the fifth week of age (Figure 5). Length of rehydration therapy and days with fever were not different among treatments (Table 3). However, calves fed high-quality colostrum were medicated for a smaller number of days as compared with those in the other treatments (Table 3). Only one calf that fed the medium-quality colostrum (TSP = 5.1 g/dL) died during the third week of age because of severe dehydration caused by diarrhea. Besides, two other calves that received the colostrum supplement died during the first week of age because of timpanism.

Rectal temperature was not affected by colostrum feeding protocols, with average value of 38.7±0.1 °C. However, there was an age effect (P<0.0001), with increasing temperature from birth up to 48 h.

Table 2 - Intake and performance of calves fed high-quality colostrum, medium-quality colostrum, or medium-quality colostrum associated with a supplement

	Colostrum feeding[1]			SEM	P-value		
	High quality	Medium quality	Medium quality + Supplement		Treatment (T)	Age (A)	T×A
Intake (g DM/d)							
Concentrate	484.8	447.1	462.6	43.2	0.8252	0.0001	0.4137
Total[2]	1085.2	1043.6	1063.2	47.2	0.8132	0.0001	0.2219
Body weight (kg)							
At birth	39.19	38.00	39.13	1.40	0.8066	-	-
56 days	64.26	63.89	66.49	3.74	0.7749	-	-
Average	48.08	47.97	48.93	3.21	0.7749	0.0001	0.4457
Weight gain (g/d)	519.8	497.6	568.8	31.3	0.5228	0.0001	0.5774
Feed efficiency[3]	0.406	0.376	0.452	0.03	0.1663	0.0001	0.4467

SEM - standard error of the mean.
[1] High-quality colostrum (>50 mg Ig/mL); medium-quality colostrum (30-40 mg Ig/mL); medium-quality colostrum (30-40 mg Ig/mL) + supplement.
[2] Considering milk replacer dilution for 12.5% solids.
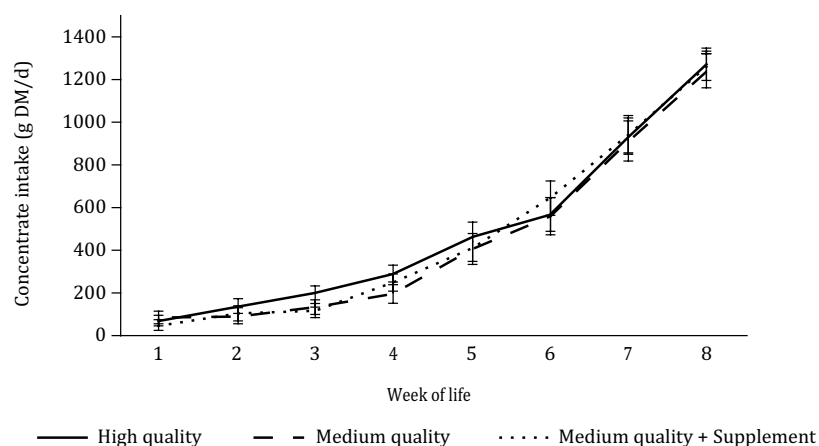[3] kg of gain/kg DM intake.

