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Symbiotic-Glyconutrient Mixture or Pasteurized Colostrum as a Strategy to Increase Health and Performance during First 30-d of Birth of Calves Reared in a Dairy Farm with Good Management Practices

Research Article

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ABSTRACT

Eighty Holstein calves born in a dairy farm with good management practices were used to evaluate the effect of treatments on calf-starter intake, growth rate, and incidence of gastrointestinal disorders during the first 30 days of birth. Calves were fed during first 6-h of birth with 4L of with unheated (RAW) or pasteurized (PST, pasteurized during 60 min at 60 ˚C) colostrum (IgG>50 g/L), followed by supplementation with or without symbiotic glyconutrients (GLY) dosed at 5 g/calve/day in the liquid diet consisting of 4 L/d of unsaleable raw whole milk during all the study. Treatments were: 1) RAW, 2) PST, 3) RAW + GLY, and 4) PST + GLY. All calves had >5.5 g/dL of total serum protein read at 12 hours of birth. Calves were assigned to individual cages. All animals had *ad libitum* access to water and chopped high-quality alfalfa hay, from the fourth day, calf-starter was offered *ad libitum*. There was no mortality nor infectious diarrhea during the experiment. Pasteurization did not affect the quality (density, pH, and IgG concentration) of colostrum. The overall noninfectious diarrhea frequency was 53.7%. A lower diarrhea frequency was noted to calves that were fed with PST when compared to RAW treatments (45 *vs.* 62.5%) without differences in the number of times (1.05 *vs.* 1.26) needed to receive treatment to stop diarrhea. Supplemental GLY did not influence the reduction of diarrhea frequency and did not improve performance. Colostrum pasteurization increased ($P<0.05$) starter intake by 28.5%, the average daily gain by 35.8%, and live weight change of 9.6%. We concluded that offering pasteurized colostrum during the first hours of life is a positive strategy to increase starter intake and growth during the first 30 days of birth in rearing calves. On the other hand, using the mixture of symbiotic/glyconutrients as feed additive did not show any advantage when offered in this type of farm.

KEY WORDS glyconutrients, Holstein calves, pasteurized colostrum, rearing, symbiotics.

INTRODUCTION

Several published studies support that increasing health and weight rate gain in the pre-weaning period results in both short and long-term positive effects (Khan *et al.* 2007; Godden, 2008). Thus, in dairy farms, a decrease in the presence of digestive disorders and rapid consumption of solid food is one of the main concerns during the first 30 days after birth (Khan *et al.* 2016). In US dairy farms, diarrhea morbidity can be as high as 30%, with mortality rates ranging from 3 to 7% during the pre-weaning period are common (Walker *et al.* 2012; NAHMS, 2014). However, in farms with poor management practices, calf mortality can reach up to 25% (Pijoan, 1997; Moran, 2011). In problematic dairy farms, one of strategies to reduce morbidity and mortality in pre-weaning calves is the use of sub-therapeutic levels of antibiotics in milk replacer. Calves receiving antibiotics in the milk replacer experienced lower morbidity compared with those that not receiving any antibiotics (Braidwood and Henry, 1990). However, there is an increasing concern regarding this practice to the development of antibiotic resistance (Landers *et al.* 2012). The study of Berge *et al.* (2005) indicates that minimizing or eliminating the use of antibiotics in the feed requires non-antibiotic alternatives, or measures to ensure adequate passive transfer of immunity. The latter has led to the incorporation of novel strategies in the rearing stage like the use of alternative feed additives such as probiotics, prebiotics, and plant extracts, or / and, treatments including the adequate management of colostrum to improve health and growth rate. Supplementation with symbiotics products, which consists of mixtures of probiotics and prebiotics that works synergistically (Radzikowski, 2017), along with glyconutrients that enhanced cell signaling, promoting general immune responses, modulation of inflammatory responses, and a generalized reduction in cellular stress (Murray, 2006) resulted in decreased mortality rates and increased live weight gain in calves presenting infectious diarrhea (López-Valencia *et al.* 2017). Furthermore, the administration of pasteurized colostrum during the first 6-h of birth has shown positive effects on health and growth rate of calves reared in farms without appropriate health management practices (Jamaluddin *et al.* 1996). Currently, there is no available information to support the advantages of the administration of pasteurized colostrum in dairy farms that apply good management practices such as an adequate sanitation system, good management of newborns, and the availability of high quality and quantity of colostrum, considering the additional cost of those practices. Given the above arguments, we propose the present study to evaluate the use of a symbiotic/glyconutrient mixture or pasteurized colostrum and the effect in the health status and performance during the first 30-d of birth of calves reared in a dairy farm currently applying good management practices.

