

Passive transfer of immunity in dairy calves with additional consumption of immunoglobulin through colostrum supplement: effects in health and performance

Saúde, desempenho e transferência de imunidade passiva em bezerras com consumo adicional de imunoglobulinas através de suplemento de colostro

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SUMMARY

The aim of this study was to evaluate the effects of additional consumption of immuneglobulins associated with high colostrum in health performance of dairy calves. After birth, 80 females were randomly assigned to two treatments: MC) high quality colostrum, supplied in a volume corresponding to 15% of birth weight; and MC+S) high quality colostrum (15% of birth weight) + colostrum supplement. Blood parameters were not affected by the additional supply of immune globulins (Ig) by colostrum supplement feeding. The concentrate and total dry matter intakes were not affected by administration of colostrum supplementation. The additional intake of Ig through the colostrum supplement did not affect the performance or feed efficiency of calves; however, animals doubled their birth weight at the end of the period, as occurred with MC fed calves. Regarding the health, additional supply of Ig did not affect the number of days with diarrhea, days with fever or medicated days. The additional intake of Ig via colostrum supplement in combination with high quality maternal colostrum have not improved immunity or affected performance and health of dairy calves.

Keywords: Blood parameters, Immunoglobulin Y, Weight gain

RESUMO

O objetivo do presente experimento foi avaliar o efeito do consumo adicional de imunoglobulinas através do fornecimento de suplemento de colostro associado a colostro materno de alta qualidade na transferência de imunidade passiva, saúde e desempenho de bezerras da raça Holandesa recém-nascidas. Após o nascimento, 80 fêmeas foram distribuídas aleatoriamente em dois tratamentos: (CM) colostro materno de alta qualidade, fornecido em volume correspondente a 15% do peso ao nascer (PN); e (CM+S) colostro materno de alta qualidade (15% PN) + suplemento de colostro. Os parâmetros sanguíneos não foram afetados pelo fornecimento adicional imunoglobulinas (Ig) através associação de suplemento com colostro materno. O consumo de concentrado e o consumo total de matéria seca (MS) não foram afetados pela administração do suplemento de colostro. O consumo adicional de Ig através do fornecimento de suplemento de colostro também não afetou o desempenho e a eficiência alimentar das bezerras, entretanto os animais dobraram o peso ao nascer ao final do experimento, assim como aqueles que receberam CM. Em relação à saúde, o fornecimento adicional de Ig não afetou os dias com diarréia, dias com febre ou dias medicados. O consumo adicional de Ig através do fornecimento de suplemento de colostro em associação ao colostro materno de alta qualidade não melhorou a imunidade, bem

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como não afetou o desempenho ou a saúde das bezerras.

Palavras-chave: Ganho de peso, Imunoglobulina Y, Parâmetros sanguíneos



INTRODUCTION

Bovine colostrum consists of a mixture of lacteal secretions and components of blood serum, rich in immune globulins (Ig) and other serum proteins, which are accumulate in the mammary gland during the prepartum dry period (Foley & Otterby, 1978). Dueto the absence of transplacental transfer of antibodies during gestation, the calves are born with undetectable serum levels of immune globulins and they depend on the consumption of colostrum to obtain maternal antibodies. A simple handling factor, such as providing high-quality colostrum in adequate time and volume, ensures a greater chance of survival. Research has shown that the adequate acquisition of passive immunity reduce the incidence of diseases, during a period that the animal will gradually activate its own immune system (Szewczuk et al., 2011).

supplements Colostrum considered an important management tool for raising health calves. These products were developed to be used in association with maternal colostrum in order to guarantee the passive immunity transfer in situations where high-quality colostrum is not available (Quigley et al., 2002). Some authors describe the use of supplements to strengthen calves' passive immunity and accelerate the immunity transfer process (Szewczuket 2011). In Brazil, commercial colostrum supplements are formulated from chicken eggs, rich in Ig called IgY and vaccinated for certain diseases Some authors argue that when supplementation is associated with maternal colostrum, it allows an increase in the amount of Ig consumed, providing benefits for the animals (Quezada-Tristan et al., 2014).

