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ORGINAL ARTICLE

Evaluation of the transfer of immunoglobulin from colostrum anaerobic fermentation (colostrum silage) to newborn calves

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ABSTRACT

Colostrum silage is an anaerobic fermentation methodology of excess farm colostrum used to conserve and provide as milk replacement for calves. The present study aimed to evaluate the levels of immunoglobulins present in bovine colostrum silage and its absorption by newborn calves. The concentration of immunoglobulins was determined in fresh colostrum and colostrum silage stored for 12 months. The absorption of immunoglobulins by calves was assessed immediately after birth and 24 h after colostrum silage intake. The immunoglobulin levels were evaluated by ELISA. The results highlighted that colostrum silage kept similar levels of immunoglobulins as the ones in colostrum *in natura*, and can be transferred to newborn calves with similar amounts to calves fed with colostrum *in natura*. It is concluded that colostrum silage keeps viable immunoglobulins, and is able to transfer passive immunity to newborn calves.

Key words: *calves, colostrum, dairy cows, immunoglobulins, newborn.*

INTRODUCTION

Colostrum contains components produced by the mammary glands and elements from the bloodstream, specially immunoglobulins (Foley *et al*. 1978; Morrill *et al*. 2012). Colostrum is produced within 5 to 7 days after giving birth. Its content is similar to blood, but it differs significantly from milk (Kehoe *et al*. 2007; Saalfeld *et al*. 2013). Colostrum plays an important role of supplying immune protection and appropriate food to the newborn calf (Ehrlich 1892; Howe 1921; Godden 2009). According to Blum and Hammon (2000) colostrum is the only source to transfer antibodies, thus being responsible for newborn protection and modulation of its immune response. Colostrum immunoglobulin absorption occurs in the neonatal intestinal epithelium in an active process in which the immunoglobulins are transferred by the enterocytes until they reach the basal membrane (Quigley 2004).

Immunoglobulin serum levels in calves might be affected by factors such as the time from birth to the first ingestion and the concentration of ingested immunoglobulins (Morin *et al*. 1997). Besides this, the passive immunity deriving from the colostrum reaches a peak of serum concentration between 12 and 48 h. After this period it tends to decrease due to catabolism (Oyeniyi & Hunter 1978; Pauletti *et al*. 2005).

Colostrum management is one of the most important management factors to determine the health and survival of calves. In case of death or communicable diseases from the mother, there is the need to keep a colostrum bank at farms to ensure the transfer of immunity to the calves.

Colostrum preservation has been studied since the mid-1940s, using colostrum immunological and nutritional potential (Allen 1944). One of the possibilities is refrigeration or freezing (Klobasa *et al*. 1988; Morrill *et al*. 2012); however, the costs are high or even unfeasible in certain regions due to the need for storage equipment (Foley *et al*. 1978). Natural acidification, with and without preservatives, makes the colostrum preservation feasible for only 28 days (Foley *et al*. 1978; Gonzales *et al*. 1978).

Contributing to a better quality and longer colostrum storage period, researchers have used anaerobic fermentation for the colostrum preservation

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(colostrum silage). This preservation allows stored colostrum for periods more than 24 months (Saalfeld *et al*. 2013). Colostrum silage does not need refrigeration, freezing or additives, reinforcing its low elaboration cost. Studies showed that colostrum silage is a feasible food for use as a milk replacement (Saalfeld *et al*. 2013). However, immunoglobulin concentration data in this food has not been established yet.

Thus, the present work aimed to study the levels of immunoglobulins present in bovine colostrum silage and its absorption by calves.

MATERIALS AND METHODS

Experiment Location

The experiments were carried out at the Farmers Training Center in Canguçu (CETAC) from EMATER-RS/Brazil. The laboratory analyses were performed at the Bacteriology Laboratory at the Technological Development Center, Biotechnology Department of UFPel. The project was approved at the Animal Experimentation Ethics Committee at UFPel under the number 23110.009613/2011–2021 CEEA 9613.

Experiment 1

Immunoglobulin quantification in cow serum, colostrum and colostrum silage

In this experiment five Holstein cows were used. Blood samples were collected immediately after the birth through jugular vein puncture using a needle (25 × 8) and BD Vacutainer® tubes (BD, Plymouth, UK). After clot retraction serum was centrifuged at 3000 × *g* for 10 min, it was separated and kept at −20°C and then analyzed by indirect ELISA described below.

Then, colostrum from the first and third milking was collected from each cow. The sample was divided for evaluation *in natura* and for silage production. *In natura* samples were stored in 100 mL bottles at −20°C until evaluation. The silage production was prepared in 500 mL polyethylene terphthalate bottles, stored for 12 months (Saalfeld *et al*. 2013). The immunoglobulin evaluation was performed by indirect ELISA described below.

Experiment 2

In vivo *transfer of immunoglobulins present in colostrum silage*

To assess the immunoglobulin absorption level by the calves, the experiment was carried out with two groups (colostrum silage group and control group). Absorption rate means: the optical density (OD) obtained from the assessment of immunoglobulins in colostrum or colostrum silage divided by the OD obtained in the serum of the animals after the administration of colostrum.