Figure 2 - Concentrate intake (g DM/d) of calves fed high-quality colostrum, medium-quality colostrum, or medium-quality colostrum associated with a supplement.
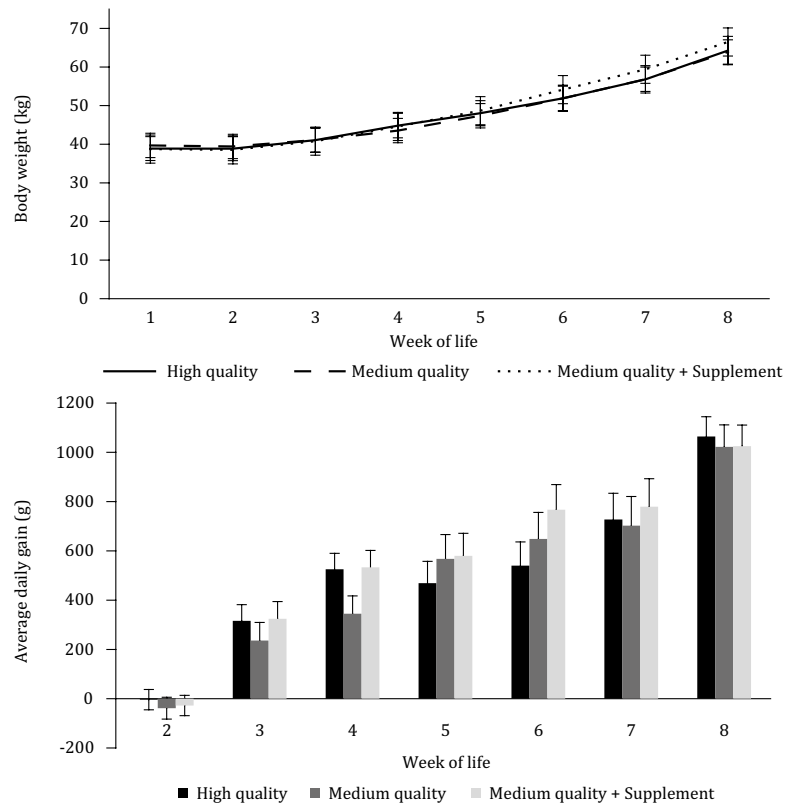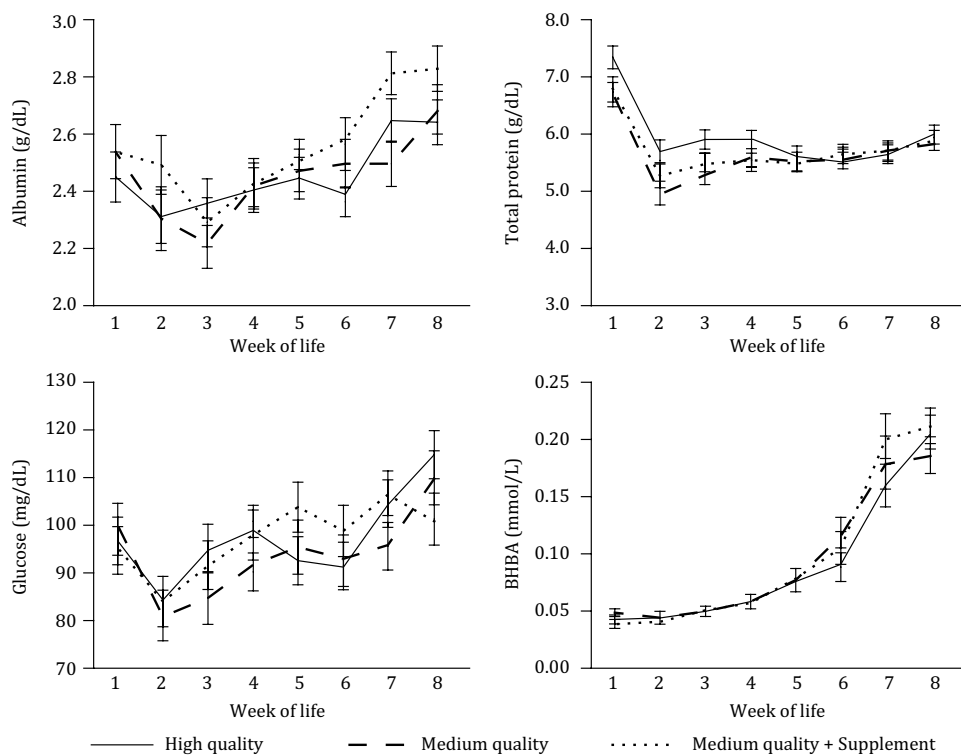
7

Passive immune transfer, health, pre-weaning performance, and metabolism of dairy calves fed a colostrum...
De Paula et al.