Provide higher doses of Ig for newborn calves has a direct impact on their

immunity system, such as higher levels of circulating Ig, reducing the risk of failure in the passive immunity transfer and consequently lower morbidity and mortality rates (Quigley et al., 2005). Research has shown the benefits of this additional Ig dose, either through higher volumes of colostrum or with supplements (Faber et al., 2005: Ouezada-Tristán et al., 2014).

The aim of this study was to evaluate the effect of the additional consumption of immune globulins through the feeding of a colostrum supplement associated with high quality maternal colostrum in the transfer of passive immunity, health and performance of newborn Holstein calves.

MATERIAL AND METHODS

The study was conducted under the protocol of the Animal Research Ethics Committee of the "Luiz de Queiroz" College Agriculture (Protocol of number 2014-18). The experiment was carried out at Fazenda Colorado, Araras, SP, Brazil, from October 2013 to February 2014. During this period, the average temperature was 25.4°C and the average air humidity was 71.1 %, according to data collected on the area. Eighty newborn Holstein female calves (birth weight = 39,83kg) were randomly distributed in one of the following treatments 1) high quality maternal colostrum fed in volume corresponding to 15% birth weight; and 2) high quality maternal colostrum (15% birthweight) + colostrum supplement (Feedtech Colostrum Supplement, DeLaval. Jaguariúna, SP, Brazil). The colostrum supplement consists of 60% soybean oil, 20% whole chicken egg powder (Salmonella Free), dextrose, vitamins and minerals, with 11% crude protein; 81% ethereal extract; 0.02% crude fiber and 5.84% ashes.



All births were supervised and all the calves were separated from the cows immediately after calving, avoiding the uncontrolled consumption of colostrum. The cows were milked and the colostrum quality was measured using a colostrometer, a manual refractometer (Sper Scientific - Model 300001) and a digital brix refractometer (Hanna Instruments - Model HI 96811). The quality of colostrum was first measured with the colostrotrometer and then with the optical and digital always refractometers. with the colostrum at the same temperature (25°C). Colostrum was only fed to the calf if its quality was greater than 50 mg Ig / mL and 21% brix. When the produced by the colostrum cow exceeded the volume for the calf, exceeding volume was frozen in a colostrum bank for supply in cases of low colostrum production or inadequate quality.

At birth, the females were identified and the navel disinfected with 7% iodine solution. Calves were weighed in an electronic scale and received colostrum in the volume corresponding to 15% birth weight, within the first 12 hours, in two meals. In the case of calves that received the colostrum supplement, the product was supplied twice, 15 mL in the first meal and 15 mL in the second meal, orally, within the 12-hour period. After this period, the females continued to receive transitional milk until they completed 48 hours, when they started receiving milk.

Blood samples were collected through jugular venipuncture by vacuum tubes without anticoagulant (Z Serum Sep. Clot Activator VACUETTE Brazil, Campinas, SP, Brazil) at 12 and 24 hours after colostrum feeding. Samples were centrifuged at 2.000 x g, for 20 min to obtain the serum, which was used to evaluate serum protein using a

protein refractometer (Instrutemp -Model ITREF 200). In addition, the excess serum was stored in plastic tubes and stored in a freezer for further analysis of total protein, albumin, γglutamyltransferase (y-GT) and alkaline phosphatase. The determination of the blood parameters was performed using (LABTEST commercial kits Diagnóstica S.A., Lagoa Santa, MG, Brazil), in Automatic System for Biochemistry _ SBA-200 (CELM, Barueri, SP, Brazil). Total protein concentrations were determined from the enzymatic kit PROTEINAS TOTAIS - Ref .: 99, by end-point spectrophotometry, with an absorbance filter of 540 nm. The albumin concentrations were determined from the enzymatic kit ALBUMIN - Ref .: 19. means of end-point bv spectrophotometry, with absorbance filter of 630nm. For the determination the concentrations of the glutamyltransferase enzyme (y-GT), the GAMA kit Liquiform - Ref .: 105 was used, using end-point spectrophotometry, with an absorbance filter of 405nm. Alkaline phosphatase concentrations were determined using enzymatic kit **FOSFATASE** ALCALINA Liquiform - Ref .: 40, by means of end-point spectrophotometry, with an absorbance filter of 590 nm. After the colostrum-feeding period (48 h), female calves were housed in individual suspended cages with free access to water and starter concentrate.