Colostrum silage group (CSG): calves, females ($n = 7$) being fed with colostrum silage. A colostrum silage sample used in the feeding of calves was sent for immunoglobulin assessment. Immediately after birth, a blood sample from the calves was collected through jugular vein puncture using a needle (25 × 8) and BD Vacutainer® tubes. After clot retraction, serum was centrifuged at 3000 × *g* for 10 min, separated and kept at −20°C to then be analyzed by the ELISA method. Then calves were fed with 500 mL of colostrum silage supplied without dilution (warmed in a water bath at 37°C), and then the next hours the calves were fed with 4 L of colostrum silage divided into two amounts of 2 L each, within an 9-h interval. After 24 h of age, a second blood sample was collected.

Control group (CG): calves, females ($n = 7$) suckling colostrum from the cow. Immediately after birth, blood from the calves was collected the same as describe above. The calves remained with the cow for 24 h suckling *ad libidum*. After 24 h of age, a second blood sample was collected from the calves as mentioned above.

Indirect ELISA

Colostrum *in natura* and colostrum silage samples was centrifuged at 8000 × *g* for 10 min for pellet precipitation. A BD 40 mm x 12 mm needle was introduced through the fat and serum was taken for assessment by ELISA. All samples were performed in duplicate.

The assessment of antibodies present in the samples was carried out using the indirect ELISA method, using as antigen a sample of *Escherichia coli* K99 from the Immunology Laboratory of the Technological Development Center, Biotechnology department from UFPel.

Plates (NUNC) with 96 cavities were used to carry out the tests. *E. coli* 2×10^8 colony-forming units/mL were suspended in carbonate-bicarbonate buffer of pH 9.6 (1/50). The plates were sensitized with 100 µL of antigen samples and incubated at 37°C for 90 min. Then, they were washed three times in phosphate buffered saline with Tween (PBST) pH 7.6 and 100 µL (1/40) of test serum was added in each well, and incubated at 37°C for 60 min. After that, it was washed three times with PBST, and the anti-bovine conjugate was added (Sigma, Jerusalem, Israel) (1/4000) and once again incubation at 37°C for 90 min was performed. Washing was done five more times with PBST, with the addition of chromogenic substrate (SIGMAFAST™ OPD) keeping the material in the dark for 15 min at room temperature. The reaction was stopped with sulphuric acid 1N, and the absorbances were measured in a microplate reader (MR 700 Microplate reader: Dynatech Labs, Chantilly, VA, USA) at 450 nm.

Statistical analysis

The analysis of variance (ANOVA) was used for statistical analysis, and the averages were compared according to Tukey test at 5% significance for comparison between samples of food and *t*-test to evaluate between calves; the program used was Statistics for Windows v. 6.0 (StatSoft, Tulsa, OK, USA).

RESULTS

OD values observed in the first milking colostrum silage (2.485 OD) did not differ ($P > 0.05$) in relation to the OD values found in the colostrum *in natura* (2.613 DO). On the other hand, the levels of immunoglobulins in the blood serum collected at birth were lower (2.236 OD) ($P < 0.05$) than the ones observed in the colostrum and colostrum silage. When the colostrum silage from the third milking was evaluated, an OD value of 0.390 was highlighted, 84.31% lower ($P < 0.05$) than the ones observed in the first milking colostrum silage (2.485 OD) (Fig. 1).

When assessing the immunoglobulin serum levels, both in the CG calves and in the CSG calves, no circulating antibody levels were detected at birth. However, 24 h after administration of colostrum *in natura* and colostrum silage these levels were elevated. In both groups the immunoglobulin absorption rate did not present any difference ($P > 0.05$) (Table 1).

DISCUSSION

The necessity of colostrum ingestion for bovines has been known for many years (Howe 1921), as they do not transfer immunoglobulins through the placenta (Kuralkar & Kuralkar 2010). The moment, quantity and quality of colostrum intake are fundamental for passive immunity in calves (Franklin *et al*. 2003). If it is impossible to feed colostrum *in natura*, the availability of other sources of immunoglobulins for calves is important. For a long time, the refrigeration of colostrum has been recommended to preserve it as food and as a source of antibodies (Morrill *et al.* 2012).

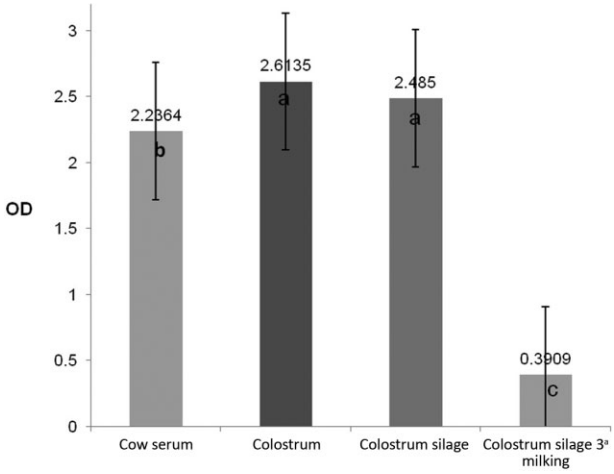

Figure 1 Optical density values observed in blood serum samples, colostrum and colostrum silage from the first and third milking after 12 months storage. Distinct averages, differing by the Tukey test ($P < 0.05$).