Figure 3 - Body weight and average daily gain of calves fed high-quality colostrum, medium-quality colostrum, or medium-quality colostrum associated with a supplement.


P-values refers to the treatment and age interaction.

Figure 4 - Albumin (g/dL; P = 0.2353), total serum protein (g/dL; P = 0.1511), glucose (mg/dL; P = 0.5292), and BHBA (mmol/L; P = 0.6210) of calves fed high-quality colostrum, medium-quality colostrum, or medium-quality colostrum associated with a supplement.

8

Passive immune transfer, health, pre-weaning performance, and metabolism of dairy calves fed a colostrum...
De Paula et al.

Table 3 - Fecal score and health of calves fed high-quality colostrum, medium-quality colostrum, or medium-quality colostrum associated with a supplement

	Colostrum feeding[1]			SEM	P-value
	High quality	Medium quality	Medium quality + Supplement		
Days with diarrhea	9.60	13.61	9.07	1.664	0.1312
Diarrhea episodes					
Number	1.35	1.52	1.26	0.143	0.4272
Duration (days)	7.20	8.38	6.68	1.132	0.5665
Days with fever	0.44	0.46	0.46	0.175	0.9920
Days of rehydration	5.2	5.9	5.0	0.96	0.7840
Days in medication	0.8a	1.0b	1.0b	0.65	0.0490

[1] High-quality colostrum (>50 mg Ig/mL); medium-quality colostrum (30-40 mg Ig/mL); medium-quality colostrum (30-40 mg Ig/mL) + supplement.
a,b - Means in the same row with different letters differ at P<0.05.
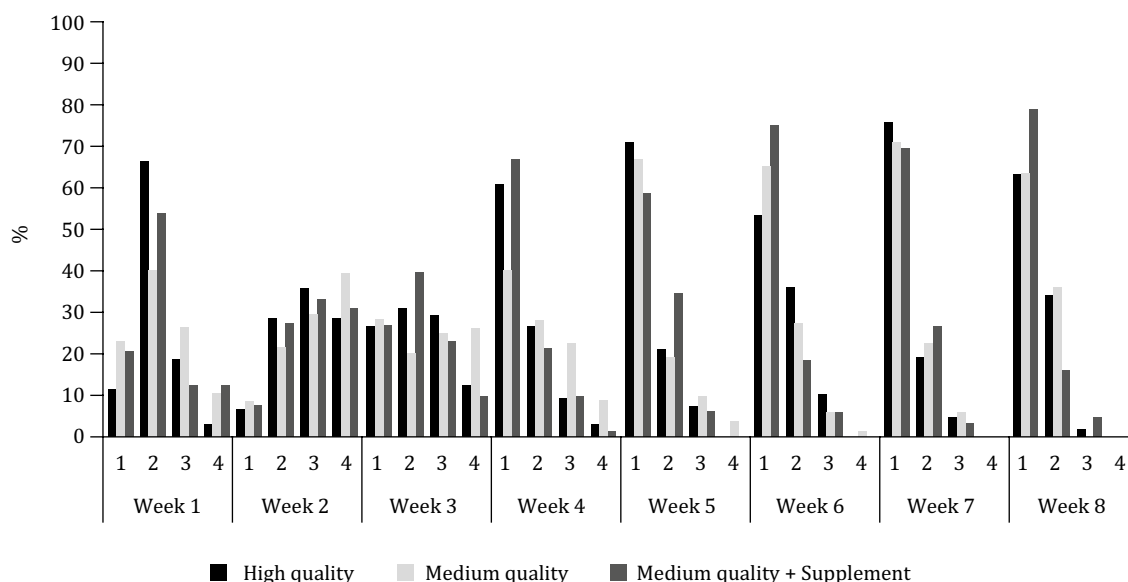
Figure 5 - Distribution of calves fed high-quality colostrum, medium-quality colostrum, or medium-quality colostrum associated with a supplement in each fecal score by week of age.