The animals received six liters of pasteurized milk daily from the farm, divided into two meals (7:00 a.m. and 3:00 p.m.). The calves received commercial concentrated feed (Rumileite, Guabi Animal Nutrition), containing 23% CP, 3.5% EE, 21% NDF, and the leftovers of the previous day were measured to establish their daily consumption of starter



concentrate. Weekly, the calves were measured with a scale weight flexible tape for performance estimation (Heinrichs & Hargrove, 1987), and at 30 and 60 days they were weighed in an electronic scale.

Fecal score evaluation was performed daily as described by Larson et al. (1977), the feces were classified according to their fluidity (1, normal, 2: soft, 3: aqueous, 4: fluid). Cases of diarrhea were considered when the animals had a score > 2. In addition, all animals were evaluated for clinical signs of diseases, especially those related to bovine respiratory diseases (BRD) and diarrhea. In the case of the development of any disease, the animals treated following were recommendations of the veterinarian responsible for the herd. All cases and treatments were recorded. Days with diarrhea were described as the number of days that the animals had a fecal score > 2; days with fever when the animal had temperature was > 39.6°C, as a cut-point suggested by the farm veterinary to treat animals; and days medicated as the time when antibiotics were administered.

The experimental design was a randomized block. All data were

analyzed as repeated measures by the MIXED of SAS (version 9.4, SAS Institute Inc., Cary, NC), according to the model (1). For the analyzes, among the 15 different covariance structures tested, the one that best fit the statistical model was chosen based on the lowest value corrected of the Akaike information criterion (AICC) (Wang & Goonewardene, 2004). For the purposes of comparison of means, the least squares method (LSMEANS) was used, with a significance level of 5%.

RESULTS AND DISCUSSION

Total serum protein concentrations, determined by refractometer or enzyme kit, were not affected by the additional supply of Ig through supplementation of maternal colostrum (P> 0.05; Table 1). On the other hand, there was a significant difference (P <0.0001) in the evaluated time points. The values observed after 24 hours of colostrum feeding were significantly higher than the values observed after 12 hours (Table 1), showing that the Ig absorption continued even after 12 hours of colostrum feeding exclusively associated with colostrum supplement.



Table 1 - Serum concentrations of passive immunity indicators in calves receiving maternal colostrum (MC) or maternal colostrum associated with colostrum supplement (MC + S)

supprement (We + 5)	Treatments					
	MC	MC+S	SEM	P-value		
Total protein, g/dl, refractometer						
12 hours	5.49^{B}	5.63 ^B	0.18	0.5907		
24 hours	6.69 ^A	6.58^{A}	0.15	0.6031		
Average	6.09	6.10	0.14	0.9399		
Total protein, g/dl						
12 hours	6.28^{B}	6.55^{B}	0.33	0.5659		
24 hours	7.50^{A}	7.15 ^A	0.22	0.2462		
Average	6.90	6.85	0.21	0.8647		
Albumin, g/dl						
12 hours	2.02	2.18	0.08	0.1467		
24 hours	2.19	2.17	0.03	0.5492		
Average	2.11	2.18	0.04	0.2821		
γglutamyltransferase, u/l						
12 hours	858.7	799.3	91.8	0.6492		
24 hours	868.9	896.7	62.9	0.7569		
Average	863.8	848.0	63.3	0.8605		
Alkalinephosphatase, u/l						
12 hours	417.2 ^A	503.7 ^A	49.8	0.2220		
24 hours	312.5^{B}	395.5 ^B	41.9	0.1650		
Average	364.9	449.6	41.9	0.1565		

SEM: Standard error of the mean.

The values reached within 12 hours of colostrum feeding would be enough to guarantee the transfer of immunity to the animals, since McGuirk & Collins (2004) considered values of serum protein higher than 5.5 g / dL as a successful transfer of passive immunity. Williams et al. (2014) fed additional Ig (172 vs 256 g) to calves through higher volume of colostrum (2 vs 4L) and observed that the animals, which ingested lower volume of colostrum and consequently lower amount of Ig, had significantly lower values of serum protein. However, this did not affect the health of the animals, since the amount offered exceeded the 100g of IgG recommended in the literature.