MATERIALS AND METHODS

All procedures and experiments followed the guidelines of approved local official guidance and protocols for welfare and good care of laboratory and farm animals under NOM (1999). The study was carried out at a commercial dairy farm located in the city of Tecate in Baja California, Mexico (32˚ 34’00’’ N 116˚ 37’59’ W). The area is 580 m above sea level and has a Mediterranean climate condition. The dairy farm has an average of 1200 births per year, applies good management practices in rearing calves, and reports an annual mortality rate of < 2% during the five consecutive years before the conduction of this study.

Animals, treatments, and sampling

Eighty newborn calves (40 females and 40 males) were used in order to evaluate the effect of colostrum pasteurization and symbiotic/glyconutrient supplementation over health status and performance during first 30-d of calves born in a dairy farm that apply good management practices.

Colostrum management

A total of 500 L of colostrum were collected into new 2.5 L plastic containers from Holstein cows during the first milking showing an immunoglobulin concentration > 50 g/L, measured with a colostrometer (Biogenics, Mapleton, OR, USA), and stored frozen at −20 ˚C until needed. Before use, frozen colostrum was thawed in a cold room at 4 ˚C for 24 h, pooled, and mixed for 20 min in a vat mixer (Zwirner Equipment, Hartsville, TN, USA). Fifty percent of the collected colostrum was transferred into new, clean 2.5 L plastic containers and frozen at −20 ˚C until needed for feeding (raw colostrum). The remaining 50% of the colostrum was pasteurized for 60 min at 60 ˚C using the Perfect Udder pasteurizer (Dairy Tech Inc., Severance, CO, USA). Once pasteurized, colostrum was cooled down, and placed in a new clean 2.5 L plastic container and stored at −20 ˚C until needed. Raw and pasteurized colostrum were heat to 40 ˚C before feeding the calves. Samples of raw and pasteurized colostrum were collected to measure pH using an Orion 261S portable pH meter (Fisher Scientific, Pittsburgh, PA, USA) and IgG concentration using the colostrometer previously described.

Calves management

After birth, calves were immediately separated from their dams, tagged, weighed, and assigned to a disinfected individual elevated cage of 100 cm wide, 200 cm long, and 50 cm high, followed by disinfection of the navel cord with a 7% iodine solution. Each animal also received a 3 mL intramuscular dose of vitamins ADE (Vigantol ADE; Bayer México) and an intranasal dose of a commercial vaccine for the prevention of infectious bovine rhinotracheitis (IBR) virus and by parainfluenza 3 (PI3) virus (TSV-2®, Zoetis México). After establishing the sex (female/male) for an even distribution of animals in all treatments, calves were fed within the first 6-h of birth with 4 L of non-heated

(RAW), or pasteurized during 60 min at 60 ˚C (PST) colostrum. Six hours after calves received full colostrum intake, individual blood samples (7 mL) were collected from each calf by jugular venipuncture using red stopper vacutainer tubes (BD, Franklin Lakes, NJ, USA) to obtain serum. Samples were centrifuged for 10 min at 3000 × *g* at 5 ˚C, and serum protein content measured using a hand-held refractometer (ATAGO, Bellevue, WA, USA).