Discussion

According to Gelsinger et al. (2014), a minimum of 100 g IgG should be offered at the first meal, up to 4 h of life, since absorption efficiency is significantly reduced during the first 6 h after birth. In the present study, animals in all treatments consumed higher doses than what is recommended by the literature, explaining the satisfactory animal performance. Animals were bottle-fed, with no calf being tubed for colostrum intake during the higher absorption efficiency period, leading to adequate passive immunity transfer, regardless of the colostrum feeding protocol. According to Besser et al. (1985), the efficiency of Ig absorption through the intestinal epithelium is higher during the first 4 h after birth, reducing with time, until intestinal closured occurs.

Total serum protein concentration during the first two days of life in the present study agrees with that found in the literature. Calves fed higher doses of Ig present higher concentrations of plasma Ig (Hopkins and Quigley, 1997), which reflects on higher TSP (Quigley et al., 2013). Only three animals, out of 14 that fed the medium-quality colostrum, presented TSP lower than 5.5 mg/dL, resulting in 14.3% of FPIT (Deelen et al. (2014). Morin et al. (1997) observed higher plasma IgG concentrations at the first

9

Passive immune transfer, health, pre-weaning performance, and metabolism of dairy calves fed a colostrum...
De Paula et al.

12 h of life for calves that received a dose of 240 g IgG by feeding high-quality colostrum, as compared with a dose of 132 g IgG by feeding poor-quality colostrum. As opposed to what was observed in the present study, these authors reported FPIT for calves fed 132 g IgG. The authors also concluded that, even though a dose higher than 100 g IgG could be given by feeding poor-quality colostrum associated or not with a supplement, calf plasma concentrations of IgG were >10 mg/dL only when animals fed high-quality colostrum. These data suggest that feeding higher volume do not compensate the low Ig concentration in the colostrum. Literature is scarce with data regarding IgY supplements associated with colostrum. Erhard et al. (1995) provided IgY from powdered eggs from vaccinated hens, associated with maternal colostrum, and reported TSP concentrations during the first 48 h of life, similar to those observed in the present study.

Rauprich et al. (2000) also reported a decrease in albumin concentration followed by an increase, with values at 48 h close to those observed at birth. Similarly, Kurz and Willett (1991) reported a postprandial decrease in albumin concentrations, which may be related to the increase in plasma volume after colostrum intake. The age effect showed by γ-GT and alkaline phosphatase concentrations is related to colostral Ig absorption, being possible to measure these enzymes as an indirect measure of passive immune transfer (Weaver et al., 2000). Kurz and Willett (1991) reported that the increase in γ-GT and alkaline phosphatase concentrations occurs 2 h after feeding, with higher values from 6 to 12 h, corroborating results from the present study. All these metabolites may be used as an indirect measure of passive immune transfer. However, contrary to expected since medium-quality colostrum was provided to one group, on average, all colostrum feeding protocols resulted in adequate passive immune transfer when cut points reported in the literature are used.

The increase in starter intake after the sixth week of age agrees with data of Khan et al. (2007), which described the step-down feeding system as a way to increase concentrate intake and adequately wean dairy calves fed higher liquid diet volumes. The increase rate was higher after liquid feed volume was reduced at the sixth week (Figure 2). In this perspective, when weaned with 56 days of age, the concentrate intake was higher than 1.2 kg DM/d. Stamey et al. (2012) recommended that calves should be consuming at least 1 kg DM/d to be adequately weaned, which was observed in the current study.

Regardless of the colostrum feeding protocol, calves lost weight during the second week of life (Figure 3), and this may be explained by the higher incidence of diarrhea during this period (Figure 5). Feed efficiency increased during the evaluation period; however, it was highest at the eighth week of age, certainly because of the higher concentrate intake associated to the increased animal performance. A higher performance was expected for calves fed higher Ig doses; however, it is possible that this advantage would be observed only in older ages, since according to the literature, feeding higher doses of Ig may positively influence the future milk production of these animals (DeNise et al., 1989; Faber et al., 2005; Szewczuk et al., 2011).