In a study providing additional Ig through a colostrum supplement associated with high-quality colostrum, contrasting the present study, Quezada-Tristán et al. (2014) observed higher values of total serum protein for calves received maternal colostrum associated with Ig from chicken eggs. The authors suggest that the IgY from the yolks was responsible for the higher values of serum protein. In addition, there is a positive correlation between protein total and serum IgG concentrations. According authors, the supply of this additional Ig was beneficial when fed with colostrum. Serum albumin concentrations were not affected the colostrums by supplementation (P = 0.2821) and were

A, B Different letters indicate statistical difference (P<0.0001).



not altered by the time of data collection after colostrum feeding (P> 0.05; Table 1). The values observed for albumin concentration agrees with the values reported by Mohri et al. (2007) for calves at this stage. According to the authors, these concentrations increase up to 3 months of age, when they stabilize and are similar to those observed in adult animals. Rauprich et al. (2000) suggested that serum albumin tends to decrease within the first 8 hours of life and from this period, it begins to increase. being correlated with colostrum supply and liver synthesis.

concentration The of glutamyltransferase (y-GT) were not affected by the additional supply of Ig via supplement (P = 0.8605; Table 1) and were not affected by collection time after colostrum delivery (P> 0.05). The lack in differences between 12 and 24h may have occurred as a response to the second colostrum meal. The concentration of y-glutamyltransferase correlates with the $(\gamma - GT)$ presented by Feitosa et al. (2001), who reported values of 840.55 U / L for calves with 24 hours after birth. Animals with 24 hours of life should present y-GT values higher than 200 U / L and animals with values lower than 50 U / L, within the first two weeks of age, are considered with failure in the process of passive immunity transfer (Weaver et al., 2000). Blum & Hammon (2000)reported that γ-GT concentrations in colostrum are higher than 30,000 U / L. Rauprich et al. (2000) report that the activity of this enzyme increases after the first meal in response to the colostrum absorption of y-GT and tends to decrease from the second to the seventh day of calves'

life. However, it is not known yet the importance of this post- prandial increase (Blum & Hammon, 2000). Weaver et al. (2000) reported that serum γ -GT can be used to confirm colostrum ingestion but does not allow an accurate assessment of serum IgG concentration in animals.

The supply of additional Ig through the association of maternal colostrum and colostrum supplement did not affect Alkaline phosphatase concentrations (P = 0.1565). However, there was an effect of the evaluation period (Table 1). The enzymatic concentrations significantly lower (P <0.05) at 24 hours of life, demonstrating that at this time the concentrations were already decreasing. Alkaline phosphatase as well as γ-GT can be used to estimate passive immunity transfer, although it has been less studied (Zanker et al., 2001). Mohri et al. (2007) also reported lower rates up to 28 days of life, when a new increase in concentrations occurs due endogenous production. to According to the authors, this enhance in the first day of life is related to the absorption of colostrum, which has concentrations of this enzyme higher than 1,000 U / L (Blum & Hammon, 2000), raging according to the amount and time of ingestion.

Starter intake and total intake were not affected by administration of colostrum supplement (P> 0.05; Table 2), but presented an age effect in both parameters (P <0.0001). Starter intake increased over the weeks (Figure 1), but at the end of the study, the calves were not consuming the adequate amounts of feed recommended by the literature to be weaned.



Table 2 - Consumption and performance of calves receiving maternal colostrum (MC) or maternal colostrum associated with colostrum supplement (MC + S)

	Treatments			P-value		
	MC	MC+S	SEM	T	A	TxA
Intake, g MS/d						
Starter intake	249.9	269.3	21.9	0.5325	0.0001	0.0842
Total (1)	999.9	1019.3	21.9	0.5325	0.0001	0.0842
Bodyweight, kg						
At birth	40.3	39.3	1.34	0.6069	-	-
At 28 days	57.0	55.9	1.34	0.5770	-	-
At 56 days	81.6	81.9	1.34	0.8938	-	-
Average total period	59.6	59.0	1.24	0.7359	0.0001	0.4820
Weightgain						
0 to 28d, g/d	556.1	553.4	27.5	0.9454	-	-
28 to 56d, g/d	839.0	886.0	27.5	0.2286	-	-
0 to 56d, g/d	697.6	719.7	22.1	0.4799	0.0001	0.2868
Heart girth gain, cm	89.1	89.1	0.61	0.9447	0.0001	0.3769
Hip width gain, cm	26.6	26.7	0.10	0.8903	0.0001	0.9554
Kg of gain/kg MS intake	0.833	0.861	0.02	0.3497	0.0001	0.2182

T: treatment effect, A: age effect, TxA: interaction Treatment x age.