Treatments

Calves that received raw or pasteurized colostrum, and showing > 5.5 g/dL of total serum protein at 12-h after birth were included in the experiment. Calves were blocked by sex and randomly assigned to receive a liquid diet consisting of 4 L/d of unsaleable raw whole milk, supplemented with or without the symbiotic/glyconutrients commercial additive (GLY), at 5 g/calf/day throughout experimental period. Then, calves were distributed equally in groups of 10 males and 10 females to each of the follow treatments: 1) RAW, 2) PST, 3) RAW + GLY, and 4) PST + GLY. The source of GLY, the symbiotic/glyconutrients formula used in this study, is a standardized mixture of probiotics, prebiotics, and glyconutrients distributed by Maxcell Global Co. LTD (Seoul, Korea), and contains a minimum of 1×10^7 CFU/g of each: *L. plantarum*, *B. subtilis*, and *S. cerevisiae*, 5% w/w of yeast cell wall ß-glucans extracted from *S. cerevisiae*, and 14% w/w of glyconutrients, mainly constituted by N-acetylglucosamine, D-xylose, and Fucose. A daily dose of GLY of 5 g was weighed using an Ohaus precision balance (Pine Brook, NJ, USA), and maintained in sealed bags until use. In groups that received GLY treatments, the GLY dose was mixed vigorously with the liquid diet and administered during the morning feeding. During the study, all animals had *ad libitum* access to water and high quality chopped alfalfa hay with a minimum of 21% crude protein (CP) and 42% neutral detergent fiber (NDF), tested at the Veterinary Medicine School-UABC. A commercial calf-starter (AL/AGPL, Tijuana, Mexico) contained, according to the label, 18% CP, 78% TDN, 0.70% Ca, and 0.18% P was offered *ad libitum* beginning at 4 d of age.

Sampling and data recording

Calf-starter refusals were collected daily at 07:50 h, weighed and stored to determine dry matter content according to an AOAC (2000) method. Calf-starter and alfalfa hay were supplemented separately, and calves monitored twice a day at 08:30 and 16:00 h to detect the presence of diarrhea. Diarrhea was identified through the quality of feces deposited in the floor or those attached to the perianal area of the calf. There were no cases of infectious diarrhea during the length of the experiments. When noninfectious diarrhea was detected, calves were treated twice a day with 500 mL of oral electrolytes (Electrodex®Calves, Pisa Agropecuaria, México) and 50 mL of kaolin-pectin (Neogen L.A., México). If diarrhea did not stop in 24 h, treatment was repeated until recovery. Each day that the calves received treatment was accounted for the record of the number of treatments calves needed to recover. At the end of the experiments, calves were weighed for two consecutive days using an electronic sale (TOR REY Electronics Inc., Houston TX, USA) before the morning feeding.

Growth performance

Average daily gains (ADG) were computed by subtracting the initial live weight (LW) from the final LW and dividing the result by the number of days in experiment (30-d). The gain-to feed ratio was computed by dividing ADG by the daily dry matter intake (DMI) of calf-starter (thus, forage consume was not considered). Live weight change was calculated by dividing final LW/initial LW.

Statistical analyses

Treatments were assigned completely at random to experimental units within each block according a general complete block design. Growth rate, starter intake and feed efficiency were analyzed with a linear model which includes μ as constant, τi and θj as fixed effects of treatment and block, and εijk as associated random error. As a linear model without interaction, the response variables Yijk are mutually independent and have a normal distribution with mean μ + τi and variance σ2. Calf was the experimental unit and sex the criterion of blocking. The analysis was carried out using the MIXED procedure of SAS software (SAS, 2004). Least squares means were compared using Tukey test. Diarrhea frequency and number of treatments needed to stop diarrhea between experimental treatments were analyzed with Chi-square test using FREQ procedure of SAS (2004). Significant differences among treatments were declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

The average ambient temperature recorded during the experiments was 15.8 ˚C, ranging from a higher of 23 ˚C and lower of 6 ˚C with an average rainfall of 28 mm. Physical properties and IgG concentration for supplemented raw colostrum and pasteurized colostrum are presented in Table 1. Values of pH and IgG for the two types of colostrum resulted within the range for colostrum defined as "good" (Bartier *et al.* 2015). The density of colostrum ranged from 1.038 to 1.062 with an average of 1.058. The density of colostrum may vary depending on the number of gestations or by the season of the year (McGrath *et al.* 2016).