Recent studies have shown that colostrum anaerobic fermentation, called colostrum silage, keeps its nutritional features and it can be used as a milk replacer for calves (Saalfeld *et al*. 2013). However, so far this food has not been studied in terms of immunoglobulin levels.

In the present study, the evaluation of calves' blood serum from the CG and CSG showed that, in both groups, the animals did not present detectable levels of circulating antibodies at birth. Twenty-four hours after colostrum silage intake the absorption rate of immunoglobulins was similar to the animals which naturally had colostrum *ad libidum* from their mothers. Although Snyder *et al*. (1974) and Foley *et al*. (1978) reported differences in the absorption levels of antibodies when fermented colostrum aerobically and frozen colostrum were used, our results differ and show that the absorption level was similar. One can suggest that the anaerobic fermentation that occurs in the silage process might cause less protein degradation and by doing so keeps antibody structures intact. If this happens it might explain the different results from our studies and others; however, we did not verified this hypothesis. In this study we observed that colostrum silage kept enough immunoglobulin to transfer antibodies to the animals with no difference in the ELISA values found in colostrum *in natura* and in colostrum silage.

Hancock (1985) reported that minimal quantities of serum immunoglobulins should be passively acquired in calves in the first hours of life may vary among authors. Even though quantifying the minimum quantity of needed immunoglobulins to provide immunity to the newborn was not the focus of the present study, it can be noticed that colostrum silage could supply the needed immunoglobulins to ensure the calves' survival, as the ones in this study fed with colostrum silage were followed up for 12 months of age and did not present health problems during their development (data not shown). Thus, this food can be used to replace colostrum as a source of antibodies.

Table 1 Antibodies levels (optical density) detected in the colostrum *in natura*, colostrum silage, animal serum and immunoglobulin transfer rate in animals in the control group and colostrum silage group

Control group				Colostrum silage group			
Calves	Optical density			Calves	Optical density		
	Colostrum	Calf serum	Transfer rate		Colostrum silage	Calf serum	Transfer rate
1	2.759	2.652	1.04	1	2.631	1.399	1.88
2	2.448	1.868	1.31	2	1.384	1.06	1.30
3	2.553	1.942	1.31	3	2.126	1.996	1.06
4	2.371	1.886	1.257	4	2.632	1.292	2.03
5	2.656	2.57	1.033	5	2.394	1.363	1.75
6	2.4675	0.986	2.5	6	1.378	0.676	2.03
7	2.646	0.757	3.49	7	1.274	0.728	1.75
Average	2.557[a]	1.808[a]	1.70[a]	Average	1.974[a]	1.216[a]	1.68[a]

Distinct averages, differing by the Tukey test ($P < 0.05$).

Similar results were reported in studies from Carlson and Müller (1977) when assessing the content of immunoglobulins between the refrigerated stored colostrum and the naturally acidified colostrum. Foley *et al*. (1978) concluded that the aerobically fermented colostrum is a potential source of antibodies for newborn calves when maternal colostrum is not available. However, the small storage period of colostrum makes it more difficult to make colostrum banks. Opposing this information, Saalfeld *et al*. (2013) demonstrated that colostrum silage is practical, economical and it can be stored for period longer than 24 months. Further, in the present study it was observed that colostrum silage kept the levels of immunoglobulins making it a good option for colostrum banks.

A decline in the level of immunogblulins in cow serum at birth in relation to colostrum *in natura* detected in this study, is in agreement with the findings of Sasaki *et al*. (1976) who state that the decline in serum concentration of immunoglobulins during the pre-natal period is associated with active removal of these antibodies by the mammary gland. Later, Foley *et al*. (1978) demonstrated that during the last 3 weeks before birth, 500 to 700 g of immunoglobulins are transferred from the blood to milk secretions.

The levels of antibodies in first milking colostrum silage were higher than the ones found in the third milking. This data agrees with Morrill *et al*. (2012) who mentioned that at birth the concentration of immunoglobulins in the colostrum is at its peak and declines at each milking in the post-natal period. Pauletti *et al*. (2005) states that the average concentration of immunoglobulins in Holstein cows starts to decline between the colostrum 12 and 24 h after the birth. The results found in this experiment suggest that colostrum first milking should be chosen for preparation of colostrum silage to be used as a source of antibodies. However, colostrum silage produced with second and third milking colostrum might be the only source of immunoglobulins and food available to the calf and should be used in emergency situations.

Conclusion

This study assesses the levels of immunoglobulins in colostrum silage used as a food milk replacer fed to calves. The merit of this work was to suggest that colostrum silage keeps levels of immunoglobulins, being able to transfer passive immunity to newborn calves.

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