¹ Considering whole milk with 12.5% of solids.

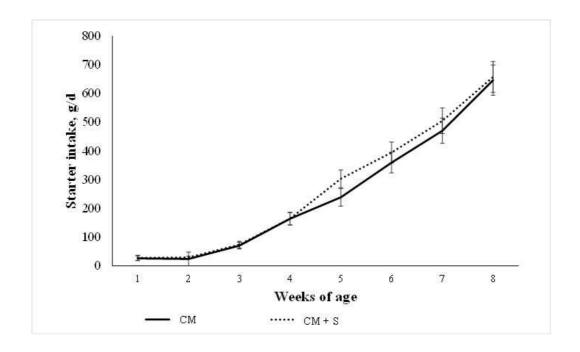


Figure 1 - Starter intake (g DM / d) of calves receiving maternal colostrum or maternal colostrum + colostrum supplement

SEM: Standard error of the mean.



A calf is prepared to be weaned when it is consuming at least 1 kg of starter concentrate per day (Stamey et al., 2012). However, in the commercial farm where the present study occurred, the weaning process occurred around tenth week of calves' guaranteeing enough time for animals to increase their consumption. Quigley (1997) & colostrum supplement associated with high-quality maternal colostrum and followed the performance of dairy calves up to their 56 days of life, receiving 4L / d of liquid diet. The authors observed that the additional Ig, similar to what occurred in the present study. did not affect animals' consumption. However, the authors reported an average intake of 400 g / day, levels higher to those reported in the present study. This factor can be explained by the lower amount of liquid diet ingested by the calves. Still, according to the authors, the total dry matter intake did not differ between treatments and was on average 917g / d. The additional consumption of Ig through the colostrum supplement also did not affect the average body weight (P = 0.7359) or the average daily weight gain (P = 0.4799). There was an age effect (P <0.0001) for these two parameters, with values increasing over the weeks (Table 2). The animals started the experiment with an average body weight of 40 kg and finish the experiment, at 56 days, with body weight higher than 80 kg, which means that the animals doubled their birth weight in this period, as recommended in the literature (Van Amburgh & Drackley, 2005). Animals receiving

maternal colostrum or an additional Ig associated with maternal colostrum, presented similar gains in the present study (Szewczuk et al., 2011). Faber et al. (2005) reported higher weight gains and higher growth rates for heifers who received additional Ig through increased colostrum feeding. In addition, the authors observed higher milk production during the first and second lactations of these animals, in response to the increased supply of colostrum.

There was no effect of supplementation on the heart girth (P = 0.9447) and in the hip width (P = 0.8903; Table 2). Other authors also did not report differences in body growth of calves receiving 250g of IgG via maternal colostrum or colostrum substitute (Jones et al., 2004). Wilson et al. (1997) performed a survey in dairy herds and observed similar values of hip width, however the hear girth was higher than those found in the present study.

Feeding efficiency was not affected by administration of colostrum supplement in combination with high-quality colostrum (P = 0.3449; Table 2). Jones et al. (2004) observed higher feed efficiency and a tendency to gain more weight during the first weeks of life in calves receiving 250g of IgG via maternal colostrum when compared to animals receiving the same dose via colostrum substitute. However, no difference was observed when the animals were weaned.

In the evaluation of the fecal score, the occurrence of diarrhea was considered when the score was higher than 2, which occurred only during the second week of calves' life (Figure 2).