Table 1 Physical properties and IgG concentration of raw colostrum and pasteurized colostrum used in the trial

Characteristics[1]	Colostrum	
	Raw	Pasteurized
Gravity density, g/L	1.048±5.10	1.052±2.85
pH	6.22±0.08	6.35±0.07
IgG, mg/dL	63.1±0.75	61.7±0.60

[1] Average of 12 samples.

Thus, the average density value of 1.058 observed in our study is within the expected range. Physical properties and IgG concentrations were similar in RAW and PST colostrum. McMartin *et al.* (2006) reported that colostrum can be safely heated up to 60 ˚C for as long as 120 min without producing significant changes in viscosity or native IgG concentration. The PST colostrum supplemented in this work was heated at 60 ˚C for 60 min, ensuring that the antibody capability to bind to foreign antigens remain unchanged despite the pasteurization process.

There was no mortality observed throughout the experiments, therefore, no data of mortality is presented in the Tables. It is well documented that one of the most important factors contributing to the mortality of pre-weaned calves is the failure in the transfer of passive immunity, which is associated with 39 to 50% of pre-weaned calf mortality (Margerison and Downey, 2005). On opposite, the survival rate during the first 30-d of age when calves receive a successful passive immune transfer is from 96 to 100% (Godden, 2008); thus, the absence of mortality in this experiment could be expected.

The frequency of noninfectious diarrhea cases and number of administered treatments required to stop diarrhea are shown in Table 2. There was no evidence of diarrhea caused by infectious agents during the experiments. All cases of diarrhea were minor and declared by a practicing veterinarian as "feed scours". The overall frequency for noninfectious diarrhea episodes was 53.7%. The frequency of noninfectious diarrhea during the first 30 days after birth is highly variable but, it is expected that calves occasionally present a mild diarrhea episode during feed adaptation (Abebaw *et al.* 2018). In our study, 80% of the diarrhea episodes were minor and occurred during the first two weeks after birth.

It is well known that the quality of dry feeds offered during the first stage of life contribute to diminishing the risk of scours episodes. As previously mentioned, the concentrate feed supplemented in this study was a commercially available mixture designed for the rearing stage, and the alfalfa provided to the calves was a good quality forage. Even when diarrheic calves that fed PST require a similar number (1.05 *vs.* 1.26; P=0.37) of treatments to stop diarrhea compared with RAW fed calves, PST subjects showed lower frequency of diarrhea when compared to RAW treatments (45 *vs.* 62.5%).

Armengol and Fraile (2016) report no significant differences in diarrhea morbidity and mortality rates between calves received raw or pasteurized colostrum. The authors attributed these results to the fact that they only include in their experiment groups of calves with total serum protein > 5.5, fed with high quality (IgG≥50 g/L), and quantity (4 L/day) colostrum. An early field trial showed that when colostrum is heat-treated at 60 ˚C for 60 minutes and used to fed calves, the animals experienced a significantly improved efficiency (15-25%) of absorption of colostrum antibodies and shown significantly higher serum IgG concentrations at 24 hours after birth when compared to calves fed raw colostrum (Johnson *et al.* 2007). The effect of pasteurized colostrum become of great relevance, especially in environments highly contaminated with pathogenic bacteria, were heat-treated colostrum can contribute to reducing morbidity and mortality rates under the experimental conditions such as the ones carried out in this study.