Ježek et al. (2011) reported higher values of hematocrit for calves up to the eighth week of life. However, values observed in the present study agree with the reference range cited by the authors, from 18 to 41% for animals during pre-weaning phase. Total serum protein concentration during the liquid feeding period was slightly lower than suggested by the literature, 6.0 and 8.5 g/dL (Luca and Reis, 2002). According to theses authors, TSP concentrations lower than 6.0 g/dL are indicative of protein intake deficiency, while higher values indicate dehydration or a chronic disease. Even though calves in the present study did not present a performance reduction, decreasing values of TSP were observed from birth to the second week, stabilizing until the sixth week, and then increased until the end of the study (Figure 4). This pattern is in agreement with the immunity window, when the calf loses its maternal antibodies acquired from the colostrum intake, and the immune system starts to respond to challenges (Hulbert and Moisá, 2016).

Results of albumin concentration corroborate those of Tothova et al. (2015), who observed decreasing concentrations of albumin during the first week, followed by increasing concentrations until 30 days of age, when experimental period ended. Glucose concentrations were expected to decrease and those of BHBA to increase as starter intake increases and rumen develops. However, glucose presented the

10

Passive immune transfer, health, pre-weaning performance, and metabolism of dairy calves fed a colostrum...
De Paula et al.

opposite behavior, increasing with age, suggesting a great participation of lactose from the milk replacer in maintaining higher levels of blood glucose, as also observed by Quigley et al. (1991). On the other hand, increasing BHBA concentration, mainly around the fifth week, suggests that calves had their rumen partially developed, allowing weaning without performance damage. According to Quigley et al. (1991), BHBA is a good predictor of rumen development as it is directly correlated to starter intake and the metabolism of butyrate by rumen epithelium.

In a survey about diarrhea incidence, Meganck et al. (2015) described lower frequency and shorter duration of diarrhea than observed in the present study. However, in agreement with this study, these authors also reported no significant differences in the percentage of diarrheic calves, regardless of the colostrum quality they fed.

Rectal temperature in all treatments was within normal values, and according to Rauprich et al. (2000), these increases in rectal temperature may be a sign of increased vitality and metabolic activity of newborn calves. Even though there were no detectable signs of passive immune transfer failure, calves fed medium-quality colostrum, associated or not with the colostrum supplement, needed more veterinary treatments than calves fed high-quality colostrum, but this did not affect performance.

Higher mortality rates were expected for calves fed medium-quality colostrum. Nevertheless, as shown by the TSP values, the Ig doses offered guaranteed the adequate passive immune transfer. Mee et al. (1996) described no differences on the diarrhea incidence or mortality rate of calves fed maternal colostrum associated or not with colostrum supplement. However, mortality rates presented by those authors were higher than what we observed in the current study, especially because of dehydration caused by diarrhea.

Conclusions

Feeding colostrum supplement associated with medium-quality colostrum has no effect on passive immune transfer, performance, nor metabolism during the liquid-feeding phase. Colostrum supplement may be used when high-quality colostrum is not available and medium-quality colostrum does not guarantee the minimal Ig dose for the newborn calf.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: M.R. De Paula, N.B. Rocha and C.M.M. Bittar. Data curation: M.R. De Paula, N.B. Rocha and C.M.M. Bittar. Formal analysis: C.M.M. Bittar. Funding acquisition: C.M.M. Bittar. Investigation: M.R. De Paula, N.B. Rocha, E. Miqueo, F.L.M. Silva, M.G. Coelho and C.M.M. Bittar. Methodology: N.B. Rocha, F.L.M. Silva and C.M.M. Bittar. Project administration: M.R. De Paula, N.B. Rocha, E. Miqueo, F.L.M. Silva, M.G. Coelho and C.M.M. Bittar. Supervision: C.M.M. Bittar. Writing-original draft: M.R. De Paula. Writing-review & editing: E. Miqueo and C.M.M. Bittar.

Acknowledgments

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11

Passive immune transfer, health, pre-weaning performance, and metabolism of dairy calves fed a colostrum...
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12

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