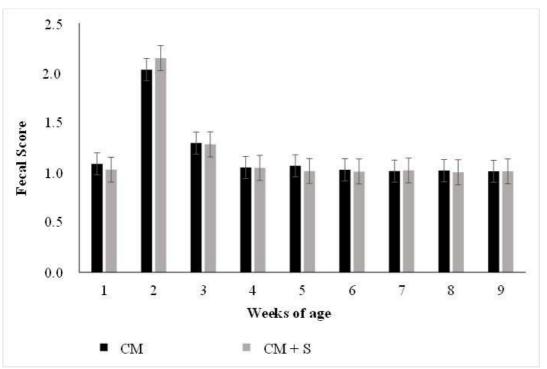


Figure 2 - Fecal score of calves that received maternal colostrum or maternal colostrum + colostrum supplement

The fecal score was not affected (P = 0.8398) by supplying the colostrum supplement in high-quality association with additional Ig supply did not affect days with diarrhea (P = 0.4907), days with fever (P = 0.2446) or days medicated (P= 0.9567; Table 3).It was expected that the additional supply of Ig would improve the animals' immunity system and would reduce cases of diarrhea. However, the fecal score was not affected by supplying the colostrum supplement in association with highquality colostrum. Meganck et al. (2015) provided colostrum of different qualities for calves in order to obtain an

colostrum. Regarding average intake of 200 g of IgG in the first 12 hours of life and did not observe differences in the percentage of calves with diarrhea. Williams et al. (2014) provided additional Ig to calves through higher volume of colostrum (2 vs 4L, 172 vs 256g Ig) and observed no difference between treatments in the occurrence of diseases, such as diarrhea and pneumonia. However, they suggest that the data should be observed with caution, since there are many external factors during the calves' rearing.

Table 3 - Health parameters of calves receiving maternal colostrum (MC) or maternal colostrum + colostrum supplement (MC + S)

	Treatm	Treatments					
	MC	MC+S	SEM	P value			
Health							
Dayswith diarrhea	2.80	3.07	0.28	0.4907			
Dayswithfever	0.93	0.62	0.19	0.2446			
Daysofmedication	2.80	2.85	0.60	0.9567			



SEM: Standard error of the mean.

In both treatments, 97% of the animals became ill for at least one day. Twenty calves that became ill from maternal colostrum group received medication, which represents that 50% of the calves needed to be treated. In the maternal colostrum group associated colostrum supplement, 43.5% of the animals were medicated. Szewczuk et al. (2011) reported that 15% of the animals that received only high-quality colostrum presented maternal respiratory diseases, whereas no case were observed in the group that received high-quality maternal colostrum associated with colostrum supplement.

In the present study, the additional supply of Ig occurred during the time that the Ig absorption is still present, however, studies indicates that benefits from additional supply may occur after this period. Vega et al. (2011) provided supplement egg-based vaccinated hens containing IgY during 14 days, after feeding colostrum for the calves. The authors reported that 20% of the supplemented animals presented diarrhea, compared to 100% of the group that did not received the supplementation. According authors, this additional Ig, besides improving the weight gain of the animals, protected them during the first 14 days of life, the critical phase of higher incidence of diarrhea. However, this action of prolonged supply is only local, acting directly on the calves' intestines, whereas the feeding of higher doses in the first hours has the main function to increase the systemic immunity.

Swan et al. (2007) reported 1.7 days of medication use for animals receiving maternal colostrum and 2.0 days of medication use for animals receiving

colostrum substitute, similar to the one found in the present study. Already, Santoro et al. (2004) reported an average of 6 days of medication for animals receiving maternal colostrum and 11 days of antimicrobial use for animals receiving colostrum supplementation.

Both groups had a 2.5% mortality rate, values lower than those were observed by Quigley et al. (2000), with rates of 16.7% mortality in animals receiving different doses of IgG from maternal colostrum (156g of IgG) or colostrum substitute (90g of IgG). Arthington et al. (2000) tested a colostrum substitute (90g of IgG) in comparison to a maternal colostrum (100g of IgG) and the authors observed mortality rates above 11% in all treatments, values higher than those found in the present study. However, the percentage of animals ill reported by some researchers (Arthington et al., 2000; Swan et al., 2007) are lower than those described in this present study, affecting less than 60% of the calves.

FINAL CONSIDERATION

The additional consumption of Ig via colostrum supplement in combination with high-quality maternal colostrum did not improve calves' performance or health. The consumption of maternal colostrum with high concentrations of Ig, at the rate of 15% of the newborn's weight right after birth, was enough to ensure a satisfactory passive immunity transfer, with no benefits from the additional supply of Ig via colostrum supplement.

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