Supplemental GLY did not show an effect on the reduction of diarrhea episodes/frequency. It has been observed in broilers and pigs that a combination of a symbiotic/glyconutrients supplement decreases the occurrence of diarrhea by reducing the oxidative stress in the small intestine (Zheng *et al.* 2017a; Zheng *et al.* 2017b). Thus, it was expected that calves fed with GLY show a lower frequency of diarrhea episodes; however, as mentioned previously, the experiments include only calves with serum total protein score > 5.5 g/dL at 12 h of birth, and then were fed with high-quality (IgG≥50 g/L) colostrum and received an appropriate amount of colostrum (4 L). Therefore, the apparent benefits on diarrhea frequency observed by the GLY supplementation on previous trials are minimized, or even undetectable, in immunoprotected animals from healthy environments. The treatments effect on the growth rate, calf-starter intake and gain-to-feed ratio are shown in Table 3. Colostrum pasteurization increased (P<0.05) the starter intake by 28.5% with and average daily gain of 35.8%. The gain-to-feed ratio was 13.8% with and increased 9.6% live weigh change. Few studies have evaluated the effects of heat treatment of colostrum over the effects on gain weight and starter intake in pre-weaning phase The results of our study are similar to those of Jamaluddin *et al.* (1996), who observed an increase of weight gain in calves fed with pasteurized colostrum compared to calves fed with raw colostrum.

Table 2 Treatments effects on cases and distribution of mechanic diarrhea

Item	Treatment			
	RAW	PST	RAW + GLY	PST + GLY
Replicas	20	20	20	20
Diarrhea cases*	11	8	14	10
Frequency, %	55	40	70	50
Total treatments	15	8	16	11
Number of times needed to receive treatment to stop diarrhea	1.37	1.00	1.14	1.10

* All cases were noninfectious diarrhea.
RAW: non-heated colostrum; PST: pasteurized colostrum (60 ˚C-60 min) and GLY: synbiotic-glyconutrients mixture (dose at 5 g/calve/day).

Table 3 Treatments effects on growth rate, calf-starter intake and gain-to-feed ratio in pre-weaning dairy calves

Item	Treatment				SEM
	RAW	PST	RAW + GLY	PST + GLY	
Replicas	20	20	20	20	
Days on test	30	30	30	30	
Live weight, kg[1]					
Initial	38.830	38.220	38.826	37.910	0.72
Final	47.150[a]	51.750[b]	47.673[a]	51.100[b]	0.88
Total gain, kg	8.320[a]	13.530[b]	8.847[a]	13.190[b]	1.09
Average daily gain, kg	0.277[a]	0.451[b]	0.295[a]	0.440[b]	0.03
DM calf-starter intake, kg	0.152[a]	0.226[b]	0.164[a]	0.217[b]	0.06
Gain-to-feed (calf-starter)	1.822[a]	1.996[b]	1.798[a]	2.027[b]	0.04
Live weight change, %[2]	1.214[a]	1.354[b]	1.228[a]	1.348[b]	0.03

[1] Initial weight registered immediately after born, final weight registered before morning liquid feed at day 30.
[2] Live weight change was calculated by dividing final LW/initial LW.
RAW: non-heated colostrum; PST: pasteurized colostrum (60 ˚C-60 min) and GLY: synbiotic-glyconutrients mixture (dose at 5 g/calve/day).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

On the other hand, Elizondo-Salazar and Heinrichs (2009) report no differences on daily weight gain (0.282 *vs.* 0.274 kg/d) between raw *vs.* pasteurized colostrum fed groups during the first 28-d of birth, but the group receiving pasteurized colostrum showed a lower average daily intake of 26%, therefore, calves fed pasteurized colostrum had 24% grater feed efficiency than the raw colostrum fed group. A possible explanation for the greater weight gains or feed efficiencies observed in calves fed with pasteurized colostrum could be explained by the fact the pasteurized colostrum promotes thinner and healthier intestinal walls, promoting higher passage rate of nutrients across the gut (Johnson *et al.* 2007). Moreover, pasteurization of colostrum can potentiate the medium-term associative or nutraceutical effects of colostrum independently of the effects on immunity and nutrient absorption (Bagwe *et al.* 2015). It has been demonstrated that colostrum contains high concentrations of a number of a variety of growth factors, which allow restoration of the intestinal mucosa (Dzik *et al.* 2017).

These include the transforming growth factor (TGF-b), epidermal growth factor (EGF), and somatomedin C (IGF-1). These growth factors play essential roles in the repair and maturation of different tissues (Playford, 2001), and these nutraceutical properties can be potentiated when colostrum is properly pasteurized and administer.

This apparent potentiated nutraceutical effects when colostrum is pasteurized is a question that should be clarified in future studies.

In our experiments, the supplementation with GLY did not shown an improvement of the performance in calves fed with the different combinations of raw or pasteurized colostrum treatments. To our knowledge, there is no information available regarding the effect of symbiotic/glyconutrient on growth efficiency in healthy calves. In a previous report, the symbiotic/glyconutrients combination significantly increased live weight gain in calves presenting infectious diarrhea (López-Valencia *et al.* 2017). Similarly, a combination of symbiotic/glyconutrients supplement increased growth performance in poultry and nursery pigs by decreasing the occurrence of diarrhea (Zheng *et al.* 2017a; Zheng *et al.* 2017b). Some properties attributed to glyconutrients include anti- inflammatory and antimicrobial effects as well as promoters of cellular integrity that increase energy efficiency and health (Murray, 2006; Timmerman *et al.* 2005).

On the other hand, glycans plays important roles in the structural conformation and bioactivity of proteins, including adhesion, targeting, folding and stability (Le Parc *et al.* 2017), which facilitate protein absorption. Additionally, the combination of symbiotic with glyconutrients have been demonstrated to reduce plasma cortisol, non-esterified fatty acids and ureic N concentration, and increase plasma glucose levels in stressed calves (López-Valencia *et al*. 2017). The results obtained here, demonstrate that the positive effect of this type of additives is non-significant in non-compromised calves reared in healthy environments. The latter can explain the inconsistent responses on health and performance of calves fed with probiotics, prebiotics, glyconutrients and their combinations, since some studies shown positive effects on health and/or performance (Signorini *et al*. 2012; Satik and Gunal, 2017; López-Valencia *et al*. 2017), but others studies fail to report any positive effect (Beauchemin *et al*. 2006; Uyeno *et al*. 2015; Tóth *et al*. 2020). Therefore, the magnitude of the response to this type of additives must be associated to the health status and welfare of calves and in their general environment.

Based on our results and those of others, it is clear that offering pasteurized colostrum during the first 6-h of birth, increases feed efficiency and live weight gain, which can have a positive effect over the economic benefits of rearing by saving in feeding and reducing the time to reach the target weaning weight. It is well documented that pasteurization of colostrum requires additional cost, handling, and maintenance, and the economic benefits of the use pasteurizing of colostrum will vary from farm-to-farm depending on fixed and variable costs of the feeding program, calf health, and environmental factors. Finally, the requirements for daily use of pasteurized colostrum include the instrumentation of protocols for pasteurization, sanitation of equipment, routine equipment maintenance and monitoring of the whole system. According to data given from a farm with 1550 milking cows and average annual milk production of 10500 kg/cow and 1200 births per year, the cost for pasteurization per liter of colostrum, including fees for pasteurizer maintenance, external services, salaries, and energy cost, is equivalent to $0.03 US dollars or 0.12 US dollars/calf, while the total cost for including GLY during 29 days was 1.02 US dollars/calf or 0.035 US dollars/d..

CONCLUSION

We concluded that, under the experimental conditions of the present study, supplementation of pasteurized colostrum is a positive strategy to increase starter intake and growth during first 30 days of birth in calves reared in dairy farms with good management practices. Pasteurization of colostrum could potentiate the medium-term associative or nutraceutical effects of colostrum independently of the effects on immunity and nutrient absorption which can have a positive effect over the economic benefits of rearing by saving in feeding and reducing the time to reach the target weaning weight. On the opposite, the use of mixtures of symbiotic/glyconutrients products as feed additives did not represent any advantage in this type of farm. The positive effects of mixtures of symbiotic/glyconutrients are non-significant in non-compromised calves reared in healthy environments.

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